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# The science, development, and commercialization of postharvest biocontrol products

Samir Droby[a,*], Michael Wisniewski[b], Neus Teixidó[c], Davide Spadaro[d], M. Haissam Jijakli[e]

[a] Dept. Postharvest Science, Institute of Postharvest and Food Sciences, ARO, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel
[b] USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430, USA
[c] IRTA, XaRTA-Postharvest, Edifici FRUITCENTRE, Parc Científic i Tecnològic Agroalimentari de Lleida, 25003 Lleida, Catalonia, Spain
[d] Dept. Agricultural, Forestry and Food Sciences (DISAFA) and AGROINNOVA Centre of Competence for the Innovation in the Agroenvironmental Sector, University of Torino, Largo Braccini 2, 10095 Grugliasco (TO), Italy
[e] Integrated and Urban Plant Pathology Laboratory, Gembloux Agro-Bio Tech, ULg, Passage des Déportés, 2, 5030 Gembloux, Belgium

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ABSTRACT

Postharvest biological control agents as a viable alternative to the use of synthetic chemicals have been the focus of considerable research for the last 30 years by many scientists and several commercial companies worldwide. Several antagonists of postharvest pathogens have been identified and tested in laboratory, semi-commercial, and commercial settings and were developed into commercial products. The discovery and development of these antagonists into a product followed a paradigm in which a single antagonist isolated from one commodity was also expected to be effective on other commodities that vary in their genetic background, physiology, postharvest handling, and susceptibility to pathogens. In most cases, product development was successfully achieved but their full commercial potential was not realized. The low success rate of postharvest biocontrol products has been attributed to several problems, including difficulties in mass production and formulation of the antagonist, the physiological status of the harvested commodity and its susceptibility to specific pathogens. All these factors played a major role in the reduced and inconsistent performance of the biocontrol product when used under commercial conditions. Although many studies have been conducted on the mode of action of postharvest microbial antagonists, our understanding is still very incomplete. In this regard, a systems approach, that takes into account all the components of the biocontrol system, may represent the best approach to investigating the network of interactions that exist. Very little is known about the overall diversity and composition of microbial communities on harvested produce and how these communities vary across produce types, their function, the factors that influence the composition of the microbiota after harvest and during storage, and the distribution of individual taxa. In light of the progress made in recent years in metagenomic technologies, this technology should be used to characterize the composition of microbial communities on fruit and vegetables. Information on the dynamics and diversity of microbiota may be useful to developing a new paradigm in postharvest biocontrol that is based on constructing synthetic microbial communities that provide superior control of pathogens.



## 1. Introduction

Biological control agents, as an alternative to the use of synthetic chemicals, have been the focus of considerable research over the last 30 years by many scientists and several commercial companies worldwide. This effort has been based on the need to reduce the use of synthetic fungicides to control postharvest pathogens on harvested agricultural commodities. The withdrawal of key fungicides, development of resistance biotypes, along with environmental and health considerations have been among the drivers for developing alternative disease management technologies that are safe and effective.

The potential use of epiphytic microbial antagonists to control postharvest pathogens was first reported back in the mid-1980s (Wilson and Pusey, 1985) and was later highlighted in several

* Corresponding author.
*E-mail addresses:* samird@volcani.agri.gov.il, samirdroby@hotmail.com (S. Droby).

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reviews that offered guidelines for isolating and selecting postharvest biocontrol agents (Wilson and Wisniewski, 1989, 1994). A key rationale used to support this approach was that, in contrast to field- and soil-based biocontrol, the postharvest environment and the disease etiology was more conducive to targeting the application of an antagonist to a commodity and maintaining its population due to controlled environmental conditions. The purpose of the current review is to evaluate the paradigms that have developed in the field of postharvest biocontrol over the past 30 years and assess their validity. More specifically, this review is aimed at reviewing the progress that has been made, examining the reasons why developed products have had such limited commercial success, and reflect on future prospects and trends. The current state of the science of postharvest biological control is discussed, challenges and obstacles are identified, and the relevance of recent advances in −omics, and their potential application to postharvest biocontrol research is presented.

Numerous microbial antagonists (yeasts and bacteria) of postharvest pathogens have been identified in laboratory, semi-commercial, and commercial studies (Droby et al., 2009). Several of these antagonists reached advanced levels of development and commercialization. Among the first generation of biocontrol products registered and made commercially available were *Candida oleophila* (Aspire, Ecogen, Langhorne, PA, US) (Blachinsky et al., 2007), *Cryptococcus albidus* (YieldPlus, Lallemand, Montreal, Canada), *Candida sake* (Candifruit, IRTA, Lleida, Spain) (Teixidó et al., 2011), *Pseudomonas syringae* Van Hall (BioSave, JET Harvest, Longwood, FL, US) (Janisiewicz and Jeffers, 1997; Janisiewicz and Korsten, 2002). Aspire, Yieldplus and Candifruit were commercialized for some years but discontinued due to business and marketing-related shortcomings. Biosave, however, still has limited use in the US market for application on fruit crops (Janisiewicz and Peterson, 2004). *Bacillus subtilis* (Avogreen, University of Pretoria, Pretoria, South Africa) was introduced in South Africa for the control of *Cercospora* spot, a postharvest disease of avocado, but did not achieve commercial success due to inconsistent results (Demoz and Korsten, 2006). More recently *C. oleophila*, (Nexy, Leasafre, Lille, France) has been developed in Belgium, and was submitted for regulatory approval in 2005 for postharvest application against wound pathogens on pome fruits, citrus, and banana (Lahlali et al., 2011). Nexy received registration approval throughout the European Union in 2013 (Massart and Jijakli, 2014). *Aureobasidium pullulans* (BoniProtect, Bio-Ferm, Tulln, Austria), has a suggested use as a preharvest application to control wound pathogens that develop on pome fruit during storage (Lima et al., 2015). Another product based on *Pantoea agglomerans* CPA-2, (Pantovital, Domca, Granda, Spain) effective against the major postharvest pathogens of pome and citrus fruits (Cañamás et al., 2008; Plaza et al., 2004; Teixidó et al., 2001) was formulated but never commercialized (Torres et al., 2014). *Metschnikowia fructicola* (Shemer, Bayer, Leverkusen, Germany) registered in Israel for both pre- and postharvest application on various fruits and vegetables, including apricots, citrus fruit, grapes, peaches, peppers, strawberries, and sweet potatoes represents a more successful example of a postharvest biocontrol product. Shemer was acquired by Bayer CropScience (Germany) and then sublicensed to Koppert (Netherlands) (Spadaro and Droby, 2016).

Interestingly, the majority of reported postharvest biocontrol agents and products are yeasts. Yeasts, in general, have high tolerance to the stressful environmental conditions prevailing before and after harvest (low and high temperatures, desiccation, wide range of relative humidity, low oxygen levels, pH fluctuations, UV radiation) and are uniquely adapted to the micro-environment (high sugar concentration, high osmotic pressure, and low pH) present in wounded fruit tissues. Additionally, many yeast species can grow rapidly on inexpensive substrates in fermenters and are therefore easy to produce in large quantities (Spadaro et al., 2010a). Moreover, in contrast to filamentous fungi, they do not produce allergenic spores or mycotoxins, and have simple nutritional requirements that enable them to colonize dry surfaces for long periods of time.

## 2. The postharvest biocontrol paradigm – looking back to move forward

Research on biocontrol of postharvest diseases has mainly focused on isolating microorganisms that are antagonistic to wound pathogens that infect a commodity during harvest and subsequent handling. Typically, pathogen spores germinate very rapidly (within 24 h) and colonize wounds that are rich in sugars and other nutrients. Therefore, it is necessary to interfere with spore germination and/or germ-tube growth in a rapid time frame in order to prevent or inhibit infections.

The discovery and development of postharvest biocontrol has been mainly pursued by plant pathologists. Early investigations to identify potential biocontrol agents basically adopted the same strategy used for finding biocontrol agents against foliar and soil-borne diseases where an isolation and screening program was designed to identify single potent antagonists. Several features of an ideal antagonist were defined by Wilson and Wisniewski (1989) and have served as the basis for many other biocontrol research programs, past and present. Rapid growth and colonization of fresh wounds by the biocontrol agent was one of the main features indicated. Following this logic, Wilson et al. (1993) designed a rapid method for screening and identifying successful antagonists. Antagonists that produced secondary metabolites inhibitory to the targeted pathogens in in vitro assays were excluded based on the assumption that indications of antibiotic production would be problematic in the registration process. Another essential feature that was defined was that the level of survival and rate of growth of the biocontrol agent on intact and injured fruit surfaces had to be sufficiently great enough to prevent pathogens from becoming established. This premise, however, neglected the fact that the introduced antagonist was not the only "player" present on the harvested commodity. Additionally, very little attention was given to the impact of different postharvest treatments on the population of antagonists and other resident microflora. Interactions between the resident microflora and the antagonists, as they were individually impacted by the other postharvest treatments, were rarely studied and are therefore poorly understood.

Droby et al. (2009) raised several reservations about the relevance of the existing paradigm for identifying antagonists that are expected to perform under "real world" situations where a wide range of wounds that serve as an infection court, exist. In the current postharvest biocontrol paradigm it is expected that a single antagonist isolated from one commodity will be effective on other commodities that vary in their genetic background, physiology, postharvest handling, and pathogen susceptibility. Perhaps this expectation or paradigm is inappropriate given our knowledge of microbial ecology and plant microbiota that has been acquired through metagenomic approaches.

## 3. Constraints and shortcomings of existing biocontrol systems

Several registered postharvest biocontrol products have been developed jointly by researchers working with commercial companies. Although product development was successful, their full commercial potential, as measured by their widespread acceptance and use, has not been realized. The low success rate of postharvest biocontrol products has been attributed to several a

factor among which is inconsistent performance under commercial conditions. Efficacy of these products must be similar to that achieved by chemical fungicides, which is in the range of 98–100% disease control. This level is seldom attained with biological control products when they are used as a stand-alone treatment. Therefore, it is important to discuss the variables that are critical to product development, performance, and viability. A schematic description of a generalized pipeline for the development of postharvest biocontrol products is presented in Fig. 1.

### 3.1. Mass production and fermentation

Economical production of large quantities of a microorganism in a formulation that ensures reasonable shelf life and maintains efficacy during large-scale testing are fundamental steps in the process of developing a commercial biocontrol product. Production and formulation processes are often conducted directly or in association with private companies and all the related research and development data is typically protected under confidentiality agreements leading to a lack of scientific references on these essential subjects.

The mass production process requires two essential steps: (1) developing an economical culture medium that provides an adequate supply of nutrients and energy for cellular metabolism, growth, and population stability, and (2) optimization of growth conditions (temperature, agitation, aeration, and pH). Current commercial production methods utilize either solid- or liquid-phase fermentation. In general, liquid-phase cultures are used for bacteria and yeasts and solid-phase cultures are used for most fungi. Optimized mass production systems have been described for some postharvest biocontrol agents, including bacteria such as *P. agglomerans* CPA-2 (Costa et al., 2001), *P. agglomerans* PBC-1 (Manso et al., 2010) and *B. subtilis* CPA-8 (Yánez-Mendizábal et al., 2012b), yeasts such as *C. sake* CPA-1 (Abadias et al., 2003a,b), *A. pullulans* (Mounir et al., 2007), and *Rhodotorula minuta* (Patiño-Vera et al., 2005), and fungi such as *Penicillium frequentans* 909 (De Cal et al., 2002), and *Epicoccum nigrum* (Larena et al., 2004).

Downstream processing of cultured microorganisms involves various steps, such as cell separation from the medium, drying, addition of bulk materials (inert ingredients), adhesives, emulsifiers and adjuvants. All these actions may adversely affect the properties of the selected biocontrol agent directly or indirectly. The need of reasonable shelf life and preserving efficacy requires the stabilization of cell viability, which can be achieved by the product being made available in: (1) a liquid state requiring refrigeration; (2) a freeze-dried state that requires the use of cryoprotectant substances during preparation, and (3) dehydrated form by drying. The latter two types of formulations can then be stored at ambient temperatures.

### 3.2. Formulation

Typically, a formulated product consists of an antagonistic microorganism (the active ingredient), an inert material that serves as a carrier, and adjuvants, such as nutrients and/or compounds, that enhance the survival of the antagonist cells or help protect them from environmental stresses such as desiccation, osmotic stress, UV radiation, and either low or high temperatures. In practice, very little literature has been reported about the formulation of postharvest biocontrol agents, and often upscaling, stabilization, and the entire formulation process in general is viewed as an art rather than a science. This is unfortunate since improvements in the formulation of biocontrol products may increase their performance under commercial conditions, and significantly increase the shelf life of the product.

Different dehydration processes have been used for formulating biocontrol agents. Freeze-drying has the advantage of maintaining high cell viability but is much more costly than other drying processes. Freeze-drying has been used to prepare Biosave (*P.* syringae), *P. agglomerans* (Costa et al., 2000), *C. sake* CPA-1 (Abadias et al., 2001a, 2001b), *Cryptococcus laurentii* (Li and Tian, 2006), *M. pulcherrima* (Spadaro et al., 2010b), and *Pichia anomala* (Melin et al., 2011).

Spray-drying is another drying method that can be used to preserve biocontrol agents in a dry state and has the advantage of being able to dry large quantities of cultures in a short time and at low cost. Only a small number of microorganisms, however, are able to survive the high temperatures used in this drying process. Only biocontrol agents that are able to produce heat-resistant endospores, such as *B. subtilis* CPA-8, are suitable for spray drying

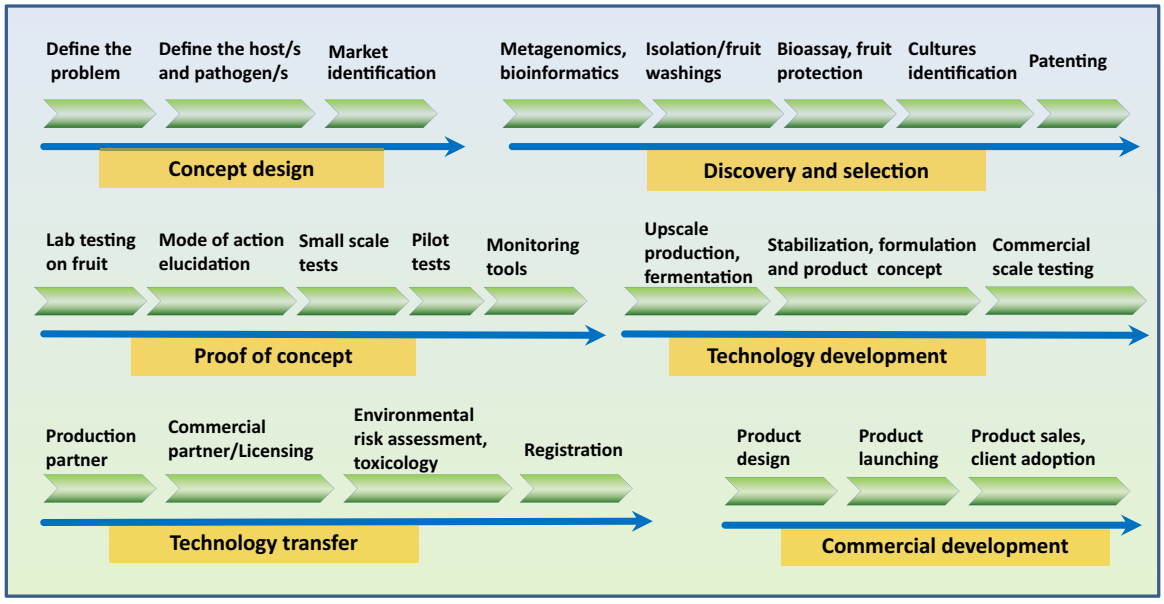


**Fig. 1.** Pipeline for development of postharvest biocontrol products.

(Yánez-Mendizábal et al., 2012a). Fluidized bed-drying is a cost-effective method of drying that can be used to dry heat-sensitive microorganisms because the drying temperatures are relatively low. Fungi such as *E. nigrum* (Larena et al., 2003) and *P. frequentans* (Guijarro et al., 2006), the yeast-like fungus, *A. pullulans* (Mounir et al., 2007), and the yeast, *C. sake* CPA-2 (Usall et al., 2009) have all been successfully dried using fluidized bed-drying. In contrast, liquid formulations are the simplest way to stabilize the viability of microbial cells. This formulation method involves storing cells in a water- or oil-based solution with different protectants and additives, typically at low temperatures. Isotonic, liquid formulations of *C. sake* CPA-1 have been reported to be a suitable alternative to solid formulations (Abadias et al., 2003b; Torres et al., 2003). Liquid formulations have also been tested with *R. minuta* (Patiño-Vera et al., 2005), *C. laurentii* (Liu et al., 2009), and *P. anomala* (Melin et al., 2011).

### 3.3. Range of activity

The narrow range of activity (hosts and pathogens) of many biocontrol agents is a serious limitation to their commercial success. In the case of postharvest biocontrol products, this problem is even more critical because the postharvest market is very limited and typically only one application of the product is used. It would be beneficial to be able to broaden the spectrum of action of these products, in terms of hosts and pathogens, and if possible extend their use to preharvest conditions. Different approaches could be used to extend the target range of a biocontrol product. For example, different preparations of the same biocontrol agent could be specifically formulated for each situation. The products BoniProtect, Blossom Protect, and Botector utilize this approach as they represent different formulations of the same biocontrol agent, *A. pullulans*. These products are specifically formulated to control postharvest diseases on pome fruit, fire blight, and *Botrytis cinerea* on grapes, respectively. Enhancing the stress tolerance of biocontrol agents has also been reported to enhance the viability of biocontrol agents during the formulation process and broaden their spectrum of action (Teixidó et al., 2011; Sui et al., 2015). In the case of *C. sake* CPA-1, it was originally developed to control postharvest diseases and later was physiologically improved to be more tolerant to osmotic stress conditions, which allowed it to be applied under field conditions and successfully control *B. cinerea* on grapes (Cañamás et al., 2011). Genetic manipulation of antagonists is also a potential approach for improving biocontrol agents and broadening their use, however, regulatory constraints and public concern about the use of genetically modified organisms (GMOs) represent a monumental hurdle to this approach.

### 3.4. Performance and consistency

Acceptable and consistent performance under commercial conditions is critical to the success of any biocontrol agent. Numerous reports have been published on various strategies and approaches that can be used to enhance the efficacy and reliability of postharvest biocontrol agents. These include combining biocontrol agents with use of salts and organic acids (Droby et al., 1997; Karabulut et al., 2001), glucose analogs (El Ghaouth et al., 2000), food additives (Droby et al., 2003; Karabulut et al., 2003; Teixidó et al., 2001), and various physical treatments (Karabulut et al., 2002; Porat et al., 2002; Zhang et al., 2008). In most cases, enhanced efficacy was demonstrated using these approaches, however, each commodity–pathogen system has its own unique features and so specific protocols will need to commercially evaluated.

## 4. An industry perspective

Concerns about food safety, including chemical residues and environmental impact, over the past twenty years have resulted in substantial regulatory changes in the use of pesticides (http://www2.epa.gov/pesticide-tolerances; http://www.ecpa.eu/page/food-safety). Regulatory restrictions are increasing on the use of a variety of chemical fungicides used to manage postharvest pathogens. Several products have been lost from the market due to the unwillingness of companies to maintain registration. Resistant biotypes of pathogens have also evolved, decreasing the efficacy of some of the existing chemicals.

In recent years, the interest of multinational chemical companies and microbial industries (such as yeast producers) in biological control technologies, including postharvest uses, has grown substantially. This is reflected in the number of acquisitions made by large, mainstream companies of small and medium sizes companies specializing in the development of green technologies for controlling plant diseases (CPM, 2010). In the case of microbial industries associated with producing yeasts for baking, brewing, and wine fermentation, interest in novel applications of their microorganisms to expanded markets is a logical extension of their business. The real question is why a multinational company would be interested in a biological control product that targets a small niche market like postharvest biocontrol. The answer is rather complex and the underlying reasons for acquiring a particular biocontrol product is difficult to determine. Given their responsibility to stakeholders, multinational chemical companies are focused on two concerns: pesticide resistance and achieving zero residues on commodities. Furthermore, they want to offer to their clients (distributors and subsequently growers) a full portfolio of existing plant protection tools, including both conventional and 'green' products.

The most difficult stage in the development of a biocontrol product is its commercialization. Commercialization is the management process that provides structure in developing and bringing a new product to market. Effective implementation of this process is needed to coordinate the gathering of information and the establishment of a project plan. The early commercialization phase is often long and fraught with a variety of difficulties, involving scientific, regulatory, business management, and marketing issues. Companies require ample information about a variety of aspects, such as market demand, market size, profit margin, and time to market, to effectively handle these issues (Bailey et al., 2009). A report published by a working group within the EU project ENDURE (Nicot et al., 2012), that was charged with analyzing the factors associated with the success of field-based biocontrol technologies against arthropod pests, diseases and weeds, stated that profit after taxes, provisions and amortization was 18% of sales for a chemical pesticide and only 2% for a biocontrol product. In the case of the postharvest market, the profit margins can be assumed to be even lower. In Europe, the size of the microbial biocontrol product market was estimated to be 52 million Euro in 2012. Currently, the biopesticide market is valued at 1.5–2.5 billion US dollars compared to 60 billion US dollars for the traditional pesticide market (http://www.research-andmarkets.com/research/7bvbnf/global_pesticide).

Fifty-two active chemical ingredients were registered in the EU between 1996 and 2000, whereas only 10 biocontrol agents were approved during the same span of time. In the past five years, however, 22 biocontrol agents were authorized in the EU and only 20 synthetic chemical pesticides. In general, there has been a significant increase in the biopesticide market worldwide, with the highest increase in Europe, which is expected to pass North America as the largest market for biocontrol products by 2018 (Anonymous, 2014). The annual worldwide annual increase in

market growth (2012–2020) is estimated to reach 12.3% for biopesticides versus 5% for chemical pesticides. Among the recently approved biocontrol products within the EU, three specifically target postharvest pathogens: *M. fructicola* strain 277 (Shemer), *A. pullulans* strains DSM 14940 and DSM 14941 (BoniProtect), and *C. oleophila* strain O (Nexy). This trend will further stimulate the development and registration of biocontrol products in Europe. Companies that have invested in these products will design marketing strategies that will increase market sales and market share in order to achieve a good profit margin. This may include adding both additional postharvest applications and/or preharvest applications registered uses for the product.

Companies may also enlarge the application of their registered product by adapting their biopesticide to new methods of application. For example, Nexy was originally developed for postharvest dipping and drenching application to fruit. In case of pome fruits, these application methods were popular when submitting the registration dossier in 2005. When the EU approval was received in 2013, however, most growers had abandoned postharvest dipping and drenching treatments in favor of preharvest treatments. Thus, nebulization of the product in fruit storage chambers could be a new postharvest method of treating pome fruits, which may require an adjustment in the formulation of the product and further education of packinghouses on how to use this method of application.

## 5. Mechanisms of action involved in biocontrol systems

Understanding the mode of action of postharvest biocontrol agents is a prerequisite for product development and registration. In general, research on postharvest yeasts and bacterial antagonists followed the traditional studies conducted on antagonists of foliar and soil borne pathogens. These studies ascribed biocontrol activity to four major modes of action: (1) competition for nutrients and space, (2) antibiotic production, (3) induction of host resistance (Droby et al., 2002), and (4) direct parasitism (Bélanger et al., 2012; Janisiewicz and Korsten, 2002). The different modes of action were recently reviewed by Liu et al. (2013) and by Spadaro and Droby (2016). Both reviews highlight important additional features of successful antagonists, including biofilm formation, quorum sensing, production of diffusible and volatile antimicrobial compounds, competition for iron, the role of oxidative stress, alleviation of oxidative damage, and the production of ROS by the host and the antagonist. Until recently, the vast majority of studies on the mode of action of either yeast or bacterial antagonists followed an approach that examined each possible mechanism separately. This approach, however, raises some critical questions: what are the effects of antagonists on wound healing and host resistance? how important and widespread are the direct effects of antagonists on pathogens? how do incidental microorganisms or mixtures of antagonists affect pathogen/antagonist interactions, and how does the nutrient/chemical composition at the wound site affect the antagonist, other microflora, the infection process, and the wound response? As initially described by Droby et al. (2009) and expanded on by Liu et al. (2013), the performance of a biocontrol agent can be seen as the result of complex mutual interactions between all the biotic (organisms) and abiotic (environmental) components of the system. Although these interactions have been the subject of postharvest biocontrol research for 30 years, our understanding is still very incomplete. When studying mechanisms of action, a systems approach should be employed to investigate the network of interactions. Such an approach, that takes into account all the components of the system, may provide the greatest understanding of biocontrol systems.

The availability of more cost-efficient, high throughput DNA/RNA and proteomic technologies, along with bioinformatics, has provided new opportunities and tools to obtain deeper insights into the mechanisms and interactions that have already been established (An et al., 2014; Kwasiborski et al., 2014). Developments in deep sequencing, transcriptomics, MS–MS proteomics, metagenomics, comparative and functional genomics can be utilized to determine changes in the physiological status of biocontrol agents, and the effect of environmental stress on its intracellular machinery (Hershkovitz et al., 2011; Hershkovitz et al., 2013; Sui et al., 2015). Changes in the level of expression of "biocontrol genes" during mass production, formulation and storage, or in response to exposure and contact with host plant tissue after application can now be more readily investigated. Massart and Jijakli (2007) reviewed the molecular techniques used to understand the mechanism of action of biocontrol agents and discussed the strategies used to study the role of various genes believed to be involved in the mechanisms of action. They concluded that the majority of studies aimed at elucidating the genetic basis and traits important for antagonistic action have focused on *Trichoderma* spp. Genes related to the production of antibiotics have been mainly studied in bacteria, such as *B. subtilis* and *Pseudomonas* spp. Very few genes involved in induction of resistance mechanisms in host plants or competition for nutrient and space have been identified in biocontrol agents. More recently, the impact of −omic technologies for understanding the various modes of action of biocontrol agents against plant pathogens was reviewed by Massart et al. (2015a,b). Whatever −omic technique (genomic, transcriptomic or proteomic) have been utilized, studies of postharvest biocontrol agents have been sparse and it is expected that greater details about interactions in the entire biocontrol system will be forthcoming.

## 6. The role of the microbiome in fruit health and disease – a new perspective

Microbial communities resident on and in plants can have negative, neutral, or beneficial effects on plant health and development (Mendes et al., 2013; Philippot et al., 2013; Berg et al., 2015). These communities colonize all parts of a plant through its entire lifecycle and marked diversity exists in communities associated with different hosts. Research on this topic is slowing moving from just describing the composition of these communities to elucidating the mechanisms involved in their assembly and function (Waldor et al., 2015).

Studies on plant microbiomes (phytobiomes) in both the phyllosphere and rhizosphere indicate that plants should be considered as "super organisms" where very diverse microbial communities provide specific functions and traits to plants (Vorholt, 2012; de Bruijn, 2013). These functions include five key features: (1) improving nutrient acquisition and growth, (2) sustaining plant growth under biotic and/or abiotic stress, (3) inducing resistance against pathogens, (4) interacting with plant or human pathogens, and (5) interacting with other trophic levels, such as insects. Soil type and plant genotype are the major parameters influencing the rhizosphere microbiome (Berg and Smalla, 2009; de Bruijn, 2013) whereas plant species and genotype are the major factors involved in defining the composition of the phyllosphere microbiome (Massart et al., 2015b). Whipps et al. (2008) published a comprehensive review of phyllosphere microbiology with special reference to microbial diversity and plant genotypes. The authors stressed the need for studies on the functional consequences of changes in microbial community structure and the mechanisms by which plants control the microbial populations on their aerial plant surfaces. The composition of microbial populations in the phyllosphere are also

influenced by environmental factors, such as, UV, humidity, temperature, geographical location (Rastogi et al., 2012; Vorholt, 2012; Rastogi et al., 2013), nitrogen fertilization (Ikeda et al., 2011), and pesticide treatments (Zhang et al., 2009; Moulas et al., 2013).

Previous studies, using plating and low-throughput molecular techniques, reported that the introduction of a biocontrol agent or a pathogen to the system had a marked impact on the plant microbiome (Teixidó et al., 1998; Zhang et al., 2008; Buddrus-Schiemann et al., 2010; Chowdhury et al., 2013; Yin et al., 2013). Erlacher et al. (2014) demonstrated shifts in the microbiota of lettuce as a result of introducing a pathogen (*Rhizoctonia solani*) and/or a biocontrol agent. The result of these studies suggests a novel mode of action for biocontrol agents, i.e. compensation for the impact of a pathogen on plant-associated microbiota. The authors speculated that this effect could originate directly from the impact of the biocontrol agent on the composition of the microbiota or indirectly by the impact of biocontrol agent on a pathogen. Compared to the application of a single species, co-inoculation with two different species of biocontrol agents caused a more pronounced impact on the microbial community structure of the cucumber rhizosphere, resulting in increased evenness and better biocontrol of *R. solani* (Grosch et al., 2012).

Harvested fresh fruits and vegetables can harbor large and diverse populations of microorganisms including bacteria, filamentous fungi, and yeasts, either as epiphytes or endophytes. Most of the work on microorganisms associated with fresh harvested commodities, however, has focused on a relatively small number of microbial species that can be easily cultured. As a result, very little is known about the overall diversity and composition of microbial communities on harvested produce and how these communities vary across produce types. Based on recent studies on this topic (Rudi et al., 2002; Ponce et al., 2008; Ottesen et al., 2009; Rastogi et al., 2012; Leff and Fierer, 2013;), a few key patterns are emerging: (1) different produce types and cultivars can harbor different levels (abundances) of specific microbial groups (Critzer and Doyle, 2010), (2) farming and storage conditions can influence the composition and abundances of microbial communities found on produce, and (3) non-pathogenic microbes can interact with and inhibit microbial pathogens found on produce surfaces (Shi et al., 2009; Critzer and Doyle, 2010; Teplitski et al., 2011). Despite this recent body of work, we still have a limited understanding of the diversity of produce-associated microbial communities, their function, the factors that influence the composition of these communities after harvest and during storage, and the distribution of individual taxa (particularly those taxa that are difficult to culture) across different commodities.

In light of the progress made in recent years in metagenomic technologies, this technology should be used to characterize the composition of microbial communities on fruit and vegetables. Metagenomic analyses are based on the amplification and sequencing of the 18S rRNA and ITS, for eukaryotes, and 16S rRNA, for bacteria. This technology, however, can still be problematic due to problems associated with PCR amplification, such as sensitivity to inhibitory compounds, primer mismatch sensitivity, lack of quantitative information, and the amplification of interfering plant organelle derived RNA sequences (Berlec, 2012).

In recent years, the use of natural and synthetic microbial communities/consortia represents an emerging frontier in the field of bioprocessing (focusing on fuel production), synthesis of high-value chemicals, bioremediation, and medicine and biotechnology (Hays et al., 2015). Microbial consortia are mixtures of interacting microbial populations that can be found in many diverse environmental niches, and can be grouped into two types: natural or synthetic. The use of a consortium has several advantages over a single species, such as efficiency, robustness, resilience to environmental stress, and modularity. Microbial consortia often have the ability to complete tasks that would be too difficult for one organism to accomplish (Pandhal and Noirel, 2014).

Massart et al. (2015a) suggested the use of microbiota-derived products or the microbiota itself, directly or indirectly, to develop novel tools for the protection of plants against pathogens. An initial approach could be the use of a synthetic or natural consortium (Gopal et al., 2013) that could be applied to a harvested commodity to see if it results in better disease control due to the expression of a variety of modes of action against the pathogen. Maintaining the right balance and diversity inside the consortium before and after its application, however, may prove to be difficult. Regulatory difficulties in registering a consortium, composed of multiple microorganisms, as a biocontrol product may also be a problem. Thus a simpler approach could be to identify a 'helper' microbial strain from the microbiota (Massart et al., 2015a). A 'helper' strain may have no biocontrol capacity but rather enhance the antagonistic activity of an existing known biocontrol agent by supporting its establishment and survival on the targeted commodity. Finally, the use of biochemical compounds derived from the culturing of a consortium that limit the development of plant pathogens could also be considered another potential technology that may be easier to register, manufacture and apply.

## 7. Concluding remarks

After more than three decades of research, the field of postharvest biocontrol has reached a crossroads and previous approaches need to be seriously evaluated, and evolving new directions need to be considered for future research and development. A review of the existing information makes it obvious that a significant gap still exists between basic research involving the discovery of a biocontrol agent and its development and implementation under commercial conditions. In recent years, a considerable volume of published research articles fall under the category of "re-inventing the wheel". In order to move a biocontrol agent from the laboratory to the market place requires many different disciplines and people with a variety of expertise.

Overall, commercial implementation of postharvest biological control products has been very limited and only comprises a very small share of the potential market. The need for alternatives to chemical fungicides, however, is still valid and the outlook for microbial biocontrol products is still very promising. In order for a biocontrol product to be viable, however, it must perform effectively and reliably, be widely accepted, have intellectual property protection (patent), and be profitable to the company that has invested the money in its development, registration, and marketing.

Significant progress has been made in understanding the various aspects of biocontrol agents that allow them to inhibit or prevent pathogen development. Collectively, the available information indicates suggests the lack of a single universal mechanism of action common to all the reported antagonists. While dissecting and characterizing the mechanisms of action involved in each biocontrol system is critical for the success of developing reliable products, the question is how can this knowledge be utilized to develop more effective products?

Biological interactions are dynamic, with dramatic changes occurring when thresholds in signaling or population levels are reached. The physiological status of the host/pathogen/biocontrol agent/other microbiota, environmental conditions, and postharvest handling all have significant but largely unknown effects on fruit/vegetable interactions with microbial communities. The realization that the microbiota is an integral and active component of harvested fruit and vegetables and is influenced by various biotic and abiotic stressors is very important for understanding all the

factors involved in the assembly and composition of a specific microbiome. The multitrophic interactions involved in postharvest biocontrol systems and the potential use of synthetic microbial communities for biocontrol of postharvest diseases should be explored. In order to overcome the scientific and technical challenges associated with developing novel biocontrol technologies based on a holistic approach, the collaboration between a wide variety of scientific disciplines is needed. Finally, collaboration between scientific researchers and companies that develop products is essential if these new technologies are to become commercially viable and relevant.

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