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

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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 studies and were developed as commercial products. The discovery and development of all these antagonists to a product followed the paradigm in which a single antagonist isolated from one commodity is expected to be effective as well on other commodities that vary in their genetic background, physiology, postharvest handling, and pathogen susceptibility. In most cases, products development was successful but their full commercial potential has not been realized. The low success rate of postharvest biocontrol products has been attributed to several factors among which mass production, formulation, physiological status of the commodity, its susceptibility to specific pathogen and application constrains played major role in the reduced and inconsistent performance under commercial conditions. Although studies on the mode of action of postharvest microbial antagonists have investigated for the last 30 years, our understanding is still very incomplete. In this regard, a systems approach should be employed to investigate the network of interactions that takes into account all the components of the biocontrol system. 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 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 fruits and vegetables. Information on the dynamics and diversity of microbiota may be useful to adopting new paradigm in postharvest biocontrol that is based on constructing synthetic microbial communities to provide superior control of pathogens.

## 1. Introduction

Biological control agents as a viable alternative to the use of synthetic chemicals has been the focus of considerable research for 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-eighties (Wilson and Pusey, 1985) and was later highlighted in several 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 applying the 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 implication on postharvest biocontrol research is presented.

Numerous microbial antagonists (yeasts and bacteria) of postharvest pathogens have been identified in both 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 Aspire™ (based on *Candida oleophila*) (Blachinsky, et al., 2007), Yieldplus™ (based on *Cryptococcus albidus*) (Janisiewicz and Korsten, 2002), Candifruit™ (based on *Candida sake*) (Teixidó, et al., 2011), and Biosave™ (*Pseudomonas syringae* Van Hall) (Janisiewicz and Jeffers, 1997). Aspire™, Yieldplus™ and Candifruit™ were commercialized for some years but discontinued due to business and marketing-related. Biosave™, however, still has limited use in the US market for application on fruit crops, potatoes, and sweet potatoes (Janisiewicz and

Peterson, 2004). Avogreen™ 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, Nexy™ (based on *C. oleophila)* was developed in Belgium, and submitted for regulatory approval in 2005 for postharvest application against wound pathogens on pome fruits, citrus, and banana (Lahlali et al. 2011). Nexy™, manufactured by Lesaffre, Inc., received registration approval throughout the European Union in 2013 (Massart and Jijakli, 2014). BoniProtect™ (based on the yeast-like fungus *Aureobasidium pullulans*), developed in Germany, has a suggested use as a preharvest application to control wound pathogens on pome fruit develop during storage (Lima et al, 2015). Another product, "Pantovital" (based on *Pantoea agglomerans* CPA-2) 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 was not commercialized (Torres et al., 2014). Shemer™ (based on *Metschnikowia fructicola*) 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) (Hershkovitz et al., 2013).

Interestingly, the vast 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 fruit 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., 2010). 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 hours) 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 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 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 is not realistic given the advances in our knowledge of microbial ecology and plant microbiomes that have been accomplished through metagenomic approaches.

## 3. Constraints and shortcoming 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 has not been realized, which can measured by its acceptance and widespread use. The low success rate of postharvest biocontrol products has been attributed to several factors 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 imperative to discuss the variables that are critical in product development, performance, and viability. A schematic description of a possible pipeline for the development of postharvest biocontrol products is presented in Fig. 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 usually 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 fermentations. 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 *Pantoea. agglomerans* CPA-2 (Costa et al., 2001), *P. agglomerans* PBC-1 (Manso et al., 2010) or *Bacillus subtilis* CPA-8 (Yánez-Mendizábal et al., 2012b), yeasts such as *Candida sake* CPA-1 (Abadias et al., 2003a), *Aureobasidium pullulans* (Mounir et al., 2007), or *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 medium, drying, addition of volume materials (inert ingredients), adhesives, emulsifiers and adjuvants. All these actions may adversely affect the properties of the selected biocontrol agent. 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 a: i) liquid state usually requiring refrigeration; ii) a freeze-dried state that requires the use of cryo-protectant substances during preparation, and; iii) dehydrating (drying) the cultures. The latter two types of formulations can then be stored at ambient temperatures.

*Formulation:* Typically, 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 low and high temperature. 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 increases 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 (*Pseudomonas* syringae), *P. agglomerans* (Costa et al., 2000), *C. sake* CPA-1(Abadias et al., 2001a, 2001b), *Cryptococcus laurentii* (Li and Tian, 2006), *Metschnikowia pulcherrima* (Spadaro et al., 2010), 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 (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,

*Aureobasidium 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. Liquid formulations are the simplest way to stabilize the viability of microbial cells. This formulation involves storing cells in water- or oil-based solutions 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), *Cryptococcus laurentii* (Liu et al., 2009), and *P. anomala* (Melin et al., 2011).

*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 becomes even more critical because the postharvest market is very limited and typically only one application of the product is necessary. 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 pre-harvest 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 Boni Protect, 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 hurdles and public concern about the use of genetically-modified-organisms (GMOs) represent a monumental hurdle to this approach.

*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. As reviewed in the introduction to this special issue (Wisniewski et

al., 2016), 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., 2002b; Karabulut et al., 2003; Teixidó et al., 2001), and various physical treatments (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 evaluate.

## 4. An industry perspective

Concerns about food safety issue, including chemical residues and environmental impact, over the past twenty years have resulted in substantial regulatory changes on the use of pesticides (http://www2.epa.gov/pesticide-tolerances; http://www.ecpa.eu/page/food-safety). Regulatory restrictions on the use of a variety of chemical fungicides used to manage postharvest pathogens is increasing. 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 development of green technologies for controlling plant diseases (CPM, 2010). In the case of microbial industries associated with producing yeast for bakery, brewery, and wine fermentation, an 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 are difficult to determine. Given their responsibility to stakeholders, multinational chemical companies are usually driven by two strategies: pesticide resistance and the objective of achieving zero residues on commodities. Furthermore, they want to offer to their clients (distributors and subsequently growers) a full portfolio of existing 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 Mio 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.researchandmarkets.com/research/7bvbnf/global_pesticide)

Fifty-two chemical active ingredients were registered in the EU between 1996 to 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 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 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: *Metschnikowia fructicola* strain 277 (Shemer™), *Aureobasidium pullulans* strains DSM 14940 and DSM 14941 (BoniProtect), and *Candida 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 applications. 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 adopt this method.

## 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, and 4) direct parasitism (Bélanger et al., 2012; Janisiewicz and Korsten, 2002). The different modes of action were recently reviewed by Spadaro and Droby (2015) and by Liu et al., (2013). 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 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: (1) what are the effects of antagonists on wound healing and host resistance? (2) how important and widespread are the direct effects of antagonists on pathogens (3) how do incidental microorganisms or mixtures of antagonists affect pathogen/antagonist interactions, and (4) 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 Jia 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

348 have been the subject of postharvest biocontrol research for 30 years, our understanding is still very
349 incomplete. When studying mechanisms of action, a system approach should be employed to
350 investigate the network of interactions. Such an approach, that takes into account all the
351 components of the system, may provide the greatest understanding of biocontrol systems.
352 The availability of more cost-efficient, high throughput DNA/RNA and proteomic
353 technologies, along with bioinformatics, has provided new opportunities and tools to obtain deeper
354 insights into the mechanisms and interactions that have already been established (Kwasiborski et
355 al., 2014; An et al., 2014). Developments in deep sequencing, transcriptomics, MS-MS proteomics,
356 metagenomics, comparative and functional genomics can be utilized to determine changes in the
357 physiological status of biocontrol agents, and the effect of environmental stress on its intracellular
358 machinery (Herschkovitz et al., 2013; Sui et al., 2015). Changes in the level of expression of
359 "biocontrol genes" during mass production, formulation and storage, or in response to exposure and
360 contact with host plant tissue after application can now be more readily investigated. Massart and
361 Jijakli (2007) reviewed the molecular techniques that have been used to understand the mechanism
362 of action of biocontrol agents and discussed the strategies used to study the role of various genes
363 believed to be involved in the mechanisms of action. They concluded that the majority of studies
364 aimed at elucidating the genetic basis and traits important for antagonistic action have focused on
365 *Trichoderma*. Genes related to the production of antibiotics have been mainly studied in bacteria,
366 such as *Bacillus subtilis* and *Pseudomonas* spp. Very few genes involved in induction of resistance
367 mechanisms in host plants or competition for nutrient and space have been identified in biocontrol
368 agents. More recently, the impact of the -omic technologies for understanding the various modes of
369 action of biocontrol agents against plant pathogens was comprehensively reviewed by Massart et al.
370 (2015). Whatever the -omic technique used (genomic, transcriptomic or proteomic), studies of
371 postharvest biocontrol agents have been sparse and it is expected that greater details about
372 interactions in the entire biocontrol system will be forthcoming.
373
374 **6. T**he role of the microbiome in fruit health and disease – a new perspective
375
376 Microbial communities resident on and in plants can have negative, neutral, or beneficial
377 effects on plant health and development (Berg et al., 2015; Mendes et al., 2013; Philippot et al.,
378 2013). These communities colonize all parts of a plant through its entire lifecycle and marked

379 diversity exists in communities associated with different hosts. Research on this topic is slowing
380 moving from just describing the composition of these communities to elucidating the mechanisms
381 involved in their assembly and function (Waldor et al., 2015).
382 Studies on plant microbiomes (phytobiomes ) in both the phyllosphere and rhizosphere
383 indicate that plants should be considered as "super organisms" where very diverse microbial
384 communities provide specific functions and traits to plants (Vorholt, 2012; de Bruijn, F., 2013).
385 These functions include five key features: (i) improving nutrient acquisition and growth, (ii)
386 sustaining plant growth under biotic and/or abiotic stress, (iii) inducing resistance against
387 pathogens, (iv) interacting with plant or human pathogens, and (v) interacting with other trophic
388 levels, such as insects. It is well established that soil type and plant genotype are the major
389 parameters influencing the rhizosphere microbiome (Berg and Smalla, 2009, de Bruijn, 2013)
390 whereas plant species and genotype are the major factors involved in defining the composition of
391 the phyllosphere microbiome (Massart et al., 2015b). Whipps et al. (2008) published a
392 comprehensive review of phyllosphere microbiology with special reference to microbial diversity
393 and plant genotypes. The authors stressed the need for studies on the functional consequences of
394 changes in microbial community structure and the mechanisms by which plants control the
395 microbial populations on their aerial plant surfaces. The composition of microbial populations in the
396 phyllosphere are also influenced by environmental factors, such as, UV, humidity, temperature,
397 geographical location (Rastogi et al., 2012, Rastogi et al., 2013; Vorholt, 2012), nitrogen
398 fertilization (Ikeda et al., 2011), and pesticide treatments (Moulas et al., 2013; Zhang et al., 2009).
399 Previous studies, using plating and low-throughput molecular techniques, reported that the
400 introduction of a biocontrol agent or a pathogen to the system had a marked impact on the plant
401 microbiome (Buddrus-Schiemann et al., 2010; Chowdhury et al., 2013; Teixidó et al., 1998; Yin et
402 al., 2013; Zhang et al., 2008). Erlacher et al. (2014) demonstrated shifts in the microbiota of lettuce
403 as a result of introducing a pathogen (*R. solani*) and/or a biocontrol agent. The result of these
404 studies suggest a novel mode of action for biocontrol agents, i.e. compensation for the impact of a
405 pathogen on plant-associated microbiota. The authors speculated that this effect could originate
406 directly from the impact of the biocontrol agent on the composition of the microbiota or indirectly
407 by the impact of biocontrol agent on a pathogen. Compared to the application of a single species,
408 co-inoculation with two different species of biocontrol agents caused a more pronounced impact on

409 the microbial community structure of the cucumber rhizosphere, resulting in increased evenness and
410 better biocontrol of *R. solani* (Grosch et al., 2012).
411 Harvested fresh fruits and vegetables can harbor large and diverse populations of
412 microorganisms including bacteria, filamentous fungi, and yeasts, either as epiphytes or
413 endophytes. Most of the work on microorganisms associated with fresh harvested commodities,
414 however, has focused on a relatively small number of microbial species that can be easily cultured.
415 As a result, very little is known about the overall diversity and composition of microbial
416 communities on harvested produce and how these communities vary across produce types. Based on
417 recent studies on this topic (Leff and Fierer, 2013; Ponce et al., 2008; Rastogi et al, 2012; Rudi et
418 al., 2002; Ottesen et al., 2009), a few key patterns are emerging: (1) different produce types and
419 cultivars can harbor different levels (abundances) of specific microbial groups (Critzer and Doyle,
420 2010), (2) farming and storage conditions can influence the composition and abundances of
421 microbial communities found on produce, and (3) non-pathogenic microbes can interact with and
422 inhibit microbial pathogens found on produce surfaces (Critzer and Doyle, 2010; Shi et al., 2009;
423 Teplitski et al., 2011). Despite this recent body of work, we still have a limited understanding of the
424 diversity of produce-associated microbial communities, their function, the factors that influence the
425 composition of these communities after harvest and during storage, and the distribution of
426 individual taxa (particularly those taxa that are difficult to culture) across different commodities.
427 In light of the progress made in recent years in metagenomic technologies, this technology
428 should be used to characterize the composition of microbial communities on fruits and vegetables.
429 Metagenomic analyses are based on the amplification and sequencing of the 18S rRNA and ITS, for
430 eukaryotes, and 16S rRNA, for bacteria. This technology, however, can still be problematic due to
431 problems associated with PCR amplification, such as sensitivity to inhibitory compounds, primer
432 mismatch sensitivity, lack of quantitative information and the amplification of interfering plant
433 organelle derived RNA sequences (Berlec, 2012).
434 In recent years, the use of natural and synthetic microbial communities/consortia represents
435 an emerging frontier in the field of bioprocessing (focusing on fuel production), synthesis of high-
436 value chemicals, bioremediation, and medicine and biotechnology (Hays et al, 2015). Microbial
437 consortia are mixtures of interacting microbial populations that can be found in many diverse
438 environmental niches, and can be grouped into two types: natural or synthetic. The use of a
439 consortium has several advantages over 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 maybe after its application, however, may prove to be difficult. The difficulty of the registering a consortium, composed of multiple microorganisms, as a biocontrol product may also be very difficult. Thus a simpler tool could consist in identifying and selecting a 'helper' microbial strain from the microbiota (Massart et al., 2015a). A 'helper' strain may have no biocontrol capacity but rather enhances the antagonistic activity of existing known biocontrol agent by enhancing its establishment and survival on the targeted commodity. Finally, the use of biochemical compounds derived from the culturing of a consortium that limits the development of plant pathogens could also be considered as another potential tool that may be easier to register, manufacture and apply.

## 7. Concluding remarks

After more than three decades of research, the field biocontrol of postharvest decay 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 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 biological control products developed for the control of postharvest diseases has been very limited and only comprise very small share of the potential market. Although, the need for alternatives to chemical fungicides 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 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 related to the ability of biocontrol agents to inhibit or prevent pathogen development. Collectively, the available information indicate the lack of a single universal mechanism of action common to all the reported antagonists. While dissecting and characterizing mechanisms of action involved in each biocontrol system is critical for the success of developing reliable products, the question is how 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 (Fig. 2). The realization that the microbiome is an integral and active component of harvested fruits and vegetables that is being 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 re based on a holistic approach, the collaboration between a wide variety of scientific disciplines is imperative. 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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Fig. 1: Pipeline for development of postharvest biocontrol products.

Fig. 2: Diagram of multiple interactions between the antagonist, the host, the pathogen and natural resident fruit microbiota.