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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 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

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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