29 Postharvest Biocontrol: New Concepts and Applications

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Overview: Biological control of postharvest products has great potential because postharvest environmental parameters such as temperature and humidity can be rigidly controlled to suit the needs of the biocontrol agent. Also, harvested commodities offer a concentrated target for the application of biocontrol agents. In this chapter we provide a personal account of the driving force behind the research and the people that were instrumental in developing postharvest biocontrol technology, the commercialization of new products and the discovery of new science and technologies to optimize the efficacy of the biocontrol process.

In the Beginning

In their book on biological control, Cook and Baker (1983) provided only one example of the biocontrol of postharvest disease of a fruit or vegetable. This was research by Tronsmo and Dennis (1977) in which *Trichoderma* was used to control *Botrytis* rot of strawberry. Subsequently, Wilson and Pusey (1985) presented their ideas on the potential of postharvest biocontrol in a feature article and documented their initial research on using a strain of *Bacillus subtilis* to control brown rot on peach, caused by *Monolinia fructicola*. This seminal work provided the initial ideas and principles that, over the ensuing 20 years, fostered a wealth of research and product development around the world. Numerous reviews have provided an account of the scientific advances that have been made in

postharvest biocontrol, as well as the problems faced in trying to develop a commercial product (Wilson and Wisniewski, 1989, 1994; Droby *et al.*, 2000, 2001; El Ghaouth *et al.*, 2004). While the reader is referred to these reviews and the primary research articles cited within these reports for details on the science of postharvest biocontrol, the present contribution will attempt to provide a personal account of the driving force behind this research and the people that were instrumental in our programme to develop postharvest biocontrol technology, products and science.

While the basic rationale underlying our research efforts was to reduce the use of synthetic chemicals on harvested commodities, our motivation was strengthened by a report by the U.S. National Research Council (NRC) (1987) that stated, 'As a class, fungicides present special difficulties because nine oncogenic compounds account for about 90% of all fungicide sales.' This report indicated that fungicides constitute 60% of oncogenic risk among all pesticides. Furthermore, it concluded that loss of the use of these chemicals would have an adverse economic impact on the production of some crops because of a lack of viable alternatives. This heightened concerns about impending problems associated with the potential decertification of some of the fungicides used to manage postharvest disease. The potential health risk associated with fungicides was further highlighted by a subsequent report by NRC (U.S. National Research Council, 1993), which documented the increased vulnerability of children to synthetic pesticides. Lastly, reports of the development of resistance to fungicides also helped to establish an urgent need to develop new, effective alternatives for managing postharvest diseases.

Although, at the time, biological control as an approach to managing plant disease did not have any major commercial success stories, we felt that the use of biological control agents in a postharvest environment held special promise. One of the major problems in applying biocontrol agents in the field is that environmental conditions can profoundly affect their survival and effectiveness. In the postharvest environment, parameters such as temperature and humidity are rigidly controlled and can be taken into account when selecting a suitable biocontrol agent. Also, harvested commodities present a more concentrated target for the application of biocontrol agents. The regulated environment, the ability to target the application of the biocontrol agent, and the high value of the harvested commodity together suggested that the use of biocontrol agents to manage postharvest disease would have an excellent chance of success.

In 1984, it was found that a strain (B-3) of *B. subtilis* was able to control brown rot of peaches caused by *M. fructicola* (Pusey and Wilson, 1984) and the organism was patented. However, it was later determined that the main mode of action of B-3 in controlling brown rot was the production of the antibiotic, iturin. It was felt that there would be resistance to the application of an antibiotic-producing microorganism on food, and commercialization of B-3 was not pursued, even though in pilot tests it demonstrated control of brown rot comparable to synthetic fungicides (Pusey *et al.*, 1988). Interestingly, from a commercial and registration standpoint, this viewpoint may not have been valid as several biocontrol products have been developed that utilize antibiotic-producing strains of *B. subtilis*.

The First Generation of Yeast Biocontrol Products

Although the postharvest environment may be especially favourable for the development of biocontrol products, a considerable investment of time and money is required to establish whether a particular organism has commercial potential. The characteristics of an ideal antagonist have been outlined in Wilson and Wisniewski (1989) and are summarized in Table 29.1. While some of these may be obvious, they deserve special consideration prior to committing substantial amounts of research personnel and monies to a project.

After the experience with B-3, two main criteria were considered paramount when we entered into the next phase of the project. First, that we wanted to identify yeast antagonists and, secondly, that the mode of action should not rely on the production of antibiotics by the antagonist. This led to the development of a selection strategy that was later adopted by postharvest biocontrol programmes around the world to identify suitable yeast antagonists (Wilson *et al.*, 1993). Rather than *in vitro* screening of organisms in Petri plates, which favoured the identification of antibiotic-producing organisms, our method involved placing washing fluids obtained from the surface of fruit into fruit wounds that were subsequently inoculated with a rot pathogen. Organisms were then isolated from the surface of wounds that did not develop infections. These were plated out and isolated. Yeasts were identified; pure cultures of potential antagonists were produced; and then each organism was screened individually in fruit wounds to assess its potential as a biocontrol agent. This method identified a number of antagonists that were studied more intensely and measured against the criteria presented in Table 29.1.

Essential to the success of establishing the science of postharvest biocontrol and developing commercial products was the collaborative relationship that was

Table 29.1.   Characteristics of an 'ideal antagonist' for the postharvest environment.

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| Genetically stable |
| Effective at low concentrations |
| Not fastidious in its nutrient requirements |
| Ability to survive adverse environmental conditions (including low temperature and controlled atmosphere storage) |
| Effective against a wide range of pathogens on a variety of fruits and vegetables |
| Amenable to production on an inexpensive growth medium |
| Amenable to a formulation with a long shelf-life |
| Easy to dispense |
| Does not produce metabolites that are deleterious to human health |
| Resistant to pesticides |
| Compatible with commercial processing procedures |
| Does not grow at 37°C and is not associated with infections in humans |
| Non-pathogenic to host commodity |

formed between us at the USDA-ARS lab (M.W. and C.W.) and scientists working with ARO at the Volcani Center in Israel (E.C. and S.D.). This collaboration began in 1985 and continues to this day. Much of this highly productive collaboration has been funded through the US–Israel Bi-national Agricultural Research Development Fund (BARD).

In the early years of the collaboration, several yeast antagonists were identified that had commercial potential. Our first yeast antagonist, strain US-7 of *Candida guilliermondii,* was originally misidentified as *Debaryomyces hansenii.* This caused some confusion in the patenting process and emphasized the need to have at least two conforming identifications by reputable yeast taxonomic services. It also emphasized the weakness of using physiological tests as the basis for making taxonomic determinations. Using the criteria outlined in Table 29.1, the decision was made, however, to abandon the commercialization of US-7 because other isolates of *C. guilliermondii* had been reported in the medical literature as pathogenic to humans. This decision was made despite the fact that US-7 showed excellent biological control activity and did not show any pathogenicity in Level I toxicology studies. Instead, we chose to focus on the commercialization of *Candida oleophila* (Strain I-182) based on its superior biocontrol activity and the fact the species did not grow at 37°C. The use of this organism was also protected by a patent.

Another critical ingredient for achieving the goal of a commercial product was the relationship that was developed with a small venture-capital company, Ecogen. This was a US-based company, with a subsidiary in Israel, interested in biological control products. It was the relationship with Ecogen that provided the bridge between theory and practice. They were able to develop a formulated product, based on growing I-182 on a low-cost substrate of industrial by-products, which had an un-refrigerated shelf-life of over 1 year (Wilson and Wisniewski, 1994). Ecogen also provided critical monetary support for conducting semi-commercial pilot tests on apples and citrus in the USA and Israel, respectively. Funding was provided through Cooperative Research and Development Agreements (CRADAs) with the USDA-ARS and similar agreements with ARO. Semi-commercial packing lines, illustrated in Fig. 29.1, allowed us to conduct large-scale studies and determine the performance of a formulated product under more realistic conditions.

The patent on *C. oleophila* was licensed to Ecogen, who handled the complete registration process with the US Environmental Protection Agency and thus the first yeast-based postharvest biocontrol product was launched under the trade name of Aspire™ beginning in 1995. After registration, commercial evaluation of Aspire™ continued in order to better understand how to adapt the use of the product to different packing-house environments and to different commodities (Droby *et al.*, 1998). This led to continued research on how to enhance the reliability and efficacy of the product and established the foundation for a second generation of postharvest biocontrol products (Droby *et al.*, 2003b; El Ghaouth *et al.*, 2004). It is important to note that a parallel but completely independent programme on postharvest biocontrol focusing on bacterial antagonists was being conducted in the USDA-ARS laboratory during this time by Dr Wojciech Janisiewicz. This effort, in collaboration with the US-based company Ecoscience, led to
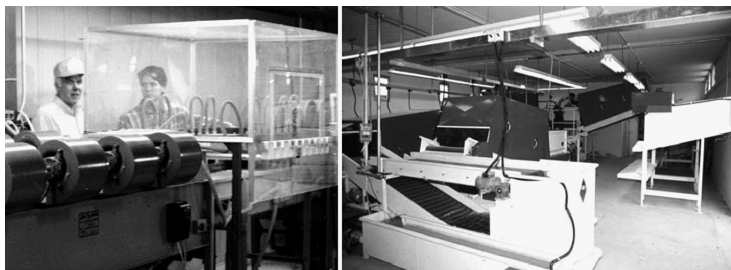
Fig. 29.1. Semi-commercial lines used to evaluate potential antagonists, formulated product and combined treatments such as UV-C light followed by antagonists. Line on left was located at the USDA-ARS facility in Kearneysville, West Virginia and the line on the right was located at the AR0, Volcani Center in Bet Dagan, Israel.

development of Bio-Save™, based on an isolate of *Pseudomonas syringae*. Readers are referred to Janisiewicz (1998) for the details on this effort.

The Science of Postharvest Biocontrol

A short review of the fundamental research we conducted related to postharvest biocontrol is presented because it played a key role in defining and shaping the direction of our programme. Our studies led to the development of key concepts, an expanded view of biocontrol, and greatly influenced the development of a second generation of postharvest biocontrol products.

A main concern was to better understand the features of an organism that made it a good biocontrol agent. In other words, what was the mechanism of action responsible for biocontrol activity? While early studies indicated that nutrient competition and the fast growth rate of our antagonists played a major role in biocontrol activity, subsequent studies indicated a much more complex interaction between the antagonist, pathogen and commodity (Wilson and Wisniewski, 1994). Two novel discoveries were the ability of the yeast to form a biofilm (Fig. 29.2) and, as illustrated in Fig. 29.3, the ability of some yeast antagonists to adhere to and parasitize pathogen hyphae (Wisniewski *et al.*, 1991). The latter report was recognized as the first reported instance of the ability of a yeast to parasitize a higher fungus. Other key factors that appeared to play a role in the efficacy of our yeast antagonists were the production of lytic enzymes by the yeast (Bar-Shimon *et al.*, 2004) and their ability to tolerate high levels of salts (Wisniewski *et al.*, 1995). The induction of resistance responses in the fruit by application of the antagonists within a wound or on the fruit surface was also a novel discovery (Wilson and Wisniewski, 1994; Droby *et al.*, 2002; El Ghaouth *et al.*, 2003).

More recently, we have used molecular approaches to examine the role of glucanases in biocontrol activity of the yeast *C. oleophila* (Yehuda *et al.*, 2003) and to enhance biocontrol activity by overexpression of antimicrobial peptides (Wisniewski *et al.*, 2003).
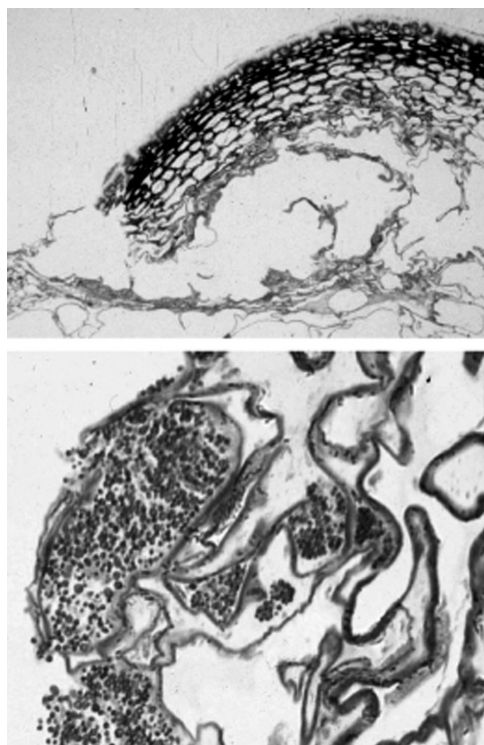
Fig. 29.2. Ability of yeast antagonists to form a 'biofilm' in wound sites of fruit. Top picture shows *Candida oleophila* forming a film along surface of wound in apple (100x). Bottom figure is an enlargement of top figure and highlights the large number of cells and yeast extracellular matrix involved in forming a biofilm.

Out of the Frying Pan and Into the Fire

By early 2000, there were three postharvest biological products available on the market: Aspire™ (limited to the USA and Israel), Bio-Save™ (limited to the USA) and YieldPlus™ (limited to South Africa). In spite of all the published fundamental and applied research on postharvest biocontrol, the commercial use of these products was, and remains, limited and accounts for only a very small fraction of the potential market. Despite this, however, it is commonly recognized that this area of biocontrol has tremendous potential for economic success. As discussed in recent reviews (Droby *et al*., 2003a; El Ghaouth *et al*., 2004), the main short-coming with the use of postharvest biocontrol products has been inconsistency in performance, especially when used as a stand-alone product in replace of synthetic fungicides. A second problem with the current generation of products is their inability to control previously established and latent infections. The reasons for these shortcomings have been reviewed by Droby *et al*. (2003a). In brief, the following factors can dramatically affect the viability and performance of postharvest biocontrol agents: (i) fermentation and formulation practices; (ii) the method by which the product is delivered to the commodity; (iii) inoculum pressure; and (iv) the physiological status of the fruit. Additionally, the strategy used to identify potential antagonists favoured the selection of organisms that exhibited protective rather than eradicative activity.
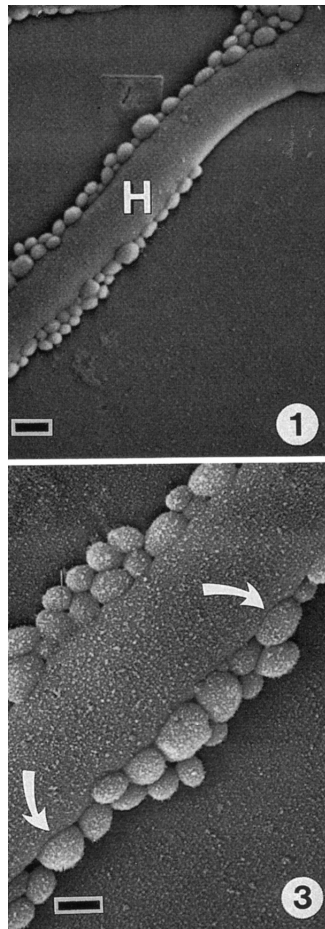
Fig. 29.3. Attachment of *Pichia guilliermondii* (Strain US-7) to hyphae of *Botrytis cinerea.* Note concave appearance of hyphal wall in lower picture. Scale = 2.5 μm.

Throughout the course of developing Aspire™ considerable research went into finding methods to enhance the reliability and efficacy of the product and other selected antagonists as well. In particular, it was our intention to find additives or physical control methods that would act synergistically with our antagonist. Initially, this involved combining the product with a low level of postharvest fungicide (Droby *et al*., 1998) or 1–2% salt solutions of calcium chloride or sodium bicarbonate and other additives commonly used in the food industry (Droby *et al*., 2003b). It was also reported that physical treatments such as hot air, curing, hot-water brushing, and combinations of the above with pressure infiltration of calcium could also increase the efficacy of antagonists (reviewed by Droby *et al*., 2003a). In collaborative research with one of us (C.S.), a pioneer in the use of low-dose UV-C light as a means of inducing host resistance to decay in harvested commodities, we also demonstrated that this approach could enhance the performance of yeast antagonists (Stevens *et al*., 1997). Combining antagonists with a sugar analogue (2-deoxy-dscp-glucose) was also suggested as an approach to

increase efficacy (Janisiewicz, 1994; El Ghaouth et al., 2000). However, due to the high cost of the sugar analogue this aspect was not pursued commercially.

## The Second Generation of Yeast Biocontrol Products

During the course of our research it was realized by one of us (CW) that if postharvest biocontrol was going to be commercially successful a broader concept of biological control would be needed. Plant pathologists have adopted the entomologists' definition of biocontrol, which involves the control of one organism with another organism. But, a plant disease is not an organism. It is a process. Therefore, we have defined the biological control of a plant disease as 'control of a plant disease by a biological process or the product of a biological process'. (See also discussion by Cook, Chapter 44 this volume.)

Using this broader definition of the biological control of plant diseases, a number of avenues become available for developing effective, commercially successful biological control products and practices: (i) the classical idea of using an antagonist; (ii) innate or induced resistance, which is a biological process; and (iii) natural antimicrobials, which are the product of a biological process. While some of these approaches are being pursued by us and others (as outlined above) without commitment to a formal paradigm, it is important to conceptualize the paradigms that drive scientific research, in order to overcome limitations and expand possibilities. This new paradigm of biological control was the primary concept that we used to develop a second generation of postharvest, biocontrol products.

In 1992, a new employee (AG) from Laval University in Quebec, Canada arrived at the USDA-ARS laboratory in Kearneysville. He brought with him a wealth of knowledge and experience on the use of chitosan as an antimicrobial compound. During his 10-year tenure here he was instrumental in the development of a second-generation postharvest biocontrol product and documenting the role of induced resistance in the mode of action of our yeast antagonists.

The main objective in developing a new product was to address the poor ability of Aspire™, and other postharvest biocontrol products, to control pre-established and latent infections. We hoped to overcome this by using a combination of natural products along with a yeast antagonist. We also decided at that time to focus on a new yeast antagonist in order to enhance patent opportunities and attract new industrial partners. These research efforts led to the development of two new products, whose main components consisted of the yeast antagonist *Candida saitoana* and a derivative of either chitosan (Biocoat) or lysozyme (Biocure). Both of the compounds had been tested worldwide and shown to have strong eradicative activity (Fig. 29.4). The two commercial products also contain other additives such as sodium bicarbonate. The additives were found to enhance control efficacy to levels equivalent to that found with available postharvest fungicides. Patents have been issued to cover this technology (El Ghaouth and Wilson, 2002; Wilson and El Ghaouth, 2002). While this research was initially conducted under a CRADA with American Cyanamid and
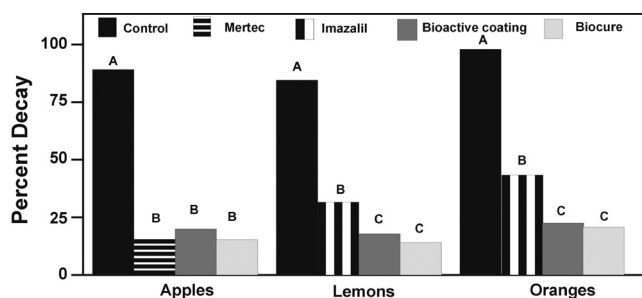
Fig. 29.4. Control of pre-established infections on apples, lemons and oranges by Biocoat (Bioactive coating consisting of the yeast *Candida saitoana*, chitosan salt and other additives) and Biocure (*Candida saitoana,* lysozyme and other additives). All fruit were wounded and exposed to decay pathogens 24 h prior to exposure to biocontrol preparation or fungicide. Mertec was the fungicide used on apples, and Imazalil was the fungicide used on citrus. Apples were stored at 18°C for 4 weeks prior to decay assessment while lemons and oranges were stored at 10°C for 28 days prior to decay assessment.

then MicroFlo (a subsidiary of BASF), the technology has now been licensed to Inova Technologies and is awaiting registration by the US Environmental Protection Agency.

A more recent product (developed by SD) has taken the approach of preventing postharvest decay by application of a yeast biocontrol agent to flowers and fruit in the field, several times throughout the growing period. This approach also addresses the problems of pre-established and/or latent infections. The product is based on the use of a heat-tolerant strain of *Metschnikowia fructicola* and is marketed under the name ProYeast-ST and ProYeast-ORG in Israel by the company AgroGreen. It has been shown to be effective against rots caused by *Botrytis, Penicillium, Rhizopus* and *Aspergillus* on strawberries (Karabulut *et al.*, 2004), grapes and citrus.

Where We Stand and Where We Go

The past 25 years have seen tremendous growth in the science and practical application of biological control of postharvest diseases. The available literature has expanded from a few publications in the early 1980s to hundreds, if not thousands, by 2005. The number of labs that conduct research in this area has also changed from 2–3 located in the USA and Israel to dozens located throughout the industrial and developing world, and several products have been made available. Our own success and influence in this field of research was a direct result of having a timely idea (i.e. being at the right place at the right time), the strong collaboration between the USDA-ARS and ARO laboratories, and the involvement of industry and their expertise and drive to develop a commercial product. International cooperation with South Africa, Brazil, Australia, Egypt,

Italy, New Zealand, Mauritania, Turkey and Uruguay, which took the form of visiting scientists, graduate students and product-evaluation arrangements, also played an important role in fostering our success and prominence. Truly, the small beginnings at the Appalachian Fruit Research Station (USDA-ARS) and the Volcani Center (ARO) blossomed into a worldwide effort.

As indicated, the use of the available postharvest biocontrol products thus far has been rather limited, given the potential market. Some of the reasons for the lack of adoption of these products have been overcome in the 'second-generation' products that are, or will soon be, available. The future success of these products will depend on market conditions. Synthetic fungicides have a long history of use, are generally easy to apply, and continue to be highly effective. Growers will only replace chemical pesticides with biologicals if there is a continued demand by consumers for pesticide-free food products. Organically grown fruit represents a large potential market for use of biological agents, since the use of synthetic fungicides is strictly prohibited. The demand for such produce has seen tremendous growth in the last decade and this does not seem to be slowing down. Importantly, new biological postharvest products must be adaptable and effective as stand-alone products, without the need for additional inputs if they are to be competitive with synthetic fungicides. Postharvest biologicals must also begin to address problems of decay management in commodities where postharvest disease is harder to control, such as stone fruits and berries. Lastly, the huge potential of providing extended decay control to the consumer, prior to and after commodity purchase, through the use of antimicrobials in modified and intelligent packaging should be recognized.

The greatest hope for a biological approach (using a broad definition of biological control) lies in a further understanding of the mechanism(s) of action of microbial antagonists and natural products, innate and induced resistance in the host, and the biology of decay pathogens. It is expected that this knowledge will lead to new, innovative approaches for controlling decay in harvested commodities and presents the best hope for the future of the biological control of postharvest disease.

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