Application of Biological Control Agents in Pre- and Postharvest Operations

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ABSTRACT

There is increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world. Regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce pesticide use. The limited number of viable alternatives to synthetic pesticides, however, is currently the major obstacle in reaching that goal. There is also the absence of effective chemicals for the control of many diseases. Some pests have developed resistance to certain pesticides. Biological control of pre- and postharvest diseases has been one of the most extensively studied alternatives and appears to be a viable technology. Research and development of biological control products for pre- and postharvest use has been on a fast track. Several commercial products are already available and others will be available in the near future. However, huge commercial successes in biological control are still rare. Currently, the main hurdle facing widespread use of postharvest biocontrol strategies is the decreasing efficacy and lack of consistency found when these methodologies are applied as standalone treatments under commercial conditions. Research into formulation technology might offer a new stimulus to help develop successful biological control agents from the vast number of strains isolated in the past decades. Combining biological, chemical, physiological, and other postharvest practices in an integrated control strategy can give excellent pest control during long term storage and transport of certain fruits. The opportunities of successful implementation of this technology is growing as information regarding various aspects related to formulation technology, application, microbial ecology, and genetics becomes available.

Key words: Biological control agents, pre-harvest, postharvest, commercial application.

1. INTRODUCTION

Biological control occurs when the number and activity of a pathogen or insect is controlled by another member of the community (Campbell, 1989) other than man. Several components of the normal microflora living on plants serve naturally to regulate the activities of some pathogens and such naturally occurring control can be enhanced by manipulation. Based on these points, a lot of pre- and postharvest methods have been employed in recent times to manipulate the natural living community in a given space or surface. Some of the preharvest methods include crop rotation, cultural practices, direct addition of antagonists, and manipulating the microflora by chemicals (Sanni et al., 2004). Microbial antagonists have been used for the control of postharvest diseases Most of the reported yeast and bacteria antagonists were naturally occurring on fruit surfaces. However, microbial biological control agents of postharvest diseases have been criticized mainly for not providing a consistent or broad-spectrum control as synthetic fungicides. Studies have been made to investigate many methods of controlling certain postharvest pathogens in order to increase ability of antagonist to control postharvest diseases, yet results are still inconsistent (Droby, 2004). Microbial biocontrol agents possess a number of important advantages over traditional chemical pesticides which make their commercial outlook particularly promising, as, in general, they are considered nonhazardous to humans and animals; biodegradable and environmentally friendly; attack specific target organisms, leaving other beneficial organisms unaffected; are easy to genetically modify; and can be commercially developed with relative ease. These advantages, however, are counterbalanced by a number of limitations which include the sensitivity of most of the currently marketed microbial control agents to adverse environmental conditions such as extreme dryness, heat, and cold; limited shelf-life; limited biocontrol efficacy in situations where several pathogens are involved in decay development; and limited effectiveness under high disease pressure (Droby et al., 1993a)

Some of the fungicides registered for postharvest use, particularly benzimidazole, are becoming ineffective due to the development of fungicide-resistant strains of postharvest pathogens. World trends are moving toward reduced pesticide use. In response, several physical and biological means have been evaluated as safer alternatives to the use of synthetic, chemical fungicides. The use of natural plant products (essential oils and plant extracts), biocontrol agents (yeast and bacterial antagonists), and non-selective biofungicides (sodium carbonate, sodium bicarbonate, active chlorine, and sorbic acid) are among the approaches currently being evaluated for the control of postharvest diseases (Droby, 2004).

Inspite of these efforts, demand for further research seems to remain high as only a small number of commercial biological control products are available on the market shelf. Such a need seems to be supported by the increasing concern about the environmental effects and safety of chemical pesticides and fungicides, as well as the absence of effective chemicals for the control of many diseases. The objective of this review is to discuss the developments in application of biological control agents as alternatives to chemical pesticides and fungicides used in the control of pests and diseases with the view to sensitize and stimulate research interest.

2. MECHANISMS OF ACTION IN THE PROCESS OF BIOLOGICAL CONTROL

A mechanism of action may be explained as the strategy used by a beneficial microorganism against a disease causing pathogen or pest. Several mechanisms of action are thought to be involved in the process of biological control. It is either thought that at least one mechanism of action is obtained (employed) by the biological control agent or more or all in order to antagonize one or several plant pathogens or pests. There are three main mechanisms of action that have been used to explain the nature of interactions between biological control agents and pathogens (Droby and Chalutz, 1994; Droby, 2004 and Sanni et al., 2004):

2.1 Competition

Nutrient or space is much likely to be unequally taken up by different seekers having different mechanisms of nutrient uptake. This would lead to one microorganism getting most of the nutrients and growing, while another has insufficient nutrients and dies. Microorganisms generally compete for carbohydrates, nitrogen, oxygen, and micronutrients such as iron.

2.2 Parasitism

This occurs when an organism for all or some part of its life derives its nutrition from a living organism of another species. A parasite usually lives in or on the body or cells of the host, which is usually harmed to some extent by the association. The term **mycoparasitism** is used in reference to the phenomenon of one fungus parasitizing another whereas the term **hyperparasitism** is used in reference to mycoparasites of fungal hosts, which are also, parasites. In general, parasites including mycoparasites are divided into necrotrophs and biotrophs; and in agreement with the necrotrophy and biotrophy concepts, necrotrophs are known to obtain their nutrition from dead host cells after killing them, whereas biotrophs do not kill their host.

2.3 Antibiosis

Antobiosis is defined as the inhibition or destruction of a microorganism by substances such as specific or nonspecific metabolites, lytic agents, or enzymes that are produced by another microorganism. Antibiotics are volatile or nonvolatile substances produced by microorganism, which operate at low concentrations: less than 10 ppm. Certain microorganisms start producing antibiotics only when a substantial quantity of substrate mainly carbon is available, while other microorganisms start producing antibiotics when the substrate availability decreases. This strategy is thought to serve in extending the general activity of certain microorganisms by preventing other microorganisms from using the remaining quantity of substrate. Hence, antibiosis seems to have an important role in the competitive ability of microorganisms that produce antibiotics.

3. POTENTIAL PRE-HARVEST BIOLOGICAL CONTROL AGENTS

Trichoderma harzianum Rifai, a widely distributed saprophytic fungus, which occurs in many soils and other natural habitat especially dead plant tissues controls damping-off and root rot caused by soil-borne pathogens including *Pythium* spp. This process has been viewed as competition mediated or facilitated by antibiosis or other forms of antagonism. Most isolates of *Clonostachys rosea (Link: Fr.)* seems to have the potential to live and reproduce as an epiphyte. The competitive ability of *C. rosea* is likely enhanced by other mechanisms of antagonism including mycoparasitism and antibiosis. C. rosea is suggested to be a destructive necrotrophic mycoparasite, which has the ability to attack a wide range of fungi and fungal structures including hyphae, spores, and sclerotia. Weeds can be attacked by arthropods, vertebrates, and pathogens (fungi, viruses, bacteria and nematodes). The weevil feeder shown in Figure 1 feeds on one particular type of weed called purple loosestrife.

There are some insects which serve as prey for other arthropods (particularly spiders and mites), and several groups of vertebrates (e.g., fish, birds, frogs, toads, bats, mice, shrews, and of course anteaters!). Insects are parasitized by various types of roundworms (nematodes) and are also attacked by a diverse group of pathogens including fungi, protozoa, bacteria, and viruses. There has been an increasing trend in the use of insects as biological control agents. This method is based on the knowledge of natural interaction between the insects and their natural enemies. Some few examples include (see Figure 1):

3.1 Predators

Beetles (order *Coleoptera*): Coccinellidae (lady beetles), Carabidae (ground beetles), and Cicindellidae (tiger beetles) are the most noteworthy families of predators. The lady beetle shown in Figure 1 is an effective predator of aphids and scale insects. A predator consumes many preys during its lifetime.

3.2 Parasites and Parasitoids

Wasps (order *Hymenoptera*): There are over 45 families of small to medium-sized wasps that parasitize other insects. The wasp shown in Figure 1 is laying its egg inside an aphid where its young will develop. Parasitoid immatures develop on or inside a host, killing it as they mature. They emerge as adults and continue the cycle.

3.3 Pathogens

Nematodes (phylum Nematoda): There are over 300 species of nematodes (in 19 families) that are known to attack insects. These nematodes are unique because they harbour symbiotic bacteria that are pathogenic to the nematode's insect host. The nematode shown in Figure 1 is just one example of a pathogen, which may kill its host. Other pathogens include fungi, protozoa, bacteria and viruses.

Several of these beneficial insects are mass produced and available for large-scale distribution with the aim to reduce pest population (see Figure 2).

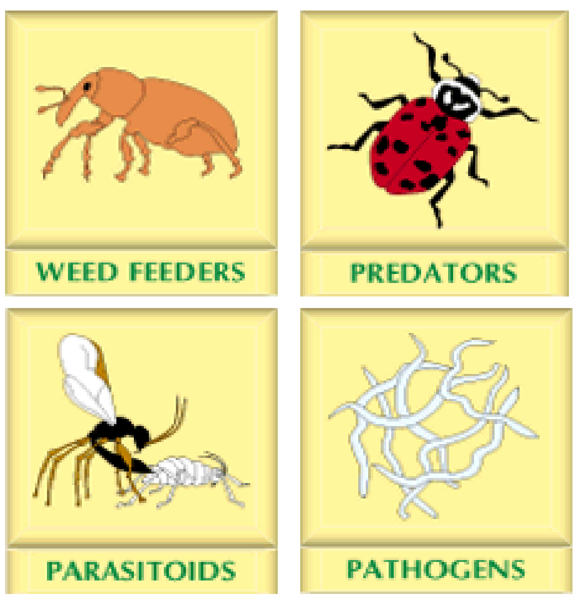

Figure 1: Four types of natural enemies for biological control
Source: Weeden (2005)

(a) (b)

Figure 2: Packages for biological control agents (a) BioBee – picture taken by author on an Israeli pepper farm; (b) Skeletal doom – a natural parasite for control of mosquito larvae

4. POTENTIAL POSTHARVEST BIOLOGICAL CONTROL AGENTS

Fresh fruits and vegetables are often washed and sanitized immediately after harvest and handled under low temperatures in controlled or modified atmosphere. This contributes to the low incidence of insect attacks. However, insect pests are more likely to constitute a major problem in grain storage rather than stored vegetables and fruits. In both cases insects are likely to interfere at earlier stages before or during harvest, although some symptoms may show up in postharvest stages. Therefore, control of such pests should be applied during the pre-harvest stages as has been indicated under pre-harvest BCAs, and thus postharvest use of BCAs against insects has been overlooked in this paper.

4.1 Bacillus spp

A natural epiphytic *Bacillus subtilis* isolated from avocados showed great promise as a biological control agent for pre- and postharvest pathogens (Wilson and Wisniewski, 1989). The organism is a natural inhabitant of the avocado leaf and fruit surfaces and dominates on these surfaces throughout the year. It has a natural ability to compete with other organisms by means of competition for space and nutrients. The organism is of particular importance due to its competitive ability against avocado fruit pathogens. The effective use of *Bacillus subtilis* and its advantages for man has been known earlier. Japan has been using *Bacillus subtilis* as an important ingredient in the fermentation of soybean, providing them a product sometimes referred to as natto, which is one of the most widely used food products in Japan (Sanni et al., 2004) The organism has also been given GRAS (Generally Regarded As Safe) clearness in the USA and has been marketed as a natural biological control product in many countries. *Bacillus* spp. have been successfully tested in field trials, postharvest applications on the use of other *Bacillus* spp. in controlling avocado postharvest diseases has revealed a significant reduction of the severity of postharvest diseases such as anthracnose and fruit rot complex, and stem-end rot.

4.2 Pseudomonas syringae

P. syringae is a nutritionally versatile organism. There are 41 pathovars of *P. syringae* which are able to cause diseases on various plants. The antagonistic strain is their non-pathogenic counterpart and is antagonistic to pathogens on many plants. It can grow well on wounded plant tissue and can control a variety of diseases on different fruits, including pome fruits, banana, and citrus fruits as well as vegetables. The fruit's surface is a natural habitat for many yeasts which dominate fruit microflora, especially close to harvest (Wilson and Wisniewski, 1994). The yeasts grow rapidly on this substrate, which has a high concentration of readily available carbon sources, such as those contained in juices leaking from wounded fruits. *P. syringae* (strain L-59-66 renamed as strain ESC- 11) can control blue mold caused by *P. expansum*, gray mold caused by *B. cinerea*, and Mucor rot caused by *Mucor spp*. on apple and pear. It can also control blue mold caused by *Penicillium italicum*, and green mold caused by *P. digitatum* on citrus fruit. Another strain (ESC-10) of this bacterium, superior in controlling decays of citrus fruit, has been isolated by EcoScience, Corp. Strain ESC-11 has been shown to reduce crown rot of banana, which is caused by a complex of fungi, including *Fusarium semitectum* and *F. moniliforme*, and it also reduced Fusarium dry rot on potato caused by *F. sambucinum*. This strain also prevented growth of the food borne pathogen, *Escherichia coli* O157:H7, in apple wounds. This pathogen can grow quickly on damaged apple tissue and consumption of unpasteurized apple cider contaminated with this bacterium has caused outbreaks of illnesses in recent years.

4.3 Yeast isolates

Yeasts appear to be particularly promising biocontrol agents since production of antibiotics probably is not involved in their activity (Droby et al., 1993b). Various observations suggest that competition for nutrients between yeasts and molds and parasitism are likely to be the main mechanisms of action. Many beneficial yeasts can effectively deplete the sugar occurring in fruit wounds (place of infection) and inhibit germination of mold propagules. Radioactively labeled sugar was taken-up more rapidly by beneficial yeast than by the mold propagules. It is postulated that the nutrient competition can play a significant role as a sole biocontrol mechanism, or it may weaken the mold propagules and predispose them to other mechanisms. Some examples of yeast isolates use as BCAs include *Cryptococcus infirmo-miniatus*, a biological control of naturally-occurring yeast, to control decay of sweet cherry either alone or in combination with a fungicide and modified atmosphere packaging (Wilson and Wisniewski, 1994). Several postharvest diseases affect sweet cherry, including blue mold (*Penecillium expansum*) and brown rot (*Monilinia fructicola*).

C. infirmo-miniatus alone did not control brown rot in air-stored cherries, but it did control blue mold, probably because the longer germination time of blue mold conidia allowed the yeast time to grow and colonize wound sites to prevent infection. A single pre harvest application of the fungicide iprodione reduced brown rot, but better control was achieved when cherry fruit were also treated with a postharvest dip in the beneficial yeast suspension. The combination of pre-harvest fungicide and postharvest biocontrol reduced decay by 88-97%. Modified atmosphere packaging also reduced brown rot, with the greatest reduction when used in conjunction with treatment with the beneficial yeast. Combining all three treatments in an integrated control strategy reduced brown rot from 41% to less than 1%. This strategy gives excellent decay control during long-term storage and transport of sweet cherry fruit (Aharoni, 2004).

Another yeast isolate from peach fruit is *Pichia membranefaciens* It is suggested that *P. membranefaciens* at $5x10^8$ cells per ml completely inhibited Rizopus rot in nectarine wounds artificially inoculated with $5x10^4$ spores per ml at 25, 15, and 30^oC storage conditions. Furthermore it is suggested that the use *P. membranefaciens* for biocontrol is compatible with several common postharvest practices including fungicide, calcium treatment, and cold storage. Other yeast isolates that have been found useful as BCAs against Botrytis storage rot in Kiwifruit are shown in Table 1. Picking wounds were inoculated with yeasts then inoculated with Botrytis spores (Cook et al., 1999).

Table 1: Incidence of Botrytis Storage Rot in Kiwifruit Treated with Different Yeast Isolates
(*4 replications of 100 fruit were used per treatment*)

Isolate	**Yeast**	**Mean percentage of rotted fruit (%)**
K52	*Kluyveromyces marxianus*	6
K58	*Kluyveromyces fragilis*	6
H80	*Hansenula capsulata*	12
K48	*Kluyveromyces fragilis*	12
S20	*Saccharomyces cerevisiae*	13
K47	*Kluyveromyces fragilis*	14
Control	Water	33
LSD (P<0 .05)		3.2

(Source: Cook et al., 1999)

5. DECREASING EFFICACY AND INCONSISTENCY IN COMMERCIAL APPLICATION

Indeed, in recent years, several private firms have been involved in the development of biocontrol products for the control of postharvest diseases of fruits and vegetables. Among these products is the AspireTNt biofungicide (Ecogen; Langhorne, PA), based on the yeast Candida oleophila, (Hofstein et al., 1994) and the EcoScience (Orlando, FL) Biosave 111 and 110, both isolates of Pseudomonas svringae that are registered and recommended for the control of postharvest decay of citrus and pome fruit. Concern has been raised regarding health and safety in relation to the mass introduction of antagonists on our food. Some of the antagonists reported to effectively control postharvest diseases have also been reported to be opportunistic pathogens on immune-compromised humans. Although this might pose an obstacle to public acceptance of this technology, these antagonists are indigenous to agricultural commodities and humans are continuously exposed to them (Whitesides et al., 1994). Even though these antagonists are introduced in large numbers to the surface of a commodity, they survive and grow only in very restricted sites on the fruit surface (surface wounds). After their introduction on the intact fruit surfaces, antagonist populations are reduced to the level of natural epiphytie microflora in a very short period of time. Thus, in spite of the rigorous tests needed to verify their safety to humans and the environment, the use of microbial antagonists to control postharvest diseases of fruits and vegetables is a commercially viable option to the use of synthetic fungicides (Droby et al., 1993b). The performance of biocontrol agents cannot by itself be expected to equal that of a synthetic fungicide. Although reports in the literature indicate that some biocontrol agents are as effective as fungicides, in most cases their performance under commercial conditions has been sometimes inconsistent. The following factors, however, may have a role in the reduced efficacy of postharvest biocontrol agents under large-scale and commercial conditions:

5.1 Fermentation and Formulation

When attempting to scale-up the production and develop a commercial formulation of a certain biocontrol agent, it is essential that the microbial cells retain their attributes as colonizers and antagonists on fruit surfaces.

5.2 Delivery Method

The type of delivery system used to apply a biocontrol agent can affect its performance. Before making a decision on the delivery system to be used to apply biocontrol agents on harvested commodities, one should examine the delivery systems currently being used for the application of fungicides - dip or dump tanks or on-line spray or drench applicators, or as a mixture with coating waxes (Fallik, 2004).

5.3 Inoculum Pressure

For postharvest biocontrol, the most important factor determining the efficacy of any microbial antagonist is the implementation of a stringent sanitation program that reduces pathogenic propagules in water systems and on rollers, belts, brushes, and packinghouse floors. As already reported, the performance of biocontrol agents is much more sensitive than that of synthetic chemicals to the effects of pathogen concentration. Chalutz and Wilson (1990), Janisiewicz (1988), and McLaughlin et al. (1990) have demonstrated that, when pathogen spore concentrations increase, biocontrol efficacy decreases. For postharvest biocontrol to be successful, packing-houses must adopt a program to minimize the exposure of fruit to pathogens.

5.4 Physiological Status of the Fruit

A principal factor that impacts the preservation of harvested commodities is the physiological status of the tissue. Once harvested, commodities are senescing rather than developing. Consequently, the susceptibility of fruit tissue to pathogen attack increases due to weakened natural defence mechanisms, as well as partial degradation of cell walls and subsequent increased leakage of solutes. Over-mature fruit is much more susceptible to fungal attack than fruit picked at optimal maturity (Boonyakiat et al., 1987).

6. BIOCONTROL EFFICACY ENHANCEMENT UNDER COMMERCIAL CONDITIONS

Recently, a bioactive coating has been developed by Wilson and El-Ghaouth (2000) which consists of the combination of complementary biological approaches for additive and/or synergistic effects. Such combinations may have greater stability and effectiveness than the use of single biocontrol agents alone. Biological control activity of antagonists can also be enhanced by several additives:

6.1 Antimicrobial Additives

Enhancing the activity of biocontrol agents could be the most important factor in their success in controlling fruit diseases and their ultimate acceptance in commercial disease management. The addition of calcium salts to yeast cell suspensions markedly enhanced the ability of *P. guilliermondii* to control postharvest diseases of apple (McLauglin et al. (1992). The biocontrol activity of isolate 182 of the yeast *C. oleophila* was enhanced by the addition of 90 or 180 mM $CaCl_2$ (Wisniewski et al. 1995). The combination of sugar analogs, such as 2-deoxy-D-glucose, with the yeast antagonists *Sporobolomvces roseus* or *C. saitoana* enhanced biocontrol against blue mold of apples (El Ghaouth et al., 2000; Janisiewicz, 1994). The addition of nisin improved biocontrol activity of the yeast *C. oleophila* (El-Nashawy et al., 1998). Many other additives, such as the GRAS (generally regarded as safe) compounds such as bioactive coating and “bioenhancer” commonly used in food industry, may enhance activity of biocontrol agents and should be considered as preferred additives.

6.2 Physical Treatments

Other ways to enhance biocontrol efficacy, and possibly ensure consistency, is to integrate the biocontrol agent with physical methods such as curing and heat treatments (Barkai-Golan and Douglas, 1991; Cook et al., 1999), ultraviolet light (Droby et al., 1993 a). Another readily applicable way to enhance efficacy is to combine the biocontrol treatment with modified or controlled atmospheres (MA/CA) and cold storage (Sugar et al., 1994). Preharvest application of biocontrol agents as a stand-alone treatment or combined with a postharvest application of the biocontrol agent may also prove to be a useful strategy in achieving improved performance against infections. This approach could be used as a tool to manipulate epiphytic populations and change patterns of surface wound colonization. Preharvest introduction of antagonists in conjunction with additional postharvest applications may prove successful in providing acceptable levels of control. To fully explore the potential of this approach, however, obtaining data on the composition of epiphytic populations before and after the introduction of a single antagonist is crucial.

6.3 Genetic Manipulation of Biocontrol Agents

While there are several approaches to improving biocontrol activity of yeast antagonists, one of the most attractive is enhancing genetic traits involved with the ability of the antagonist to inhibit establishment and development of the pathogen at the infection court. A molecular approach would be useful in achieving this goal and would allow full exploitation of the potential of these yeasts.

7. FORMULATIONS DESIGN FOR BIOCONTROL AGENTS

To be most effective, antagonists of plant disease and food spoilage should be genetically stable, effective at low concentrations, easy to culture and amenable to growth on an inexpensive medium, effective against a wide range of pathogens in a variety of systems, prepared in an easily distributed form, non-toxic to humans, resistant to pesticides, compatible with other treatments (physical and chemical) and non-pathogenic against the host plant. Under ideal conditions, such as in the laboratory and screen houses, antagonists have been found to completely protect plants and stored fruits and vegetables from pathogens. However, in field conditions where proper deployment of the antagonist appears to be crucial, disease control is likely to be less successful. Commercial successes in preharvest biological control are therefore still rare. Critical factors include moisture, nutrient availability, and pH. If the deployment system can meet the needs of the antagonist, successful colonization is more likely. Careful selection of an aggressive strain of the antagonist is also important. Under these circumstances therefore, research into formulation technology may offer a new stimulus to help develop successful biological control agents from the vast number of strains isolated in the past decades. It is recommended that a rational formulation design should offer the following advantages for biological control agents: easy handling, protection from biotic and abiotic stress factors, enhanced shelf life, controlled release (controlled by environmental conditions and formulation materials) and enhanced efficacy in the soil. Especially amending capsules with certain nutrients, water-retaining substances, and drying additives are expected to enhance efficacy. Depending on the formulation problem different types of formulations can be developed.

Unlike the control of tree, field crop, or soilbome diseases, successful commercial control of postharvest diseases of fruits and vegetables must be extremely efficient, in the range of 95 to 98%. As of today, such levels of control can be reached by biofungicides only when supplemented with low levels of synthetic chemical fungicides. However, by employing several biological, chemical, and physical avenues, either singly or in combination, the efficacy of microbial antagonists may be significantly increased.

8. CONCLUSIONS

Innovative biological control strategies should take into consideration the growing concern over contamination of the produce with human pathogens, as well as plant pathogens. The time is ripe to integrate biocontrol agents with one or more physical treatments such as heat treatments, controlled and modified atmospheres, natural biocides, and food-grade preservatives. Such an integrated approach will probably provide adequate control levels comparable to those achieved by chemical fungicides. To achieve this goal, the fruit and vegetable industry should adopt certain changes in packing and sorting lines required for successful implementation of an integrated control strategy. Molecular approaches may prove useful in developing biocontrol agents with enhanced biocontrol activity and set directions for full exploitation of the genetic potential of these antagonists. Yeast antagonists selected for their biocontrol activity appear to be very well adapted to growth and colonizing fruit surface and especially fruit wound sites. This feature suggests that these yeasts provide an excellent means of delivering bioactive compounds, such as fungal cell-wall-degrading enzymes, directly to areas where pathogen propagules are most likely to germinate and infect the tissue. Combining the ability of these yeasts to rapidly colonize wound sites with enhanced constitutive production of antifungal proteins may prove to be a useful strategy for improving biocontrol effectiveness.

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10. REFERENCES

Aharoni, N. 2004. Packaging, MAP and CA. Lecture notes. International Research and Development Course on Postharvest Biology and Technology, The Volcani Center, Israel.

Campbell, R. 1989. Biological control of microbial plant pathogens. Cambridge University Press, Cambridge. 211pp.

Barkai-Golan, R. and Douglas, J.P. 1991. Postharvest heat treatment of fresh fruits and vegetables for decay control. Plant Dis., 75:1085.

Boonyakiat, D., Chen, P.M., Spotts, R.A., and Richarsdson, D.G. 1987. Effect of harvest maturity on decay and postharvest life of d'Anjou pear. Scientia Hort., 31:131-139.

Chalutz, E. and Wilson, C.L., 1990, Postharvest bioeontrol of green and blue mold and sour rot of citrus by *Debaryomyces hansenii.* Plant Dis., 74:134-137.

Cook, D.W.M., Long, P.G., and Ganesh, S. 1999. The combined effect of delayed application of yeast biocontrol agents and fruit curing for the inhibition of the postharvest pathogen *Botrytis cinerea* in Kiwifruit. Postharvest Biol. Technol., 16:233-243.

Droby, S. 2004. Non-chemical treatments in postharvest. Lecture notes. International Research and Development Course on Postharvest Biology and Technology, The Volcani Center, Israel.

Droby, S. and Chalutz, E. 1994b. Mode of action of biocontrol agents for postharvest diseases.

In: Biological Control of Postharvest Diseases of Fruits and Vegetables: Theory and Practice, C.L. Wilson and M.E. Wisniewski, Eds., CRC Press, Boca Raton, FL, pp. 63-75.

Droby, S., Chalutz. E., Horev, B., Cohen L., Gaba, V., Wilson, C.L., and Wisniewski, M.E. 1993a. Factors affecting UV-induced resistance in grapefruit against the green mold decay caused by *Penicillium digitatum*. Plant Pathol., 42:418-424.

Droby, S., Chalutz, E., Wilson, C.L., and Wisniewski, M.E. 1993b. Biological control of postharvest diseases: a promising alternative to the use of synthetic fungicides. Phyto-parasitica. 20:149-153.

El-Ghaouth, A., Wilson, C.L., and Wisniewski, M. 1998. Ultrastructural and cytoehemical aspects of the biological control of *Botrytis cinerea* by *Candida saitoana* in apple fruit. Phvtopathology; 88:282-291.

Fallik, E. (2004). Chapter 24: Hot Water Treatments for Control of Fungal Decay on Fresh Produce. In: Microbiology of Fruits and Vegetables. (Sapers, G.M., Gorny, J.R. and Yousef, A.E. Eds). CRC Press, Inc., Boca Raton, Florida.

Hofstein, R., Fridlender, B., Chalutz, E., and Droby, S. 1994. Large scale production and pilot testing of biocontrol agents of postharvest diseases. In: Biological Control of Postharvest

Diseases of Fruits and Vegetables: Theory and Practice, C.L. Wilson and M.E. Wisniewski. Eds., CRC Press, Boca Raton FL, 182 pp.

Janisiewicz, W.J. 1988. Biological control of diseases of fruit. In: Biocontrol of Plant Diseases, Vol. II, K.G. Mukergi and K.L. Grag, Eds., CRC Press, Boca Raton, FL, pp. 153-165.

Janisiewicz. W.J. 1994. Enhancement of biocontrol of blue mold with nutrient analog 2-deoxy-D-glucose on apples and pears. Appl. Environ. Microbiol., 60:2671-2676.

McLaughlin, R. J., Wisniewski, M.E., Wilson, C.L., and Chalutz, E. 1990. Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. Phytopathology, 80:456-461.

McLaughlin, R. L., Wilson, C. L., Droby, S., Ben-Arie, R., and Chalutz, E. 1992. Biological control of postharvest diseases of grape, peach, and apple with the yeast *Kloekera apiculata* and *Candida guilliermondii*. Plant Dis. 76:470-473.

Sanni, L., Almasri, M., Fan, Z., Mjahed, J and Khumlert, R. 2004. The use of biological control agents. Unpublished Group Project. International Research and Development Course on Postharvest Biology and Technology, The Volcani Center, Israel.

Sugar, D., Roberts, R.G., Hilton, R.J., Reghetti, T.L., and Sanchez, E.E. 1994. Integration of cultural methods with yeast treatment for control of postharvest decay in pear. Plant Dis., 78:791-795.

Weeden, C.R., Shelton, A. M., Li, Y and Hoffmann, M.P. 2005. Biological control. A guide to natural enemies in North America. http://www.nysaes.cornell.edu/ent/biocontrol/

Whitesides, S.K., Daoust, R.S., and Gouger, R.J. 1994. Commercialization of biological control agents: an industry perspective. In: Biological Control of Postharvest Diseases of Fruits and Vegetables: Theory and Practice, C.L. Wilson and M.E. Wisniewski, Eds. CRC Press, Boca Raton FL, 182 pp.

Wilson, C.L. and Wisniewski, M.E. 1989. Biological control of postharvest diseases of fruits and vegetables: an emerging technology. Ann. Rev. Phytopathol., 27:425-441.

Wilson, C.L. and Wisniewski, M.E. 1994. Biological Control of Postharvest Diseases of Fruits and Vegetables: Theory and Practice. CRC Press, Boca Raton, FL, 182 pp.

Wisniewski, M.E., Droby, S., Chalutz, E., and Eilam, Y. 1995. Effects of Ca+ and Mg+ on *Botrytis cinerea* and *Penicilium expansum* in vitro and on the biocontrol activity of *Candida oleophila*. Plant Pathol., 44:1016-1024.