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Review article

BACTERIA AND FUNGI AS BIOCONTROL AGENTS AGAINST POME FRUIT POSTHARVEST PATHOGENS

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ABSTRACT
Pome fruits are highly susceptible to postharvest decomposition by various fungi. The most common decays resulting in tremendous economic losses are caused by *Penicillium expansum*, *Botrytis cinerea*, *Monilinia fructicola*, *Colletotrichum* spp., *Mucor piriformis* and *Rhizopus* spp. In addition to vast economic losses due to fungal decomposition of these fruits, some decay-causing pathogens (*i.e. P. expansum*) produce toxic secondary metabolites which have adverse health effects on humans. Mainly, synthetic fungicides have been used to protect fruits from postharvest pathogens. In order to reduce the use of these chemicals and consequently, lessen their adverse effects on human health and the environment and to find ways to control pathogen strains that have acquired resistance to such fungicides, scientists have been in quest of alternative methods for controlling postharvest pathogens. Biocontrol agents (BCAs) are a promising alternative to chemical fungicides. Several bacteria, yeasts and moulds including members of the *Bacillus*, *Pantoea*, *Pseudomonas*, *Candida*, *Cryptococcus*, *Filobasidium*, *Kloeckera*, *Metchnikowia*, *Pichia*, *Rhodotorula*, *Sporobolomyces*, *Aureobasidium* and *Trichoderma* genera were proven to possess biocontrol activities against various postharvest pathogens. Combinations of BCAs as well as application of a BCA following another treatment produced increased protective effects against the pathogens. This review summarizes the specific activities of several BCAs and their advantages and shortcomings as means of controlling postharvest decays of pome fruits.

KEY WORDS: postharvest decays, pome fruits, biocontrol, bacteria, yeasts, moulds.

INTRODUCTION

Many fungal species attack and cause spoilage of pome fruits (apples, pears, quinces, *etc*.) in the field and after harvest in storage, during marketing and at the consumers' hands (El-Ghaouth *et al*., 2004; Pierson *et al*., 1971). The main mode of controlling field and postharvest fruit diseases involves the utilization of synthetic chemical pesticides. In more recent years there is a trend to turn away from such synthetic substances due to their toxicity and negative effects on human health and the environment (Eckert *et al*., 1994); additionally, pathogens often acquire resistance to these chemicals and can no longer be controlled by the traditional fungicides (Holmes and Eckert, 1999; Spotts and Cervantes, 1986; Xiao and Campbell, 2007). An attractive alternative is the utilization of biological control. Biocontrol is the use of live organisms, which, when applied to the plants or plant crops simultaneously with the disease-causing organisms, suppress the growth of the pathogen and therefore, lessen the impact of the disease (Eilenberg, 2006). These protective organisms are collectively referred to as biological control agents (BCAs). In order for a BCA to be effective, it should possess certain attributes such as the ability to grow well in the same environment (ecological fitness) where the pathogen grows and causes the disease, it should have similar nutritional requirements as the pathogen and most importantly, the BCA should not inflict disease on the crop that it is supposed to protect. Additionally, the biocontrol agent and its metabolites should not be toxic or pathogenic to humans and animals. The use of live organisms to protect crops from pathogenic ones has been considered for over three decades. Many biocontrol agents have been studied, but a much lesser number of them have been used on a commercial scale in various parts of the world to control a number of plant pathogenic organisms in the field (Johnsson *et al*., 1998; Stockwell *et al*., 2010). BCAs have also been tried for the protection of various fruits against postharvest pathogens with varying degrees of success. Applications of *Aureobasidium pullulans* on strawberries before storage, for instance, resulted in 70% reduction of spoilage by *Rhizopus*, while application of an antibiotic-producing *Pseudomonas corrugata* on wounded peaches and nectarines was effective in controlling postharvest brown rot caused by *Monilinia fructicola*. Similar effects against brown rot were observed when *Pseudomonas cepacia* (*Burkholderia cepacia*) was used at a concentration of 10^5 cfu/wound (Lima *et al*., 1997; Smilanick *et al*., 1993).

Several microorganisms possessing biocontrol capabilities against pome fruit postharvest pathogens have also been studied over the last three decades. Most of the research involving BCAs has been focused on the suppression of spoilage of pome fruits by *Penicillium expansum*, *Botrytis cinerea*, and *Rhizopus* spp. Some examples of such research include the studies on *Cryptococcus laurentii*, *Candida oleophila* and *Pseudomonas syringae* (ESC-10) as antagonists against gray mould (*B. cinerea*) of apples, the research on the potential of yeasts belonging to *Cryptococcus*, *Debaryomyces*, *Filobasidium*, *Rhodosporidium*, *Rhodotorula*, *Sirobasidium*, *Sporidiobolus*, *Tremella*, *Trichosporon* and *Yarrowia* genera as BCAs for controlling postharvest spoilage of pome fruits by *B. cinerea*, and the studies on *Cr. Laurentii*, *Metschnikowia pulcherrima*, *C. oleophila*, and *P. syringae* as means of controlling blue mould (*P. expansum*) decays in apples (Filonow *et al*., 1996; He *et al*., 2003; Janisiewicz and Marchi, 1992; Janisiewicz *et al*., 2001; Janisiewicz and Korsten, 2002; Mercier and Wilson 1994; Roberts 1990; Sugar and Spotts 1999; Zhang *et al*., 2006). This review summarizes the bio-protective capabilities of various bacterial and fungal BCAs studied and/or utilized for the control of fungi causing postharvest spoilage in pome fruits.

Microorganisms with biocontrol activities against postharvest pathogens of pome fruits

An excellent postharvest biocontrol agent must have several characteristics including inability to grow at 37°C and cause human or animal disease, stability, high efficacy at low concentrations, feasibility to be produced easily and inexpensively, adaptability to a formulation with good shelf life, ease of dispersion, possessing modes of biocontrol other than production of antibiotics, ability to survive at low temperatures and controlled atmospheres, exerting efficacious control against the target postharvest pathogen(s) (preferably having inhibitory activity against a wide spectrum of pathogens), inability to cause disease on the treated commodity and resistance to chemical pesticides. Although it is difficult to find an ideal biocontrol agent with all these characteristics, several bacterial and fungal strains have exhibited good antagonistic properties against various postharvest fruit pathogens. Among the most extensively-studied BCAs are *Pseudomonas* spp., *Aureobasidium pullulans*, *Trichoderma* spp. and certain members of the yeast genera *Cryptococcus*, *Candida*, *Metschnikowia*, *Pichia*, *Rhodotorula* and *Sporobolomyces* (Table 1). A description of the efficiencies of these organisms is presented in the following pages.

Bacteria

1. *Bacillus* species

Several *Bacillus* strains showed potential as BCAs for the protection of pome fruits against postharvest spoilage. *Bacillus* sp. T03-c from preserved bean curd (Furu) and *Bacillus* sp. C06 (*B. amyloliquefaciens*) from cheese starter were antagonistic to *M. fructicola* and reduced brown rot decays of artificially-infected apple fruits by more than 50% as compared to the pathogen-only-inoculated control fruits (Zhou *et al*., 2008). Various strains of *Bacillus megaterium* were also found to possess biocontrol properties against pre- and postharvest pathogens. Abanda-Nkpwatt *et al*. (2006) reported that a *B. megaterium* isolate from strawberry leaves exhibited good biocontrol against *B. cinerea in vitro*. Poleatewich (2010) studied the biocontrol potential of *B. megaterium*, strains A3-6 and Ae-1 on Golden Delicious and Rome Beauty apples pre- and postharvest. Pre-harvest application of the bacteria reduced leaf and fruit scab (caused by *Venturia inaequalis*). The biocontrol effects continued after harvest and suppressed biter rot (*Colletotrichum acutatum*) development in apples during storage. The biocontrol activity was improved when these BCAs were re-applied to the fruits after harvest. Postharvest application of strain A3-6 or Ae-1 in combination with chitosan caused a significant reduction in blue mould rot and improved the control of biter rot in Golden Delicious and Rome Beauty apples. Scanning electron microscopy studies showed that *B. megaterium* A3-6 cells were attached to *C. acutatum* hyphae and that the hyphae were damaged at the points of attachment. Another *Bacillus*, *B. mycoides* strain A1-1, as well as *Brevibacillus laterosporus* isolate FLS-1 also induced significant reduction of bitter rot and blue mould lesion size on Golden Delicious and Rome Beauty apples (Poleatewich, 2010).

2. *Burkholderia* species

Burkholderia cepacia (*P. cepacia*) was studied and found to have inhibitory activity against some plant and postharvest pathogens such as *B. cinerea* and *P. expansum* on apples and pears (Janisiewicz *et al*., 1991; Parke, 2005). *B. cepacia* products, Blue Circle and Deny, were developed and registered with the United States Environmental Protection Agency (USEPA) as biocontrol agents. Due to the fact that some *B. cepacia* strains are human pathogens, however, in 2004 EPA issued a new rule revoking all *B. cepacia* strains as BCAs (USEPA, 2004). *Burkholderia gladioli* was evaluated by Scuderi *et al*. (2009). In this study, cell suspensions and cell-free culture filtrates of three *B. gladioli* strains were tested *in vitro*. Both, cells and cell-free filtrates, showed varying degrees of inhibition against *P. expansum*, *B. cinerea* and *Aspergillus flavus* among other postharvest pathogens. Application of cell suspension had greater inhibitory effect on pathogens than cell-free culture filtrates. Complete inhibition of *P. expansum* inoculated on apple wounds and incubated for 9 days at 20°C was achieved by one of the strains (DISTEF-G).

3. Lactic acid bacteria (LAB)

Lactic acid bacteria recovered from fresh fruits and vegetables were tested for antagonistic activities against *P. expansum* and *B. cinerea* by Trias *et al*. (2008). This study revealed that some LAB isolates possessed moderate biocontrol activity against the pathogens. A *Weissella cibaria* (strain TM128) reduced infection levels in artificially-wounded and inoculated Golden Delicious apples by 50%. Antimicrobial compounds found in cell-free supernatants of some bacterial cultures were mainly organic acids, while some bacteria also facilitated the formation of hydrogen peroxide.

Zhou *et al*. (2008) tested several bacterial strains including *Lactobacillus* strains P02 (recovered from Kimchi-cabbage) and C03-b (from Ricotta cheese) for antagonistic activity against *M. fructicola*. These investigators surface

sterilized and artificially wounded Jonagold and Golden Delicious apples and subsequently, inoculated the wounded fruits with 20 µl/wound of a suspension containing a potential biocontrol agent and the pathogen (1.0×10^7 cfu/ml biocontrol agent and 1.0×10^4 *M. fructicola* conidia/ml). The treated apples were incubated at 22-24ºC under 85% relative humidity (RH). The incidence and severity of the disease was observed after 5 days of incubation. Both strains showed antagonism against the pathogen. *Lactobacillus* strain (P02) reduced the incidence of brown rot of apple fruit by more than 80% and the lesion size by over 90%, while *Lactobacillus* sp. C03-b reduced incidence of decays by 53% and lesion size by about 47%.

4. *Pantoea agglomerans*

Various past studies have shown that effective control of postharvest apple and pear diseases could be achieved by application of *Pantoea agglomerans*. Francés *et al*. (2006) studied the effect of *P. agglomerans* EPS125 on various postharvest pome fruit fungal pathogens. These investigators used Golden Delicious apples and Blanquilla pears in their experiments and found that *P. agglomerans* was efficient in controlling blue mould and *R. stolonifer* on apples and pears, and *B. cinerea* on apples. The degree of biocontrol depended on the pathogen aggressiveness and the concentration of the BCA. Nunes *et al*. (2002a) studied the effects of *P. agglomerans* (strain CPA-2) on the growth of the above pathogens on Golden Delicious apples. These investigators inoculated various concentrations of *P. agglomerans* along with the pathogens, *P. expansum* or *B. cinerea*, onto the wounds of artificially-damaged apples and incubated the treated fruits at 20ºC and ~85% RH for 1 week. The conclusion of this study was that *P. agglomerans* was a potent antagonist of the pathogens examined. The highest control of *P. expansum* and *B. cinerea* was achieved when *P. agglomerans* was used at a concentration of 8.0×10^7 cfu/ml. The antagonistic effect was further enhanced when *P. agglomerans* (8.0×10^7 cfu/ml) was applied in combination with *C. sake* (2.0×10^7 cfu/ml) at a 50:50 proportion. The efficacy of *P. agglomerans* against *P. expansum* and *B. cinerea* was also studied on apples stored at 1ºC and ~90% RH for 120 days in air or 140 days under reduced oxygen. *P. expansum* decays of fruits in cold storage (1ºC) in air and under low oxygen (1% O_2-2% CO_2) were reduced by 81 and 100%, respectively. Under these conditions, control of *B. cinerea* by *P. agglomerans* was similar to that of 0.5% of the fungicide, imazalil (Nunes *et al*., 2001).

Nunes *et al.* (2001) also studied the efficacy of *P. agglomerans* (CPA-2) for controlling postharvest diseases of pears and reported that this organism was very effective against *B. cinerea, P. expansum* and *R. stolonifer* grown on pears. These investigators inoculated wounded Blanquilla pears with 2.0×10^7, 8.0×10^7 or 1.0×10^8 cfu/ml of *P. agglomerans* along with a pathogen mould (*B. cinerea, P. expansum* or *R. stolonifer*) at concentrations of 1.0×10^3, 1.0×10^4 or 1.0×10^5 spores/ml and stored the treated fruits at 20ºC for 7 days or at 1ºC for 60 days. *P. agglomerans* grew well on the fruit wounds at room temperature and cold storage but did not flourish on the surface of intact fruits perhaps due to inability to derive enough nutrition from the intact skin of the fruit. At 20ºC, the antagonist completely inhibited *P. expansum* and *R. stolonifer* and significantly reduced *B. cinerea* lesion size. *Pantoea* was also very effective against *B. cinerea* and *P. expansum* in cold storage. Its inhibitory effect was comparable to that of commercial doses of imazalil.

5. *Pseudomonas* species

Several pseudomonads have been assessed and found to possess biocontrol properties against various plant and postharvest pathogens. They are suitable as BCAs because they can utilize many compounds present in plant exudates as sources of nutrition, they can survive in the environment under relatively harsh conditions and they grow exponentially in pome fruit wounds protecting the fruit from fungal pathogen infections and decay (Lungtenberg *et al*., 1999; Janisiewicz, 2012). The ability and degree of biocontrol is species- and strain-dependent. Experiments by Etebarian *et al*. (2005) showed that *Pseudomonas fluorescens* (strain 1100-6) significantly suppressed the incidence and size of rots caused by *P. expansum* on apples after 11 days of incubation at 20ºC and 25 days at 5ºC when it was inoculated onto apple wounds 24-48 h before the pathogen. The levels of *P. fluorescens* were increased during the incubation period from 6.95 at inoculation time to 9.12 log 10 cfu/wound after 25 days at 5ºC. Cell-free supernatants of *P. fluorescens* cultures also reduced colony size of *P. expansum*, indicating that the organism perhaps excretes antifungal compound(s).

Certain *Pseudomonas syringae* strains such as ESC-10 (from apple fruit) and ESC-11 (from apple leaf) have been extensively studied and commercialized as biocontrol agents. ESC-10 is sold under the commercial names BioSave 10 LP, BioSave 100 and BioSave 1000, while ESC-11 (formerly called *P. syringae* strain L-59-66) is marketed as BioSave 110 and BioSave 11 LP (Stockwell and Stack, 2007). Comparisons of BioSave formulations to chemical fungicides and alternative decay control treatments have shown that these formulations are highly efficient in limiting postharvest fruit spoilage. Sugar and Basile (2007) examined various non-fungicide treatments for the inhibition of postharvest decay in winter pears including BioSave 110, chitosan, sodium bicarbonate and peroxyacetic acid (StorOx), and thiabendazole (TBZ) (Mertect 340F) as chemical fungicide control. Postharvest treatments were applied to wounds of Bosc pears and the treated fruits were stored at 0ºC for 4 months. Although all treatments exerted significant control of pear decay, the lowest decay was observed in fruits treated with sodium bicarbonate and BioSave 110. Decay control by these methods was comparable to that facilitated by TBZ. Sodium bicarbonate and chitosan, however, caused fruit injuries and could not be recommended for decay control in pears. BioSave 10 LP and BioSave 11 LP were also applied to pome fruits after harvest to control storage pathogens (Stockwell and Stack, 2007). Use of BioSave 10 LP reduced growth of blue and gray mould decays in Golden Delicious apples by over 60% while BioSave 11 LP effected 67-97% control of these diseases (Janisiewicz and Korsten, 2002). BioSave 11 LP exerted 100% biocontrol of blue mould on Ace Spur Delicious and Pink Lady apples in cold storage (Janisiewicz *et al*., 1992). The

latter strain was also very effective against gray mould rot in Anjou, Bosc and Bartlett pears bringing about 100, 78 and 100% decay control, respectively when the treated fruits were stored at ambient temperature. The control of blue mould on the same pear varieties by BioSave 11 LP was 93, 69 and 41% in cold storage and 84, 46 and 40% at ambient storage for Anjou, Bartlett and Bosc pears, respectively (Janisiewicz and Peterson, 2004). In another study, Errampalli and Brubacher (2006) tested BioSave 10 LP and cyprodinil (a reduced risk fungicide) for their efficacies in controlling blue mould in Empire and McIntosh apples in cold storage (~2ºC) or at 20ºC. These investigators used a bacterial concentration of 1.4 x 10^8 cfu/ml or 20 µg/ml cyprodinil. These concentrations were proven effective against TBZ-sensitive and TBZ-resistant *P. expansum* strains when the inoculated fruits were kept in cold storage for 30 days and subsequently at 20ºC for 6 days. Cyprodinil was effective when it was applied at the same time or after pathogen inoculation, while *P. syringae* was more effective when it was co-inoculated with the pathogen.

Yeasts

Several members of the *Candida*, *Cryptococcus*, *Filobasidium*, *Kloeckera*, *Metchnikowia*, *Pichia*, *Rhodosporidium*, *Rhodotorula*, *Sporobolomyces*, and *Saccharomyces* genera were assessed and proven to possess biocontrol activity against various pome fruit postharvest pathogens. A description of the biocontrol capabilities of several such yeast species is given in the following pages.

1. *Candida* species

Candida oleophila has been extensively studied as BCA for postharvest control of fungal pathogens to reduce fruit losses in storage. The antagonistic activity of *C. oleophila* against the gray mould causative agent, *B. cinerea*, was investigated by Mercier and Wilson (1994). These researchers applied 20 µl of a *C. oleophila* (strain 182) cell suspension (~1.0 x 10^6 cells/ml) to freshly-inflicted wounds of Red Delicious apples and 2 h later infected the wounds with 20 µl of a *B. cinerea* conidial suspension (1.0 x 10^5 cfu/ml); subsequently, they stored the treated fruits at 4 and 18ºC for 20 and 10 days, respectively. Observations made after the incubation periods revealed that *C. oleophila* successfully colonized the wounds of the fruit and exercised significant gray mould control. Co-inoculation of *C. oleophila* with some natural fungal flora of apples (*e.g. Aureobasidium pullulans* or *Sporobolomyces roseus*) did not affect its colonization ability and in some cases improved the antagonist effect against the pathogen. Mercier and Wilson (1995) investigated the biocontrol activity of *C. oleophila* (isolate 182) on wounded apples with various levels of moisture in the wound area and reported that this antagonist exercised efficient control of gray mould decay on wounded apples stored at 18ºC. Moisture level in the wound area affected yeast proliferation. Application of the yeast to fresh wounds (high moisture) resulted in a 32-fold increase of yeast population in the first 24 h and increased the degree of biocontrol of the pathogen (*B. cinerea*), while air-drying the wounded apples for 24 h (prior to the BCA application) resulted in significantly lower yeast growth and lower degree of *B. cinerea* inhibition. Wisniewski *et al*. (1995) studied the effects of Ca^{2+} and Mg^{2+} on the biocontrol activity of *C. oleophila* (strain 182) against *B. cinerea* and *P. expansum*. These investigators found that the biocontrol activity of the yeast was increased when 90 or 180 mM of $CaCl_2$ was added and that Ca^{2+} ions were inhibitory to mould spore germination. When the $CaCl_2$ concentration increased from 25-175 mM, the spore germination and germ tube growth was decreased in both moulds tested. The pectinolytic activity of *B. cinerea* and *P. expansum* were also inhibited by $CaCl_2$ with a greater effect on *P. expansum*. *C. oleophila* was commercially utilized under the name 'Aspire' in the U.S. for postharvest biocontrol of fungal pathogens to reduce fruit losses in storage. This product however, is no longer available (Droby *et al*., 2009). Another *C. oleophila*-based product under the name 'Nexy' was later developed in Belgium for use as a postharvest biocontrol agent against *P. expansum* and *B. cinerea* on apples and pears (Janisiewicz, 2010; Kegley *et al*., 2010).

Candida ciferrii stain 283 was assessed as an antagonist of *P. expansum* on harvested Red Delicious apples and was found to reduce blue mould decays by 80% when the inoculated fruits were stored at 25ºC for 7 days or at 5ºC for 28 days. The severity of mould decay was also significantly reduced by the application of this yeast (Vero *et al*. 2002). Scherm *et al*. (2003) studied the effects of two *Candida guilliermondii* strains (3-C-1b and F1) on *P. expansum* inoculated on apples. During this study, Golden Delicious and Fuji apples were surface-disinfected, wounded and inoculated with *C. guilliermondii* (1.0 x 10^6 cells/wound); two hours after the yeast application, the fruits were artificially-infected with *P. expansum* (1.0 x 10^4 conidia/wound) and stored at 25ºC under ~85% RH for 1 week. Both strains had significant antagonistic capabilities against *P. expansum* reducing lesion diameter on Golden Delicious apples by ≥75%. Combination of *C. guilliermondii* with $CaCl_2$ increased pathogen inhibition sometimes up to 100%.

Candida membranaefaciens was tested by Gholamnejad and Etebarian (2009) *in vitro* and *in vivo* (on apples) for antagonism against *P. expansum*. These investigators used yeast cell suspensions, cell-free culture filtrates and volatile culture metabolites in their experiments and found that the highest inhibition was facilitated by the volatile metabolite fraction followed by cell-free filtrates. Yeast cell suspensions were also inhibitory to spore germination and germ tube growth. The degree of inhibition depended on the yeast cell concentration; at a concentration of 1.0 x 10^7 cfu/ml, *P. expansum* was completely inhibited *in vitro* and on apples. The antagonistic activity of the yeast was enhanced by the addition of 2% $CaCl_2$ to the suspension. The potential of *Candida sake* as a biocontrol agent against *P. expansum* was investigated by Usall *et al*. (2000).These investigators artificially inoculated harvested Golden Delicious apples with *C. sake* (strain CPA-1) and the pathogen before cold storage. This treatment caused over 80% reduction in lesion diameter and 50% reduction in the incidence of decays. Higher biocontrol was achieved when the inoculated fruits were stored under controlled atmospheres (Teixido *et al*., 1999). *C. sake* has been utilized in the formulation of 'Candifruit', a biocontrol product developed in Spain and used for controlling

decays of pome fruits (Abano and Sam-Amoah, 2012; Janisiewicz, 2010; Nunes, 2012).

2. *Cryptococcus* species

Past studies showed that some cryptococci possessed substantial antagonistic activities against various postharvest pathogens. Several *Cryptococcus* strains (*Cryptococcus albidus* strain HRB2, *Cryptococcus infirmo-miniatus* strain YY6, *Cryptococcus laurentii* strain HRA5, *etc*.) were studied by Chand-Goyal and Spotts (1996) for antagonism against moulds causing pear diseases. *Cr. albidus* strain HRB2, *Cr. infirmo-miniatus* strain YY6 and *Cr. laurentii* strain HRA5 were effective against blue mould; *Cr. laurentii* HRA5 and *Cr. infirmo-miniatus* YY6 were also inhibitory to gray mould, while *Cr. infirmo-miniutus* strain YY6 was the most efficient in controlling *Mucor* rot. This strain, when inoculated onto wounded Bosc pears (about 5.0 x 10^6 cfu/wound), was also effective in reducing blue mould rot for 60 days at 0ºC. The incidence of infection was reduced by more than 70% and the lesion size decreased from about 21 mm to less than 4 mm (Sugar and Spotts, 1999). Another *Cr. albidus* strain (CBS No. 604.94) was highly effective against *B. cinerea* and *P. expansum* in apples and pears; this BCA was commercialized and registered in South Africa under the brand name YieldPlus (Anchor Yeast, South Africa) (Haissam, 2011). Filonow *et al*. (1996) tested *Cryptococcus humicola* NRRL Y1266 *in vivo*. According to these investigators, *Cr. humicola* applied to wounds of Golden Delicious apples, which were subsequently inoculated with *B. cinerea* spores and incubated at 22-24ºC for 7 days, reduced the incidence of mould growth by 89% and the lesion diameter from over 27 mm to 1.1 mm as compared with the control apples. The severity and incidence of gray mould on apples treated with *Cr. humicola* was substantially lower than on apples treated with the pesticide benomyl. Research on *Cr. laurentii* showed that several strains of this species were efficient antagonists of postharvest pathogens. He *et al.* (2003) tested a *Cr. laurentii* strain (isolated from soil collected from a pear orchard) for its antagonistic activity against *P. expansum*. These researchers inoculated the test yeast and the pathogen on wounds of Red Fuji apples, incubated the treated fruits under various conditions and determined that *Cr. laurentii* possessed biocontrol activity and that certain factors such as Fe^{2+} concentration in the yeast growth medium, inoculation timing and incubation temperature affected the degree of biocontrol. The highest pathogen inhibition was observed when the yeast was growing in a medium containing 2.5 µmol/l Fe^{2+} and it was suggested that perhaps there was some competition for iron that facilitated inhibition of *P. expansum* spore germination. The size of the mould decay was positively-correlated with the incubation temperature and inversely-correlated to yeast concentration inoculated onto the fruit wounds. A concentration of 3.5 x 10^7 *Cr. Laurentii* cells/ml was sufficient to inhibit the growth of *P. expansum* (1.0 x 10^4 spores/ml) for over 2 weeks when incubated at 15ºC. The timing of the antagonist inoculation also played a pivotal role in the degree of biocontrol exhibited by this yeast. Application of the antagonist 12 h or longer prior to inoculation of the pathogen resulted in complete protection of the apples against the pathogen (He *et al*., 2003). *Cr. laurentii* BSR-Y22 (from soil) reduced gray mould spoilage substantially in artificially-infected Golden Delicious apples. The apples were co-inoculated with *B. cinerea* and its antagonist yeast and incubated for 1 week at 22-24ºC. The incidence of *Botrytis* growth after the incubation period was reduced by 74.4% and the diameters of the lesions were about 1.5 mm while respective lesions on pathogen-only-inoculated apples were larger than 27 mm (Filonow *et al*., 1996). In a study by Roberts (1990), *Cr. laurentii* RR87-108 (1.0 x 10^4 - 1.0 x 10^5 cells/wound) was inoculated onto punctured wounds of Golden Delicious apples along with *B. cinerea* conidia (10 µl/wound of a suspension containing 2.0 x 10^4 spores/ml) and the fruits were incubated at various temperatures (5, 10, 15, or 20ºC) for 12 days. The results of this study showed that this strain was a strong antagonist of *B. cinerea* at all temperatures tested and its effect was similar to that of the fungicide benomyl. A *Cr. laurentii* strain isolated from the surface of pear from an unsprayed orchard was studied by Zhang *et al*. (2006). These investigators tested the organism for its ability to control the growth of *P. expansum* on artificially-wounded pears. The fruit wounds were inoculated with 20 µl of a *P. expansum* conidial suspension (1.0 x 10^5 spores/ml), air-dried for 2 h and subsequently, inoculated with 30 µl of a *Cr. laurentii* suspension (1.0 x 10^8 cells/ml). Then the fruits were stored at 25ºC for 6 days. This experiment showed that the antagonist yeast effected a significant reduction of both, percentage of decayed fruits and size of lesions. Biocontrol was enhanced when the antagonist was combined with microwave treatment (2450 MHz for 2-3 min). Sugar and Spotts (1999) tested two *Cr. laurentii* strains (HRA5 and 87-108) among other yeast species for their effects on the growth of TBZ-sensitive and TBZ-resistant *P. expansum* on Bosc pears. Both strains exhibited strong biocontrol traits against the mould pathogen reducing its occurrence on the artificially-inflicted pear wounds by 94-96% and the size of the lesions from about 22 mm to less than 1 mm. These strains had significantly higher antagonistic activities than those of the commercial biofungicides, Aspire and BioSave 11. Other *Cr. laurentii* strains (*e.g. Cr. laurentii* BSR-Y7 and *Cr. laurentii* EB-4D-Y4) were antagonistic to *B. cinerea* grown on Golden Delicious apples to much lesser degrees (Filonow *et al*., 1996).

3. *Filobasidium floriforme*

Filobasidium floriforme NRRL Y7454 was found to substantially reduce the incidence of spoilage in Golden Delicious apples by gray mould. Decay incidence was reduced by 75.6% and the lesion size was about 1/9 that on the untreated control apples after 1 week of incubation at 22-24ºC. The pathogen suppression achieved by this strain was comparable to that of the chemical fungicide, chlorothalonil and the well accepted biocontrol agent, *Sporobolomyces roseus* FS-43-238 (Filonow *et al*., 1996).

4. *Kloeckera* species

Some *Kloeckera* strains have also been tested in the past for biocontrol activity and a few were found to be antagonistic to postharvest pathogens of apples. Four strains of *Kloeckera apiculata* (including strain 138, isolated from the surface of grapes) were evaluated for biocontrol activity against *B. cinerea* and *P. expansum* by

McLaughlin *et al*. (1992). These investigators inoculated artificially-wounded apples with a suspension containing 1.0 x 10^8 cells/ml of *K. apiculata* (50 µl/wound) and, after 2 h at 24-26ºC, they infected the fruit wounds with *P. expansum* (20 µl/wound of a 1.0 x 10^4 spores/ml suspension) or *B. cinerea* (20 µl/wound of a 1.0 x 10^5 spores/ml suspension) and stored the treated fruits at 24ºC for 1 week. The tested yeast strains were effective in reducing decays caused by both, *B. cinerea* and *P. expansum*. Their biocontrol efficacies increased when the yeast cell suspensions were made in 2% $CaCl_2$. Spadaro *et al*. (2008) tested *Hanseniaspora uvarum* (*K. apiculata* asexual stage) for biocontrol activity against *P. expansum* artificially-inoculated on four apple cultivars (Golden Delicious, Stark Delicious, Granny Smith and Royal Gala) after harvest. The infected apples were stored at room temperature and in cold storage. The results of this study suggested that *H. uvarum* exerted significant biocontrol of *P. expansum* on Golden Delicious apples.

5. *Metschnikowia* species

Certain *Metschnikowia* strains have exhibited biocontrol activity against postharvest pathogens of pome fruits. In general, the most effectual BCAs were strains residing in orchards and on fruit surfaces. *Metschnikowia andauensis* PBC-2 (isolated from apple fruit) was studied by Manso and Nunes (2011) for its ability to inhibit *P. expansum* on various apple cultivars and Rocha pears. This organism (at concentration of 1.0 x 10^7 cells/ml) was able to reduce blue mould decays on both apples and pears. *M. andauensis* was also very effective against *R. stolonifer* and *B. cinerea* artificially-inoculated on Golden Delicious apples and exercised excellent control of *P. expansum* in semi-commercial trials under cold storage. The mechanism of biocontrol action of this organism is not known. *Metschnikowia fructicola* was also found to possess significant biocontrol activity. This organism is registered in Israel under the name 'Shemer' and has been used for the control of postharvest diseases of various fruits and vegetables (Droby, 2006; Blachinsky *et al*., 2007; Kurtzman and Droby, 2001).

Metschnikowia pulcherrima has been frequently isolated from orchards and various aerial parts of apple trees (leaves, growing apples, apple bunds and floral parts) and is one of the most frequently isolated organisms from wounded apples and mature grapes (Davenport, 1976; Chamberlain *et al*., 1997). Therefore, its potential as a biocontrol agent has been explored in the past couple of decades. Janisiewicz *et al*. (2001) tested eight *M. pulcherrima* strains originating from an unmanaged orchard for their antagonistic activities against *P. expansum* on artificially-wounded Golden Delicious apples. These investigators reported that all tested strains grew similarly well on laboratory media at 1ºC, but had differing growth rates at 0ºC and different degrees of resistance to diphenylamine (DPA), an antioxidant used to control scalding of apples in storage. All strains exhibited a high biocontrol potential against *P. expansum* and significantly reduced decays on Golden Delicious apples stored for 1 month at 1ºC followed by 1 week at room temperature. Furthermore, these researchers reported that the strains under study did not produce killer toxins that would be active against *Saccharomyces cerevisiae* NCYC 1006 (a strain sensitive to these toxins) (Farris *et al*., 1991). Saravanakumar *et al*. (2008) studied *M. pulcherrima* strain MACH1 for its ability to inhibit the growth of *B. cinerea*, *Alternaria alternata* and *P. expansum* on apples stored at 1ºC for 8 months. *In vitro* and *in vivo* tests showed that this yeast under low iron concentrations retarded the growth of the mould pathogens through iron depletion. In another study by Conway *et al*. (2004), two strains of *M. pulcherrima* (SD1-D9 and FMB-24H-2) were tested for their capabilities to control *P. expansum* and *C. acutatum* on Golden Delicious apples. The fruits were artificially wounded and inoculated with both mould pathogen and one of the *M. pulcherrima* strains and subsequently, one lot was refrigerated at 0ºC while another was heated at 38ºC for 4 days. After the heat treatment, all apples were stored at 0ºC for 4 months followed by 2 weeks at 20ºC. Both yeast strains had a strong inhibitory effect on *C. acutatum* during the 4-month storage at 0ºC, but they were not effective during storage at 20ºC; the biocontrol activities of these yeast strains were weaker against blue mould during cold storage than on *C. acutatum*. Janisiewicz *et al*. (2003) had also investigated the effects of *M. pulcherrima* (T5-A2) and heat treatment (38ºC for 4 days) against *C. acutatum* and *P. expansum* inoculated on artificially-wounded Golden Delicious apples and found that this yeast was more efficient in controlling *C. acutatum*, while heat was more effective against *P. expansum*. Additionally, *M. pulcherrima* was very efficient in inhibiting *P. expansum* growth on Stark Delicious, Granny Smith and Royal Gala apples (Spadaro *et al*., 2008).

6. *Pichia* species

Pichia angusta (formerly *Hansenula polymorpha*), *Pichia guilliermondii*, *Pichia anomala*, and *Pichia fermentans* have shown biocontrol activities against various plant pathogenic fungi. *P. angusta* was studied by Fiori *et al*. (2008) for its ability to control *B. cinerea*, *P. expansum* and *M. fructicola* in stored apples. These investigators used seven strains of the yeast from the Ancona Yeast Collection (ANY-32, ANY-34, ANY-38, ANY-39, ANY-41, ANY-43, and ANY-67). Yeast suspensions (1.0 x 10^8 cells/ml) were inoculated onto artificially-inflicted wounds (10 µl/wound) of otherwise sound Golden Delicious apples. The postharvest pathogens, *P. expansum*, *B. cinerea* and *M. fructicola*, were inoculated onto the wounds of the apples (~1.0 x 10^4 spores/wound) 2 h after yeast application and the fruits were incubated at 25ºC for 8 days as described by Giobbe *et al*. (2007). Subsequently, the lesion diameters were measured and compared to lesion size of the control, which was inoculated with the pathogen but not with the yeast antagonist. Significant reduction of lesion size facilitated by *P. angusta* was observed in all apples inoculated with *B. cinerea* or *M. fructicola*, but the yeast was not very effective against *P. expansum*. Apples inoculated with the latter pathogen had smaller decays than respective controls, but the differences were not significant (Fiori *et al*., 2008). McLaughlin *et al*. (1992) reported that *P. guilliermondii* was effective against *B. cinerea* inoculated onto wounds of Golden Delicious apples and an enhancement of the biocontrol activity of *P. guilliermondii* was brought about by the addition of $CaCl_2$. These investigators tested several salts

and found that calcium ions had the greatest effect while Mg^{2+} was ineffective. *P. guilliermondii* was also studied as a biocontrol agent against *P. expansum* by Gholamnejad and Etebarian (2009) in *in vitro* and *in vivo* experiments (on apples). Yeast cell suspensions, cell-free culture filtrates as well as volatile culture metabolites were assessed for activity against the pathogen; the highest inhibition was facilitated by the volatile metabolites followed by cell-free filtrates. Yeast cell suspensions at a concentration of 1.0 x 10^7 cfu/ml completely inhibited *P. expansum in vitro* and on apples. A negative relationship between yeast cell concentration and pathogen spore germination and germ tube growth existed. The antagonistic activity of the yeast was further increased when 2% $CaCl_2$ was added to the suspensions. Friel *et al*. (2007) tested several *P. anomala* strains on wounded Golden Delicious apples and observed that strain K effected an over 95% reduction in lesion diameter when the yeast (1.0 x 10^5 cfu/wound) was applied to the wounds 24h before infection with *B. cinerea* (5.0 x 10^4 spores/wound). Another strain (KH6) had slightly lower effect causing about 90% lesion reduction under the same as above conditions. Haissam (2011) also reported that *P. anomala* strain K was antagonistic to *B. cinerea* on apples. This strain inhibited *B. cinerea* spore germination. Possible mechanisms of action were presumed to be competition for nutrients and mycoparasitism since *P. anomala* filtrates contained endo- and exo-β-1, 3-glucanase activities. *P. fermentans* 726, on the other hand, was effective against brown rot (*M. fructicola*) of Golden Delicious and Renetta apples, when co-inoculated with the pathogen onto artificially-inflicted wounds of the fruits. The fruits were stored at 25ºC and 85% RH for 1 week. *P. fermentans* population increased from 6.0 x 10^5 to 1.0 x 10^7 cfu/wound within the first 4 days of incubation and formed a thin film over the wounds. This film probably prevented the mould spores from coming in contact with the fruit tissue and deriving the necessary nutrition for germination and proliferation. Cell-free culture filtrates of the organism had no inhibitory effect (Giobbe *et al*., 2007).

7. *Rhodosporidium toruloides*

Rhodosporidium toruloides NRRL Y1091 was studied by Filonow and his co-workers (1996) for its effect on the growth of *B. cinerea* on wounded Golden Delicious apples. These investigators demonstrated that *R. toruloides* had strong antagonistic activity against the mould pathogen and reduced the mould growth incidence by 86.7% and the lesion size from 27.8 mm to 1 mm. Pathogen inhibition achieved by this strain was similar to that of chlorothalonil and the well accepted biocontrol agent, *S. roseus* FS-43-238.

8. *Rhodotorula* species

Rhodotorula glutinis has exhibited antagonism against *P. expansum* and *B. cinerea*. *Rh. glutinis* HRB6 was studied by Sugar and Spotts (1999) as a BCA for controlling blue mould decay in Bosc pears after harvest. These investigators artificially wounded the fruits and inoculated 40 µl of a yeast suspension containing 1.0-1.5 x 10^8 cells/ml onto each wound (~5.0 x 10^6 cells/wound) and subsequently, added 40 µl of *P. expansum* spore suspension (1.0 x 10^4 cfu/ml) to each wound and refrigerated the treated fruits at 0ºC for 2 months. At the end of the storage period, the fruits were examined for incidence of visible infection and lesion size. Lesion diameters were recorded and compared to respective lesions on the control (not inoculated with the BCA yeast) fruits. This strain was found to facilitate significant reduction in percent infections and size of lesions caused by both TBZ-sensitive and TBZ-resistant strains of *P. expansum.* The same strain was also evaluated by Chand-Goyal and Spotts (1996) and was found to be a strong antagonist of *P. expansum* and *B. cinerea*, capable of controlling blue and gray mould rots in Bosc pears after harvest. Application of the yeast in combination with TBZ (15 µg/ml) significantly improved decay control. *Rh. glutinis* produced a siderophore, rhodotorulic acid, which was at least partially responsible for its biocontrol activity (Calvente *et al*., 1999).

Other *Rhodotorula* species (*Rhodotorula aurantiaca* and *Rhodotorula minuta*) were also investigated and found to possess notable biocontrol activities. Two *Rh. minuta* strains, YCL6 and YCL7, were capable of significantly reducing the size of *P. expansum* lesions in Bartlett pears, but did not reduce blue mould decay incidence (Chand-Goyal and Spotts, 1996). *Rh. minuta* was also effective against *Colletotrichum* and had good tolerance to low temperatures, retaining high viability after 6 months at 4ºC. This trait is very important for BCAs destined for postharvest applications on fruits (Patiño-Vera *et al*. 2005). According to Chand-Goyal and Spotts (1996), *Rh. aurantiaca* strain YCL5 reduced incidence of blue mould on Bartlett pears by 50% and lesion diameter from 35.5 mm to 15.8 mm. Experiments with d 'Anjou pears showed that this strain was efficacious in reducing severity of blue mould rots, but only reduced disease incidence by 8%.

9. *Saccharomyces* species

Several *Saccharomyces* strains isolated from food products were screened for biocontrol activity against the brown rot-causing mould, *M. fructicola*, by Zhou *et al*. (2008). The yeasts and the pathogen were co-inoculated on artificially-inflicted wounds of Jonagold and Golden Delicious apples (approximately 2.0 x 10^2 *M. fructicola* conidia and 2.0 x 10^5 cfu of a yeast isolate per wound) and the treated fruits were incubated for 5 days at 22-24ºC in a chamber with 85% RH. At the end of this experiment, it was determined that three strains, *Saccharomyces delbrueckii* A50, *Saccharomyces cerevisiae* YE-5 and *S. cerevisiae* A41 showed biocontrol activity against *M. fructicola*. *S. delbrueckii* A50 reduced brown rot incidence by over 80% and lesion size by more than 68%, while *S. cerevisiae* YE-5 and *S. cerevisiae* A41 reduced disease incidence by 52 and 54%, respectively.

10. *Sporobolomyces* species

Some *Sporobolomyces roseus* strains were reportedly efficient in inhibiting postharvest gray and blue mould decays on apples. Janisiewicz *et al*. (1994) tested *S. roseus* FS-43-238 isolated from pear for antagonism against postharvest pathogens of apples and found that this strain had high capacity in inhibiting growth of *B. cinerea* (gray mould) and *P. expansum* (blue mould). The degree of inhibition depended on the concentrations of the yeast antagonist and pathogens. When this yeast was drop-inoculated onto the wounds of the fruits (25 µl/wound)

with cell suspensions containing 7.9 x 10^6 and 6.3 x 10^5 cfu/ml (for *P. expansum* and *B. cinerea*, respectively) and with 20 µl/wound of a pathogen spore suspension containing 1.0 x 10^4 spores/ml and incubated at 22°C for 1 week, it reduced the incidence of blue mould rot from 100% to 0% and gray mould decays from 78% to 0%. This biocontrol agent also decreased decays of wounded apples dipped in yeast-pathogen (*P. expansum* or *B. cinerea*) suspensions and stored at 1°C for 3 months. Filonow *et al*. (1996) tested three *S. roseus* strains (FS-43-238, ATCC 28988 and ATCC 24257) for antagonistic activity against *B. cinerea*. These investigators inoculated artificially-wounded Golden Delicious apples with the test yeasts and the pathogen and stored the treated fruits at 22-24°C for 1 week. After the incubation period, all three strains had shown inhibitory influence on the pathogen, but the ATCC strains had a greater antagonistic effect than the FS-43-238 strain. Both ATCC strains reduced decay incidence by over 70% and lesion diameters by 84 and 95% for ATCC 28988 and ATCC 24257, respectively. All *Sporobolomyces* strains tested by these researchers performed better than the fungicide benomyl. *Sporidiobolus salmonicolor* (*Sporobolomyces salmonicolor* anamorph) ATCC 623 under the same experimental conditions caused a 37% reduction of decay incidence and reduced the lesion diameter from 27.8 mm to 9.4 mm (66%). This strain also performed better than benomyl (Filonow *et al*., 1996). Experimental work with a *S. roseus* mutant resistant to 2-deoxy-D-glucose (2-DOG) demonstrated that this organism was very effective against *P. expansum* when used simultaneously with 2-DOG (Janisiewicz, 1994). In this study, Golden Delicious apples and Anjou pears were wounded and subsequently, wounds were inoculated with the *S. roseus* mutant alone or in combination with 2-DOG (25 µl/wound of a suspension containing the antagonist yeast plus 4 mg of 2-DOG per ml) and the pathogen. The results of the experiment showed that addition of 2-DOG significantly increased control of decays by *P. expansum*.

Moulds

1. *Aureobasidium pullulans*

The yeast-like fungus *Aureobasidium pullulans* is a widespread saprophyte commonly found in phyllosphere and carposhere. This organism is able to withstand desiccation conditions and survive relatively high irradiation. It has exhibited substantial biocontrol activity against several postharvest pathogens and has been utilized to control decay of various fruits (*e.g.* apples, cherries, peach and strawberries) caused by the postharvest pathogens *P. expansum*, *B. cinerea*, *R. stolonifer* and *Monilinia* spp. (Mounir *et al.*, 2007; Bencheqroun *et al*., 2007; Lima *et al*., 1997; Ippolito *et al*., 2000; Schena *et al*., 2003). The ability of *A. pullulans* (isolate LS-30) to control postharvest fruit decays was investigated by Castoria *et al*. (2001). These researchers conducted *in vivo* experiments on artificially-wounded Royal Gala apples infected with *P. expansum* or *B. cinerea*. The fruits were surface-disinfected in 1% commercial bleach and subsequently, inoculated with an *A. pullulans* suspension (3.0 x 10^6 cfu/wound); two hours after the BCA inoculation, a pathogen spore suspension was applied to the wounds (3.0 x 10^2 spores/wound) and the treated fruits were incubated at 20°C and 95% RH for 6 days. This strain reduced decay incidence by 76.5 and 88.5% for *B. cinerea* and *P. expansum*, respectively. Vero *et al*. (2009) tested 10 *A. pullulans* strains (recovered from apples held in cold storage for several months) for biocontrol activity against the blue and gray mould pathogens. Results of this study showed that one *A. pullulans* strain (ApB), in particular, caused significant reduction of blue and gray mould decay incidence and severity on Red Delicious apples stored at 0-1°C for 3 months. According to various past studies, *A. pullulans* utilizes a combination of biocontrol mechanisms such as competition for nutrients, induction of host resistance and production of lytic enzymes (chitinases, glucanases and proteases) (Bencheqroun *et al*., 2007; Ippolito *et al*., 2000; Zhang *et al*., 2010; Zhang *et al*., 2012). Castoria *et al*. (2001) studied the mechanisms of biocontrol action of *A. pullulans* strain LS-30 and concluded that the main mechanism was competition for nutrients, but lytic enzymes such as exo-chitinase and β-1, 3-glucanase were also detected in the apple wounds inoculated with this organism. *A. pullulans* strain ApB also produced the lytic enzymes, chitinase and β-1, 3-glucanase. Additionally, this strain generated hydroxamate-type siderophores, which probably contributed to its biocontrol efficacy (Vero *et al*., 2009). An *A. pullulans*-based product has been developed in Germany and used under the name "BoniProtect" for the control of storage diseases of apples in some European countries (Janisiewicz, 2010; Trapman *et al*., 2010; Weiss *et al*., 2006).

2. *Trichoderma* species

Trichoderma spp. (*Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma reesei* and *Trichoderma virens*) have been tried and commercially used as biocontrol agents against various plant and postharvest pathogens (Harman *et al*., 2004; Rocco and Perez, 2001). Quaglia *et al*. (2011) reported a significant reduction of percent infectivity and lesion diameters of *P. expansum* decays by various *Trichoderma* species (*T. harzianum* strains T22 and T67, *T. atroviride* P1 and *T. reesei* T34) when this pathogen was artificially inoculated on wounded Golden Delicious apples and stored for 6 days at ~21°C. In a study by Bordbar *et al*. (2010), two *T. virens* strains (T6 and T8) were tested for antagonistic activity against *P. expansum in vivo* (on Golden Delicious apples). The BCA was applied to fruit (2.0 x 10^5 conidia/wound) 4 h prior to pathogen (2.0 x 10^3 conidia/wound) inoculation and the treated fruits were stored at 20°C for 8 days. Both *Trichoderma* strains effected significant decrease in fruit lesions caused by the pathogen at both, day 4 and 8 of incubation, but strain T8 had a greater antagonistic effect. Peroxidase, catalase and β-1, 3-glucanase activities as well as phenolic compound levels increased in fruits inoculated with *P. expansum* and the antagonist strains. Increased enzyme activities and phenolic compounds probably contributed to biocontrol activity of these organisms. Batta (2007) evaluated *T. harzianum* strain Th2 (isolated from soil from a vegetable garden) formulated in an invert emulsion and in non-formulated form (as suspension in sterile de-ionized water) as an antagonist of pome fruit postharvest pathogens. In that study, Th2 was inoculated on artificially-wounded Golden Delicious apples and

pears. The antagonist concentration applied to each wound was 25 µl of a suspension containing 9.6 x 10^8 conidia/ml or 25 µl of an invert emulsion with 4.6 x 10^8 conidia/ml. The pathogens, *R. stolonifer* (strain RS1) isolated from infected strawberry, *B. cinerea* (strain BC1) from infected apple fruit, or *P. expansum* (PE8) originating from infected pear fruit were inoculated onto the wounds of the fruits (25 µl/wound of suspensions containing over 2.0 x 10^6 spores/ml) 1 h after the antagonist application and the fruits were stored at 20 ±2ºC or 30±2ºC for 3-4 days. *T. harzianum* Th2 facilitated significant reduction of lesion diameters of *R. stolonifer* in apples and pears and diminished lesions of *B. cinerea* and *P. expansum* in pears. This organism protected unwounded fruits for up to 2 months and reduced lesion diameter up to 86%. Spore suspensions of this *Trichoderma* strain in sterile de-ionized water had a lesser effect on the pathogens than spores formulated in invert emulsion. *Trichoderma* spp. develops a kind of symbiotic relationship with plants. They derive nutrition from plant exudates and excrete effector proteins into the plant tissue; these proteins trigger an increase in plant defense (*e.g*. increase of Ca^{2+} levels), which in turn results in resistance to pathogens (Yedidia *et al*., 2000; Woo *et al*., 2009). Besides induction of plant defense responses, other biocontrol mechanisms such parasitism, competition for space and nutrients, and production of antibiotics were observed in *Trichoderma.* Trichodermas can parasitize on some plant and postharvest pathogens such as *B. cinerea* and *P. expansum* where they can cause hyphal destruction of the pathogens and consequently, limit plant diseases and postharvest decays *(*Bordbar *et al*., 2010*;* El-Naggar *et al*., 2008; Hai, 2012). Schuhmacher *et al*. (2009), on the other hand, reported the production of several antifungal peptaibiotics by *T. atroviride*. Elad *et al*. (1999) studied the biocontrol mechanisms of *T. harzianum* T39 against *B. cinerea* and found that this BCA restrained pathogen growth possibly through competition for nutrients and space and suppression of the pathogen's hydrolytic enzymes - cutin esterase, exo- and endo-polygalacturonase, pectin methyl esterase and pectate lyase. These investigators also noted that a serine protease produced by T39 was responsible for suppressing *B. cinerea* infectivity. Although the antagonistic effects of *Trichoderma* spp. are potent against some pome fruit pathogens, their application to fruits after harvest is not very favorable because of their possible adverse effects on the fruit natural protective flora and due to their strong and unpleasant odors which may make the fruits undesirable for consumption.

Use of combination of biocontrol agents and other treatments for higher antagonistic effectiveness

Use of combinations of BCAs (*e.g.* utilization of certain bacterial and yeast strains in pairs), or integration of BCAs with other treatments to increase antagonistic power and broaden the spectrum of activity has also been examined in recent years. Some examples of successful such combinations are given below.

Use of mixtures of biocontrol agents

1. *Candida sake* and *Pantoea agglomerans*

C. sake was found to be more effective against postharvest apple pathogens when used in combination with the bacterium *P. agglomerans* (Viñas *et al*., 1998). Nunes *et al*. (2002a) reported that the best combination of these antagonists was at a 50:50 proportion of *C. sake* (2.0 x 10^7 cfu/ml): *P. agglomerans* (8.0 x 10^7 cfu/ml). When the inoculated fruits were incubated at room temperature (20ºC) for 7 days, this combination completely inhibited the growth of the pathogens on pears and reduced the severity (lesion size) of *P. expansum* decay on Golden Delicious apples by more than 95%. The incidence (percentage of infected wounds) of the disease on the latter product was also significantly reduced (from 100% to 6%). According to these investigators, the same mixture (at the 50:50 proportions) also exerted the best biocontrol against *P. expansum* and *B. cinerea* on artificially-infected, wounded apples and pears incubated at 1ºC for 60 days.

2. *Sporobolomyces roseus* and *Pseudomonas syringae*

Use of a mixture of *S. roseus* and *P. syringae* at a 50:50 proportion improved biocontrol of *P. expansum* on wounded Golden Delicious apples as compared with experiments in which only one of the two BCAs was used against the pathogen. The unique biochemical requirements of the two *P. expansum* antagonists (broad nitrogen utilization by *P. syringae* and multi-source carbon use by *S. roseus*) allowed them to flourish on apple wounds and perhaps excluded the pathogen from acquiring the necessary nutrients for spore germination and growth (Janisiewicz and Bors, 1995).

3. *Cryptococcus laurentii* and *Metschnikowia pulcherrima*

The combined effects of the yeasts *Cr. laurentii* ST4-E14 and *M. pulcherrima* FMB-24H-2 were studied by Conway *et al*. (2005). These investigators artificially infected wounds of Golden Delicious apples with *P. expansum* and subsequently, inoculated the yeast antagonists onto the same wounds (individually or in combination) and they observed that over 95% of the apples had no signs of decay after 2 months of storage at 1ºC. After 4 months at 1ºC, 67% of fruits inoculated with *Cr. laurentii* and 56% of fruits inoculated with *M. pulcherrima* were still undamaged; higher decay control was achieved when both yeasts were simultaneously added to the wounds.

BCAs combined with low amounts of synthetic fungicides or other chemicals

Combinations of biocontrol agents with reduced amounts of commonly used postharvest fungicides were found to have higher efficiencies in controlling storage pathogens. Combination of *P. syringae* with cyprodinil was more efficient in controlling *P. expansum* in artificially-infected Empire and McIntosh apples than either treatment alone (Errampalli and Brubacher, 2006). *Cr. laurentii* (strains HRA5 and 87-108), *Cr. infirmo-miniatus* YY6, or *Rh. glutinis* HRB6 combined with 100 ppm TBZ completely inhibited the growth of TBZ-sensitive *P. expansum* on wounds of Bosc pears (Sugar and Spotts, 1999).The combined effects of low level of TBZ (15 µg/ml) with *Cr. laurentii* HRA5, *Cr. infirmo-miniatus* YY6, or *Rh. glutinis* HRB6 were also evaluated by Chand-Goyal and Spotts (1996). The results of this study showed that combination of the two treatments resulted in significant reduction of pear disease after harvest.

Other chemicals such as ammonium molybdate, calcium salts, 2-deoxy-D-glucose (2-DOG), etc. were also shown

to improve biocontrol activity of certain BCAs. Combination of *C. sake* with ammonium molybdate resulted in increased control of postharvest decays in pome fruits, while use of 2-DOG in combination with *Candida saitoana* improved the control of postharvest apple decays caused by *P. expansum* and *B. cinerea* (El-Ghaouth *et al*., 2000b; Nunes *et al*., 2002b). A study conducted by Janisiewicz (1994) showed that addition of 2-DOG (up to 4 mg/ml) caused a significant increase in the antagonism of a *S. roseus* mutant against blue mould postharvest spoilage in Golden Delicious apples and to a lesser degree in Anjou pears. Simultaneous use of *C. oleophila* (strain 182) and 90 or 180 mM of $CaCl_2$ enhanced postharvest control of *P. expansum* and *B. cinerea.* Calcium ions inhibited the germination of spores and the production of pectinolytic enzymes by these pathogens (Wisniewski *et al*., 1995). Combination of other *Candida* BCAs such as *C. guilliermondii* strains (3-C-1b and F1) and *C. membranaefaciens* with $CaCl_2$ also resulted in increased control of decays caused by postharvest pome fruit pathogens (Gholamnejad and Etebarian, 2009; Scherm *et al*., 2003). Application of a 2% $CaCl_2$ on apple wounds simultaneously with *K. apiculata* or *P. guilliermondii* also enhanced biocontrol of *P. expansum* and *B. cinerea* by *K. apiculata* and significantly increased the antagonistic activity of *P. guilliermondii* against *P. expansum* (Gholamnejad and Etebarian, 2009; McLaughlin *et al*., 1990; McLaughlin *et al*., 1992). Sugar and Basile (2007) employed various pre- and postharvest treatments in an effort to minimize decay of Bosc pears in storage and found that the best spoilage control was the use of $CaCl_2$ in the orchard followed by postharvest treatment with BioSave 110 and storage in LifeSpan modified atmosphere packaging. Poleatewich (2010) studied the combined effects of bacteria (*B. megaterium* A3-6 or Ae-1, *B. mycoides* A1-1, or *B. laterosporus* FLS-1) and chitosan on *C. acutatum* (causative agent of bitter rot) and *P. expansum* (cause of blue mould) inoculated on Golden Delicious and Rome Beauty apples and found that bitter rot and blue mould decays were significantly lower on the treated apples than on the pathogen-only-inoculated controls; a synergistic effect existed when a combination of *B. mycoides* (isolate A1) or *B. megaterium* (isolates A3-6 and Ae-1) and chitosan was applied to wounded Golden Delicious apples. Additionally, an improvement of *C. saitoana* biocontrol activity against moulds causing postharvest decays in apples by the addition of glycochitosan was reported by El-Ghaouth *et al*. (2000a). A *C. saitoana* product was developed by Neova Technologies (Abbotsford, British Columbia, Canada) and is used for the biocontrol of postharvest diseases in fruits (Droby *et al*., 2009; Abano and Sam-Amoah, 2012).

BCAs integrated with other treatments

The protective effect of *M. pulcherrima* T5-A2 combined with heat treatment (38ºC for 4 days) against *P. expansum* and *C. acutatum* was studied by Janisiewicz *et al*. (2003). These investigators inoculated the antagonist along with the pathogens on Golden Delicious apples and stored the treated apples at 0.5ºC under controlled atmospheres (1.1% O_2 and 1.8% CO_2) for 2 or 4 months, or 4 months under the above conditions followed by 2 weeks at room temperature. The results of the study showed that the pathogens were controlled better when apples were treated with hot air (38ºC) for 4 days prior to inoculation with the pathogens and the antagonistic yeast. Conway *et al*. (2004) also examined the effects of combining *M. pulcherrima* (ST1-D9 or FMB-24H-2 strains) with heating at 38ºC for 4 days on Golden Delicious apple decays caused by *P. expansum* and *C. acutatum* and found that combination of either strain with heat treatment eliminated the postharvest decays by the two pathogens. Conway *et al*. (2005) treated wounded Golden Delicious apples with heat (38ºC for 4 days) and subsequently, inoculated the wounds with *P. expansum* and the yeast antagonists, *Cr. laurentii* strain ST4-E14 and *M. pulcherrima* strain FMB-24H-2 (alone or in combination) and stored the treated fruits at 1ºC for 4 months plus 2 weeks at 20ºC. After the incubation period, it was determined that complete inhibition of *P. expansum* decay was achieved with this treatment. Use of microwave treatment followed by the application of the biocontrol agent, *Cr. laurentii*, to control blue mould rot in pears was investigated by Zhang *et al*. (2006). These researchers studied the effect of microwave treatment (2450 MHz for 2 min), *Cr. laurentii* application as well as the combination of the two methods on natural decay of pears stored at 2ºC for 60 days and subsequently kept at 20ºC for 15 additional days and found that the antagonistic effects were stronger when the antagonist was combined with microwave treatment. Combination of the two treatments on pears artificially-wounded and inoculated with *P. expansum* also resulted in enhanced reduction of decays. This combination reduced the incidence of fruit infections from a 100% to 20% and the lesion size from 2.81 mm to 1.1 mm. The effects of biocontrol yeasts combined with controlled atmospheres (CA) have also been investigated. Usall *et al*. (2000) used *C. sake* strain CPA-1 alone or in combination with CA to inhibit blue mould decays on apples. These investigators inoculated wounded Golden Delicious apples with the pathogen (*P. expansum*, 1.0×10^4 spores/ml) and various concentrations of *C. sake* and stored the treated fruits at 1ºC under various O_2 - CO_2 atmospheres for 60 days. Results of this experiment showed that better decay control was achieved when *C. sake* application was integrated with use of CA than either treatment alone. The highest reduction in lesion size (97%) was noted when the fruits were treated with a *C. sake* suspension containing 2.4×10^6 cells/ml and stored under a CA consisting of 3% O_2 and 3% CO_2. Under the same conditions the incidence of blue mould decays was reduced by 93%. Tian *et al*. (2002) also studied the combined effects of CA and yeast antagonists on the reduction of *B. cinerea* and *P. expansum* decays of apples and pears. These investigators artificially wounded and inoculated Golden Delicious apples and Jingbai pears with the BCAs *Cr. albidus* or *Trichosporon* sp. and the pathogens, and stored the inoculated fruits under various CA conditions at 1ºC for 40 days. The mould pathogens were better controlled in apples than in pears; the inhibitory effects were stronger against *B. cinerea* than on *P. expansum* and were more pronounced in storage under increased CO_2 concentrations. Although many BCAs (alone or in combination with other biocontrol organisms or integrated with chemical or heat treatments) were found to exert significant decay control in fruits after harvest,

they have some major shortcomings such as inability to control already established infections, failure to retain high viability and substantially proliferate under harsh conditions, and short shelf life in formulations destined for easy application (Droby *et al*., 2009; Nunes, 2012; Sharma *et al*., 2009). Search for better, more efficient BCAs and/or improvement of the ones currently used should be the focus of future studies.

Biocontrol mechanisms employed by various BCAs

The mechanisms of biocontrol utilized by various BCAs have not been well elucidated. Modes of action believed to be responsible for the biological control of plant and postharvest pathogenic fungi include microbial antagonism and induced plant resistance to the pathogen. The first category encompasses parasitism, competition for nutrients and space between BCAs and pathogenic organisms, and antibiosis. The second category includes induction of plant defense mechanisms.

Parasitism

Parasitism is facilitated through the production of various types of cell wall-degrading enzymes (CWDEs) which enable the entrance of the antagonist organism into the hyphae of the pathogen (Chet and Baker, 1981; Poleatewich, 2010). Such enzymes include cellulases, glucanases, and chitinases (exo- and endochitinases). Bacteria known to produce CWDEs are *B. cereus*, *B. megaterium*, *P. agglomerans*, *Arthrobacter*, *Enterobacter cloaceae*, *Micromonospora carbonacea*, various actinomycetes, etc. (Chernin *et al*., 1995; El-Tarabily *et al*., 1996; Pleban *et al*., 1997; Valois *et al*., 1996). Strong attachment of the BCA to the pathogen cells is necessary for parasitism. Poleatewich (2010) used scanning electron microscopy to elucidate the mechanism of postharvest biocontrol of *C. acutatum* by *B. megaterium*, isolate A3-6. This researcher observed that the bacteria were attached to the hyphae of the pathogen and that the hyphae were damaged and collapsed at the points of attachment.

Several biocontrol yeasts and some moulds were also shown to have the ability to attach to pathogens' hyphae and produce CWDEs. *P. membranaefaciens* was able to attach to *P. expansum*, *R. stolonifer* and *M. fructicola* hyphae and had high glucanase and exo-chitinase activity in the presence of fungal hyphae, while *P. guilliermondii* showed high β-1, 3-glucanase activity, which could possibly cause hyphal wall degradation of the pathogen (Chan and Tian, 2005; Jijakli and Lepoivre, 1998). Wisniewski *et al*. (1991) reported that *P. guilliermondii* strongly attached to *B. cinerea* hyphae and that the hyphae were deformed and partially degraded at the points of yeast attachment while non-antagonistic yeasts attached loosely and did not cause degradation of the hyphae. This BCA also attached tightly to *P. expansum* hyphae and caused hyphal damage when the two organisms were co-cultured (Widiyastuti, 2008). El-Ghaouth *et al*. (1998), on the other hand, observed a strong attachment of *C. saitoana* to *B. cinerea* hyphae and deformation of hyphae in the areas of attachment.

Some examples of mould on mould parasitism are the invasion and destruction of *Rhizoctonia solani* by *Trichoderma* spp. and the antagonistic degradation of *Sclerotinia* spp. by *Sporidesmium sclerotivorum* and *Coniothyrium minitans* (Adams and Fravel, 1993; Jones *et al*., 2004). Lytic enzymes such as exo-chitinase and β-1, 3-glucanase were found in apple wounds inoculated with *A. pullulans* suggesting that this organism may cause some pathogen cell wall destruction (Castoria *et al*., 2001; Marusich *et al*., 1997). Zhang *et al*. (2010) reported the production of various lytic enzymes (chitinases, glucanases and proteases) as possible factors contributing to biocontrol efficiency of *A. pullulans* PL5, while Zhang *et al*. (2012) demonstrated that an alkaline protease from this organism was effective against *B. cinerea* and *P. expansum* grown on apple fruit. *Trichoderma* species also produce chitinases which facilitate cell wall destruction of the moulds they parasitize on (Lorito, 1998; Lorito *et al*., 1996; Liu *et al*., 2004). Haran *et al*. (1996) reported that *T. harzianum* strain T-Y exercises mycoparasitism through differential expression of its chitinolytic enzymes (β-1, 4-*N*-acetylglucosaminidases and endo-chitinases).

Competition for nutrients and space

Competition for nutrients between plant pathogens and BCAs has been explored in various studies. Filonow *et al*. (1996) reported competition for nutrients as one of the mechanisms of controlling *B. cinerea* by various yeast species including *Cr. humicola*, *F. floriforme*, *R. toruloides* and *S. roseus*. When high numbers of the biocontrol organism(s) colonize the fruit wounds, they restrict pathogen access to nutrients necessary for its spore germination and growth, thus inhibiting fruit spoilage (Nunes 2012). The main biocontrol mechanism utilized by *A. pullulans* was deemed to be competition for nutrients (Castoria *et al*., 2001). Bencheqroun *et al*. (2007) tested *A. pullulans* (strain Ach1-1) for modes of antagonism against *P. expansum* grown on apples and determined that competition for nutrients, particularly amino acids, was the main mechanism of action. Strong attachment of BCAs to pathogen hyphae is also believed to restrict the pathogen's access to nutrients and therefore, inhibit its spore germination and growth (El-Ghaouth *et al*., 2004; Sharma *et al*., 2009). Additionally, excretion of nutrient-capturing substances can result in depletion of certain nutrients and consequently, retard or inhibit pathogen growth; an example is the competition for iron through the excretion of iron-chelating compounds (siderophores) by fluorescent *Pseudomonas* species. Siderophores have high affinity for ferric ions, thus causing depletion of iron and consequently, growth restriction of the mould pathogens (O'Sullivan and O'Gara, 1992). Competition between the BCA and the pathogen for nutrients and space was also observed in *T. harzianum* T39 (Elad *et al*., 1999). Competition for space can occur when the antagonist forms a well-established biofilm on the wounds and punctures of fruits before the spores of the pathogen come in contact with the fruit surface (Elad *et al*., 1999; Li *et al*., 2011). Therefore, application of the BCA before the pathogen comes in contact with the fruit is important in pathogen inhibition. Storage at room temperature for several hours after BCA inoculation was found to increase biocontrol activity. That time period possibly enabled the BCA to colonize the wound, proliferate and reach significantly-increased viable counts and consequently, prohibit the pathogen spores from getting access to fruit wounds and germinate (Nunes *et al*., 2008).

Antibiosis

Another mechanism of action is the excretion of antifungal substances. Filonow *et al*. (1996) applied dilute, cell-free sucrose solutions, in which various biocontrol yeasts were previously cultured, to apple wounds and observed that some of these solutions substantially inhibited conidial germination of *B. cinerea*. Other examples of antibiotic production by antagonist microorganisms are the antifungal compounds produced by *B. subtilis*, *B. cepacia* and various *Pseudomonas* and *Trichoderma* species (Degenkolb *et al*., 2008; Kadir *et al*., 2008; Mukherjee *et al*., 2012; Pusey *et al*., 1988).

Studies on the mode of biocontrol action of *B. cepacia* revealed the production of a wide spectrum of antifungal compounds (Parke, 2005). *B. cepacia* strain B23 (formerly *P. cepacia*) was investigated by Kadir *et al*. (2008) and was found to produce pyrrolnitrin and other antifungal substances which were inhibitory to moulds like *Colletotrichum gloeosporioides*. *Bacillus* spp. (*e.g. B. subtilis*) achieved biocontrol through the production of antibiotics such as iturin (Pusey *et al*., 1988). Several *Pseudomonas* strains also produce antibiotics, thus protecting fruits from the invasion and spoilage by plant and postharvest pathogens (MicrobeWiki, 2011). Certain biocontrol *P. fluorescens* strains, for instance, produce several antibiotics including pyrrolnitrin, pyoluteorin and 2, 4-diacetylphloroglucinol which facilitate pathogen growth inhibition (Loper *et al*., 1997; Nowak-Thompson *et al*., 1999; Stutz, 1986; Whistler *et al*., 2000).

Trichoderma spp. produce peptaibiotics - linear peptides containing unusual amino acids such as α-aminoisobutyric acid (aib) - and semi-volatile pyrones which exert antibiotic activity against various pathogens (Degenkolb *et al*., 2008; Mukherjee *et al*., 2012; Szekeres *et al*., 2005). *T. atroviride* ATCC 74058, for instance, produces 20 Aib peptaibiotics (Schuhmacher *et al*. 2009). A subgroup of peptaibiotics, peptaibols (non-ribosomal, short peptides with high number of non-standard amino acids) produced by many *Trichoderma* species are strong antifungal compounds (Degenkolb *et al*., 2008; Mukherjee *et al*., 2012). Pyrones such as 6-pentyl pyrone (produced by *T. atroviride* and *T. harzianum*) were also shown to have antifungal activity against various pathogens including *Fusarium oxysporum* and *Bipolaris* (Mathivanan *et al*., 2008; Mukherjee *et al*., 2012; Reithner *et al*., 2005; Rubio *et al*., 2009), while a gliotoxin from *T. virens* was inhibitory to *B. cinerea* spore germination (DiPietro *et al*., 1993). Concerns about using BCAs producing antibiotics to manage postharvest diseases in fruits (and in foods in general), however, have resulted in rejecting commercialization of certain efficacious BCAs. Such example is *B. subtilis* strain B-3 which was not commercialized because it mainly controlled *M. fructicola* through the production of the antibiotic, iturin (Pusey *et al*., 1988).

Induction of resistance to pathogen

Some biocontrol organisms have the ability to induce resistance to pathogens in plants or harvested fruits by educing their defense response. Plant/fruit defense response includes production of substances inhibitory to pathogen CWDEs, phytoalexins, ethylene, phenolic compounds and active oxygen species (*e.g.* superoxide anion O_2-) as well as strengthening of the cell walls of the host (Jamalizadeh *et al*., 2011; Karunaratne, 2011; Kumar *et al*., 2011). Induction of resistance is a complex mechanism involving several biochemical reactions which bring about changes in host tissue and production of pathogenesis-related proteins (Kloepper *et al*., 1992; Van Loon, 1997). Stimulation of phytoalexin production in wounds of fruits facilitated by *Candida famata* was reported by Arras (1996), while an increased resistance to *P. digitatum* initiated by *C. oleophila* (applied to wounded or intact grapefruits) was demonstrated by Droby *et al*. (2002). Increased production of super oxide anions and hydrogen peroxide by *C. oleophila* (in the presence of *M. fructicola*) on wounded and unwounded apple and citrus fruits was reported by Macarisin *et al*. (2010), whereas induction of systemic resistance in apple facilitated by *C. saitoana* was reported by El-Ghaouth *et al*. (2000c). More research is needed in order to further elucidate the mechanisms of biocontrol. Better understanding of these mechanisms will enable us to select more efficacious BCAs and develop appropriate methods for application of the antagonists. Elucidation of the modes of biocontrol will also aid in improving the biocontrol traits of BCAs through genetic engineering.

Economic feasibility

The development and commercialization of a BCA (biofungicide) is a long process consisting of many steps such as isolation and screening of the BCA, determining the mode of biocontrol action and improving efficacy, toxicology, production and scale-up production, formulation, semi-commercial trials, pilot trials, commercial trials, and registration. Although time-consuming and expensive, commercialization of biological control of postharvest diseases could be feasible because the conditions of fruit storage (*i.e.* temperature and humidity) can be regulated at such levels to favor survival and good performance of the biocontrol agent (Wisniewski *et al*., 2007). Economic feasibility is also possible since high numbers of the biocontrol agents can be readily produced and efficiently applied to stored fruits. The fact that the commodities to be treated are concentrated in relatively small areas ensures the need for smaller amounts of the BCA to be utilized making the treatment more economical. The biocontrol would be more cost-effective, if BCAs with high and wide spectrum efficacies are available (*e.g.* a BCA with inhibitory effects against several postharvest pathogens of the same commodity). Some obstacles exist in the production of very efficacious BCAs because some organisms tend to lose viability during processing and storage. More research should focus in enhancing the biocontrol strategies so that organisms like non-spore-forming bacteria do not lose viability during formulation and storage (Fravel, 2005). Once the technical obstacles have been surpassed and a BCA is proven effective against certain pathogens in a laboratory setting, it needs to be moved into a commercial scale. At this point some challenges are encountered with the registration of the new biological control agent with the USEPA and state regulatory agencies. This process includes environmental safety testing which can be lengthy and expensive (Harman, 2000). Another economic drawback of biopesticides is the fact that most of the existing formulations consist of lyophilized cells that need

freezer storage. This preparation process and storage are costly. It would be more economical if the biocontrol agents could be maintained in high-titer suspensions at refrigeration temperatures. Some microorganisms in high-titer suspension, however, lose viability rapidly and consequently, lose biocontrol efficacy. Research is needed to improve long term refrigerated storage or find less expensive means for formulation and storage of BCAs.

TABLE 1. Microorganisms with biocontrol activity (antagonism) against pome fruit postharvest pathogens

Antagonist	Strain(s)	Target pathogen	Crop	Reference
Bacteria				
Bacillus amyloliquefaciens	C06	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
Bacillus megaterium	A3-6, Ae-1	*Colletotrichum acutatum*	Apples	Poleatewich, 2010
		Penicillium expansum	Apples	Poleatewich, 2010
Bacillus sp.	T03-c	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
Bacillus mycoides	A1-7	*Penicillium expansum*	Apples	Poleatewich, 2010
		Colletotrichum acutatum	Apples	Poleatewich, 2010
Brevibacilus laterosporus	FLS-7	*Colletotrichum acutatum*	Apples	Poleatewich, 2010
		Penicillium expansum	Apples	Poleatewich, 2010
Burkholderia cepacia		*Penicillium expansum*	Apples, pears	Janisiewicz *et al*., 1991; Parke, 2005
		Botrytis cinerea	Apples, Pears	
Burkholderia gladioli	DISTEF-G	*Penicillium expansum*	Apples	Scuderi *et al*., 2009
		Botrytis cinerea	In vitro	Scuderi *et al*., 2009
Lactic acid bacteria				
Lactobacillus sp.	P02, C03-b	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
Weissella cibaria	TM128	*Penicillium expansum*	Apples	Trias *et al*., 2008
		Botrytis cinerea	Apples	Trias *et al*., 2008
Pantoea agglomerans	EPS125	*Penicillium expansum*	Apples, pears	Frances *et al*., 2006
		Rhizopus stolonifer	Apples, pears	Frances *et al*., 2006
		Botrytis cinerea	Apples	Frances *et al*., 2006
	CPA-2	*Penicillium expansum*	Apples, pears	Nunes *et al*., 2002; Nunes *et al*., 2001
		Botrytis cinerea	Apples, pears	
		Rhizopus stolonifer	Apples, pears	
Pseudomonas fluorescens	1100-6	*Penicillium expansum*	Apples	Etebarian *et al*., 2005
Pseudomonas syringae	ESC-10	*Penicillium expansum*	Apples	Janisiewicz and Korsten, 2002
		Botrytis cinerea	Apples	Janisiewicz and Korsten, 2002
	ESC-11	*Penicillium expansum*	Apples	Janisiewicz and Peterson, 2004;
		Penicillium expansum	Pears	Janisiewicz *et al*., 1992
		Botrytis cinerea	Pears	Sugar and Basile 2007;
Yeasts				
Candida ciferrii	283	*Penicillium expansum*	Apples	Vero *et al*., 2002
Candida guilliermondii	3-C-1b, F1	*Penicillium expansum*	Apples	Scherm *et al*., 2003
Candida membranaefaciens	-	*Penicillium expansum*	Apples	Gholamnejad and Etebarian, 2009
Candida oleophila	182	*Botrytis cinerea*	Apples	Mercier and Wilson, 1994; Mercier and Wilson , 1995
		Penicillium expansum	Apples	Wisniewski *et al*., 1995
Candida saitoana	-	*Penicillium expansum*	Apples	El-Ghaouth *et al*., 2000
		Botrytis cinerea	Apples	El-Ghaouth *et al*., 2000
Candida sake	CPA-1	*Penicillium expansum*	Apples	Usall *et al*., 2000; Teixido *et al*., 1999
Cryptococcus albidus	HRB2	*Penicillium expansum*	Apples, pears	Chand-Goyal and Spotts, 1996
	CBS No. 604.94	*Penicillium expansum*	Apples, pears	Haissam, 2011
		Botrytis cinerea	Apples, pears	Haissam, 2011
Cryptococcus humicola	NRRL Y1266	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
Cryptococcus infirmo-miniatus	YY6	*Penicillium expansum*	Apples, pears	Chand-Goyal and Spotts, 1996
		Mucor	Apples, pears	Chand-Goyal and Spotts, 1996
Cryptococcus laurentii	-	*Penicillium expansum*	Apples	He *et al*., 2003
	HRA5	*Penicillium expansum*	Pears	Sugar and Spotts, 1999; Chand-Goyal and Spotts, 1996
		Botrytis cinerea	Pears	Chand-Goyal and Spotts, 1996
		Mucor piriformis	Pears	Chand-Goyal and Spotts, 1996
	BSR-Y22	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
	RR87-108	*Botrytis cinerea*	Apples	Roberts, 1990
	87-108	*Penicillium expansum*	Pears	Sugar and Spotts, 1999
	-	*Penicillium expansum*	Pears	Zhang *et al*., 2006
Filobasidium floriforme	NRRL Y7454	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
Kloeckera apiculata	138	*Penicillium expansum*	Apples	McLaughlin *et al*., 1992; Spadaro *et al*., 2008
		Botrytis cinerea	Apples	McLaughlin *et al*., 1992
Metschnikowia	PBC-2	*Penicillium expansum*	Apples, pears	Manso and Nunes, 2011

andauensis		*Botrytis cinerea*	Apples	Manso and Nunes, 2011
		Rhizopus stolonifer	Apples	Manso and Nunes, 2011
Metschnikowia pulcherrima	MACH1	*Penicillium expansum*	Apples	Saravanakumar *et al*., 2008
		Botrytis cinerea	Apples	Saravanakumar *et al*., 2008
		Alternaria alternata	Apples	Saravanakumar *et al*., 2008
	SD1-D9	*Penicillium expansum*	Apples	Conway *et al*., 2004
		Colletotrichum acutatum	Apples	Conway *et al*., 2004
	T5-A2	*Colletotrichum acutatum*	Apples	Janisiewicz *et al.* 2003
		Penicillium expansum	Apples	Janisiewicz *et al.* 2003
	FMB-24H-2	*Penicillium expansum*	Apples	Conway *et al*., 2004
		Colletotrichum acutatum	Apples	Conway *et al*., 2004
Pichia angusta	ANY-32, ANY-34, ANY-38	*Botrytis cinerea*	Apples	Fiori *et al*., 2008
		Monilinia fructicola	Apples	Fiori *et al*., 2008
Pichia anomala	K	*Botrytis cinerea*	Apples	Friel *et al*., 2007; Haissam, 2011
	KH6	*Botrytis cinerea*	Apples	Haissam, 2011
Pichia fermentans	726	*Monilinia fructicola*	Apples	Giobbe *et al*., 2007
Pichia guilliermondii	87	*Botrytis cinerea*	Apples	McLaughlin *et al*., 1992
	-	*Penicillium expansum*	Apples	Gholamnejad and Etebarian, 2009
Rhodosporidium toruloides	NRRL Y1091	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
Rhodotorula aurantiaca	YCL5	*Penicillium expansum*	Pears	Chand-Goyal and Spotts, 1996
Rhodotorula glutinis	HRB6	*Penicillium expansum*	Pears	Sugar and Spotts, 1999
	HRB6	*Botrytis cinerea*	Pears	Chand-Goyal and Spotts, 1996
		Penicillium expansum	Pears	Chand-Goyal and Spotts, 1996
Rhodotorula minuta	YCL6	*Penicillium expansum*	Pears	Chand-Goyal and Spotts, 1996
	YCL7	*Penicillium expansum*	Pears	Chand-Goyal and Spotts, 1996
Saccharomyces delbrueckii	A50	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
Saccharomyces cerevisiae	YE-5	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
	A41	*Monilinia fructicola*	Apples	Zhou *et al*., 2008
Sporobolomyces roseus	FS-43-238	*Penicillium expansum*	Apples	Janisiewicz *et al*., 1994
		Botrytis cinerea	Apples	Janisiewicz *et al*., 1994 ; Filonow *et al*., 1996
	ATCC 28988	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
	ATCC 24257	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
Sporidiobolus salmonicolor	ATCC 623	*Botrytis cinerea*	Apples	Filonow *et al*., 1996
Moulds				
Aureobasidium pullulans	LS-30	*Penicillium expansum*	Apples	Castoria *et al*., 2001
		Botrytis cinerea	Apples	Castoria *et al*., 2001
	ApB	*Penicillium expansum*	Apples	Vero *et al*., 2009
		Botrytis cinerea	Apples	Vero *et al*., 2009
Trichoderma atroviride	P1	*Penicillium expansum*	Apples	Quaglia *et al*., 2011
Trichoderma harzianum	T22	*Penicillium expansum*	Apples	Quaglia *et al*., 2011
	T67	*Penicillium expansum*	Apples	Quaglia *et al*., 2011
	Th2	*Penicillium expansum*	Pears	Batta, 2007
		Botrytis cinerea	Pears	Batta, 2007
		Rhizopus stolonifer	Apples, pears	Batta, 2007
Trichoderma reesei	T34	*Penicillium expansum*	Apples	Quaglia *et al*., 2011
Trichoderma virens	T6	*Penicillium expansum*	Apples	Bordbar *et al*., 2010
	T8	*Penicillium expansum*	Apples	Bordbar *et al*., 2010

CONCLUSION

Many bacterial and fungal strains have been studied to date and several were proven to possess biocontrol activity against various postharvest pathogens. Combinations of such strains (*i.e*. the use of *P. agglomerans* in conjunction with *C. sake*) as well as application of a BCA following another treatment (*e.g*. use of reduced amounts of fungicides or heat treatment) resulted in increased protective effect against the pathogens. Despite the promising biocontrol properties of certain strains, some problems associated with the use of BCAs to control postharvest diseases have been encountered. Inconsistency in performance when used as the only treatment for the management of postharvest diseases and inability to control the growth of already established pathogens are the two major shortcomings of biocontrol agents. To overcome these problems improvements must be made in the various stages of their production and application. Additionally, research should be directed towards finding new biocontrol agents (perhaps with eradicative instead of protective activity) and improving the antagonistic traits of existing BCAs possibly through genetic modifications. The use of genetically modified organisms (GMOs), however, faces two major challenges – consumer acceptance and governmental policy – which must be surpassed in order for such organisms to be used as BCAs in food commodities.

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