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STATE OF ART AND FUTURE PERSPECTIVES OF BIOLOGICAL CONTROL OF POSTHARVEST FRUIT DISEASES

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Abstract

Synthetic fungicides, when admitted, are the primary means to control postharvest diseases of fruits. Biological control using antagonists has emerged as one of the most promising alternatives to chemicals. During the last twenty years, several biocontrol agents have been widely investigated against different pathogens and fruit crops. Many biocontrol mechanisms have been suggested to operate on fruit including antibiosis, parasitism, induced resistance and competition. Trying to extend the use of the biofungicides, there have been deep studies on application of antagonists mixes, preharvest use, and integration with chemical and physical means of protection. The formulation and application methods are key issues for the efficacy and final success of the commercial product. The new molecular techniques are useful tools in the characterisation of the microorganisms and enhancement of their biocontrol capabilities, through genetic engineering. Although a huge number of scientific papers published on biological control, this method at the moment should be viewed as an important component of an integrated disease management scheme if a significant and permanent reduction of pesticide use is our goal. Anyway scientists, growers and consumers alike must accept the fact that BCA's are usually not as effective as pesticides.

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1. Introduction

Fruits are an important part of the human diet, because they supply essential nutrients such as vitamins, minerals, and they are important to human health and well-being, for their contents in antioxidants and anticancer substances. An increasing awareness by consumers that diet and health are linked has resulted in a greater consumption of fruits. At the same time, consumers are also more concerned about the safety of the fruits they eat, and they ask for food free from pesticide residues, toxins and pathogens.

Losses due to pests and diseases attacks on fruit in field and during storage, transit, and commercialisation steps, before reaching the consumer, are not easily assessed, but can result in 25% of the total production in industrialised countries (Harvey, 1978). In developing countries damages are often higher, exceeding 50%, because of the lack of adequate storage structures (Eckert and Ogawa, 1985). The high water content of plant products, such as fruit, is one of the features that makes them more susceptible to pathogens attack, since they are in orchard (Harvey, 1978). Another factor favourable to pathogenic fungi, particularly to the necrotrophic ones, is the presence during storage on the plant organs of wounds, often produced during harvest and transport of fruit, which represent an ideal way of access for microorganisms.

Synthetic fungicides, when admitted, are the primary means to control postharvest diseases. However, several reasons, such as the growing public concern over the human health conditions and the environmental pollution associated with pesticide usage in orchards (Wilson and Wisniewski, 1994), the development of fungicide resistant strains of postharvest

71 pathogens (Romano et al., 1983; Spotts and Cervantes, 1986) and the lack of reregistration of
72 some of the most effective fungicides (Gullino and Kuijpers, 1994) have encouraged the
73 search of alternative approaches.

74 Biological control well fits with the concept of sustainable agriculture, because it mostly
75 exploits natural cycles with zero or reduced environmental impact. Among the biological
76 strategies adoptable in postharvest, the induction of resistance in the fruit, the use of plant or
77 animal products with a fungicidal activity, and, above all, the application of antagonistic
78 microorganisms can be considered. Biological control using antagonists (Wilson and
79 Wisniewski, 1994) has emerged as one of the most promising alternatives, either alone or as
80 part of an integrated pest management to reduce pesticide use.

81 The postharvest environment represents a particular sector for the development of biological
82 measures. Peculiar difficulties are present in the control of postharvest diseases (Chalutz and
83 Droby, 1998): the disease control level required is extremely high (also 95-98%); the
84 nutritional safety imposes special care to the direct use of living microorganisms on food
85 products; the potential market to employ a biofungicide expressly developed for postharvest
86 use is relatively small. On the other side, the possibilities of success for postharvest biological
87 means can be numerous (Chalutz and Droby, 1998): the storage conditions partially
88 controlled, such as temperature and humidity, can switch the host-pathogen-antagonist
89 equilibrium towards the antagonist and the laboratory trials and results have a higher
90 possibility to be transferred into practice; the application site of the antagonist, which is the
91 fruit, is limited, permitting an increase of the biocontrol agent (BCA) efficacy and avoiding
92 the presence of some interfering factors; finally the high value of fruit can justify a treatment
93 with a product relatively expensive.

94 During the last 20 years, several biocontrol agents have been exploited and widely
95 investigated against different postharvest fungal pathogens (*Alternaria*, *Botrytis*,

96 *Colletotrichum*, *Monilia*, *Penicillium*, *Rhizopus* spp.) on different host species. Many of the
97 first studies were aimed at the study of the mode of action and the evaluation of the efficacy
98 of some potential biocontrol bacteria, such as *Brevibacillus subtilis*, producer of antibiotics
99 (Pusey et al., 1986). The consideration that the application of bacterial antagonists on the fruit
100 was not commercially acceptable, at least in the short period, brought to switch the interest on
101 antagonists using modes of action different from antibiosis. Wilson and Wisniewski (1994)
102 indicated the characteristics of an ideal antagonist: genetic stability, efficacy at low
103 concentrations and against a wide range of pathogens on various fruit products, simple
104 nutritional requests, survival in adverse environmental conditions, growth on cheap substrates
105 in fermenters, lack of pathogenicity for the host plant and lack of production of metabolites
106 potentially toxic for humans, resistance to the most frequently used pesticides, compatibility
107 with other chemical and physical treatments. Yeasts, between the potential BCAs, possess a
108 good number of the mentioned features and, during the last years, research focused on the
109 selection and study of these microorganisms.

110 At present, three products containing *Pseudomonas syringae* Van Hall, active against
111 *Botrytis*, *Penicillium*, *Mucor* and *Geotrichum* spp., named Bio-Save 100, Bio-Save 110 and
112 Bio-Save 1000 and commercialised by EcoScience Corp. (Janisiewicz and Jeffers, 1997), and
113 a product containing *Candida oleophila* Montrocher, effective against *Botrytis* and
114 *Penicillium* spp., named Aspire and commercialised by Ecogen Inc. (Hofstein et al., 1994),
115 are available on the market for postharvest protection. Other yeast species have been
116 extensively tested and could be registered in relatively short times. Among the
117 microorganisms under development, there are antagonistic strains belonging to the species
118 *Aureobasidium pullulans* (Ippolito et al., 2000), *Candida saitoana* (El-Ghaouth et al., 1998),
119 *Candida sake* (Teixido et al., 1998) and *Metschnikowia pulcherrima* (Spadaro et al., 2001a).

2. Mechanisms of action

Information on the mechanisms of action for most of the antagonists investigated is still incomplete, because of the difficulties encountered during the study of the complex interactions between host, pathogen, antagonist and others microorganisms present in the site of interaction. However a good understanding of the mode of action is essential to develop appropriate formulation and methods of application, and to obtain registration.

Several possible biocontrol mechanisms have been suggested to operate against post-harvest rots on fruit including antibiosis, parasitism or direct interaction with the pathogen (extracellular hydrolases), induced resistance and competition for nutrients and space (Droby and Chalutz, 1994).

2.1. Antibiosis

Some of the most active bacteria are producer of antibiotics, whose action, at least partially, influences these BCA's effectiveness. *B. subtilis* was able to produce iturin, a powerful antifungal peptide (Gueldner et al., 1988) but also gramicidin S (Edwards and Seddon, 2001), *Pseudomonas cepacia* showed to synthesise pyrrolnitrin, that used alone can control *Botrytis cinerea* and *Penicillium expansum* attacks on pome fruit (Janisiewicz et al., 1991). Also from these observation, it was possible to develop phenylpyrroles fungicides (Gehmann et al., 1990). Often, over antibiosis, other mechanisms are present: *Penicillium digitatum* strains resistant to pyrrolnitrin are still inhibited by *P. cepacia* (Smilanick and Denis-Arrue, 1992). The main concerns, related to the use of antibiotics in food products, regard the selection of human pathogens resistant to these chemicals and the likely rapid development of resistance in fruit pathogens. Even if antibiotic producers are able to control wound infections occurred before antagonist application or latent infections, at the moment there are not such antagonists

registered for use on food.

2.2 Competition

Others selected microorganisms, particularly yeasts, act mainly establishing with the pathogen a competition for space and/or utilisation of some nutrients (Piano et al., 1997; Filonow, 1998; Spadaro et al., 2001a). Yeasts can successfully compete with the pathogen, inhibiting its growth but often leaving it alive. Also some bacteria act for competition, for example *Enterobacter cloacae* against *Rhizopus stolonifer* on peaches (Wisniewski et al., 1989). In the competition for space yeasts are helped by the formation of an extracellular polysaccharide capsule that can promote adhesion to fruit surface (Andrews et al., 1994). Competition for nutrients was demonstrated for *Pichia guilliermondii* against *P. digitatum* cocultivated on synthetic means (Droby et al., 1989): the addition of exogenous nutrients resulted in a reduced efficacy, because antagonists offered better results when nutrients were scarce. A rapid multiplication and colonisation by antagonist cells in the wound was elucidated in various interactions (Droby et al., 1989; Smilanick and Denis-Arrue, 1992; Piano et al., 1997). Recent studies on the repartition of radiolabelled glucose between the antagonist yeasts *Sporobolomyces roseus* or *Cryptococcus laurentii* and the pathogen *B. cinerea* point out a strong sugar assumption by the BCAs, that blocks fungus conidial germination for nutrients deprivation (Filonow, 1998). In fruit wounds, nutrients competition is likely extended to other nutrients, such as nitrogen compounds low concentrated. Janisiewicz et al. (2000) recently have developed a non-destructive method using tissue culture plates with cylinder inserts containing defusing membrane at one end to study competition for nutrients without competition for space.

2.3 Parasitism

Antagonist and pathogen can interact also through a direct parasitism. Wisniewski et al. (1991) observed a strong adhesion *in vitro* of *P. guilliermondii* antagonist cells to *B. cinerea* mycelium, perhaps due to a lectin link. Such adhesion is blocked by exposure to compounds able to alter the protein integrity and the respiration process. Moreover *P. guilliermondii* shows an high activity of β -1,3-glucanase enzyme, that could be associated to pathogen link and result in the degradation of the fungal cell walls (Jijakli and Lepoivre, 1998). Also *Aureobasidium pullulans* in apple wounds produces extracellular exochitinase and β -1,3-glucanase, that could play a role in the biocontrol activity (Castoria et al., 2001). El-Ghaouth et al. (1998) found, through ultrastructural and cytochemical studies, that *Candida saitoana* yeast cells, when cultivated together with *B. cinerea* mycelium, link fungal hyphae that show cytological damages, such as papillae and other protuberances in the cell wall, and degeneration of the cytoplasm. Finally some yeasts, such as *S. roseus* and *C. laurentii*, when applied, are able to reduce, on apple carposphere, the conidial adhesion and germination of *B. cinerea*, that is favoured by butyl acetate, volatile aroma produced by the fruit (Filonow, 2001).

2.4 Induced resistance

Some BCAs can interact with the host tissue, particularly the wounds, increasing the cicatrization processes (Droby and Chalutz, 1994). Several antagonistic yeasts are as much effective as applied before pathogen inoculation. This observation has brought to suppose that yeast cell application could induce resistance processes in the fruit skin. Some *Candida* strains are able to cause chemical and osmotic changes in apple tissues, favouring antagonist settlement (McLaughlin et al., 1990). A *P. guilliermondii* strain stimulates the production of ethylene, an hormone able in grapefruit to activate the phenylalaninammonium-lyase (Wisniewski et al., 1991), enzyme involved in the phenols, phytoalexins and lignins synthesis.

A phytoalexins accumulation (scoparon and scopoletin) was noted in citrus fruit treated with yeast cell (Rodov et al., 1994). In addition to control decays, *A. pullulans* can cause on apple fruit a transient increase in β -1,3-glucanase, chitinase, and peroxidase activities starting 24 h after treatment (Ippolito et al., 2000).

3. Extension of use

Potential biocontrol agents often have some significant limitations: they have a reduced action range, because they partially act on specific hosts against well defined pathogens on particular environmental conditions. A method to select antagonist with a broader spectrum of action, preferably for commercial development, is the carrying out of initial efficacy tests on various pathogens and fruit species (Wilson et al., 1993; Lima et al., 1999).

3.1 Antagonist mixtures

In the enhancement of a biocontrol system, work could focus on a promising approach, which is the development of antagonist mixtures. There is a superior probability to have an effective biological control with a mixture of several antagonists, complementary and non competitive, than with only one. Such mixture has some peculiar advantages (Janisiewicz, 1998): over the broadening of the spectrum of action (different fruits, cultivars and ripening stages), it can increase the efficacy (less biomass necessary) and offer a higher reliability, permitting a reduction in the application times and treatment costs. Moreover, it permits the combination of different genetic traits, avoiding genetic engineering. The evaluation of casual combinations of antagonists isolated from apple fruits and leaves resulted in the selection of a mixture of *P. syringae* and *S. roseus*, superior to both BCAs applied alone in the control of the Penicillium rot (Janisiewicz and Bors, 1995).

221

222 3.2 Preharvest use

223 One of the major obstacles to the development of postharvest BCAs is their inability to
224 control previously established infections, such as latent infections. Field application of the
225 BCAs may enable early colonisation of the fruit surfaces, protecting from these infections
226 (Ippolito and Nigro, 2000). To be successful in preharvest application, potential antagonists
227 should be able to tolerate low-nutrient availability, UV rays, high temperature and dry
228 conditions. Leibinger et al. (1997) applied in preharvest a mixture of a yeast *A. pullulans* and
229 a bacterium (*B. subtilis*) obtaining a level of control of *P. expansum* and *B. cinerea* on apple
230 similar to fungicides. Teixido et al. (1998) applied unmodified and low water activity tolerant
231 cells of *Candida sake* before harvest to control blue mould of apples, obtaining similar
232 disease control.

233

234 3.3 Integrated use

235 Biological means of control can not at the moment solve all the problems of postharvest rots
236 during fruit storage and they must be considered instruments to be used in combination with
237 other methods of control in an integrated vision of disease management. For example,
238 biocontrol agents can be combined with waxes and fungicides applied not only in post but
239 also in pre-harvest (Pusey, 1994). In some laboratory and semi-commercial trials the efficacy
240 of the BCA *P. guilliermondii* was consistently increased by the addition of small
241 concentrations of imazalil or thiabendazole, reaching the levels of control of the fungicide
242 alone (Droby et al., 1993). Antagonists, if employed together with fungicides, have to be
243 tolerant or they request the development of resistant strains. Yeasts generally are tolerant to
244 many of the fungicides used in postharvest: *Metschnikowia pulcherrima* (Spadaro et al.,
245 2001a) is tolerant to relative high concentrations of benzimidazoles (benomyl and

thiabendazole) and dicarboximides (vinchlozolin and procymidone). Moreover microorganisms need to survive to thermal, hygrometry and atmospheric storage conditions. The efficacy of *M. pulcherrima* against *B.cinerea* and *P. expansum* is increased when fruits are stored at low temperatures optimal for host, respect to storage at 23°C, because the antagonist is more adaptable than the pathogens to cold temperatures (Spadaro et al., 2001a). Among the strategies evaluated during the last years, it is to remember the combination of biological treatments with other techniques alternative to chemicals: thermotherapy (Barkai-Golan and Phillips, 1991), ultraviolet rays (Chalutz et al., 1992), animal and plant natural products (Aharoni et al., 1993), calcium infiltrations (Janisiewicz et al.,1998), sodium bicarbonate (Teixido et al., 2001) or ethanol (Spadaro et al., 2001b). Calcium chloride infiltrations combined to antagonist application on apples increases control of *P. expansum* after 3 and 6 months of storage at 1°C, compared to simple biological treatment (Janisiewicz et al., 1998). A even more integrated approach, experienced on “Gala” apples (Conway et al., 1999) consists of a heat treatment (4 days at 38°C), followed by a calcium chloride (2%) infiltration and a *Pseudomonas syringae* cell suspension application. The result of the three treatments carried out together was a reduction of the *Penicillium* rots (91%) higher respect to calcium, antagonist, or heat treatments applied alone. The efficacy of *Pantoea agglomerans* for the control of green mould was improved when combined with sodium bicarbonate, or baking soda (Teixido et al., 2001), a common food additive, allowed with no restrictions for many application by European and North American regulations, and listed as approved ingredients on products labelled as “organic”. The combination of sodium bicarbonate, or ethanol, or acybenzolar-S-methyl with *M. pulcherrima* cell suspension and heat treatment was also studied by Spadaro et al. (2001b). Ethanol occurs naturally in fruit and many other food products, and its toxic effects on spores of fungal pathogens have been reported: the flesh treated with the alcohol was significantly firmer and

injury to the fruit did not occur.

It is possible to conclude that the possibilities to apply antagonists in the context of integrated control are very extensive.

4. Improvement of biocontrol agents

4.1 Formulation and application

Differently from soilborne or open field pathogens, where a disease control of 70-80% can be considered satisfying, postharvest disease control requests a higher level of efficacy and more consistent results. Even considering the most effective BCA's studied until now, they rarely reach the levels of efficacy of the fungicides. It is therefore necessary an increase in their antagonistic activity to achieve a practical use.

A rapid, efficient and cheap mass production of the antagonist, generally by fermentation, is a key issue. The efficacy of many antagonists of wound pathogens is directly related to the number of antagonist propagules inoculated (Hofstein et al., 1994), so that a really simple way to increase the effectiveness is the application of a higher number of cells. To scale-up a laboratory fermentation process to an industrial level, it is fundamental to find the nitrogen and carbon sources that provide maximum biomass production and minimum cost of media, whilst maintaining biocontrol efficacy. Costa et al. (2001) have studied yeast extract, dry beer yeast, sucrose, and molasses as possible substrate for the production of the biocontrol agent *P. agglomerans*.

A correct formulation can be decisive in the improvement of the efficacy and extension of the product shelf-life, facilitating the storage for periods of time commercially acceptable (Janisiewicz and Jeffers, 1997). The application of adjuvants can protect and stimulate the establishing of the antagonist on the host surface. The addition of calcium salts increases the

activity of several antagonistic yeasts (Janisiewicz et al., 1998). The addition of glycerol and trealose to the culture means augmented the osmotic tolerance and control capability of *C. sake* against *P. expansum* on apple (Janisiewicz, 1998). Sodium alginate, carboxymethylcellulose and chitosan, are adhesivants and can be added to yeast cell suspension, to increase the activity of the formulate. The three adhesivants have been added to a strain of *M. pulcherrima* (Piano et al., 1998) significantly increasing the efficacy against grey rot on apple. Chitosan has also a fungistatic activity demonstrated against the main postharvest pathogens of strawberry (El-Ghaouth et al., 1992). Recently, El-Ghaouth et al. (2000a) developed a biocontrol product called "bioactive coating" consisting of a unique combination of an antagonist with glycolchitosan, a chemically-modified chitosan. The bioactive coating made it possible to exploit the antifungal property of glycolchitosan and the biological activity of the antagonist. Moreover, when applied as a pretreatment, sodium carbonate enhanced the efficacy of the bioactive coating (El-Ghaouth et al., 2000b).

Also application systems have a fundamental role in the determination of the final results. The coatings most often applied to citrus fruit contain shellac, which is a purified product of the hardened resinous secretion of the scale insect *Kerria lacca*. Having developed coating formulations before biological control, it is important to test the suitability of such products for antagonistic applications. McGuire (2000) found that, after 3 or 4 months storage, *C. oleophila* survival was more efficacious when applied in the shellac than when applied by preliminary immersion and subsequent drying of fruit prior to shellacking. On the contrary, Chalutz and Droby (1998) had noted that the application of antagonistic yeasts in aqueous suspension before waxing results in an efficacy much more consistent respect to a unique application of antagonists and wax.

Another issue to face in the commercial production of biocontrol agents is the storage, that should be as long as possible. The BCA's should have a storability of at least 6 months and

preferably of 2 years (Pusey, 1994). Abadias et al. (2001a) found that freezing at -20°C was the best method to preserve the viability of *C. sake* cells after freeze drying. Survival of the cells was higher using 10% skim milk as a protectant, and increased by using other appropriate protectants, such as lactose, glucose, fructose or sucrose. Skimmed milk was also the best rehydration medium with 1% peptone (Abadias et al., 2001b). However the efficacy of freeze-dried cells was significantly lower than that of fresh cells.

The transition from fungicide use to employment of biological means will be as easier as more flexible are the biofungicides.

4. 2 *The nutritional environment*

Temperature, humidity and gas composition in warehouse are optimised to guarantee a high fruit quality during storage and should not be changed but it is possible to manipulate the chemical environment to favour the antagonist. The addition of nutrients preferably metabolised by the antagonist and difficultly by the pathogen was suggested in several antagonist-pathogen interactions (Janisiewicz, 1998). The application of two nitrogen compounds, L-asparagine and L-proline (Janisiewicz et al., 1992), and an analogue of sugar, 2-deoxy-D-glucose (Janisiewicz, 1994) showed a consistent increase of the control of *P. expansum* on apple. Both aminoacids stimulated *P. expansum* germination but slowed mycelial growth. Also L-glutamine added to *M. pulcherrima* showed a direct influence on the yeast, because its application with the antagonist contributed to reduce Botrytis rot, while without yeast, it was ineffective (Piano et al., 1998). The sugar analogue 2-deoxy-D-glucose could be a useful additive to antagonistic microorganisms, provided that it has a fungicidal action on the major postharvest pathogens of apple and peach fruit (El-Ghaouth et al., 1997) and that the antagonist is resistant to its toxic effects. Recently the sugar analogue was found to be compatible with the antagonistic yeast *C. saitoana* and effective against apple and citrus

fruit decay (El-Ghaouth et al., 2000c).

Nutritional composition can influence not only density and competitiveness of the population, but also the production of metabolites crucial in many control systems, such as antibiotics (Gueldner et al., 1988) and cell wall degrading enzymes (Wisniewski et al., 1991). The form and concentration of nitrogen and carbon sources can therefore be important factors in the synthesis and secretion of key compounds in the biocontrol mechanism.

4.3 Manipulation of antagonists

It is possible to increase the ecological competence of microorganisms manipulating antagonists with techniques of mutagenesis (physical or chemical) or of sexual recombination, through protoplast fusion or continued culture.

Because nitrogen seems to be a limiting substance, it was noted that biocontrol increases adding some aminoacids. Therefore biocontrol strains with a superior capability of exploitation of nitrogen compounds present, or with a higher transport or metabolism rate of the limiting factor, could be developed (Janisiewicz, 1998). Some phenolic compounds, among them benzoic or chlorogenic acid, present at the wound sites, influence negatively the colonisation by yeasts; it is therefore conceivable trying to obtain strains resistant to these phenolic compounds (Bizeau et al., 1989). Mutants that use new substrates, not metabolised by the pathogen, to provide a nutritional advantage, could also be induced (Janisiewicz, 1998).

With genetic engineering techniques, features for the exploitation of plant products or additives applied together with the antagonist, for fungicide resistance, for carposphere colonisation in storage conditions, for synthesis of compounds favouring antagonism (antibiotics or siderophores), could be transferred into the potential antagonistic microorganisms (Pusey, 1994). Because one mechanism involved in the biocontrol of

postharvest fruit pathogens is mycoparasitism, lytic enzymes such as chitinases (Chernin et al., 1997), proteases and glucanases (De la Cruz et al., 1995) produced by bacterial and fungal microorganisms, could be inserted in the potential antagonists to improve the degradation of the pathogen cell walls, resulting in death or inhibition of growth of the attacked fungus. For biocontrol of soilborne pathogens, for example, a chitinase gene, isolated from *Serratia marcescens*, was introduced into the endophytic bacterium *Pseudomonas fluorescens* to improve the control of *Rhizoctonia solani* on bean (Downing and Thomson, 2000). Another idea could be the insertion of the gene for amylase under constitutive promoter in some BCA's, because many fruits are rich in amid and antagonists could use effectively this carbon source having a consistent advantage. First experiments of transformation have been successful: *M. pulcherrima* was transformed with the green fluorescent protein gene (Nigro et al., 1999), *C. oleophila* was transformed with β -glucuronidase gene (Chand-Goyal et al., 1998), and histidine auxotrophs of *C. oleophila* were transformed with *HIS3*, *HIS4* and *HIS5* genes (Chand-Goyal et al., 1999). All these studies were accomplished only to obtain variants of the antagonistic strains with a genetically stable marker to expedite studies on the ecology of the yeasts on the fruit surface, but are really useful to put successive insertions of useful genes right. In all cases the transformed antagonists maintained the biocontrol capability.

5. Molecular characterisation

New molecular technologies improving the preliminary selection of biocontrol agents and the monitoring of field applied antagonists may greatly facilitate the selection and screening of yeast isolates having a superior antagonistic activity. Morphological and cultural characteristics alone are not sufficient to distinguish between strains of the same species and do not allow the exploitation of any possible naturally available genetic variability. Moreover

the effectiveness of the preharvest strategy depends to a large extent upon the survival of the antagonists in competition with other microorganisms on the surface of fruits. A study of the ecology of the BCA's is therefore necessary but often complicated by the existence of other microorganisms on the fruit that are morphologically similar to the antagonistic strain. Molecular approaches can help to differentiate a biocontrol agent from an epiphytic microorganism. Between the technologies already used to obtain DNA fingerprintings the random amplified polymorphic DNA (RAPD) and the arbitrarily primed PCR (AP-PCR) are to remember (Schena et al., 1999; 2000). Both these methods are based on the amplification of random genomic DNA fragments by arbitrarily selected PCR primers and have the major disadvantage that they are very sensitive to the reaction conditions, DNA quality and PCR temperature profiles, which limit their applications.

New techniques more robust and reliable, such as amplified fragment length polymorphism (Vos et al., 1995; De Barros et al., 1999), based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA, and PCR amplification of the ITS regions of the ribosomal DNA (Masih et al., 2001) can be applied on potential biocontrol agents.

6. Conclusions

From this review, it appears that some significant progress has been made toward biological and integrated control of postharvest diseases on fruits. Some biofungicides are already on the market in a few countries, and will probably become more widely available as they are registered in more areas. Other BCA should reach the market soon. Postharvest conditions provide an ideal niche for BCAs since they are less subject to sudden climate changes, and are often equipped with a sophisticated system of climate control.

It is unrealistic to assume that perfect conditions for the development of BCA's will always prevail in the warehouse, and as a result, biofungicides will rarely stand alone as a complete measure of disease control under all conditions. For this reason, scientists, growers and consumers alike must accept the fact that BCA's are usually not as effective as pesticides. The success of biological control greatly depends in a change of mentality of the consumer, not anymore willing to have fruit with satisfying exterior aspects, but with an inner quality. Biological control should be viewed at the moment as an important if not essential component of an integrated disease management scheme if a significant and permanent reduction of pesticide use is our goal.

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