Chapter 10
Utilization of Yeasts in Biological Control Programs

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Contents

Abstract In an agricultural environment, the native flora is replaced by a commercial crop and consequently the native microbiota also undergoes changes and, no seldom, species with antagonistic action against pathogens are eliminated. The lack of natural competitors may result in an outburst of diseases or herbivores that will feed upon the growing crop. Several strategies such as: chemical control, pathogen resistant cultivars and biological control may be used to avoid economical loses in the crop. Biological control protocols are based on the assumption that in an undisturbed environment outbursts of diseases are seldom due to the presence of naturally occurring antagonists and therefore, the introduction/augmentation of antagonism in a disturbed environment will control the disease. A successful agent for biological control has to hold several characteristics such: antagonism against pathogens, well know biology, specificity, be ease to produce and apply, be safe to the environment. Yeast may present all of those characteristics and are used in several biological control protocols. We will discuss in this chapter the basic concepts of biological control, the use of yeasts as biological control agents and describe the commercial products that use yeasts for biological control.

Keywords Biological control, environment, competitors, antagonists, antagonism

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10.1 Introduction

The concept of biological control correlates well with the sustainable agriculture strategies because both exploit the natural biological cycles in search of crop production with reduced environmental impact (Spadaro and Gullino, 2004). Ecophysiological aspects of pathogen, host and their interaction with the biological control agent (BCA) are the fundamental information for the development of biological control strategies of plant disease since the core of this technology is the manipulation of the ecological interaction among the host, pathogen and BCA towards the decrease of pathogen damage. Population control in a natural habitat acts by different mechanisms such as environmental resistance and biological antagonism. Although abiotic environmental control is hard to achieve, it can effectively diminish pathogen damage in a crop. Among the techniques used for environmental control there are changes in temperature (green-house, solarization), pH, (soil correction), salinity, radiation incidence, humidity, as well as decrease in the availability of nutrients in the substrate and introduction of toxic compounds. Biotic control of pathogens can be achieved by the introduction or augmentation of existent antagonists in the ecological niche of the pathogens. Biotic antagonism limits growth by competition for space or food, parasitism, predation, inhibition of growth through the production of toxic substances and by producing environmental modification. These factors influence microbial activities and play very important roles in determining the spatial and temporal dynamics of microbial populations. Usually, the biotic activities modify the environment and these modifications change the community structure.

Microbial ecology examines the diversity and activity of microorganisms in Earth's biosphere (Xu, 2006). In a broad view, microbial ecologists organize and group microorganisms in specific metabolic categories related to its energy source and generation. Yeasts are ubiquitous unicellular fungi widespread in nature and colonize terrestrial, aerial and aquatic environments and also plant and animals surfaces, where the successful colonization is intimately related to their physiological adaptability to a highly variable environment (Rodrigues et al., 2006). Distribution of yeasts in nature is partially determined by nutritional characteristics of substrate, biogeographical characteristics and dispersal agents (Lachance et al., 2003; Pimenta, 2001).

All those natural characteristics should be taken in consideration when applying those organisms in a program of disease control and management. Yeasts are particularly interesting microorganisms in a Biological Control programs because they are relatively easy to produce and maintain and have several characteristics that can be manipulated in order to improve its use and efficiency.

Yeasts do not occur randomly throughout the biosphere, and each yeast community may be defined by its habitat (Lachance and Starmer, 1998). Yeast species may be defined as generalists or specialists, depending on their habitat occupation and physiological profiles. Generalist yeast has the ability to utilize diverse carbon compounds and due to this they can survive and grow in different environments. Specialist yeasts have a simple physiologic profile and obtain energy solely from few carbon compounds and this limitation restricts their habitat amplitude. However, a simple physiologic profile allows faster growth. In contrast, the complex physiology, found in generalist yeasts allows a great range of food supply, but that leads to slow colonization (Morais et al., 1995; Abranches et al., 2000; Rosa and Péter, 2006). Depending on the metabolism type of a BCA yeast, a different strategy for applying a biological control program should be devised. Generalist yeasts are easier to maintain, and because they use several different media for growth they are generally more suitable for industrial production. However, because they are found in various environments, specificity towards a particular pathogen is not frequent. Such metabolic pattern is desired for a BCA to be used against opportunistic or non-specialist saprophytic pathogens. Specialist yeasts, on the other hand, are restricted to fewer environments and are more prone to exploit such environments quickly and with less inter-specific competition. These BCAs are more suitable for the development of specific pathogen control programs or to be applied preventively. However, they are usually more difficult to produce and use for biotechnological applications.

The transmission of infectious diseases is an inherently ecological process involving interactions among at least two, and often many species (Keesing et al., 2006). In an agricultural condition, a commercial crop replaces the native microorganisms, consequently the native microbiota is modified or completely changed. As a result, no seldom species with antagonistic action against pathogens are eliminated. The lack of natural competitors may result in an outburst of diseases or herbivores that will feed upon the growing crop. The knowledge of such environment and biotic changes are capital for the development of biological control strategies. In situations where populations of related host species grow sympatrically but isolated from other populations of both hosts, cross-species disease transmission can have great influences on disease dynamics and patterns of pathogen persistence (Carlsson-Granér, 2006).

Recently, there has been renewed interest in the potential effects of diversity on disease risk, partly because of the interest in identifying and evaluating utilitarian functions of biodiversity. Various empirical and modeling investigations have suggested that increased species diversity could reduce disease risk due to genetic variability. However, in particular situations, some studies propose an increase in disease risk caused by varying numbers of vectors and hosts (Keesing et al., 2006).

The usual approach for solving an agronomic problem is to focus in the disease and study the interaction of pathogen and host alone. Such view could inhibit the development of alternative control strategies that could be more efficient and environmentally safe. Designing and using a biological control protocol is an exercise of manipulating ecological conditions in a commercial environment. Unfortunately, the knowledge of ecological interactions in such environments is scarce. Therefore, is necessary an effort to understand the ecology of natural environments, the changes caused by agronomic use and apply this knowledge in developing strategies to decrease disease pressure in a crop. The efficient use of a biological control strategy is a challenge that should be pursued by the conjoint effort of the ecologist and the agronomist.

An important characteristic in commercial crops is the search for efficiency in production. Efficiency is usually measured by the volume of production. The factors that may decrease production such as diseases and pests are normally neglected and view as secondary. Strategies based of increased efficiency have generated high productive crops, but having special nutritional and environmental demands. Usually such crops do not cope well with environmental challenges such as diseases and pests. To overcome this problem, farmers compensate such inadequacy with the use of chemical soil correction and pesticides. The use of genetically improved cloned seeds surely has increased the volume of production by cultivated area for many crops, but at the same time, generated problems related to low genetic variation. The losses due to microbiological food deterioration may reach up to 5 to 20% of the crop yield in developed countries. In tropical areas the losses may be even higher, reaching up to 50% in countries where modern techniques for food storage and transport are not implemented (Eckert and Ogawa, 1985; Chand-Goyal and Spotts, 1997; Varma and Dubey, 2001; Janisiewicz and Korsten, 2002).

The classical way to approach disease control is based on exploiting aspects of the disease triangle theory. The disease triangle summarizes disease as an interaction among host, pathogen and the environment (Fig. 10.1).

Biological control protocols add a biotic factor to the equation, that will interact with the pathogen directly (antagonistic effect), with the host by changing its resistance characteristics (induced resistance) or through environmental changes (nutrient depletion, pH change, etc.) (Jeger and Spence, 2001).

Different plant substrates can be attacked by different pathogens, and the type of plant species can influence the BCA efficacy. Consequently, fruits, seeds, leaves, and flowers can be targets for pathogens and biocontrol agents. Therefore, the attack and protection can happen at different times of cultivation, from planting to post harvesting. Normally fruits have high sugar concentration and low pH. Leaves surfaces are poor in complex nutrients. However, stems, roots and seeds can offer a great and diverse supply of nutrients. With this, the biological controller needs to have a physiological profile compatible with host resources and also the ability to colonize the substrate.

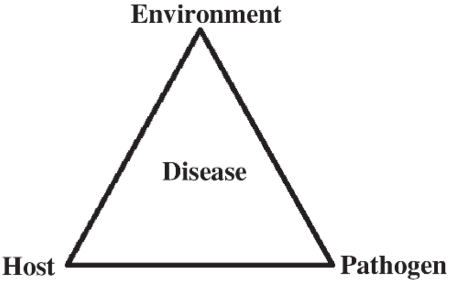

Fig. 10.1 Graphic representation of disease triangle

Since the 1960's the storage times of fruits have been increasing considerably and post-harvest diseases are today a major cause of economical losses. Actually, there are over a hundred thousand types of plant diseases and about eight thousand species of pathogenic fungi described, but only 100 fungal species are responsible for the majority of post harvest ones. All plants can be infected by one or more fungal species and some fungi can infect several plant species. Plant disease is the damage of cells or tissues, and result in development of symptoms by the pathogenic agent or environmental condition. Disease involves morphological or physiological modifications but also alterations in integrity or behavior. These modifications can result in partial damage or death of the plant or of its parts (Agrios, 1997; Tripathi and Dubey, 2004).

The post harvest storage time of fruits increased mainly due to new technologies for temperature and humidity control and the use of fungicides. Fungicides have been efficient in decreasing losses by deterioration of food, but also generated the increase of public health concerns and environmental problems mainly due to the carcinogenic and/or teratogenic proprieties of the compounds, and by their cumulative toxicity (Janisiewicz and Korsten, 2002). Therefore, the development of new environmentally friendly and healthy technologies is necessary. Among the possibilities, biological control of post-harvest diseases is particularly suitable. Generally, biological control is harmless to the public and if applied correctly will not cause any environmental damage.

Among the microorganisms that can be utilized as biocontrol agents, yeast has several characteristics such as ability for fast colonization and survival on the fruit surface for a long period of time and in different environmental conditions, that make them a good candidate for the development of biological control protocols (Droby et al., 1999, 2003). Different yeasts species are being utilized as biocontrol agents, for example: Candida oleophila and Pichia membranifaciens is commercially used for the control of Botrytis cinerea post harvest rot in apples (Jijakli and Lepoivre, 1998); Debaryomyces hansenii against Penicillium digitatum on the decay of grapefruit; Pichia guillermondii against Botrytis, Rhizopus, Penicillium and Alternaria on decaying tomato fruits; Cryptococcus laurentii, Cr. flavus and Cr. albidus for control of Mucor in pears; Candida sake against B. cinerea and Rhizopus nigricans on apple decay (Masih et al., 2000).

In this chapter we will discuss yeast ecology applied to biological control of postharvest disease of fruits and seeds, including the inhibition of aflatoxin production and possible probiotic proprieties of yeasts. We will bring to discussion different strategies of disease control, the methods for evaluating biological control efficiency and the perspectives for research and development in the field.

10.2 Postharvest Diseases

Postharvest diseases are those that may start in the field and develop during the cropping, transportation, packing and storage. The concept of disease in vegetables is not only applied to the plants or parts of plants that are affected by pathogenic agents in the field, but also to deterioration of vegetable products, such as grains, roots, fruits, wood, during transportation, storage or in consumption phase. Usually, the most tender and succulent products, such as fruits, have the greatest susceptibility to the attack of pathogenic fungi because they have a high water and nutrient content. Fruits and flowers attacked by postharvest disease usually cause direct losses to the producer and to the seller, due to loss of quality or quantity of these products and are particularly pernicious because the loss occurs after the investment in the production, transport and storage is made (Agrios, 1997; Leggott and Shephard, 2001; Mercier and Jiménes, 2004).

A relatively small number of fungi and bacteria cause these diseases. Postharvest diseases are normally caused by primary parasites, like Rhizophus, Penicillium and Erwinia, that actively attack living tissues of vegetables, provoking degradatives lesions that usually serve as entrance to secondary infection. Lesions produced by mechanical injury during harvesting, transportation, storage and commercialisation of the fruits are also an entrance door for the pathogen. Facultative or accidental pathogens are saprophytic organisms and normally attack soft organs used as nutrient reserves. These pathogens are considered primitive parasites since they exhibit great severity and low specificity (Obagwu and Korsten, 2003; Stange et al., 2002). Spreading of the pathogen occurs normally from fruit to fruit within the storage area (Agrios, 1997; Huang et al., 2000).

Traditionally, the postharvest diseases are controlled by fungicides. However, the appearance of resistant varieties of pathogens as well as the difficulties of the implementation of protocols that guarantee the safe use, has made more and more problematic the application of these substances. The main problems involved in fungicide utilization are related to environmental pollution and public health concerns especially due to its carcinogenic and/or teratogenic properties (Harman et al., 1996; Masih et al., 2001; Janisiewicz and Korsten, 2002; Mercier and Jimenes, 2004).

10.3 Biological Control

Biological control is a natural phenomenon that occurs widely in the environment. It consists in growth control of a population or community by one or more antagonistic organisms. This control is established by a reciprocated influence of pathogen, host and environment and it potentially happens with all organisms and species, since all types of living forms have a pathogen. Biological control is spread on Earth and many species belonging to different taxa may take part on the maintenance of ecological equilibrium. However, anthropic environments such as plantations or cities have a different and normally less diverse community of organisms. According to Keesing et al. (2006), the lack of diversity may lead to ecological imbalance that may allow some species to increase in number and niche area while other populations are eliminated or reduced, increasing the possibility of appearance of epidemic disease.

The idea that some species can reduce the populations of others considered noxious is very old. Probably, the Chinese were the first to use a biological control strategy. They used a predacious ant Oecophylla smaragdna for the control of a herbivorous Lepidoptera in citrus crops, as early as the century III A. C. (Parra et al., 2002; Santos and Del-Claro, 2002). However, the first well documented case of success of classic biological control was the introduction of Rodolia cardinalis from Australia to California in 1,888 to control the white greenfly, Icerya purchasi. After two years of its introduction, C. cardinalis totally controlled the insect (van den Bosch et al., 1982). Biocontrol initiatives were first used to control insects, acaroids and weeds, but afterwards, its use became wider and other invertebrate, phytopathogens and also some vertebrates are now considered targets. Nowadays, biological control is used in several agronomical problems, among them the control of postharvest diseases.

The growing interest in the consumption of foods free of fungicides has led producers worldwide to demand new alternatives of control of diseases (Spadaro and Gullino, 2004). In this context, biological control of pathogens grows as an alternative, especially the biological control of postharvest diseases, and it represents a promising alternative to total or partial substitution of chemical pesticides (Janisiewicz, 1991; Harman et al., 1996; Chand-Goyal and Spotts, 1997).

Biological control is the use of several ecological interactions between pathogen and a BCA to decrease economical loss. The ecological interactions employed in the reduction of plagues and pathogens are: competition, parasitism, production of antibiotics, induction of resistance in the host and predation, being common the presence of more than one type of interaction (Bernard, 1999; Schoeman et al., 1999; Bapat and Shah, 2000; Qin et al., 2003, 2004).

Competition is an interaction among populations resulting in decrease in the number of individuals and may be classified in:

- Competition by mutual inhibition - when two populations inhibit actively each other.
- Competition for resource - in which each population affects negatively the other, in an indirect way, in the dispute for a limited resource, as space or nutrient.
- Antibiosis - in which a population is inhibited and the other is not affected, being usually mediated by antibiotics.
- Parasitism and predation are associations in which a population affects negatively another through a direct attack, depending on the other population as food or habitat.

Despite the ecological mechanism of disease control, most of the biological control protocols using microorganisms are developed similarly.

The classical biological control development starts with the isolation of naturally occurring strains of microorganisms associated with the target vegetable to obtain one or more antagonistic species in Nature. This step is followed by experiments to select the antagonist, the multiplication of the antagonist in laboratory and multiple inoculations in field using different and usually high concentrations of the antagonist. Even though this strategy is widely used, the isolation of antagonists in nature is time consuming and not ensures commercial applicability. Frequently, the isolated microorganism after several cycles of multiplication in laboratory, loss its efficacy and environmental fitness, diminishing its use in the field. To increase the time of use of a particular BAC, several strategies have been developed, as a result of our better understanding of microorganism ecology and physiology. The integrated biological control is an example of such improvement. Integrated biological is an association of classical biological control with a GRAS (Generally Regarded As Safe) substance.

Among others, sodium bicarbonate, sodium carbonate, ethanol, ascorbic acid, acetic acid, lactic, benzoic, sorbic are considered as GRAS and used in integrated biological control protocols (Kang et al., 2003; Gamagae et al., 2003; Irtwange, 2006). This method, differing from the classical biological control, has presented similar results to chemical control. Most of the GRAS substances if used solely, presents some sort of disease control. Sodium bicarbonate has been used in orange disinfection since 1920 (Obagawu and Korsten, 2003). However, the use of this substance in high concentrations can produce burn-like lesions, depending on the type of treated fruit. The use of sodium carbonate and bicarbonate alone has controlled partially the citrus pathogens P. expansum, P. digitatum and P. italicum. However, its use is preventive and not curative, because sodium bicarbonate seems to act as fungistatic and it probably produces some poisonous effect in the spores (Palou et al., 2002; Gamagae et al., 2003; Yaoa et al., 2004). Therefore the use of sodium bicarbonate alone has not been capable to reduce in an effective way the incidence and severity of the lesions and it is not indicated as main strategy of control of citrus fruits pathogens (Smilanick et al., 1999). However, when sodium bicarbonate is applied in consortium with a BCA, the efficiency of control increases significantly.

10.4 Yeasts for Postharvest Control of Pathogens in Fruits

According to Cook et al. (1996), microorganisms are a vast resource still little explored for the control of plagues and phytopathogens. Among them, yeasts are particularly suitable for use as BCAs due to their ability of fast colonization of the vegetable surface, maintaining viability for long periods of time under different environmental conditions (Cartwright and Spurr, 1998; Droby et al., 1999, 2003; Janisiewicz et al., 2001). Yeasts and bacteria can prevent deterioration of foods during the stockpiling through competition with the pathogen for space and food (Wilson and Wisniewski, 1989; Roberts, 1990; Wisniewski et al., 1991; Avis and Belanger, 2002).

Pathogen control by yeasts, in pre- or postharvest diseases have been demonstrated extensively (Paulitz and Bélanger, 2001; Irtwange, 2006; Punja and Utkhede, 2003). Generally most of the antagonistic yeasts are obtained from the epiphytic microbiota associated with flowers and fruits and display a range of activities such as competition for nutrients and space, production of antagonistic substances and predation (Goyal and Spotts, 1996; Piano et al., 1997). Young (1987) documented antagonistic interactions mediated by the production of soluble proteic molecules by yeasts that provoke disruption of cell membrane and cell wall. Starmer et al. (1987) concluded that the production of killer toxins is a strategy to eliminate competitor strains of the same or different species. The term predacious yeast was introduced by Lachance and Pang (1997) to describe certain yeast species that produces small feeding appendages or haustoria that penetrate and kill other yeast cells. Parasitism and degradation of hyphae of the pathogenic fungi by antagonistic yeasts have been linked to the adherence of the yeast cells to fungal hyphae and to high production of glucanase derived from different carbon sources (Odum, 1988; Chand-Goyal and Spotts, 1997; Lewis and Larkin, 1998). Among the antagonistic relationships among yeasts and other microorganisms, the production of antibiotic substances is not common. On the other hand, competition for resources is often observed. For example competition for L-proline, observed between the yeasts Candida membranifaciens and Cryptococcus laurentii, and Penicillium expansum can be responsible for the reduction of infection of apples by this pathogen (Blum et al., 2004).

Jijakli and Lepoivre (1998) showed that Candida oleophila that is found in fruits, and Pichia membranifaciens that is usually isolated from a great variety of habitats, specially fermented substrates, actively eliminated Botrytis cinerea from apples. According to Masih et al. (2000) Debaryomyces hansenii, an ubiquitous yeast can act against Penicillium digitatum in decaying grapefruit. Experiments showed that the ubiquitous yeast Pichia guilliermondii is effective against Botrytis, Rhizopus, Penicillium and Alternaria in decaying tomato fruits. The yeasts Cryptococcus laurentii, Cr. flavus and Cr. albidus are usually found in foliar surfaces and could potentially are used for control of Mucor in pears. Also, Candida sake, a yeast commonly isolated from fermentations, soil and water seems to act against B. cinerea and Rhizopus nigricans, which are the main causes of postharvest disease on apple.

Several yeasts used as BCAs have shown more effective results in control of phytopathogens when inoculated simultaneously with sodium bicarbonate that is a recognized as GRAS by the US FDA (United States Food and Drug Administration). Saccharomycopsis schoenii, a predacious yeast, was tested as a biological control agent against Penicillium italicum, P. expansum, and three strains of P. digitatum causing post harvest decay in oranges. In an integrated biological control test, treatment with S. crataegensis associated with sodium bicarbonate, resulted in no decay 96 h after the treatment (Pimenta, 2004).

10.5 Yeasts for Aflatoxin Inhibition in Food

Peanuts, coffee, corn and others substrates are often invaded by Aspergillus flavus and/or other mycotoxigenic fungi causing damage. Biodeterioration of seeds and grains in the field and during storage limits the stockpiling and reduces the nutritional value of those foods (Reddy and Shetty, 1992; Prado et al., 1999; Sarimehmetoglu et al., 2004; Bittencourt et al., 2005; Erdogan, 2004).

One of the most important effects of postharvest diseases of seeds and grains is mycotoxin accumulation. Mycotoxins are almost certainly the main non-infectious dietary risk factor associated with food. Mycotoxins are secondary metabolites of molds that exert toxic effects on animal and humans. The toxic effect of mycotoxin on animal and human health is referred to as mycotoxicosis, the severity of which depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals to which the individual is exposed. Acute mycotoxicosis can cause serious and some times fatal diseases (Peraica et al., 1999). The toxic effects are mainly localized in liver as manifested by hepatic necrosis, bile duct proliferation, icterus and hemorrhage. Chronic toxicity in birds is characterized by loss of weight, decline in feed efficiency, drop in egg production and increased susceptibility to infections. The incidence of hepatocellular tumors, particularly in ducklings, is considered to be one of the serious consequences of aflatoxicoses (Krogh, 2004). Ergotism and the poisoning due to consumption of mushrooms are the most largely known examples of mycotoxicosis. The magnitude of this problem is exemplified by the consumption of moldy grains in Russia during the Second World War, when a sudden appearance of lesions in the skin, hemorrhage, bankruptcy of liverwort led to countless deaths of soldiers and animals. Similar symptoms were observed in thousands of birds dead poisoned by toxins present in peanut feed in the middle of the 60 decade (Christensen and Kaufmann, 1965; Agrios, 1997; Rastogi et al., 2004; Rasooli and Abyaneh, 2004; Keller et al., 2005).

Different mycotoxicosis result in serious diseases and can lead to death. Filamentous fungi produce these toxins mainly in stocked seeds and processed foods. Usually, the infection of the seeds occurs in the field or during the initial phases of the storage (Prado and Oliveira, 1996; Shephard and Leggott, 2000; Batista et al., 2003; Blesa et al., 2003). The contamination of foods with aflatoxins is more frequent in tropical and subtropical areas, where the climate favors the development of toxigenic fungi (Sabino et al., 1986, 1989; Whitaker, 2003). The disease control for molds in grains, vegetables and other plant products depends on certain precautions and conditions that can be applied before and during cropping and also during the storage (Prado et al., 1995; Prado and Oliveira, 1996; Widstrom et al., 2003). The grains should be protected from mechanical damages, in order to reduce the access of the mold to the internal tissue of the vegetable. Some strategies of reduction of toxins in foods have reached positive results, such as pasteurization, fermentation, addition of substances and filtration, among others. However, the demand for products "in nature" and the fact that many of these procedures result in undesirable alterations in the foods, such as, loss of nutritious substances and organoleptic alterations, led to several studies aiming to establish new control procedures (Leggott and Shephard, 2001; Dorner et al., 2003).

Pimenta (2004) showed that the concomitant inoculation of Saccharomycopsis schoenii in peanuts infected with Aspergillus flavus resulted in 73.5% decrease in the accumulation of aflatoxin in the grain. The same study observed that the amount of aflatoxin produced varied according to the concentration of mold cells. Previous studies had already pointed the importance of the concentration of spores of A. flavus for toxin production (Odamtten et al., 1987; Sharma et al., 1980; Chulze et al., 1999). Usually aflatoxigenic molds produce more toxins when the initial inoculum is about 10^3 spores/ml. Measures to reduce or increase this optimal spore concentration would also reduce toxin production. On the other hand, control measures that would reduce populations to near 10^3 spores/ml would probably amplify toxin production. Another important aspect of control is that the inoculation of an antagonistic filamentous fungi or yeast, added to a toxigenic population lower than 10^3 spores/ml would also increase the number of cells and consequently lead to an increase in toxin production. The studies have shown that that inoculation of BCAs must use concentrations up to 10^6 cells/ml and that any procedure leading to reduction of the toxigenic population must reduce the initial population to levels below 10^1 cells/ml (Pimenta, 2004).

Another potential use of yeasts against toxigenic pathogens is as probiotics. Probiotic microbes are defined as those which upon ingestion in adequate amounts confer health benefits to the host by improving its intestinal microbial balance (Buzzini and Vaughan-Martini, 2006). When ingested as viable cells by the individual intoxicated with aflatoxin, the yeasts reach the gastrointestinal tract and lead to a return to the equilibrium of the microbiota and improve the complexing of toxins or their inducing agents. The use of live cells of Saccharomyces cerevisiae was capable of minimizes the histotoxicity of aflatoxins in mice (Baptista, 2001; Baptista et al., 2005; Pennacchia et al., 2006).

10.6 Control Strategies

The best strategy for the establishment of a biological control program consists in the utilization of ecological interactions that already exist in nature, including interactions between the crop and microorganisms. It is necessary therefore to search microbial species occurring in the geographic region or associated with the target plant. The yeast or other biocontrol microorganism should be obtained from the phylloplane (leaf surfaces), fruit surfaces, inner plant structures (endophytic microorganisms) or from soil. These protocols avoid the risk associated with introduction of exotic organisms and increase the chance of selecting an organism already suitable to survive and grow in the environmental conditions present in the application area.

The application of the biocontrol agent by inoculation should be made accordingly to the cultural traits of the crop to be protected. In situations of postharvest disease control, the inoculation with a massive population of the biocontroller should be made after harvesting but before storage to prevent pathogens from infecting fruit wounds immediately after harvesting. Wounds are normally the preferred infection route for most of the postharvest pathogens. Once the pathogen gains the interior of the fruit or grain it is virtually unreachable for chemical or biological control. Therefore, the protective inoculation should be made before any chance of wounding or fungal infection. Avoiding wounds is difficult in most cases, since the act of harvesting fruits could produce injuries. In some cases a preharvest application should be considered (Janisiewicz and Korsten, 2002).

The association of biological control with physical control methods has also had success. An example of this combination is a prestorage treatment to control disease and plagues associated with modified methods for the ripening of the vegetable products. Reduction of humidity, use of heat or cold, modification of the atmosphere, irradiation treatments are among the most employed physical methods for diminishing storage loss (Leverentz et al., 2000; Pimenta, 2004). Low temperatures are recognized as very efficient to increase storage time because they delay physiological processes associated to the ripening and also reduces fungal growth. Humidity reduction preserves commodities by reducing pathogen metabolism but it is an expensive method compared to other methodologies. Treatments using heat generation (hot air at 38°C for 4 days) eliminate insects and phytopathogenic microorganisms. However, the greater limitations to the use of heat refer to the lack of residual protection against recontamination by opportunistic parasites and to injury pit can provokes in the host together with acceleration of the ripening process of several fruit species. Modified atmosphere inhibits the normal aerial growth of the mycelia and greatly prevents sporulation of pathogens. These alterations can be done by a reduction in O_2 and increase in CO_2 concentration, which reduce the ethylene synthesis delaying the ripening of the fruits. Ozone addition also reduces microbial proliferation. Ozone does not deposit a persistent residue on the product, and it is accepted by many organic growers' organizations (Palou et al., 2003; Moraes et al., 2006).

Induction of systemic resistance is a mechanism that operates through the activation of multiple defense proteins. Some BCA can interact with the host tissue particularly with wounds, increasing scarring processes and stimulating host tissue to produce enzymes and other substances that prevent pathogen growth, such as α-1,3-glucanase, chitinase, peroxidase, salicylic acid, terpenoids, and others (Punja and Utkhede, 2003; Spadaro and Gullino, 2004; Liu et al., 2005). An efficient BCA should present some characteristics, like fast growth, genetic stability, efficacy at low concentrations against a wide range of pathogens on various plant products, survival in adverse environmental conditions, growth on cheap substrates, lack of pathogenicity for the host plant and absence of production of metabolites potentially toxic to humans, resistance to the most frequently used pesticides and compatibility with other chemical and physical treatments (Spadaro and Gullino, 2004).

The time of endurance of BCA on substrate is indispensable. Once this period is determined, it will point to the viability and need for reinoculation protocols, although in most cases, reinoculation is not accessible or viable (Pimenta, 2004).

10.7 Conclusions/Tendencies

The transition from chemical to biological control is now evident and a combination of economic, political, and environmental factors has probably contributed to this change. There is increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world. Regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce pesticide use. Biological control of postharvest diseases has been one of the most extensively studied alternatives and appears to be a viable technology. Several commercial products are already available and others will be available in the near future. During the past ten years, over 80 biocontrol products have been marketed worldwide and this reflects a growing demand for biocontrol strategies in crop production and storage (Chand-Goyal and Spotts, 1997; Irtwange, 2006).

The yeast Candida oleophila (strain I182) and two strains of a bacteria (Pseudomonas syringae) are registered as Aspire (Ecogen, Inc., Langhorne, PA), BioSave-100, and BioSave-110 (EcoScience Corp., Worchester, MA), respectively, for the control of postharvest diseases of citrus and apple fruits. The integrated control using biological agents associated with one or more physical and chemical (GRAS) treatments such as heat treatment, controlled and modified atmospheres, sodium bicarbonate, calcium chloride, and foodgrade preservatives will probably provide adequate control levels comparable to those achieved by traditional chemical fungicides.

This is an effective method for control of postharvest diseases that seems to be safe and possesses negligible risk to human health and the environment. The biological control of plant diseases using saprophytic, naturally occurring microorganisms which do not produce antifungal compounds, do not grow at human body temperature, and are consistent in controlling the target disease is a safe way to reduce postharvest losses (Chand-Goyal and Spots, 1997). The biological control is an important method for the postharvest control, considered not alone but as part of a multivariate strategy to be applied for reduction of pollution and intoxication. Phytopathogens are associated with plantations since the appearance of agriculture and they will endure along with the cultivation of vegetables by men. The major idea of a biological control program is the return to an equilibrium between species in modified environments (crops), and the reduction in use of toxic compounds in food reached satisfactory standards of food safety (Leverentz et al., 2000; Vivekananthan et al., 2004).

Recently, food safety is one of the most important restrictions to international trade and creates non tariff barriers between countries. The United States and European Union are the most important markets for fruits, and so the most rigorous consumers for safe foods (Skogstad, 2001). Regulators agencies in the United States, Canada, and Europe tend to be favorable towards biological pesticides and they encourage companies to register these products. For example, the costs of registration of biofungicides are lower than for chemical pesticides, and in many cases, registration is unnecessary. Biological products automatically enter a fast-track review process that speeds up registration. This initiative will probably be followed by other countries (Paulitz and Bélanger, 2001). Scientists, growers and consumers alike must accept the fact that BCAs are usually not as effective as pesticides, however, the benefits to the environment and public health compensate a less appealing appearance. The success of biological control greatly depends on influencing the consumer to prefer inner quality to outward appearance (Spadaro and Gullino, 2004).

The use of yeasts in postharvest biocontrol formulations apparently presents advantages over other organisms. Yeasts are easy to cultivate, fast growing and readily found in a variety of substrates and conditions. The use of genetic engineering and other tools of DNA manipulation may, in the near future, increase the use of yeasts to protect fruits and other foods but also to add desirable flavors, higher nutrient content and probiotic properties to foods.

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