Chapter 11
The Use of Psychrophilic Antarctic Yeast in the Biological Control of Post-harvest Diseases of Fruits Stored at Low Temperatures

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11.1 Introduction

The current primary strategy used to control the post-harvest losses of fruits and vegetables is the utilization of massive amounts of chemical fungicides (Pimenta et al. 2009; Zhou et al. 2014; Spadaro and Droby 2016; Usall et al. 2016a; Ferreira et al. 2018). However, there is now a global trend towards a reduction in the use of toxic chemicals, mainly due to an increased concern with their negative effects on public health and the environment. Thus, the need for alternative strategies such as biological control is becoming more evident (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a). Research on the biological control of post-harvest diseases, using antagonistic yeasts, has received considerable attention from researchers in the last decades and represents one of the approaches used to guarantee the quality and safety of vegetables, grains, and mainly fruits (Droby et al. 2016). This chapter discusses how, among the different groups of microorganisms used as biological control agents, yeasts have been the most used mainly for promoting the control of several phytopathologies, both under pre-harvest and post-harvest conditions. The use of these microorganisms is a promising alternative for the total or partial replacement of the use of chemical pesticides (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a).

Yeasts that are naturally found colonizing plant surfaces are termed epiphytic and represent the main group of yeasts used to manage post-harvest diseases.

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However, other sources, such as seawater, soil samples, and plant exudates, are being studied to obtain antagonistic microorganisms. The main intention in exploring new sources of isolation is to identify yeasts that may develop in stressful environments and have different mechanisms of action (Liu et al. 2013). This study aims to better adapt the adverse conditions to which they can be subjected when used as a biocontrol agent (Liu et al. 2013).

The use of microorganisms isolated from cold environments such as those from the Antarctic, Tibetan, polar sea, and other low temperature regions may favour the development of biocontrolled yeasts previously adapted to cold which present a higher tolerance to this stress abiotic. This feature favours their use as biocontrollers in fruits that are stored and/or transported in cold chambers (Wang et al. 2010a, b; Lutz et al. 2012; Vero et al. 2013; Hu et al. 2017).

Several investigations have already been carried out to identify and to evaluate potential biocontrol agents isolated from cold environments. However, the work in this line of research is still limited, especially in the Antarctic region (Wang et al. 2010a; Lutz et al. 2012; Vero et al. 2013; Arrarte et al. 2017; Hu et al. 2017). Therefore, the purpose of this chapter is to discuss the use of psychrophilic yeasts as biological control agents for the management of post-harvest diseases of fruits stored at low temperature.

11.2 Post-harvest Diseases of Fruits Stored at Low Temperature

Post-harvest losses of fruits and vegetables caused by fungi are the main causes of economic losses in food production. It is estimated that about 20–25% of these products are lost during storage in developed and developing countries; these losses may be even higher and exceed 50% (Lima et al. 2015; Spadaro and Droby 2016; Usall et al. 2016a). Strategies to reduce these losses are very important because there is an increase in the global demand for food and areas of agricultural expansion are becoming limited. Fungal degradation of food takes a toll not only on the economy but also on the health of the population, since many pathogens can release toxic substances in food or directly cause infections in consumers (Wilson 2013; Dukare et al. 2018).

Rigorous sanitation, fungicide use, cold storage, modified atmosphere application, and other physical and/or chemical tools are used to reduce or delay the development of pathogens (Sangorrín et al. 2014). Most fruits have a very limited shelf-life after harvest if they are kept at normal storage temperatures. For this reason, refrigeration is one of the most used techniques for the preservation of fresh fruits, since it reduces metabolism, decreases weight loss, slows senescence, and delays development of some deteriorating fungus species (Usall et al. 2016b).

Some species of phytopathogenic fungi can grow rapidly under low temperatures, such as *Alternaria alternata*, which can degrade papaya, apple, and various horticultural crops (Troncoso-Rojas et al. 2014). *Penicillium expansum* is a psychrophilic fungus able to grow at 0 °C, which decays mainly apples, grapes, and pears during cold storage (Morales et al. 2010; Zoffoli and Latorre 2011). *Penicillium digitatum* and *Penicillium italicum* are the main agents of blue rot and green rot, post-harvest diseases affecting citrus, and can grow during refrigerated storage of fruits (Plaza et al. 2003; Pimenta et al. 2008; El-Otmani et al. 2011). *Cladosporium herbarum* and *Cladosporium cladosporioides* cause post-harvest disease in grapes stored at 0 °C (Zoffoli and Latorre 2011). *Botrytis cinerea fungus* is capable of degrading strawberries at low temperatures (0–5 °C) (Feliziani and Romanazzi 2016).

However, other fungi causing post-harvest diseases can grow at temperatures just above 0 °C, such as *Monilinia fructicola* (1 °C), the most economically important pathogen for stone fruit such as plums and peach; *Aspergillus niger* (11 °C); *Colletotrichum gloeosporioides* (9 °C), which causes rot in papaya, mango, and citrus; *Colletotrichum musae* (9 °C), anthracnose agent in bananas; and *Botryodiplodia theobromae* (8 °C) and *Ceratocystis paradoxa* (5 °C), disease agents in bananas and pineapples (Sommer 1985; Usall et al. 2016b). In the case of mesophilic phytopathogenic fungi, refrigeration is able to limit growth and prevent disease development. However, for those who are called psychrophiles, cold storage is not sufficient to ensure satisfactory fruit preservation (Barkai-Golan 2001). In view of this, control alternatives that are complementary to or that increase the efficiency of the technologies already used to reduce or to delay the development of post-harvest pathogens can contribute to a more efficient way to the integrated control of these phytopathologies.

11.3 Biological Control

Chemical control methods using fungicides are conventionally the most widely used treatment for the prevention and control of fungal decomposition in fruits during field and post-harvest handling. They are consistently used with systems management practices and storage at low temperatures. However, the use of many synthetic fungicides has been reduced in the last decade due to several factors such as environmental pollution, selection of resistant pathogens, occurrence of outbreaks of diseases considered as secondary, reduction of populations of beneficial microorganisms, and presence of toxicity to human and other animals (Pimenta et al. 2009; Spadaro and Droby 2016; Usall et al. 2016a; Zhou et al. 2014; Dukare et al. 2018; Ferreira et al. 2018).

As a result, the management of fruits has been changing, due to growing consumer concern with impacts on public health and the environment. Moreover, the legislation has become more restrictive and rigorous regarding the registration and application of chemicals and minimum residues tolerated in food, especially at the post-harvest stage. For this reason, there is an ongoing search for alternative control methods, such as biological approaches that include the use of antagonistic microorganisms (Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a). In contrast to chemical treatments, the use of biological control through the use of microbial antagonists does not leave toxic residues in the fruits, but generally requires integrated action with other control methods (Lima et al. 2015; Droby et al. 2016; Spadaro and Droby 2016; Usall et al. 2016a).

According to Cook and Baker (1983), biological control involves a reduction in the inoculum and disease-causing activities caused by a pathogenic organism, using one or more other organisms. According to Lima et al. (2000), the basic premise of biological control is the maintenance of the population density of pest or pathogen species associated with agriculture at economically and ecologically acceptable levels. During the post-harvest period, biological control can completely replace or act in association with chemical control, making food storage more sustainable and safe and reducing the pathogen inoculum or the intensity of disease symptoms (Mondino and Vero 2006).

There are two basic sources of microorganisms that can be used as microbial antagonists in the control of post-harvest diseases. First is the use of microorganisms that occur naturally in the product (fruit and vegetable surface), and second is the artificial introduction of antagonistic microorganisms to the pathogens obtained from other substrates (Sharma et al. 2009). Numerous microorganisms, such as yeast and bacteria, have been used in laboratory, semi-commercial and commercial studies as antagonists. However, the potential of yeasts as post-harvest biocontrol agents has been widely demonstrated in the scientific literature (Droby et al. 2009, 2016).

Another aspect associated with traditional biological control is integrated biological control, which involves the use of an organism antagonistic to the pathogen to be controlled using Generally Regarded As Safe (GRAS) compounds, which contain micro-biostatic or microbiocidal chemicals. Strategies combining micro-biostatic and microbiocidal approaches can produce a synergistic effect and potentiate the results in comparison to the classical biological control and have, therefore, also been widely explored by researchers in the field (Pimenta et al. 2012).

11.4 Yeasts as Biocontrol Agents

Yeasts are defined as fungi belonging to *Ascomycota* or *Basidiomycota* phyla, whose sexual state has no fruiting bodies and vegetative growth occurs by budding or fission. They are predominantly unicellular, immobile microorganisms. Most of them are saprobic and some are opportunistic parasites (Miller 1979; Boekhout and Kurtzman 1996; Lachance and Starmer 1998; Kurtzman et al. 2011).

The ability of these microorganisms to assimilate a wide variety of organic compounds expands their dispersal and survival capacity in different ecological niches in terrestrial (plants, soil, animals), air, and aquatic environments (lakes, rivers, seas) (Phaff and Starmer 1987). They have several characteristics that make them good candidates as biocontrol agents, such as a high nutrient utilization capacity, which allows them to proliferate rapidly (Lima et al. 1997, 2000; Spadaro et al.

2004); the production of extracellular polysaccharides that increase their ability to survive in several environments, thereby restricting space for the development of phytopathogenic agents (Mendéz an Mondino 1999); and tolerance for the fungicides frequently used during post-harvest (Spadaro et al. 2004). There are two basic sources of microorganisms that can be used as microbial antagonists in the control of post-harvest diseases. First is the use of microorganisms that occur naturally in the product (fruit and vegetable surface), and second is the artificial introduction of antagonistic microorganisms to the pathogens obtained from other substrates yeasts in the control of post-harvest diseases due to the fact that these organisms are the major components of the microbial community on the leaf and fruit surfaces (Wilson et al. 1993). Yeasts may be effective control agents because they are phenotypically more adapted to these niches and are skilled in the colonization and competition for space and nutrients (Filonow 1998). Another advantage of the use of yeasts in the biocontrol of plant diseases is consumer acceptance, which is due to the fact that yeasts are widely used for food and beverage production (Wisniewski et al. 2016).

In the case of epiphytic microorganisms, due to the physical and environmental conditions (humidity, temperature, luminosity) of the phylloplane being quite variable, the colonization process is influenced by a series of specific factors. In the leaves, for example, the important factors are the thin layer of air that covers the surface of the leaves and retains vapours of water emitted from the stomata and the veins (Axtell and Beattie 2002), release of simple sugars 'leached' from the interior of the plant by means of small lesions or glandular trichomes (Mercier and Lindow 2000; Lindow and Brandl 2003), and the deposition of exogenous nutrients such as pollen and insect secretions (Warren and Dias 2001; Fokkema et al. 1983). The production of surfactants by some microorganisms reduces the hydrophobic effects of plant cuticles on the water scattering and diffusion of substrates (Bunster et al. 1989; Hutchison et al. 1995). Other microorganisms are producers of substances that affect the transport of ions in plant cell membranes (Hutchison et al. 1995). For these reasons, epiphytic microorganisms are also plant growth promoters and have been extensively studied for use in the biocontrol of post-harvest diseases in fruits and vegetables (Wilson and Wisniewski 1989) and in the control of foliar diseases (Lee et al. 2017). Yeasts are known to efficiently colonize the epiphytic environment, which could antagonize the introduction and development of plant pathogens (Buck and Burpee 2002; Fonseca and Inácio 2006).

Yeasts are particularly interesting microorganisms for use in a Biological Control program because they are relatively easy to grow and have several characteristics that can be manipulated to improve their use and efficiency (Pimenta et al. 2009; Sharma et al. 2009; Janisiewicz et al. 2011). Yeast species have been used as biological control agents to control *B. cinerea*, causal agent of grey mould in grapes and strawberries; *P. digitatum* in grapes; *P. italicum* and *P. digitatum* in citrus; *Botrytis*, *Rhizopus*, *Penicillium,* and *Alternaria* in tomato; and *B. cinerea* and *Rhizopus* fungi, which cause post-harvest diseases in apples (Mehrotra et al. 1996; Jijakli and Lepoivre 1998; Guetsky et al. 2001; Masih et al. 2001; Jijakli and Kupper et al. 2013) (Table 11.1). Despite the knowledge about some functions of yeasts in the environment, much remains to be discovered, especially on the mechanisms of

Table 11.1 Yeasts used as a biocontrol agent obtained from several sources of insulation

Antagonist yeast	Disease (pathogen)	Fruits	References
Aureobasidium pullulans	Grey mould (*Penicillium expansum*)	Apple	Vero et al. (2009)
Candida sake	Grey mould (*Botrytis cinerea*), Penicillium rot (*Penicillium expansum*)	Apple	Wilson et al. (1993)
	Penicillium rot (*Penicillium expansum*)	Apple	Vinas et al. (1996)
	Rhizopus rot (*Rhizopus nigricans*)	Apple	Vinas et al. (1998)
	Penicillium rot (*Penicillium expansum*)	Apple	Usall et al. (2001)
	Penicillium rot (*Penicillium expansum*)	Apple	Torres et al. (2006)
	Penicillium rot (*Penicillium expansum*)	Apple	Morales et al. (2008)
Candida oleophila	Penicillium rot (*Penicillium expansum*)	Apple	El-Neshawy and Wilson (1997)
	Anthracnose (*Colletotrichum gloeosporioides*)	Papaya	Gamagae et al. (2003)
	Grey mould (*Botrytis cinerea*)	Peach	Karabulut and Baykal (2004)
	Penicillium rots (*Penicillium digitatum*, *Penicillium italicum*)	Citrus	Lahlali et al. (2004, 2005)
Candida guilliermondii	Grey mould (*Botrytis cinerea*)	Nectarine / peach	Tian et al. (2002)
	Grey mould (*Botrytis cinerea*)	Tomato	Saligkarias et al. (2002)
Candida ciferrii	Blue mould (*Penicillium expansum*)	Apple	Vero et al. (2002)
Cystofilobasidium infirmominiatum	Blue mould (*Penicillium italicum*)	Citrus	Vero et a. (2011)
Cryptococcus laurentii	Bitter rot (*Glomerella cingulata*)	Apple	Blum et al. (2004)
	Grey mould (*Botrytis cinerea*)	Peach	Zhang et al. (2007a)
	Rhizopus rot (*Rhizopus stolonifer*)	Strawberry	Zhang et al. (2007b)
Debaryomyces hansenii	Green and blue mould (*Penicillium digitatum*, *Penicillium italicum*)	Citrus	Singh (2002)
	Rhizopus rot (*Rhizopus stolonifer*)	Peach	Mandal et al. (2007)
	Blue rot (*Penicillium italicum*)	Lemon	Hernández-Montiel et al. (2010)
	Anthracnose (*Colletotrichum gloeosporioides*)	Papaya	Hernández-Montiel et al. (2018)

(continued)

Table 11.1 (continued)

Antagonist yeast	Disease (pathogen)	Fruits	References
Metschnikowia fructicola	Blue mould (*Penicillium expansum*) and grey mould (*Botrytis cinerea*)	Apple	Spadaro et al. (2002, 2004)
	Botrytis rot (*Botrytis cinerea*)	Grapes.	Kurtzman e Droby (2001)
Pichia guilliermondii	Blue mould (*Penicillium expansum*)	Apple	McLaughlin et al. (1990)
	Grey mould (*Botrytis cinerea*)	Apple	Janisiewicz et al. (1998)
	Green mould (*Penicillium digitatum*)	Citrus	Chalutz and Wilson (1990)
	Rhizopus rot (*Rhizopus stolonifer*)	Grape	Chalutz et al. (1988)
Pichia guilliermondii	Grey mould (*Botrytis cinérea*)	Tomato	Chalutz et al. (1988)
	Rhizopus rot (*Rhizopus nigricans*)	Tomato	Zhao et al. (2008)
Saccharomyces cerevisiae	Athracnose (*Colletotrichum musae)*	Banana	Zhimo et al. (2016)
Saccharomycopsis schoenii	Blue mould (*P. expansum* and *P. italicum*)	Citrus	Pimenta et al. (2008)
Rhodotorula glutinis	Blue mould (*Penicillium expansum*)	Apple	Calvo et al. (2007)
	Grey mould (*Botrytis cinerea*)	Apple	Zhang et al. (2009)
	Blue rot (*Penicillium expansum*)	Pear	Zhang et al. (2008)
Rhodotorula mucilaginosa	Grey mould (*Botrytis cinerea*)	Strawberries	Zhang et al. (2013)

action of most biocontrol agents (Rosa-Magri et al. 2011). These biological agents can act in complex interactions between the host (fruit), pathogen, antagonist, and environment (Fig. 11.1). The main interaction processes are: mycoparasitism, lytic enzyme production, predation, resistance induction, competition for space and/or nutrients, among others. Often more than one mechanism of antagonism is involved in the process of action of biological control agents (Pimenta et al. 2008; Di Francesco et al. 2015; Lima et al. 2015; Droby et al. 2016; Spadaro and Droby 2016).

Among the mechanisms of action, competition for space and nutrients (e.g. carbohydrates and nitrogen sources) is considered the main way in which yeasts suppress the development of pathogens. For this reason, the biocontrol agent must have the ability to grow rapidly and efficiently and remain viable in the substrate to be protected in order to limit the establishment of the pathogen in the fruits (Nunes et al. 2001). These mechanisms have already been demonstrated in several studies as developed by Zhang et al. (2010) when using *P. guilliermondii* against *B. cinerea* in apples and Lutz et al. (2012) when evaluating the mechanisms of action

Fig. 11.1 Improved strategy for the selection of Antarctic antagonist yeasts to control post-harvest strawberry diseases and the mechanism of their action**.** (**1**) and (**2**) Isolation of antagonist yeasts of angiosperms from Antarctica; (**3**) Biological control test in strawberries containing the antagonistic yeast and the pathogen; (**4**) Pathogen developing the disease without the presence of yeast; (**5**) Yeasts promoting biological control in strawberry. (Source: authors)

associated with the biocontrol capacity of four yeast strains (*Cryptococcus albidus* strains NPCC 1248 and NPCC 1250, *Pichia membranifaciens,* and *Vishniacozyma victoriae*) against *Penicillium expansum* and *Botrytis cinerea* in pears; and the application of *Debaryomyces hansenii* yeast in the control of *Penicillium digitatum* in grapes (Droby et al. 1989) and *Aureobasidium pullulans* (Janisiewicz et al. 2000).

Mycoparasitism was a mechanism of action observed in the studies conducted by Zhang et al. (2010); Arras et al. (1998) found that *P. guilliermondii* caused changes in the hyphae of the fungi *Botrytis cinerea* and *Penicillium digitatum*. Predation was observed by Pimenta et al. (2008) when studying the activity of the yeast *Saccharomycopsis schoenii* against the fungus *P. digitatum*, which is a pathogenic agent in oranges; the authors observed that hyphae and spores of the pathogen were predated by the yeast.

Antibiosis is the production of metabolites that are generated by the yeast antagonists which have inhibitory effects on the growth or germination of the pathogen through the production of antifungal compounds such as peptides and volatile metabolites. Some yeast strains may produce extracellular proteins or toxins called killer toxins, which are lethal to sensitive microbial cells (Spadaro and Droby 2016). Kupper et al. (2013) evaluated the antagonistic potential of *Saccharomyces cerevisiae* against *P. digitatum* and observed inhibition of fungus growth by the production of inhibitory substances. The production of volatile compounds was observed by Arrarte et al. (2017) who demonstrated that the psychrophilic yeast *Candida sake* played a significant role in reducing the growth of *P. expansum* in apples stored at low temperatures. Rosa-Magri et al. (2011) investigated the production of killer toxins as control mechanisms and verified that the yeasts *Torulaspora globosa* and *Candida sublineola* controlled the growth of *Colletotrichum graminicola*.

The production of lytic enzymes may also play an important role in biocontrol activity. The production of chitinase, β-glucanase, and chitosanases, which are the main components of the wall of fungal cells, antagonistic yeasts that have high enzymatic activity related to β-1,3-glucanase and chitinases (endo and exo), could cause lysis of the cell wall of phytopathogenic agents (Di Francesco et al. 2015). However, there is also evidence that biocontrol agents have the ability to induce disease resistance by activation of fruit defence enzymes such as phenylalanine ammonia-lyase (PAL), peroxidase, and polyphenoloxidase (Di Francesco et al. 2015; Liu et al. 2013). In works by Wang et al. (2010a, b), inhibitory actions promoted by enzymes of the β-1,3-glucanase (GLU), phenylalanine ammonia (PAL), peroxidase (POD), and polyphenoloxidase (PPO) were observed to be responsible for the control of pathogens. Hernandez-Montiel et al. (2010) also found that strains of *Debaryomyces hansenii* inhibited the growth of *Penicillium italicum* by detecting β-1,3-glucanase production and protease activity. In a more recent work, Hernandez-Montiel et al. (2018) also observed that the same enzymes were associated with the biocontrol capacity of *D. hansenii* against *Colletotrichum gloeosporioides*.

Biological control using cold-adapted yeasts has been cited as an important alternative to the use of synthetic chemical fungicides for the management of post-harvest fruit rot (Santos et al. 2004; Nally et al. 2012; Sriram and Poornachandra 2013; Sukorini et al. 2013; Lopes et al. 2015; Zhimo et al. 2016).

11.5 Antarctic Psychrophilic Yeasts as Biocontrol Agents in Post-harvest of Fruits

Antarctica is a continent that presents a set of characteristics that make it inhospitable for the survival of many organisms, because it has the coldest and driest climate on the planet. Despite this, Antarctica presents enormous biological diversity (Ruisi et al. 2007; Yergeau and Kowalchuk 2008; Onofri et al. 2008; Rosa et al. 2010; Margesin and Miteva 2011). Among the organisms adapted to these environmental conditions are microorganisms and among them the yeasts which may present great potential for use as biocontrol agents (Rosa et al. 2010; Nascimento et al. 2015). In addition, the microbial diversity in this environment is at an early stage of discovery. (Ruisi et al. 2007).

The yeast communities that inhabit the Polar Regions can be classified as endemic or cosmopolitan. Although some endemic species have psychrophilic behaviour, most of them are psychro-tolerant yeasts that can adapt and grow over a wide temperature range. Connell et al. (2008) studied the diversity of yeasts isolated from the soil in the Antarctic region and found that 89% of the isolates were composed of basidiomycetes. De garcia et al. (2007) observed that the genus *Cryptococcus* was the most frequently isolated, followed by cold-adapted *Leucosporidium and Rhodotorula*. In a study conducted in glacial rivers of melted water in Argentine Patagonia, Buzzini et al. (2017) obtained different genera of yeasts in the Antarctic region, the most prevalent being: *Debaryomyces*, *Metschnikowia*, *Pichia*, *Cystobasidium*, *Dioszegia*, *Filobasidium*, *Glaciozyma*, *Holtermanniella*, *Malassezia*, *Mrakia*, *Naganishia*, *Papiliotrema*, *Phenoliferia*, *Sporidiobolus*, *Tausonia*, and *Vishniacozyma.*

Yeasts that develop in extremely cold environments are interesting as biological control agents for post-harvest diseases because they naturally produce compounds capable of allowing them to grow under extremely low temperature conditions such as those observed in cold storage chambers (Buzzini et al. 2012). Many species of psychrophilic yeasts were considered to be good antagonists against pear post-harvest pathogens (Lutz et al. 2012; Hu et al. 2015). An isolate of soil samples collected in Tibet identified as *Rhodotorula mucilaginosa* have presented biocontrol potential against fungi *Penicillium expansum* in pear (Hu et al. 2015). Using different criteria, *Rhodosporidium paludigenum*, obtained from cold sea water, showed remarkable activity against *Alternaria* rot in cherry tomatoes (Wang et al. 2008).

The knowledge of the diversity of yeasts in the Antarctic environment is recent (Connel et al. 2010; Carrasco et al. 2012; Godinho et al. 2013; Furbino et al. 2014; Zhang et al. 2014). Vero et al. (2013) examined the potential of cold-adapted yeasts isolated from Antarctic soils to manage post-harvest disease of fruits. *Leucosporidium scottii* isolate (At17) was identified as a good biocontrol agent producing volatile antifungal substances that inhibit apple pathogens (Vero et al. 2013). Arrarte et al. (2017) demonstrated that the production of volatile compounds (VOCs) by a psychrophilic *Candida sake* strain isolated from water and soil samples from King George Island in the subantarctic region showed potential use as post-harvest biocontrol agents in apples stored at low temperature. In a previous work, Robiglio et al. (2011) showed that the microbiota associated with pears stored in the cold showed better biocontrol performance against post-harvest deterioration fungi than conventional yeast. Vero et al. (2011) demonstrated that *Cystofilobasidium infirmominiatum* (PL1), selected as cold-adapted yeast, was able to control blue and green mould in oranges during storage at 5 °C. Lutz et al. (2012) successfully used the yeasts *C. albidus*, *P. membranifaciens,* and *V. victoriae* on *P. expansum* and *B. cinerea* in pears when fruits were stored at 0 °C for 100 days.

Thus, several investigations using yeasts isolated from the Antarctic region, as well as in other cold environments, have demonstrated that this approach may present a great potential for prevention against the deterioration of fruits by phytopathogenic fungi during post-harvest and storage (Sangorrín et al. 2014).

11.6 Strategies for Success of Yeasts as Biocontrol Agents

Tests for the use of biocontrol agents still focus mainly on research laboratories. The success of formulations based on these microorganisms depends mainly on the level of control performed for the target disease. Thus, the agents' efficacy needs to be evaluated, first in a pilot, then in a semi-commercial scale, and finally in commercial studies under different conditions of storage and packaging (Nunes 2011; Dukare et al. 2018). Once the previous stages have been successful, the next step involves regulatory licensing and regulatory approval. Regulatory approval of biofungicide formulations is generally based on the effectiveness of disease control and safety assessment of the formulated product and involves numerous research and development stages which should include the following steps (Dukare et al. 2018):

- Proof of post-harvest rot or disease;
- Isolation and characterization of native strains of pathogens involved;
- Isolation and selection of microorganisms/yeasts that are possible biocontrol agents;
- Testing of the ability to control the site of action;
- Identification of the biocontrol agent;
- Determination of the mechanisms of action;
- Toxicity test;
- Large-scale production.

In the last decades, many microorganisms have been identified for use as agents of biological control of post-harvest diseases in fruits. However, only few have been formulated and marketed effectively (Droby et al. 2009; Abano and Sam-Amoah 2012). This scarcity in products has been the focus of much discourse, and among the main reasons identified for this limitation are the small number of companies involved in the development of organic products, the small size of the post-harvest market, and the necessary expenses and time required for the selection and registration of products (Droby et al. 2009; Abano and Sam-Amoah 2012).

Table 11.2 Examples of biocontrol products based on yeast antagonists

Biocontrol products	Yeast base	Fruit	Target pathogens	Country	In use
Shemer	*Metschnikowia fructicola*	Table grape, strawberry, sweet potato	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Netherlands	Yes
Boni protect	*Aureobasidium pullulans*	Pome fruit	*B. cinerea*, *Penicillium* sp., *Monilinia* sp.	United States	Yes
Nexy	*Candida oleophila*	Pome fruit	*B. cinerea*, *Penicillium* sp.	Belgium, USA	Yes
Aspire	*Candida oleophila*	Citrus	*B. cinerea*, *Penicillium* sp.	United States	No
Candifruit	*Candida sake*	Pome fruit	*B. cinerea*, *Penicillium* sp.	Spain	No
Yieldplus	*Cryptococcus albidus*	Apples and pears	*B. cinerea*, *Penicillium* sp., *Mucor* sp.	South Africa	No
Pro Yeast-ST	*Metschnikowia fructicola*	Strawberries, grapes, and citrus	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp.	USA	Yes
Pro Yeast-ORG	*Metschnikowia fructicola*	Strawberries, grapes, and citrus	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp.	USA	Yes
Biocoat	*Candida saitoana* + chitosan	Grapes and sweet cherries	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Israel	Yes
Biocure	*Candida saitoana* + lysozyme	Grapes and sweet cherries	*Botrytis* sp., *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp.	Israel	Yes

Source: Wisniewski et al. (2016), Spadaro and Droby (2016)

However, several yeast-based products have already reached advanced stages of development and are available on the market (Table 11.2). These products were registered for use in post-harvest against various fungal pathogens in fruits and vegetables. For example, the Shemer product was developed based on the yeast *Metschnikowia fructicola* NRRL Y-27328 (Droby et al. 2009; Dukare et al. 2018), initially registered in Israel for both pre-harvest and post-harvest application. It is used to control deterioration caused by fungal genera such as *Aspergillus*, *Botrytis*, *Penicillium,* and *Rhizopus* (Blachinsky et al. 2007). This product was acquired later by Bayer CropScience (Germany) and licensed to Koppert (Holland). BoniProtect, a product developed in Germany and produced by Bio-Ferm (Australia), is based on two antagonistic strains of *Aureobasidium pullulans* and is used for the control of developing pathogens in lesions of apples during storage (Spadaro and Droby 2016).

Nexy, based on a strain of *Candida oleophila* which was developed in Belgium and registered throughout the European Union, is produced by Lesaffre Company (Spadaro and Droby 2016). Aspire is made from another strain of *Candida oleophila*. It is among the first products made using yeast. Aspire was registered as a commercial formulation in 1995, and it is commercially developed by the Ecogen Corporation for use in the United States and Israel (Droby et al. 1998). Candifruit is produced from the *Candida sake* yeast; it was registered in Spain by the company IRTA/Sipcam-Inagra. Another bioproduct, Yieldplus, based on the yeast *Cryptococcus albidus,* was registered in South Africa and is being produced by Lallem Company (Janisiewicz and Korsten 2002; Dukare et al. 2018).

However, Aspire, Candifruit, and Yieldplus have been withdrawn from the market due to a number of reasons, including low and inconsistent trading efficiency, low profitability, and difficulties in market penetration because of the fact that small companies do not have the resources available to sustain product development and marketing (Spadaro and Droby 2016).

Pro Yeast-ST and Pro Yeast-ORG, both developed from *M. fructicola* yeast, are products developed by BASF and licensed by Inova Technology for use in the United States (Sharma et al. 2012). A new generation of biofungicides such as Biocoat and Biocure has also been developed for some years, being produced by AgroGreen. They present *Candida saitoana* yeast in their composition, but also chemical substances of natural origin such as chitosan and lytic enzymes are added in order to potentiate the protective action and to optimize the control effects. The combination of yeasts with other methods of control may be a promising approach to overcome disadvantages in the activity of the biological control agent, increasing its effectiveness. The combination of biocontrol agents with heat treatments, cooling, use of GRAS substances, and other conservation treatments can produce a synergistic effect and increase levels of control of pathogens at levels similar to those achieved with chemical fungicide treatments (Droby et al. 2009; Usall et al. 2009; Sharma et al. 2012).

The commercial use of biofungicides is limited and represents only a very small fraction of the potential market (Usall et al. 2001). To expand the use of biocontrol products, Sangorrín et al. (2014) suggest that research must evolve to integrate the use of agents into a production approach with greater efficiency and to obtain a greater quantity of effective microorganisms. Thus, the development of new commercial products will be more feasible if the microbial antagonists have a wide scope of application, perform reliably under a variety of conditions, and are adaptable to different packaging and processing systems. In addition, evaluation using different post-harvest practices, modes of application, and storage conditions is also required (Janisiewicz and Korsten 2002).

Another important consideration would be the selection of yeasts adapted to cold temperatures for the development of new bioproducts. Because these yeasts are adapted to extreme conditions, they may present greater resistance and adaptation compared to species present in tropical environments. Since the determination of cardinal temperatures (minimum, optimal, and maximum) for the growth of a specific biocontrol agent should be considered, psychrophilic or psychro-tolerant microorganisms present potential for use as biocontrol agents, especially in cold rooms (Sangorrín et al. 2014).

This strategy has still been little explored and no biofungicide has been developed or registered for this purpose so far. Thus, if more species of yeast adapted to cold are identified, their use as biological control agents for the management of post-harvest diseases should be expanded and more successful strategies may be achieved. Refrigeration is still the main method of preserving and prolonging the shelf-life of fresh food. This fact makes evident the potential of using yeasts adapted to cold as possible biological control agents to optimize the production and conservation of fresh fruits (Sangorrín et al. 2014).

11.7 Conclusions and Perspectives

Prolonging the availability and shelf-life of post-harvest of fruits stored under refrigeration often requires application of a chemical fungicide to prevent decompositions caused by fungi growing under this condition. An alternative approach that has not been explored yet is the use of yeasts from extreme environments such as Antarctica or other cold environments. Thus, because they are adapted to such conditions, these yeasts can present a greater resistance and adaptation and facilitate the process of development of biological control agents for use under storage conditions in cold rooms.

According to Sangorrín et al. (2014), not only the control by psychrophiles biocontrolled yeasts of post-harvest diseases of fruits should be investigated but also the potential to prevent diseases during the storage of tubers, and other nonedible species such as flowers and ornamental plants should be explored. It is also important to highlight other possible areas of application such as the use in supermarkets and domestic refrigerators in order to extend the life of a greater diversity of products.

The psychrophiles yeast selection system for application in biocontrol is still a vast field, and it is likely that several new studies will be developed using only strains adapted for cold conditions. However, although there are some products of yeast-based biological control on the market, no product developed with yeasts adapted to cold is available. This methodology should become a practice adopted in the future for the development of new biofungicides suitable for the refrigerated fruit market.

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