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Microbial Biocontrol of Post-harvest Fungal Rot in Apples: Current State of the Science

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ABSTRACT

Our study consists of a careful literature review carried out with the aim of better understanding the models developed in the field of biocontrol of post-harvest fungal rot in apples (PHFRA) over the past two decades. It aims, more specifically, to shed light on the progress made by examining the products developed, their nature, their target pathogens, their effectiveness, their modes of action and the stage of their development. The post-harvest biocontrol of apples has made remarkable progress during the last twenty years of research. Several products (yeasts, bacteria, filamentous fungi and actinomycetes) have been selected. Some are already marketed, others are at different stages of development. However, several points limit the optimal use of microbial antagonists in the bio-management of post-harvest apple rots as an alternative to chemicals. It is, in fact, still necessary to develop appropriate formulations of these microbial biocontrol agents, to better study their mechanisms of action, to test them under commercial conditions and against a broad spectrum of pathogens and hosts. However, although sometimes considered less effective than chemical treatments, biocontrol products based on microorganisms have major advantages for an application in an integrated post-harvest apple protection strategy.

1. Introduction

The apple is among the most cultivated and consumed fruits in the world. Its production has greatly evolved over the past 60 years, from around 17 million tonnes (MTn) in 1961 [1], to more than 86 MTn in 2018 [2]. Apple is marketed in more than 100 countries, both in developed countries and in emerging, transition or developing economies [1]. Apple is characterized by: (1) its ease of cultivation and consumption, (2) the extent of its commercial varieties (6000 varieties worldwide) [3], (3) its long storage times, (4) its lower production and transport costs, (5) its nutritional value (very rich in water (84.3%), in sugars (12.6%), in vitamins C and E and in mineral salts, mainly magnesium) and its intrinsic health capital (Great source of dietary fiber (2 to 3g / 100g) [4] and polyphenols which are the main source of the high antioxidant potential of this fruit [5]. All these factors have made the apple a global fruit [1].

However, like most fresh fruits intended for human consumption, the apple is subject to constraints that may limit its marketing. Among these constraints, those of a
sanitary and phytosanitary nature, observed since the mid-1990s, have gained increasing weight [1].

Post-harvest rots are the major constraint that compromises the profitability of the apple sector worldwide. The losses they cause are considerable and can reach up to 25% of the total harvest in developed countries [6], and 50 to 60% in developing countries [7,8].

Several species of fungi are responsible for these rots. They belong to different genera including, *Penicillium*, *Botrytis*, *Monilinia*, *Rhizopus*, *Botryosphaeria*, *Alternaria*, *Aspergillus*, *Fusarium*, *Gloeosporium* and *Mucor* [9-13]. But the most important losses are, mainly, caused by the species: *Botrytis cinerea*, *Penicillium expansum* [14-16] and species of the *Gloeosporioides* group [6].

Although several approaches have been proposed for the management of these diseases, chemical control, in pre-or post-harvest application, is still the most widely used method [6,19,20]. However, growing concerns about fungicide residues in fruit [21-23], the development of resistant strains among pathogens [9,24-26], as well as the environmental risks linked to their continuous use, have stimulated the search for alternative control strategies, safe and effective, but less harmful to human health and more respectful of the environment. One alternative to control post-harvest fungi has been implemented through the application of medicinal and aromatic plant extract. In recent decades, researchers have evaluated the effects this natural compound against main fungi responsible for rotting apples in storage, interesting results have been obtained [12]. However, the most attractive alternative method for the control of post-harvest apple rots remains the biocontrol using microbial antagonists (yeasts, bacteria, filamentous fungi and actinomycetes) [6,8,17-27].

The use of microbial agents in the control of post-harvest apple rot has been the subject of considerable researches, over the past 20 years, and has experienced remarkable progress. Several antagonists have been isolated and tested against the main post-harvest diseases of apples and their efficacy has been well established [6,28-33]. Some antagonists have been formulated and marketed, others are currently at different stages of development [6,34,35]. Most of these antagonists have been isolated from the surface of apples [14,36-39], but also from other sources, including soil and seawater [11,32,40,41].

The present work aims to give a complete overview of the use of microbial antagonists (fungi, bacteria and Actinomycetes), as biological control agents against various pathogenic fungi causing post-harvest rots in apples and to shed light on the progress made by examining the products developed, their nature, their target pathogens, their effectiveness, their modes of action and the stage of their development. As well as the approaches used to improve their effectiveness in the biocontrol of (PHFRA).

2. The Marketed Microorganisms Used in Biocontrol of Apple Post-Harvest Rots

Many microbial antagonists, mainly yeasts and bacteria, have been identified and selected by researchers, many of which had reached advanced levels of development and commercialization. The first generations of registered and commercialized antagonists are shown in Table 1.

| Antagonists | Trade name | Firm | Reference |
|-------------|------------|------|-----------|
| *Aureobasidium pullulans*, Strains:DSM 14940; DSM 14941 | BoniProtect | BIO-FERM GmbH, Austria | Lima et al. [45] |
| *Cryptococcus albidus* | YieldPlus | Anchor Yest, Cape Town, South Africa | Mari et al. [45] |
| *Candida sake* | Candidfruit | Sipcam-Inagra, Valencia, Spain | Teixidó et al. [46] |
| *Candida oleophila* (Strain O) | Naxy | BioNext sprl, Belgium | Lahlali et al. [47] |
| *Candida oleophila* (Strain 1-182) | Aspire | Ecogen, Inc. Langhorne, PA, United States | Blachinsky et al. [48] |
| *Metschnikowia fricticola* | Shemer | Bayer/Koppert, Germany | Spadaro and Droby [49] |
| *Bacillus subtilis* | Avogreen | University of Pretoria, Pretoria, South Africa | Demoz and Korsten [50] |
| *Pantoea agglomerans strain CPA-2* | Pantovital | Domca, Granada, (Spain) | Cañamás et al. [51]; Plaza et al. [44]; Nunes et al. [52]; Teixidó et al. [53] |
| *Pseudomonas syringae Van Hall* | Bio-Save | JET Harvest, Longwood, FL, United States | Janisiewicz and Korsten [54]; Janisiewicz and Jeffers [55] |

3. Mechanisms of Action Involved in Applicable Post-Harvest Diseases Biocontrol Systems

Understanding the modes of action involved in biocontrol systems is a prerequisite for the development of post-harvest biological control agents and their registration [15]. In fact, it improves the performance and reliability of biocontrol through the development of appropriate for-
mulations and application methods [27]. Most research on fungal and bacterial antagonists has attributed to biological control four main modes of action: (1) Nutrients and Space Competition, (2) antibiotic production, (3) induction of host resistance, and (4) direct parasitism [8]. Competition for space and nutrients is the main mode of action of microbial antagonists against post-harvest apple fungi [53, 54]. Additional mechanisms of action have been explored, most recently, including: (1) biofilm formation, (2) Quorum detection, (3) Production of diffusible and volatile antimicrobial compounds (4) Competition for iron, (5) Induction of tolerance to oxidative stress, (6) The production of reactive oxygen species (ROS) by the host and the antagonist [11,27].

In studies carried out on the biocontrol of fungal rot in apples, the mode of action of antagonists is rarely studied and when it is, it is not well understood. On the other hand, In the vast majority of studies, each mechanism is generally examined separately. However, it is rare that only one mechanism is involved in the suppression of a disease [27,55-59]. Table 2 presents some modes of action reported in the literature on the biological control of (PHFRA).

Table 2. Modes of action of post-harvest apple biocontrol antagonists

| Mode of action | Antagonist | Pathogenic | Reference |
|---------------|------------|------------|-----------|
| Induction of resistance in the host | Debaryomyces hansenii AH4b | Monilia fructicola | Czarnecka et al. [60] |
| | Streptomyces rochei A-1 | Botryosphaeria dothidea | Zhang et al. [61] |
| | Rhodosporidium paludigenum | P. expansum | Lu et al. [62] |
| | Candida oleophila | B. cinerea | Liu et al. [63] |
| | Candida guilliermondii | B. cinerea | Zhang et al. [65] |
| | Candida saitoana | B. cinerea | El-Ghaouth et al. [67] |
| | Candida saitoana (iso-late US-240, NRRL Y-21022) | P. expansum | de Capdeville et al. [68] |
| | Aureobasidium pullulans L47 | B. cinerea | Ippolito et al. [69] |
| | | P. expansum | |
| Induction of tolerance to oxidative stress caused by (ROS) | C. oleophila (I-182) | P. expansum | Liu et al. [70] |
| | M. fructicola | B. cinerea | Liu et al. [71] |
| | Cryptococcus laurentii LS-28 | P. expansum | Macarisin et al. [72] |
| | Rhodotorulla glutinis LS-11 | B. cinerea, P. expansum | Castoria et al. [73] |
| Nutrient competition | Nitrates | C. guilliermondii, Strains: 3C-1b and F1 | P. expansum | Scherm et al. [74] |
| | Sugars and nitrates | Metschnikowia pulcherrima 2.33 and 4.4 | B. cinerea | Piano et al. [75] |
| | Amino acids | Cryptococcus laurentii | B. cinerea | Filonow [76] |
| | | Sporobolomyces roseus | |
| | | M. fructicola | A. alternata | Saravanakumar et al. [77] |
| | | A. pullulans Ach 1-1 | P. expansum | Krimi Bencheqroun et al. [78] |
| | | A. pullulans 1113-5 | P. expansum | El Guili et al. [79] |
| | | Rahnella aquatilis | B. cinerea | Calvo et al. [80] |
| | | A. pullulans LS-30 | P. expansum | Castoria et al. [81] |
| | | B. cinerea | | |

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| Iron competition (production of siderophores) | M. pulcherrima \( M. \) fruticula | B. cinerea, \( P. \) expansum, \( A. \) alternata | Saravanakumar et al. [74] |
| Space competition | \( P. \) guilliermondii | B. cinerea | Zhang et al. [65] |
| | \( R. \) glutinis | \( P. \) expansum | Calvente et al. [80] |
| Nutrients and space competition | \( Pichia \) anomala | B. cinerea | Kwashiborski et al. [81] |
| | C. oleophila, \( O \) | B. cinerea \( P. \) expansum | Massart et al. [82] |
| Biofilm training on injury | \( Pichia \) fermentans 726 | M. fruticola | Giobbe et al. [83] |
| | M. fruticola, \( BHO126, GS88, GA102, \) and \( GS37 \) | \( P. \) expansum \( B. \) cinerea | Spadaro et al. [84] |
| Antibiotic production | \( P. \) fluorescens strains: 1-112 and 4-6 | B. cinerea | Wallace et al. [58] |
| | Bacillus subtilis 9407 | B. dothidea | Fan et al. [85] |
| | Bacillus sp. ( \( \text{UYBC38} \) ) | B. cinerea | Rabosto et al. [86] |
| | Bacillus subtilis | B. cinerea | Ongena et al. [87] |
| | Saccharomyces cerevisiae M25 | \( P. \) expansum | Scermin et al. [71] |
| | Trichoderma harzianum | \( P. \) expansum | Batta [89] |
| | \( B. \) amyloliquifaciens BUZ-14 (Production of Iturin) | \( P. \) expansum | Calvo et al. [90] |
| Production of enzymes | proteases | \( T. \) harzianum | Deng et al. [91] |
| | chitinases, glucanases | \( Amycolatopsis \) sp (isolat 521) | Colletotrichum gloeosporioides | Sadeghian et al. [40] |
| \( \beta-1,3\)-glucanase | \( C. \) oleophila | \( P. \) expansum | Guerrero et al. [92] |
| production of volatile organic compound (VOCs) | \( C. \) sake | B. cinerea \( P. \) expansum \( A. \) alternata | Arrarte et al. [91] |
| | \( A. \) pullulans (strain: \( L1 \) et \( L8 \)) | \( B. \) cinerea Colletotrichum acutatum \( P. \) expansum | Francesco et al. [93] |
| | \( Muscodor \) albic | \( P. \) expansum \( B. \) cinerea \( A. \) teniissima \( A. \) arborescens | Mari et al. [94] |
| Involvement of several mechanisms | Production of (VOCs) and direct contact | Candida pyralidae and \( Pichia \) kluyveri | Mewa-Ngongang et al. [96] |
| Nutrients/space competition, Biofilm and antibiotic production | \( P. \) fluorescens, Strains: 1-112, 2-28 and 4-6 | B. cinerea | Wallace et al. [58] |
| Competition for space ; direct contact ; induction of \( \beta-1,3\)-glucanase in apple ; production of glucanase in apple wounds ; antifungal action of wall chitin | \( Rhodosporidium \) fluviale | B. cinerea | Sansone et al. [57] |
| Competition for space and nutrients ; (VOCs), secretion of extracellular lytic enzymes ; inhibition of spore germination | \( Aureobasidium \) pullulans GE17 \( \) and \( \) Meyerozyma guilliermondii KL3 | Agirman and Erten [95] |
4. Microbial Antagonists Used in the Biocontrol of Apple Fungal Rots in Storage

In our study, we identified and analyzed the results of 125 research studies investigating the potential of microbial antagonists in the biocontrol of apple fungal rot. Among these antagonists, yeasts occupy by far the first place with 86 studies and 28 species, followed by bacteria (27 studies and 9 species) then filamentous fungi (10 studies and 2 species) then actinomycetes with only 2 studies and 2 species (Figure 1). Most of these strains have shown significant potential in the fight against the main fungal agents of post-harvest apple rot.

4.1 Fungal Antagonists in Post-harvest Apple Rot Control

In the field of research, considerable efforts have been devoted to the identification of fungi capable of effectively controlling post-harvest fruit and vegetable diseases \[53,97,98\]. Several antagonistic fungi have shown the ability to protect many fruits against *Botrytis cinerea*, *Penicillium expansum*, *Monilinia fructicola* and *Rhizoctonia* \[99, 100\] and other fungi responsible of apple rots in storage. They are mostly yeasts some are filamentous fungi (Figure 1).

4.1.1 Yeasts

Yeasts have great potential as post-harvest biocontrol agents \[11\]. They have, in fact, a high tolerance to stressful environmental conditions prevailing in pre-and post-harvest (low and high temperatures, desiccation, broad spectrum of relative humidity, low oxygen levels, pH fluctuations, UV radiation). They are also adapted to the micro-environments present in the tissues of injured fruit (high sugar content, high osmotic pressure and low pH). They can also grow quickly on affordable substrates, in fermenters and are therefore easy to produce in large quantities \[101\]. Finally, note that yeasts also have simple nutritional requirements that allow them to colonize dry surfaces for long periods, and unlike filamentous fungi, they do not produce allergenic spores or mycotoxins \[102\]. Isolates of 41 yeast species belonging to 12 different genera: *Candida*, *Pichia*, *Aureobasidium*, *Metschnikowia*, *Rhodotorula*, *Cryptococcus*, *Leucosporidium* and *Saccharomyces*, *Sporobolomyces*, *Rhodosporidium*, *Debaryomyces* and *Wickerhamomyces* have been tested for their biocontrol capacity against the main agents of post-harvest fungal rotting of apples. The most studied genera are in order of importance *Candida*, *Pichia*, and *Aureobasidium*. Come second, *Rhodotorula*, *Cryptococcus* and *Metschnikowia*, then third *Rhodosporidium* and *Saccharomyces*. The other genera are very rarely cited in literature (Figure 2).

Figure 1. Relative importance of different microbial groups reported in the biocontrol of post-harvest fungal rot in apple

Figure 2. Number of yeast species in the genera reported in the literature on bio-control of (PHFRA)

(1) Genus Candida

Seven yeast species of the genus *Candida*, including *C. oleophila* \[45,92,103-105\], *C. sake* \[36,41,106,107\], *C. diversa* \[11,108,109\], *C. guilliermondi* \[110\], *C. membranfaciens* \[39\], *C. saitoana* \[64\] and *C. pyralidace* \[98\], have been reported to be effective biological control agents for post-harvest fungal apple rot. *Candida oleophila* is one of the most studied species of genus *Candida*. \[103\] have shown the efficacy against *P. expansum* and *B. cinerea*, In vitro and in vivo experiments. The researchers have also been able to improve its efficacy against these two pathogens, both in vitro and in vivo on injured fruit, by addition of 0.4% of nisin to the antagonist suspension. Guerrero et al., \[92\] evaluated three strains of *C. oleophila* (L06, L07 smooth and L07 rough) against *P. expansum* the three strains allowed a significant reduction in the incidence of the disease by 72-82% on “Golden Delicious” apple fruits. Another strain, *C. oleophila* (strain O) has been isolated.
from apple (cv. Golden delicious) and selected for its high and reliable antagonistic properties against *P. expansum* and *B. cinerea*, the two of the most devastating pathogens of harvested apples [29]. Strain (O) was used to control blue rot by Lahlali and Jijakli [104], who have demonstrated that the addition of skim milk, sucrose or sorbitol to the O strain of *C. oleophila* can significantly improves its survival on the surface of apples by 80.8%, 42.26% and 37.27%, respectively, and improve the effectiveness of biological control of *P. expansum*.

Another speciess, *Candida sake*, has been approved for the control of *P. expansum*, *B. cinerea* and *Rhizopus nigricans* [39]. The antagonistic potential of several strains belonging to this speciess has been demonstrated. Arrarte et al., [41] tested several psychrotrophic, non-penicilloytic yeasts from Antarctica as potential biocontrol agents against several apple pathogens. 34 of them were able to maintain the incidence of rot caused by *P. expansum* and *B. cinerea* at less than 25% on apples stored at a temperature of 0 to 1°C. These researchers, also demonstrated that one strain, C. sake (strain 41E), isolated from marine water, was also effective, in vivo, in the biocontrol of *P. expansum* on “Red Delicious” apples. The antagonistic potential of strain C. sake 41E was approved in a later study [111], in wich It was also shown that it could also reduce the concentration of patulin in apple juice by almost 80% at room temperature without adsorbing mycotoxin on its cell walls. The biocontrol potential against *P. expansum* was also demonstrated in other strains, C. sake (CPA-1) [106] and C. sake (CPA-2) [107].

Another speciess, *C. diversa*, has also been reported as an effective biological control agent against *Botrytis cinerea* of apples in post-harvest [105, 106, 109].

Strains of *C. guilliermondii* have also been studied for the biological control of grey and blue rot in apples by McLaughlin et al., [110] who demonstrated that the efficacy of biocontrol was directly related to the concentration of the pathogen’s spores and the cellular concentration of antagonistic yeasts. Scherm et al., [71] tested the efficacy of two strains of *C. guilliermondii* (F1 and 3C-1B) in the biocontrol of blue rot in In Vivo trials on apple “Golden delicious” and “Fuju” variety. The isolates resulted in a significant reduction in lesion diameter to (14 -38%) and (17 -27%) on the Golden delicious and Fuji variety respectively.

In the study conducted by Ghomamnejad et al., [39], Three strains, of another speciess, *C. membranfaciens* (A2, A4 and A5) were isolated from the surface of apples and were evaluated, In Vitro, for the control of apple blue mould caused by *P. expansum*. All 3 yeast strains inhibited the growth of *P. expansum*. The inhibition ranged from 20.6 to 78.9%. In In Vivo trials, the three strains caused a significant reduction in the diameter of the lesions on apples stored at room temperature (20 °C) and cold (5 °C).

El-Ghaouth et al., [64], in turn, studied the efficacy of another speciess of genus *Candida*: *C. saitoana* against *B. cinerea* apple rot, and showed that the application of this yeast 48 h and 72 h before inoculation with *B. cinerea*, reduced the diameter of the lesions by 50 and 70% respectively, but, when applied at the same time or 24 hours after inoculation with the pathogen, it had no effect.

Another speciess, *C. pyralidae*, was recently tested by Mewa-Ngongang et al., [96] against several post-harvest fungal apple rot (*B. cinerea*, *C. acutatum* and *R. stolonifer*). Cell suspension has shown growth inhibition activity of up to 100% against all species of pathogens tested. A 100% inhibition against the germination of both three species was observed on the grape pomace extract agar (GPA) plates. The apple bioassay demonstrated the ability of *C. pyralidae* to control spoilage caused by *B. cinerea*, *C. acutatum* and *R. stolonifer*, by 100, 43 and 52% respectively

(2) Genus *Pichia*

*P. anomala* is by far, the most studied speciess of the genus Pichia in the biocontrol of fungal rot of apples (6 research studies among the 14 devoted to the genus). One strain of *Pichia anomala* (strain K) was selected by Jijakli and Lepoivre [112] for its great biocontrol activity against infection by *B. cinerea* and *Penicillium Sp.*, on injured Golden Delicious apples. Jijakli et al., [113] were able to achieve a good level of protection against *P. expansum* in apple, by spraying strain K 12 days before harvest, the level of protection was higher than the post-harvest chemical treatment but remained below the level of protection obtained by standard pre-harvest chemical treatments. Lahlali et al., [114] tested and evaluated the efficacy of this strain against *P. expansum* in the laboratory and in the field. Very high levels of protection and final yeast densities were obtained when the initial concentration applied was $1 \times 10^6$ cfu ml$^{-1}$. The level of protection correlated positively with the density of yeast determined on the wounds and was influenced by the humidity on the surface of the apple. Jijakli [6] tested another strain *P. anomala* (K) and reported that treating injured sites with 50 μl of yeast suspension (strain K) (107 CFU / ml) was sufficient to completely inhibit the development of rot induced by *B. cinerea* and *Penicillium sp.* at 5 and 25 °C. Lahlali et al., [115] have evaluated, in vitro and in vivo, the influence of artificial UV-B radiation on strain K of *P. anomala*, and on its potential for controlling fruit diseases after harvest. The
in vitro 50 and 90% lethal dose values were 0.89 and 1.6 KJ/m², respectively, whereas lethal values in vivo were 3.2 and 5.76 KJ/m², respectively. They also tested the effect of protective substances against UV rays on the efficacy of *P. anomala* strain K in the biocontrol of post-harvest diseases of apples. Several substances (congo red, tryptophan, riboflavin, lignin, casein, gelatin, folic acid, tyrosine) have been tested alone or as a mixture. The results showed that, with the exception of lignin and folic acid, none of the compounds or mixtures significantly increased the capacity of the K strain to control post-harvest *P. expansum* on the injured apples.

Another species of genus *Pichia* which has also been well reported in literature in the biocontrol of fungal rot of apples was *P. caribbica*. The effectiveness of this species, in controlling post-harvest blue mold in apples has been demonstrated by Cao et al., [116]. This species reduced significantly the incidence blue mold rot in apples treated compared to controls, and the higher the concentration of *P. caribbica*, the better the effectiveness of the biocontrol. *P. caribbica* significantly controlled the development of apple rot after storage at 20 °C for 35 days or at 4 °C for 45 days. Likewise, spore germination and growth of *P. expansum* were significantly inhibited by *P. caribbica* in *in vitro* assays. The effect of this yeast on the breakdown of patulin produced by *P. expansum* has also been determined in *in vitro* tests. In another study, Mahunu et al., [117] also showed that *P. caribbica* significantly reduced the incidence of blue mold on apples after 10 days of storage at 20 °C and 95% RH. The efficacy of *P. caribbica* in the biocontrol of blue mold in apples was also evaluated by Zhang et al. [118], who showed that yeast causes a reduction in the severity of the disease by almost 50% compared to the control. In addition to the antagonistic effect of *P. caribbica*, the effect of this yeast on the breakdown of patulin produced by *P. expansum* has also been determined in vivo. After incubation with *P. caribbica* at 20 °C for 15 days, the production of patulin by *P. expansum* inside the apples was significantly reduced compared to the control [116].

One of the species of the genus *Pichia* which is also well reported in the literature in the biocontrol of fungal apple rot in storage is *P. guilliermondii*. Scherm et al., [71] tested the efficacy of a strain *P. guilliermondii* (SA) in the biocontrol of blue rot in *in vivo* trials on Golden delicious apples. The isolate resulted in a significant reduction in lesion diameter of almost 50%.

Zhao and Yin [119] reported the application of *P. guilliermondii* at a dose of 1 × 10⁶ CFU mL⁻¹ can effectively control the rots caused by *B. cinerea*, *P. expansum* and *C. gloeosporioides*, on “Red Fuji” apples while now good physical and chemical quality of the fruit.

Mokhtarnejad et al., [120] have developed two effective formulations of *P. guilliermondii* with inorganic (talk, kaolin) and organic (Rice bran, wheat bran) carriers, and the viability of the yeast cells in formulations stored at 4°C and 24°C. Results showed that yeasts cells could survive at organic and inorganic carriers for more than 6 months. The storage at 4°C gave the highest number of viable cells for all formulations examined. The usefulness of powder formulations of *P. guilliermondii* with attention to biocontrol efficacy has been indicated.

Three other species of the genus *Pichia*, little reported in the literature, have also shown some efficacy in biocontrol of post-harvest fungal diseases of the apple. These are: *P. fermentans*, *P. angusta* and *P. kluyveri*: *P. angusta* was tested, and for the first time, by Fiori et al., [121], and eight isolates showed significant biological control activity against *B. cinerea* and *M. fructicola*, while the efficacy against *P. expansum* was low. Genus *P. kluyveri*, on the other hand, was tested, in a recent study [96] against several Fungi (*B. cinerea*, *C. acutatum* and *R. stolonifer*), and showed, In vitro, a 100% inhibition against the spore germination of all fungal species, In vivo, a 38 and 22% growth inhibition of *B. cinerea* and *R. stolonifer*, when it almost completely inhibited the growth of *C. acutatum* [96].

(3) *Genus Aureobasidium*

*A. pullulans* is the only species of this genus that has been reported in the literature, in the biocontrol of post-harvest apple fungal rots. But several strains of this species have been selected for their antagonistic power against the main agents of apple rot in storage. Leibinger et al., [28] have evaluated, in laboratory tests, on “Golden delicious” apples, the effectiveness of two strains of *A. pullulans* (CF10 and CF40) against *P. expansum*, *B. cinerea* and *Pezicula malicorticis* agents of post-harvest rots apples. Each of the two strains, applied at a dose of 10⁷ CFU ml⁻¹) allowed a significant reduction in the size and number of lesions caused by the three pathogens, The effectiveness of these two strains of *A. pullulans* was more important on *B. cinerea* and *P. expansum*. In experiments conducted by Castoria et al., [79], another strain, *A. pullulans* LS-30 significantly reduced the infection caused by *B. cinerea* and *P. expansum* on apples by 76.5% and 88.5% respectively.

Achbani et al., [14] were able to isolate from the surface of the apples (Golden Delicious variety) two other strains of *A. pullulans*, Ach 1-1 and 1113-5, who showed a high antagonistic power (> 80%) at 25 °C, against *P. expansum* and *B. cinerea*. El Hammouchi et al., [122] have developed molecular markers and a semi-selective
medium (PDA medium supplemented with (0.5 mg L\(^{-1}\) euparen, 1mg L\(^{-1}\) sumico, 2.5 mg L\(^{-1}\) hygromycin B, 30 mg L\(^{-1}\) streptomycin sulfate and 1 mg L\(^{-1}\) cycloheximide), allowing the identification and the quantification of the two strains Ach 1-1 and 1113-5 of \textit{A. pullulans}.

Significant production of strain Ach 1-1 yeast biomass (10\(^6\) g dry wt \(^{l-1}\)) was obtained in 48 h in a fed-batch fermentor, with a glucose solution \cite{123}. The biomass produced was dried in a fluidized bed dryer with a final viability of 62%. After 7 months at 4°C, the viability was 28% of the initial value. The formulated yeast was also evaluated for its antagonistic activity against \textit{P. expansum} at pilot scale. A protection level of 89% was achieved with the biomass preparation at 1\(^{10}\) c.f.u. ml\(^{-1}\) after 28 and 7 days for apples stored respectively at 5 and 25°C \cite{123}.

Another strain \textit{A. pullulans} (GE1 7), has been reported recently for its ability to inhibit the mycelial growth of \textit{P. expansum} by 83.4% \cite{129}.

Still within the genus \textit{Aureobasidium}, Sukmawati et al., \cite{13}, Screened two strains of \textit{Aureobasidium} sp. nov. (T3 and T4) that had the capacity as biological control agents against the growth of \textit{Aspergillus brasiliensis}. Based on in vivo antagonistic activity tests on damaged apples, the T4 isolate (50% rot incidence; 25% disease severity) has shown greater capacity as biological control agents for \textit{A. Brasiliensis} compared to isolate T3 (incidence of decay 100%; severity of the disease 25%), while T3 were able to reduce decay symptoms in apples inoculated with another pathogen species \textit{A. flavus}. The ability of the two isolates to reduce the growth of \textit{A. Brasiliensis} was better than that of synthetic fungicide Dithane M-45 0.3% (incidence of caries 100%; severity of the disease 44%).

(4) Genus \textit{Rhodotorula}

Two species of this genus have shown antimicrobial activity against the fungi responsible for post-harvest rot of the apple: \textit{R. glutinis} and \textit{R. mucilaginosus}. \textit{R. glutinis} was also found to be effective against apple rots caused by \textit{B. cinerea} and \textit{P. expansum} \cite{124}. Both pathogens were completely inhibited by \textit{R. glutinis} applied at doses of 1 \(\times\) \(10^8\) and 5 \(\times\) \(10^8\) CFU ml\(^{-1}\), respectively. The effectiveness of \textit{R. glutinis} (CF35 strain) in the biocontrol of post-harvest rot of apples caused by \textit{P. expansum}, \textit{B. cinerea} and \textit{Pezicula malicorticis}, was evaluated, in laboratory tests, on “Golden delicious” apples beforehand stored for more than 8 weeks at 2°C and 95 % relative humidity \cite{28}. \textit{R. glutinis}, at a dose of \(10^7\) CFU ml\(^{-1}\), significantly reduced the size and number of lesions caused by all three pathogens, after storage for 4 weeks at 4°C. The reduction was more marked when the antagonist was applied. The efficacy of another strain of \textit{R. glutinis} (strain HRB6) as a biological control agent for apple blue rot was also demonstrated under semi-commercial and commercial storage conditions \cite{125}. Another strain of \textit{R. glutinis} (LS-11) has been also reported to be very effective (80 % reduction in blue rot compared to the control) against blue mold in apples \cite{126}.

In addition to its antagonistic effect, \textit{R. glutinis} is also known for its ability to reduce the accumulation of mycotoxins, including patulin, in infected fruit \cite{107,124,127}.

For \textit{R. mucilaginosus}, Li et al., \cite{129} have found that this strain has biological control efficacy against \textit{P. expansum} in apples. Two strains (A1 and A7 strains), inhibited the growth of \textit{P. expansum} in vitro from 31.5 to 89.1 % and caused significant damage reduction in apples stored at 5°C and 20°C \cite{139}.

Yang et al., \cite{129} tested the biocontrol efficacy of a strain of \textit{R. mucilaginosus} isolated from the surface of peach blossoms against \textit{P. expansum} on apples of the “Fuji” and demonstrated that inoculation of apples with a suspension of \textit{R. mucilaginosus} of (1 \(\times\) \(10^4\) CFU ml\(^{-1}\)) caused a 50 % reduction in disease incidence and a 20 % reduction in fruit lesion diameter. Recently Sukmawati et al., \cite{13} screened strain of \textit{R. mucilaginosus} T1, isolated from Cerbera manghas L., who exhibited in In vitro test the potentiel ability to act as biocontrola gents for two destructive molds in apples, \textit{Aspergillus brasiliensis} and \textit{Aspergillys flavus}. In vivo, T1 reduced the growth of pathogens, thereby reducing apple rot (decay incidence 25%; disease severity 6.25%). Compared to Dithane M-45 synthetic fungicide (0.3%) the potentiel ability of T1 was was much better.

(5) Genus \textit{Cryptococcus}

Tree species of the genus \textit{Cryptococcus} were repported in litterature for biocontrol of (PHFRA), they are, in order of importance \textit{C. laurentii}, \textit{C. albidos} and \textit{C. infirmominiatus}. \textit{C. laurentii}, has been shown to be effective in post-harvest biological control of apple rot \cite{130} it allowed a significant decrease in the incidence of the disease to 41.6% compared to 100% in the Control. The diameter of lesions also decreased from 22.8 mm in the control to 12.4 mm. The efficacy of \textit{C. laurentii} (strain LS-28) against several apple rot agents (\textit{Penicillum, Rhizopus, Botrytis and Aspergillus}) was reported \cite{128}. LS-28 reduced rot from 80 to 100 % compared to the control, on apples stored 4-6 days at 20°C, and reduced \textit{Penicillum} rot at 95% compared to the control on apples stored for 60 days at 4°C\cite{126}.

Blum et al., \cite{38} tested \textit{C. laurentii} isolated against \textit{P. expansum}, \textit{Glomerella cingulata} and \textit{P. malicorticis} and found an effectivenss in the biocontrol comparable to that of synthetic fungicides such as thiabendazole and iprodione. The same result was obtained by \cite{31} Blum et al., using isolat \textit{C. laurentii} (36) on “Fuji” and “Gala” apples stored
in the laboratory conditions (15-20 °C et 60-70 % RH). In cold storage (1 °C et 90-95 % RH), isolat (36) was as effective as several systemic fungicides against *P. expansum*.

*C. albidos* has also been shown to be effective against *P. expansum* rot [123]. Fan and Tian [132] proved the capacity of *C. albidos* at a concentration (1 × 10⁸ CFU / ml), to completely inhibit the decay of *B. cinerea* and *P. expansum* on “Fuji” apples stored at 23 and 1 °C, especially when it was applied after or simultaneously with the pathogens.

The efficacy of the species *C. infirmominiatius* (strain YY6) was demonstrated against apple blue rot under both semi-commercial and commercial storage conditions [125].

(6) Genus Metschnikowia

Two species have been reported in the biocontrol of (PHFRA). The first is *M. fructicola*, their efficacy, against apple rot caused by *P. expansum* has been reported [87,133]. Strain *M. fructicola* AL27, was as effective as chemical fungicide in the biocontrol of *P. expansum* on apples of different cultivars (“Golden Delicious”, “Granny Smith”, “Red Chief” and “Royal Gala”) stored at (22 ± 1 °C for 7 days) and at (1 ± 1 °C for 56 days), but its control potential was higher on “Golden Delicious” apples. The second species is *M. pulcherrima*. The efficacy of two strains from this species (2.33 and 4.4), was tested against *B. cinerea* under different storage conditions. Both strains inhibited the growth and germination of *B. cinerea* spores, but the biocontrol effectiveness was strongly dependent on the concentration of the antagonist and the time of its application [72]. The efficacy of other isolates (GS37, GS88, GA 102 and BIO126) of the yeast *M. pulcherrima* against *B. cinerea*, *P. expansum*, *Alternaria sp.*, and *Monilia sp.*, on apple fruits, has been demonstrated [84]. In this study, all four strains were able to completely inhibit *Monilia sp.* after storage at 23 °C, as well as *B. cinerea* and *P. expansum* after cold storage (at 4 °C). In another study, BIO126 strain proved to be very effective in controlling blue and gray molds (Reduction in lesion diameter of 56.6% and 97.2%, for *P. expansum* and *B. cinerea*, respectively) [167]. This strain has the added benefit of not growing at temperatures of 37 °C or higher, which is important from a toxicological point of view [167]. Two other strains of *M. pulcherrima* MACH1 and a GS9 also showed efficacy in the biocontrol of *P. expansum* on apples of different cultivars (“Golden Delicious”, “Granny Smith”, “Red Chief” and “Royal Gala”) stored at (22 ± 1 °C for 7 days) and at (1 ± 1 °C for 56 days) [133].

(7) Genus Saccharomycies

Only one species *S. cerevisiae* was implicated in biocontrol of (PHFRA). Scherm et al., [71] tested In Vivo the efficacy of strain *S. cerevisiae* M25 in the biocontrol of blue rot, on “Golden delicious” and “Fuji” apples and resulted in a significant reduction in lesion diameter by 100 %, but its capability was significantly inhibited with the addition of nitrates or sugars (maltose and raffinose).

Another train, *S. cerevisiae* (YE-7), was tested in the biocontrol of blue mould on “Golden delicious” apples and showed significant reduction of the incidence of disease and patulin accumulation in the tissues of rotten apples by 48% and 42.6%, respectively, compared to control. This, when yeast is applied as pre-treatment or simultaneous treatment. Late treatment of pathogen-infected apples with YE-7, did not reduce patulin accumulation in rotten tissue compared to other treatments [134].

(8) Genus Rhodospiridium

A marine yeast, *R. paludigenum*, previously considered effective in the biocontrol of various post-harvest fruit diseases [135-137]. This species showed ability to control blue mould in apples reducing apple rot by 80% after 5 days of incubation at 25 °C and inhibiting mold infections at high concentrations (1 × 10⁷ and 1 × 10⁶ mL⁻¹ cells) [62].

The effect of a strain *R. paludigenum* as also evaluated on post-harvest blue mold and patulin accumulation in apples stored at 23 °C [138]. *R. paludigenum* was able to control post-harvest decay in apples and to remove patulin in vitro effectively, by both biological degradation and physical adsorption. However, the application of the yeast at a high concentration (10⁷ cells per ml) enhanced patulin accumulation in fruit after 7 days of storage 24.2 times compared to the controls.

(9) Other Genera (Leucosporidium, Debaryomyces, Wickerhamomyces)

A strain of yeast species, *Leucosporidium scottii* At17, isolated from the soil, was found to be effective against blue and grey rot of apple caused by *P. expansum* and *B. cinerea* [52]. This strain was selected for its ability to form biofilms on the surface of apples and for its resistance to fungicides commonly used in post-harvest, which suggests its use in an integrated pest management combined with low doses of fungicides.

Recently, biocontrol potential of different strains of *Debaryomyces hansenii* species against *M. fructicola* was demonstrated both in In vitro and In vivo trials [90]. One strain (K12a) showed a high in vitro biocontrol activity, inhibiting mycelium growth by 69.5%, as compared to control fungal cultures. K12a and another, strain (All4b) reduced significantly brown rot on apple fruits by 85.1% and 70%, respectively, in comparison to infected fruits, which did not receive any pre-treatment [90].

The species *Wickerhamomyces* also showed a high potential for biocontrol of anomalous (BS91) against brown rot, resulting in an inhibition of mycelium growth of
66.08% compared to the control, and a reduction in brown rot of apple by 70.02%, compared to fruit treated only with the pathogen [143].

4.1.2 Filamentous Fungi

In addition to yeasts, antagonistic filamentous fungi are also considered to be very promising agents that can be involved in integrated post-harvest disease management strategies [139]. Their use in biological control against post-harvest diseases of apples however remains very limited, probably due among other things to: (1) Their biocontrol potential is much slower than that of chemical fungicides; (2) their need for high humidity for their spore germination, development and sporulation; (3) their sensitivity to UV radiation. Two genera have been reported for their biological control potential against apple fungal rots, Trichoderma and Muscodor

(1) Genus Trichoderma

Trichoderma harzianum is the only species of genus Trichoderma reported in the literature as biological control agents for (PHFRA). T. harzianum was used to control fungal diseases of apple, caused by Alternaria alternata, P. expansum (blue rot), B. cinerea (grey rot), M. fructigena (brown rot), weak diseases caused by Pythium species and Rhizoctonia sp. [99,140-143].

Batta [143] attempted a formulation of T. harzianum conidia using an inverse emulsion (water-in-oil formulation) based on coconut and soybean. Treatment with conidia of T. harzianum formulated at a concentration of 6.10^7 conidia ml^{-1}, significantly reduced the diameter of lesions on apples, inoculated by P. expansum, to 17.5 mm compared to 34 mm for controls (emulsion products, distilled water) and 26 mm for treatment with unformulated T. harzianum [143]. Dipping trials of injured apples in a suspension of T. harzianum conidia showed a significant preventive effect of T. harzianum conidia formulated against B. cinerea and P. expansum [89]. A biopesticide based on T. harzianum strain “TrichoPAL1” was produced with a suitable formulation of water-in-oil invert emulsions based on soybean oil (28.50%) coconut oil (19.50%) [144]. The efficacy test of this biopesticide against post-harvest mold (B. cinerea and P. expansum) on fresh apple fruit indicated a significant reduction in the diameter of the mold lesion on fruit stored at 20 °C ± 1 °C and, an extended protection of two and a half months for apples stored under controlled and semi-commercial conditions. Cheng et al. [145] identified an amino acid oxidase (Th-L-AAO), produced by T. harzianum ETS 323, and showed that Th-L-AAO effectively inhibits, in vitro, the growth of hyphae of B. cinerea, causes cytosolic vacuolization in hyphae and led to a lysis of hyphae. Th-L-AAO treatment also showed direct disease control against B. cinerea in vivo. Apple fruit inoculated with B. cinerea and treated with Th-L-AAO showed significant inhibition of B. cinerea-induced lesions after 6 days. The untreated apples displayed a 12-fold increase in the lesion radius relative to the treated. More recently, it was demonstrated that the strain (TRIC8) of T. harzianum had potential as a biocontrol agent for the control of post-harvest decay of “Granny Smith” apple caused by P. expansum and M. fructigena by reducing lesion diameter of this fungi by 69.73% and 97.13% respectively [145]. Deng et al., [91] studied biocontrol activity of recombinant aspartic protease P6281 from Trichoderma harzianum (rP6281), expressed in Pichia pastoris, against several pathogenic fungi, including B. cinerea and demonstrated rP6281 ability to control the grey mold rot on apples. Transmission electron microscopy revealed that rP6281 efficiently damages the cell wall of this pathogenic fungi.

(2) Genus Muscodor

The species M. albus is an endophytic fungus who produces a mixture of VOCs that are lethal to a wide variety of plant and human pathogenic fungi and bacteria [146].

It was reported that biofumigation for 24 h with a culture of M. albus grown on autoclaved rye grain completely controls blue (P. expansum) and grey mold (B. cinerea) of apple [147]. It was also reported that M. albus volatiles had a significant effect on the germination, growth and survival of B. cinerea and P. expansum [95]. Their results clearly showed that increasing the weight of M. albus-colonized grain from 0.25 to 1.25 g·L^{-1} had a significant effect on the ability of M. albus volatiles to inhibit spor germination of the two fungi.

4.2 Bacterial antagonists in control of (PHFRA)

In the biological control against post-harvest diseases of apples, bacterial antagonists hold second place in terms of importance after yeasts. Several bacteria have been identified as playing an important role as biological control agents against many phytopathogenic fungi [99,148-150]. Among the 125 researches included in our study 27 (20.8%) was devoted to bacterial antagonists (Figure 1). Nine species belonging to 6 different genera have been studied for their biocontrol power of post-harvest fungal diseases of apples (Figure 3). The species that have aroused the most interest from researchers are Pseudomonas Syringae, P. fluorescense, Pantoea agglomerans, Baccillus amylace-faciens and B. Subtilis (Figure 3).
Figure 3. Biocontrol bacterial species of (PHFRA) and the number of related studies

Among the various bacteria for biocontrol of post-harvest diseases of the apple, *Pseudomonas syringae* seems to be the most used and the most reported in literature. The results obtained with regard to its effectiveness are indeed very promising. The strains of *P. syringae* van Hall have been marketed under the name of BioSave (sold by EcoScience) approved in the United States, provided biological control against blue rot (*P. expansum*), grey rot (*B. cinerea*) and Mucor’s rot of pome fruits, including apples and pears [153,154]. A strain of *P. syringae* designated MA-4 originally isolated from the surface of apple leaves collected from eastern Ontario and which has been shown to be effective against *R. stolonifer* [155], was also effective against blue (*P. expansum*) and gray (*B. cinerea*) rots in post-harvest apples, especially when combined with cyprodinil, a commercial fungicide [155]. Another strain (*P. syringae* CPA-5), also showed great ability in the biological control of *P. expansum* and *B. cinerea* on cold-stored “Golden Delicious” apples, and a great capacity to colonize the surfaces of apples under cold storage conditions [156].

Another *Pseudomonas* species, *P. fluorescens*, has been reported to be effective in the biocontrol of grey rot caused by *Botrytis* sp. [155]. Furdermore, Etebarian et al. [156], evaluated one isolate of *P. fluorescens* (1100-6), as a potential agent for the biocontrol of blue mold in apple caused by *P. expansum* or *P. solitum*. This isolate decreased the growth of *Penicillium* spp., and produced large areas of inhibition in double agar culture tests. The metabolites it produces decreased the colony area of *Penicillium* isolates from 17.3% to 78.5%. Similarly, applied 24 or 48 hours before inoculation with *Penicillium* spp., it significantly reduced the severity and incidence of apple rot, after 11 days at 20°C for *P. expansum*, and after 25 days at 5°C for both species (*P. expansum* and *P. solitum*). these researchers [156] also reported that isolate (A506), a microbial pesticide marketed for the control of fire blight agent *Erwinia amylovora* [157], could potentially be used as an effective biocontrol agent for post-harvest disease control in apples.

*Pantoea agglomerans* is also another well-reported species in the AFRS biocontrol (Figure 3). Several strains of *P. agglomerans* have been reported to be effective against apple rot caused by, *B. cinerea*, *R. stolonifer*, *P. expansum* [151,152,153,154,155].

In laboratory tests, a high level of control of those three fungi was also obtained with *P. agglomerans* strain CPA-2 [83]. In tests under semi-commercial conditions, this strain allowed the reduction of blue mold by 81 and 100% on apples stored at 1 °C in ambient air in an oxygen-poor atmosphere, respectively, and it was as effective as imazalil in controlling gray mold [83]. The efficacy of this same strain against blue mold has also been proven on “Golden Delicious” apples, stored at 1°C [107]. These researchers even showed the capacity of this strain to control the accumulation of patulin in the treated apples. Their work has, however, shown the ineffectiveness of this strain in controlling the disease and the production of patulin on apples previously stored in ambient atmosphere. Still against blue mold, another strain, *P. agglomerans* EPS125, was shown to be effective when tested on apples, “Golden Delicious”, stored under controlled atmosphere (0 °C, 2% O2 and 2% CO2) and apples “Granny Smith” stored at 20°C [148]. This strain has, in fact, reduced rot from (12.5-14.1%) to (2.5-2.6%), on “Golden Delicious” apples and (80-73% ) to (7-8%) on “Granny Smith” apples, with an efficiency of (80-81%) and (91-88%), respectively.

Two other interesting species of the genus *Bacillus* were reported for their biological control potential against post-harvest apple rot fungi, *B. subtilis* and *B. amyloliquefaciens*.

*B. subtilis* applied to injured apples has been reported to reduce fruit rot caused by *Botrytis cinerea*, *Alternaria alternata*, *P. expansum* and *Pezicola malicorticis* [28,159].

Several strains of *B. amyloliquefaciens* were reported for their high potential in AFRS biocontrol. Strain 9001 isolated from healthy apple from an infested orchard was assessed as having biological control activity against ring rot in apples in vitro and in vivo [158], its application both in the field during the growing season and in post-harvest results in a significant reduction of disease incidence of within the storage period of 4 months at room temperature. Another strain of (*B. amyloliquefaciens* BUZ-14) has also been reported for its biological control potential against *P. expansum* in apples [156]. Preventive treatment with this strain reduced the incidence of *P. expansum* on apples from 100% to 20%. Effectiveness of the strain BUZ-14 against *P. expansum* was also demonstrated by Calvo et
and study revealed iturin as a key metabolite in the inhibitions with an In vivo MICs of 33.9 μg mL⁻¹. However, the study showed that this strain had no effect in controlling other rots on apples, including gray and brown rots.

Recently, biocontrol potential of the B amyloliquifaciens SF14 against M. fructicola was demonstrated both In vitro, In vivo and in Semi-commercial large-scale trials[161]. Results obtained in semi-commercial trial, were not significantly different from both commercial bacterial strains B. subtilis Y1336 and P. agglomerans P10c, but significantly lower than that of thiophanate-methyl fungicide.

The only species of the genus Rahnella reported in the literature in the biocontrol of (PHFRA) is R. aquatilis. This epiphytic bacteria was isolated from the surface of fruit and apple leaves, and tested for its antagonistic properties against P. expansum and B. cinerea In vitro and In vivo on “Red Delicious” apple. Bacteria inhibited, by direct contact, spore germination of the two pathogens and reduced incidence of the two diseases by almost 100% on apples stored at 4°C and by only 60% and 0% for the diseases caused by and B. cinerea respectively on apples stored at 15°C [160].

Other bacterial species were also reported in literature for biocontrol of (PHFRA) including Weissella cibaria [162] and Alcaligenes faecalis [161].

Weissella cibaria is a lactic acid bacteria. One isolate of this species, W. cibaria (TM128), was able to to decrease blue rot infection levels, on “Golden Delicious” apples, by 50% [162].

Use of Alcaligenes species for post-harvest management was reported for phytopathogen fungi on some stored fruits [161,163,164]. Strain A. faecalis ACBC1, selected from among the species highly effective for M. fructigena, agent of brown rot of fruit, has shown great potential for preventing brown rot both in vitro, by strongly inhibiting mycelial growth of than in vivo, by significantly reducing the severity of the disease. It has also been confirmed effective in a large-scale semi-commercial trial [161].

4.3 Actinomycetes used in the Biocontrol of Post-harvest Apple Rot

Actinomycetes, represent potential agents for the biocontrol of several plant diseases [165,166,167,168]. However, few studies have investigated their antagonistic effect against phytopathogenic post-harvest apple fungi. We will cite two studies here. In the first, the antagonistic activity of more than 100 Actinomycetes against C. gloeosporioides, the causative agent of apple bitter rot was evaluated [40]. Their In vitro bioassays revealed that six of the isolates had significant inhibitory effects against the mycelial growth of the pathogen. In vivo post-harvest experiments indicated that the six antagonists inhibited significantly the rotting of apples, either by inhibiting the appearance of the disease on healthy fruit, or by preventing the spread of lesions on diseased fruit. Among the tested antagonists, one isolate who was the most effective in in vitro bioassays was identified as Amycolatopsis (Pseudonocardiaceae family). The second study [61] shows that treating apples with strain Streptomyces rochei A-1 induces their resistance to ring rot caused by B. dothidea, and reduces the area of lesion and the incidence of the disease by 65.4% and 27.1%, respectively, compared to the control, after storage at 25 °C for 7 days. Treatment with Streptomyces rochei A-1 also improves significantly the activities of peroxidase, superoxide dismutase, catalase and phenylalanine ammonia-lyase, and strongly inhibits lipid peroxidation [63].

5. Improvement the Effectiveness of Microbial Biological Control of (PHFRA)

Over the past 30 years, new approaches have aimed at developing biocontrol systems of (PHFRA) in order to overcome existing limitations, such as the great variability in the efficacy of antagonists, reduced spectrum of activity of this antagonists and their use for preventive action only. In our study we will develop two main approaches: Combination of several biocontrol agents (Antagonistic mixtures) and Combination of microbial biological control with other control methods.

5.1 Combination of Several Biocontrol Agents

In most studies on biological control of post-harvest spoilage agents, the effect of each control agent is considered individually [169]. To improve the effectiveness of biocontrol of post-harvest diseases to acceptable levels, and to broaden the spectrum of action of antagonists, researchers have studied the combination of several control agents. Most of their works showed that the combined use of at least two antagonists exhibit more effective control of post-harvest rots on many fruits than antagonists applied alone [170,171]. Over the past three decades several combinations of microbial biocontrol agents have been tested for their antagonistic potential against the main fungi responsible for apple rot after harvest. Some researchers have combined strains belonging to the same species [92], others have combined strains belonging to different species but of the same genus [125,172]. Several researchers have tested
combinations between strains belonging to different genera but from the same group \cite{28,29,170,173}. Finally, other researchers tested combinations between strains belonging to different groups such as bacteria combined with fungi \cite{28,174}. Very recently, Zhimo et al., \cite{175} explored a natural probiotic microbial consortium (commercial kefir grains), composed with 7 bacterial ans 4 yeast genera, in biocontrol of apple blue rot.

The results of all this research differ from one study to another and from one combination to another but they were, generally, very encouraging (table 3)

### 5.2 Combination of Microbial Biological Control with Other Control Methods

In the literature, several alternative methods have been combined with biocontrol in order to improve the fight against post-harvest diseases of apples. Some researchers have combined biocontrol with a physical treatment such as heat treatment of apples \cite{119,176}, or their storage under modified or controlled atmosphere \cite{186,178-180}, others have combined biocontrol by antagonists with treatments of a chemical nature, using: (1) GRAS substances including calcium chloride CaCl2 \cite{39,132,178,180-182}, sodium bicarbonate (BCS) \cite{176,180,183} or ethanol \cite{176,184} or (2) Host defense elicitors or substances delaying the senescence of apples, such as: glycine betaine \cite{118,185}, phytic acid \cite{117,129}, ascorbic acid \cite{186,187}, cytokinin N6-benzyladenine \cite{188}, indole acetic acid (IAA) \cite{189,190}, glycolchitosan \cite{191}, salicylic acid \cite{130}, iprodione \cite{132}, L-serine and L-aspartic acid \cite{157}, or fungicides at low rates \cite{192,193}, including cyprodinyl \cite{153} and thiabendazole \cite{125}.

The results of scientific researches cited in our study (Table 4), showed that combined treatments improve the effectiveness of antagonists against the main post-harvest agents of apples, which is often comparable to that of conventional fungicide treatments.

| Pathogen | Combined Biocontrol agents | Results achieved | Reference |
|----------|-----------------------------|------------------|-----------|
| P. expansum | P. syringae + Sporobolomyces roseus | bicontrol by the mixture (M) was more effective than by each antagonist individually | Janisiewicz et Bors \cite{176} |
| P. expansum | C. laurentii + C. infirmo-miniatus | Biocontrol as effective as chemical treatment with thiabendazole at a high dose of 528 μg / ml. | Chand-Goyal and Spotts \cite{125} |
| Pezicula malicorticis | M1: A. pullulans (CF10 and CF40) and one strain of R. glutinis (CF35) 
M2: B. subtilis (AG704 and HG77) and A. pullulans strain CF10 | M1 completely suppressed gray and blue rot to the same extent as Euparen ; M2 completely eliminated brown rot and significantly reduced blue rot ; M1 and M2 improved biocontrol against P. malicorticis | Leibinger et al. \cite{28} |
| P. expansum | M1: R. glutinis SL 1 + C. albidas SL 43 
M2: R. glutinis SL 30 + C. albidas SL 43 
M3: R. glutinis SL 1 + C. laurentii SL 62 | M1 and M2 (synergistic power against P. expansum) ; M3 (Synergistic power B. cinerea) ; none of the (M)s was effective against both molds at the same time. | Calvo et al. \cite{174} |
| P. expansum | M. pulcherrima, + C. laurentii | The two antagonists were more effective when mixed. | Conway et al. \cite{177} ; Janisiewicz et al. \cite{171} |
| P. expansum | M1: R. aquatilis-R. glutinis ; M2: R. aquatilis-C. laurentii (106 cell / ml) | M1 was more effective (inhibited the two mold and reduced diseases incidence to zero) R. glutinis was strongly stimulated by the presence of R. glutinis. | Calvo et al. \cite{172} |
| P. expansum | C. oleophila (L06 + L07 smooth + L07 rough) | (M) was as effective as the chemical treatment with (fludioxonil + ciprodinil) at a dose of 1g L-1. | Guerrero et al. \cite{192} |
| P. expansum | Meyerozyma guilliermondii KL3 and A. pullulans GE17 (108 cells ml-1) | (M) inhibited spore germination of pathogen from 86% versus 82% for GE17 alone ; KL3 was ineffective against blue mold | Agirman and Erten \cite{199} |
| P. expansum | commercial kefir grains (fresh and milk-activated forms) 7 bacterial + 4 yeast genera | Effective inhibition of the P. expansum | Zhimo et al. \cite{175} |

Table 3. Biocontrol of (PHFRA) by microbial antagonist mixtures
### Table 4. Biocontrol of (PHFRA) by combining microbial antagonists with other control methods

| Pathogen | Biocontrol agents and combined treatments | Results achieved | Reference |
|----------|------------------------------------------|------------------|-----------|
| **Biocontrol combined with physical treatment.** | | | |
| B. cinerea, M. piriformis P. Expansum | *P. fluorescens* (Isolats 1-112, 2-28 et 4-6) + AC (1,5% CO2 + 1,2% O2; 1°C) | Under AC the 3 isolates were as effective as BioSave® against gray mold and blue mold on “Ambrosia” apples | Wallace et al. [180] |
| P. expansum B. cinerea et C. gloeosporioides | *P. guillermondii* + hot air treated apple (38°C during 96 h) | Total inhibition of infection of apples by the three pathogens | Zhao et Lin [119] |
| | M. fructicola pretreated by mild heat shock (HS) (30 min at 40°C) | -Greater biocontrol activity -Faster growth rate in apple wounds stored at 25 °C -Better tolerance of to oxidative stress | Liu et al. [177] |
| | *Cryptococcus laurentii* and chitosan | combination of chitosan and *C. laurentii* resulted in a synergistic inhibition of the blue mold rot, being the most effective at 0.1% of chitosan with low viscosity (12 cP). | Yu et al. [185] |
| | *M. pulcherrima* BIO126 + dipping Golden delicious apples in deionised water at 50°C for 3 and 10 minutes | BIO126 combined with hot water provided a good control of the pathogen at 23°C (29.2% of reduction) and 4°C (38.2%). | Spadaro et al. [176] |
| | *M. pulcherrima* ST1-D9 and FMB-24H-2+4 day at 38% (Pommes Golden delicious) | 100 % apple protection. | Conway et al. [177] |
| | *C. saitoana* + Chitosan | Additive effects compared with the two treatments used alone. No evidence of synergistic effect | de Capdeville et al. [65] |
| P. expansum B. cinerea Trichosporon sp. and C. albidos + AC (3% O2 et 3% CO2) or (3% O2 et 8% CO2) at 1°C | Significant improvement in biocontrol against the two pathogens | Tian et al. [179] |
| P. expansum C. sake CPA-1 + storage under AC (3% O2 et 3% CO2) “Golden delicious” apples | Reduction of the incidence of the disease by 97% (storage under AC against 40% (Storage in air). | Usal et al. [106] |
| P. expansum P. syringae + 4 day at 38°C “Gala” apples | Reduction in the incidence of decay by 70% to 25% for biocontrolalone | Conway et al. [178] |
| **biocontrol combined with a chemical treatment (inorganic salts and plant extracts)** | | | |
| P. expansum | *Meyerozyma guilliermondii* YS-1, *Meyerozyma caribbica* YS-3, C. albidos YS-4 or *Cryptococcus sp.* YS-5 + CaCl2 (2%) on “Golden delicious” apple | Decays on yeast + CaCl2-treatment were substantially smaller (74-77%) and (49%-73%) lower than those on apples treated with pathogen alone after 1 and 2 weeks of incubation, respectively. | Tournas and Katsoudas [194] |
| | Pseudomonas fluorescens (Isolat 4-6) + CaCl2 or + BCS or + Salicylic acid (SA), | In vitro, antagonist + chemical additives has not improved its effectiveness against pathogens. On “Ambrosia” apples, isolate 4-6 + BCS had efficacy against the three pathogens comparable to that of the fungicide Scholar®, after 15 weeks in cold storage at 1 °C. | Wallace et al. [180] |
| | *Sporidiobolus pararoseus* Y16 + glycine betaine (GB) | S. pararoseus amended with 1 mMGB reduces the diameter of blue rot lesions on apples, reduces spore germination and germ tube length of *P. expansum*. But affects the flavonoid content and the pH of apples. | Abdelhai et al. [185] |
| | *Candida oleophila* combined with CaCl2 | The combined treatment significantly improves biocrotoland significantly reduces the activities of chitinase and β-1,3-glucanase. | Cai et al. [181] |
| **P. expansum** | **P. caribbica** treated with Glycine Betaine (GB) | GB decreased the incidence of the disease from 48.8% (untreated yeast) to 32.1% and improved growth and stress tolerance of the yeast | Zhang et al. [118] |
| **P. expansum** | **P. caribbica** + phytic acid (0,2% v/v) | Significant improvement in phytic acid control | Mahunu et al. [117] |
| **P. expansum** | **R. mucilaginosa** + phytic acid (4 µmol) | Reduction of the incidence of the disease from 66.1% to 62.5% (apples treated with phytic acid) | Yang et al. [129] |
| **P. expansum** | **P. caribbica** treated with ascorbic acid | Improvement of the yeast biocontrol activity of its growth and its tolerance to oxidative stress | Li et al. [116] |
| **P. expansum** | **Rhodotorula mucilaginosa** (A1) at 20°C + CaCl2 at different concentrations | Reduction of the lesion area to (185.1 -1738.1) mm², depending on the concentration of CaCl2, against 2452.84 mm² (control) | Gholamnejad et al. [39] |
| **P. expansum** | **C. guilliermondii** and **P. membranaefaciens** + calcium solution to 2% (*In vitro* and *In vivo*). | Decreased spore germination rate and cell growth of the pathogen; reduction in the incidence and severity of the disease. | Gholamnejad and Ebabarien [112] |
| **B. cinerea** | **P. expansum** | 100% reduction in the incidence of gray and blue rot (treated yeast) compared to 23% and 25%, respectively (untreated yeast). | Yu et al. [111] |
| **P. expansum** | **M. pulcherrima** or **C. laurentii** + calcium solution to 2% | The SBC improved the effectiveness of *M. pulcherrima* but not *C. laurentii*. | Conway et al. [173] |
| **P. expansum** | **P. syringae**+ Cyproidinil (Fungicide) at different concentrations. | A combined treatment was more effective than either *P. syringae* or cyproidinil alone. | Errampalli and Brubacher [192] |
| **P. expansum** | **M. pulcherrima** (BIO126) + ethanol (20%) or SBC (5%) | Significant improvement in biocontrol against *P. expansum* on Golden delicious apples stored at 23°C. | Spadaro et al. [176] |
| **P. expansum** | **C. guilliermondii** (3C-1b and F1) + CaCl2, (11 g l⁻¹), ammonium molibdate (6.17 g l⁻¹), Na₂CO₃, (20 g l⁻¹) or 2-deoxy-D-glucose | Enhancement of biocontrol of the two strains efficacy by all GRAS substances on “Fujji” ans “Golden delicious” apples | Scherm et al. [71] |
| **P. expansum** | **P. syringae** (MA-4) + Cyproidinil in different doses (from 2,5 to 20 µg/ml) | Increased repression of the disease to 35% to 91% compared to 11% with the MA-4 strain alone. | Zhou et al. [151] |
| **P. expansum** | **C. albicans** + iprodione at 50 ppm a.i. on Fuji apples | Better control with the addition of iprodione | Fan et Tian [132] |
| **P. expansum** | **P. syringae** + CaCl2 (2%). | Reduction in incidence to almost 89% compared to 25% with the antagonist alone. | Conway et al. [178] |

**biocontrol combined with several treatments of different nature.**

| **P. expansum** | **M. pulcherrima** + **C. laurentii** + several combinations of SBC | Improved biocontrol on Golden delicious apples. | Janisiewicz et al. [171] |
| **P. expansum** | **M. pulcherrima** +C. laurentii +BCS + AC (1,4 kPa O2 et 3 kPa CO2 during 2 or 4 month at 1 °C) | The combination of all of these treatments completely eliminated the rot of *P. expansum* on “Golden Delicious” apples. | Conway et al. [173] |
| **P. expansum** | **M. pulcherrima** T5-A2 +1-méthylcyclop propane (1-MCP) | 1-MCP increases bitter and blue rot but the combination (Antagonist + hot air + AC) effectively controls the two rots on apples even on those treated with 1-MCP. | Janisiewicz et al. [199] |
| **M. pulcherrima** | Colletotrichum acutatum | Air heated to 38 °C for 4 days + AC (1,1% O2, 1,8% CO2) “Golden Delicious” apples | Janisiewicz et al. [199] |
| **B. cinerea** | **P. anomala** (K)(105 ufc/ml)+ 1,3-glucane (2g/l)+ CaCl2(20g/l) | Significant improvement in the percentage of protection of Golden delicious apples against *B. cinerea* (up to 100%) | Jijakli et al. [113] |
| **P. expansum** | **P. syringae** +4 days à 38°C +CaCl2 at 2% | 91% reduction in decay compared to 25% with the antagonist alone. | Conway et al. [178] |
6. Discussion and Conclusion

Post-harvest rots are the main constraint that affects the profitability of the apple industry worldwide. The losses they cause are considerable and can reach up to 30% of the harvested fruit. Although several approaches have been proposed for the management of these diseases after harvest, chemical control applied before or post-harvest, remains the most used method. However, growing concerns about fungicide residues in fruits, the development of resistant strains among pathogens, as well as the environmental risks associated with their continued use, have spurred the search for safe and effective alternative strategies. Among these strategies, biological control based on the use of beneficial microorganisms or the metabolites they produce to control the development of diseases has been the most studied.

During the last three decades, a considerable research effort has been devoted to the isolation and identification of bacteria, yeasts and other fungi which effectively control the main post-harvest pathogens fungi of apples. Currently, considerable information is available with respect to their efficacy, their application under storage conditions, their mixture with safe substances, or according to the formulation.

In all of the research studies included in our project that exceed 190 studies, microbial antagonists have often demonstrated their effectiveness against the main post-harvest rot agents in apples by expressing fungicidal or fungistatic activity against them. Under certain conditions, some of these antagonists have even enabled total inhibition of the growth of one or more pathogenic fungi.

The experience accumulated over all these years of research has therefore been crowned with great success. However, there are still some limitations that hinder the development of microbial biological control of postharvest fungal rot in apples.

(1) Most of the research conducted was carried out under laboratory conditions:

In most works, the researchers isolated strains from different sources, then examined their antagonistic power against generally a single pathogen of apple rot in conservation, rarely against several. Note here that the species *P. expansum* and *B. cinerea* were the most targeted by researchers, certainly because of the enormous damage they cause worldwide to apples in conservation.

The study of the antagonistic power of the isolated strains is done first by in vitro tests where the isolates are confronted with the pathogen in Petri dishes containing a culture medium. Following these tests, the most efficient isolates are selected (first screening). In others, these potential antagonists are also tested in vivo on artificially injured apples. It is important to note here that little research has studied the potential effect of the type of injury (size, depth, etc.) on the biological control power of the antagonist agent. In addition, these in vivo tests rarely targeted multiple varieties of apples at the same time.

However, many efforts are made to determine the optimal dose and the appropriate time to apply the biocontrol agent and also to study the action of certain external factors such as temperature and humidity, on the effectiveness of the biocontrol. Most of the published research has not gone beyond this stage. The screening of strains via in vitro and in vivo tests is certainly an essential step in the development of antagonists, but this type of tests is, in fact, only the first link in a very long chain leading to the development of biopesticides in appropriate formulations, safe for human health and the environment, and can be marketed.

(2) The effectiveness of control agents (PHFRA) is not very high:

In all the studies carried out on the biological control of post-harvest diseases of apple, the efficiency rarely reaches 100% and the efficacy of the same biocontrol agent is not stable and often varies from one test to another, certainly due to various biotic (host species, pathogenic species) and abiotic (temperature, humidity, relative humidity) factors.

The discovery and development of these biocontrol agents have been based on the paradigm of isolating a single antagonist that is effective against several different postharvest pathogens and was expected to be effective on different commodities that vary in their genetic background, physiology, pathogen susceptibility, and pre- and postharvest management practices [175]. This paradigm has resulted in several limitations, including inconsistent efficacy and a narrow range of biocontrol activity on specific hosts or pathogens. These shortcomings constitute one of the serious limitations which hinders their commercial success [31,35].

Several approaches have been suggested for improving the biocontrol efficacy of postharvest biocontrol agents. Attempts to enhance efficacy have included the use of mixture of different fungal antagonists in combination. Many researchers have reported that such mixtures has been shown to be more effective in controlling the post-harvest rot of apple and many other fruits, than any antagonist applied alone [176-173]. One approach to enhance the biocontrol efficacy that provides a new outlook to postharvest biocontrol is to broaden the spectrum of action of biological control agents by utilizing compatible microbial consortia instead of single antagonists which...
could comprise natural or synthetic mixtures of interacting microbial populations that thrive in many diverse environmental niches [175].

Attempts to enhance efficacy have included also the combinations of biological control with a variety of other alternative treatments, such as combining a biological control agents treatment with physical means (heat, hot water brushing), natural and food-grade chemicals, and different packaging techniques [196-198]. Those alternative treatments result in an additive or even synergistic effect to improve the control of fruit decomposition [196]. An appropriate combination of alternative control measures can provide long-term commercially acceptable control of post-harvest disease in apples and may help reduce dependence on fungicides [173]. Some of them have direct effects on pathogens, while others can act indirectly by increasing the resistance of fruits to pathogens or by delaying their senescence [196]. The results of scientific researches cited in our study (Table 4), showed that combined treatments improve the effectiveness of antagonists against the main post-harvest agents of apples, which is often comparable to that of conventional fungicide treatments.

Other attempt to enhance efficacy of bio-control agents was to extend their activity under pre-harvest conditions [199]. Researchers propose, to improve the effectiveness of post-harvest biocontrol agents for apples, to start treatment at the pre-harvest stage to guarantee colonization of wounds by the biocontrol agent before infection by the pathogen [104,113]. Such pre-harvest application would have numerous benefits, such as decreasing the level of damages, which can occur during the post-harvest treatment. However, the development of a formulations allowing the use of antagonistic microorganisms, both before and after harvesting, is an area which has been less widely explored.

(3) There is little knowledge about the durability of a control methods for apple protection:

The prolonged and massive use of antagonists in particular, those which have a mode of action by antibiosis, can lead to the development of resistance in phytopathogenic fungi to natural fungitoxic molecules [200]. However, there are few studies on the long-term effect of biological control agents of (PHFRA). The persistence of the efficacy of a control method in space and time is an important factor in the success of biocontrol. It is therefore necessary to develop knowledge concerning the possible erosion of this effectiveness, which will result in identifying types of biological control agents with lower risk of efficacy loss, i.e., modes of action of biological control agents that does not favor the selection of resistant isolates in natural populations of plant pathogens [201].

(4) The modes of action of biological control agents of (PHFRA) are not yet well elucidated:

In a large number of studies carried out in the field of biocontrol of (PHFRA), mechanism involved in the antagonistic action of the selected strains against the pathogens tested is not identified, and when it is, each mechanism is generally examined separately. However, as already mentioned in paragraph 3, it is rare that only one mechanism is involved in the suppression of a disease. Biological control agents have, in fact, a very high specificity vis-à-vis the target disease, which must certainly require the combination of several agents for the biological control of post-harvest diseases of the apple.

In addition, the laboratory tests, which are the only tests used in the majority of studies carried out on the biocontrol of (PHFRA), lead to the selection of certain modes of action only, such as antibiotics or direct parasitism. Strains with other modes of action may not be selected. Laboratory tests, in fact, do not allow the expression of all the mechanisms that an agent can involve in its pathogenic action.

Understanding the mode of action of microbial control agents is essential to achieve optimum disease control. Also understanding the mode of action is important to be able to characterize possible risks for humans or the environment and risks for resistance development against the antagoniste [202].

Although many studies have been conducted on the mode of action of post-harvest microbial antagonists, our understanding is still very incomplete, and further investigations are still required.

Advanced microbiological, microscopic, biochemical and molecular techniques are currently available and can be used effectively to improve our knowledge of the mechanisms of action of microbial antagonists [131].

(5) Biocontrol research of (PHFRA) does not take into account all the interactions that the biological control agent can have:

Most of the research that has been done in bio-control of (PHFRA) does not support all of the interactions that the biocontrol agent can have, namely the interaction with host tissue, with the pathogen, the Microbial communities on plant surfaces and the environment, which are essential for developing the biological control system. Until now, scientific approaches have focused on the different components of these interactions but separately.

Special attention has been paid in recent years to the importance of the microbial communities (microbiome) present in and on plant tissues and which plays an essential role in the health and physiology of fruit after it is harvested [203].
Spadaro and Droby, mentioned in their literature review [27], the importance of epiphytic microflora in impacting disease control through their interactions with host plants, pathogens, and biological control agent, in a quadritrophic interaction system, and recommended to take into consideration all the components of this quadrithrophic relationship when studying mechanisms of action in postharvest biocontrol. Droby and Wisniewski, [20] proposed using plant improvement or genetic modification of plants to intentionally modulate the composition of the microbiome and its function, by recruiting disease antagonists and plant growth promoters which improve the plant health and the quality of harvested products, and thus try to develop natural or synthetic consortia that can be used to prevent post-harvest diseases and reduce physiological disorders in harvested products.

To conclude, the biological control against post-harvest fungal diseases of apples is very promising, the results obtained so far are very encouraging. Compared to the use of fungicides causing ecological and health hazards in food chain, use of bio-control agents will be useful not only in minimizing the loss to farmers but also in reducing the fungicidal residue in apple fruits used for human consumption. The application of new technologies such as meta-omic technologies will open up new research opportunities which will certainly improve the understanding of post-harvest biological control and will make it possible to overcome the weaknesses which still hamper the development of biological control of (PHFRA).

But whatever progress may be made, biological control of (PHFRA) can only be important in the context of a modern integrated pest management strategy where it is essential to reconcile and coordinate the use of biological control agents with other means of control, including chemical control.

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