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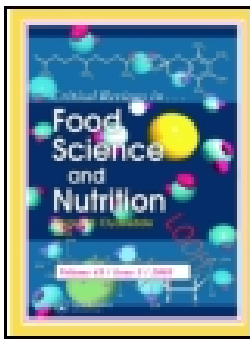
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Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review

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ABSTRACT

Fungal diseases result in significant losses of fruits and vegetables during handling, transportation and storage. At present, post-production fungal spoilage is predominantly controlled by using synthetic fungicides. Under the global climate change scenario and with the need for sustainable agriculture, biological control methods of fungal diseases, using antagonistic microorganisms, are emerging as ecofriendly alternatives to the use of fungicides. The potential of microbial antagonists, isolated from a diversity of natural habitats, for postharvest disease suppression has been investigated. Postharvest biocontrol systems involve tripartite interaction between microbial antagonists, the pathogen and the host, affected by environmental conditions. Several modes for fungistatic activities of microbial antagonists have been suggested, including competition for nutrients and space, mycoparasitism, secretion of antifungal antibiotics and volatile metabolites and induction of host resistance. Postharvest application of microbial antagonists is more successful for efficient disease control in comparison to pre-harvest application. Attempts have also been made to improve the overall efficacy of antagonists by combining them with different physical and chemical substances and methods. Globally, many microbe-based biocontrol products have been developed and registered for commercial use. The present review provides a brief overview on the use of microbial antagonists as postharvest biocontrol agents and summarises information on their isolation, mechanisms of action, application methods, efficacy enhancement, product formulation and commercialisation.

1. Introduction

Pathogen infections result in considerable spoilage of fruits and vegetables during their postharvest handling, distribution and storage and reduce shelf-life. Recent studies by various international organisations have shown that globally, about 33% of the total fruit and vegetable production are wasted (FAO, 2011; OECD, 2014). In India, postharvest losses of fresh fruits and vegetables account for 4.6–15.9% (Jha et al., 2015). Fungal spoilage is primarily responsible for significant losses during storage. Infections of fruits by fungal pathogens, in the field as well as after harvest, result in postharvest spoilage/decay. In the developing world, these losses are often more severe due to inadequate cold storage and transportation amenities. Further, even in developed countries, the pathogenic decay of fruits and vegetables during handling and storage may account for up to 20–25% (Sharma et al., 2009). The high levels of losses due to fungal pathogens are related to high moisture levels, increased nutrients, low pH values, and intrinsic decay resistance after harvest (Droby et al., 1992).

In addition to quality deterioration and economic losses, fruits infected with fungal pathogens pose an impending health risk since several fungal genera, such as *Aspergillus,*

Alternaria, Fusarium, and *Penicillium* produce mycotoxins. For example, *Penicillium expansum*, an etiological agent of blue mold in a variety of harvested fruits, produces numerous potential carcinogenic metabolites including citrinin, patulin and chaetoglobosins (Andersen et al., 2004). Other mycotoxins such as aflatoxins, ochratoxins, alternaria and fumonisin are also produced in fruits and vegetables contaminated with fungal genera such as *Aspergillus, Alternaria* and *Fusarium* (Sanzani et al., 2016).

Traditionally, fungus-induced postharvest spoilage is mainly controlled through the use of chemical fungicides, which are applied either in the field or after harvesting (Vitoratos et al., 2013). Frequently, chemical control is coupled with efficient postharvest and cold chain system management practices. However, the use of many synthetic fungicides in postharvest disease control has been curtailed in the last decade due to the following reasons: (i) emergence of pathogen resistance to many key fungicides; (ii) development of new pathogen biotypes; (iii) lack of effective alternative fungicides; (iv) increasing levels of fungicide residues in agricultural produce; (v) toxicological problems related to human health and, vi) negative environmental impacts (Droby, 2006). Therefore, the global trend is

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shifting towards the search for safer and ecofriendly alternative approaches to control postharvest diseases and decay.

Among the different approaches to control postharvest disease and decay caused by pathogens, biological control via antagonistic microbes is an emerging and attractive option (Dukare et al., 2011; Liu et al, 2013). The application of antagonist microbes in postharvest disease control offers certain advantages in comparison to synthetic fungicides such as; (a) no toxic residues; (b) environmental friendliness; (c) safer application method (d) easy to deliver; and (e) economically to produce (Bonaterra et al., 2012). During the last few decades, a number of bacterial and fungal antagonists for the control of postharvest pathogens of tropical, subtropical and temperate fruit crops were identified as biocontrol agents for commercial use (Droby et al., 2009). A substantial amount of information is also available with respect to the commercial production of biocontrol antagonists, including formulation, fermentation, handling and storage (Wisniewski et al., 2007).The primary step for the application of biocontrol agents on a commercial scale is the adequate formulation followed by extensive field trials. Pilot studies using liquids as well as dry formulations are also reported (Melin et al., 2007; Long et al., 2007; Mokhtarnejad et al., 2011).

This review presents a comprehensive understanding of microbial antagonist-mediated postharvest biocontrol systems, including mechanisms of their biocontrol actions, efficacy improvement, effective formulation development and application of the commercial products. The scope of DNA-based studies and technologies in postharvest biocontrol systems is also discussed.

2. Key postharvest pathogens and postharvest disease development

Postharvest disease development and decay of fruits and vegetables takes place due to several fungal infections. Fungal genera of *Alternaria, Aspergillus, Botrytis, Fusarium, Geotrichum, Gloeosporium, Monilinia, Penicillium, Mucor* and *Rhizopus* are the major postharvest pathogens responsible for fruits and vegetables diseases (Barkai-Golan, 2001). The disease symptoms build up in fungal infected horticultural commodities during the transportation and storage.

Several biotic/abiotic stresses, including ripening, harvesting, and mechanical injuries often activate the postharvest disease development. The process initiates when fungal pathogens germinate and penetrate the host tissue cuticle through wounds and injuries (Alkan and Fortes, 2015). Pathogens also enter through the lenticels, pedicel-fruit interphase and sometimes reside endophytically in the stem ends. The pathogens penetrate directly also in the host cuticle throughout the fruit growth period. Several fungal genera (*Alternaria, Botrytis, Botryosphaeria Colletotrichum, Lasiodiplodia, Monilinia* and *Phomopsis*) reside quiescently at the initial introduction site of unripe fruits (Prusky et al., 2013). These remain inactive and unidentified by visual examination during the storage until the fruits ripen. When the fruit begin to ripe, fungal pathogens grow aggressively. During the growth process the pathogenic fungus kill the host tissues necrotrophically and take nutrients from the host, leading to decomposition of the tissues and decay initiates. The pathogenic fungi may live dormant either endophytically (e.g., *Alternaria, Lasiodiplodia, Phomopsis* and other) or hemibiotrophically

(e.g., *Colletotrichum*) on fruit tissue till ripening. The intrinsic disease resistance mechanism protecting the fruits from fungal attack becomes weak or inefficient during the ripening and then fruits become vulnerable to fungal attacks (Prusky et al., 2013). Therefore, postharvest disease control becomes vital to prevent quantitative and overall quality losses of the harvested crop.

3. Fundamental of microbial antagonists use

Antagonism is a phenomenon whereby action of the antagonistic organisms suppresses or interfere the normal growth, development and activity of phytopathogens occurring in its vicinity. Such organisms can control the insect-pests and pathogens of the horticultural crops and referred to as "Biological Control Agents" (Heydari and Pessarakli, 2010). Large number of microbial antagonists possessing antagonistic activity against pre-harvest and postharvest pathogens have been reported. These microorganisms produce pathogen-specific antifungal compounds/ metabolites, which inhibit the growth and metabolism of pathogens. These organisms prevent, inhibit or kill the propagules of pathogen growing on fruit and thereby control the further possible spoilage of fruits during storage. Antifungal microbial agents employ both direct and indirect inhibitory mechanisms in biological suppression of fungal pathogen growth. The antagonistic microbial agents used for control of fungal pathogens belong to several taxonomic groups including bacteria, yeast and filamentous fungi.

In the last decades, diverse microbial species have been isolated, identified and artificially deployed as biocontrol agents on several horticultural commodities (Wisniewski et al., 2016). Microbial mediated postharvest disease suppression is achieved by employing naturally occurring epiphytic antagonistic microflora already exist on surfaces of fruits (Sobiczewski et al., 1996) and exogenous application of the selective microbes possessing antagonistic activity. The role of naturally occurring microbial antagonists in suppressing disease growth revealed in a study when occurrence of decay incidences were more in the washed fruits in comparison to the unwashed fruits (Chalutz and Wilson, 1990). Extraneous application of the antagonistic microbes is effective method of postharvest disease control in comparison to the use of naturally occurring epiphytic microflora.

3.1 Sources of microbial antagonists

Most of the microbial antagonists are naturally present on apparently endemic to fruit and vegetable surfaces. Among them, many antagonists have been isolated and identified as suitable biocontrol agents for the control of postharvest pathogens (Vero et al., 2011; Janisiewicz et al., 2013). Apart from the fruit surface, microbes can be obtained from other closely related or unrelated sources, such as the phyllosphere (Kalogiannis et al., 2006), roots (Long et al., 2005) and soil (Zhao et al., 2012). The yeast *Rhodotorula glutinis* (strain Y-44), obtained from the tomato phyllosphere impedes the growth of *Botrytis cinerea*, an etiological agent of grey mold on tomato leaves and fruits (Kalogiannis et al., 2006). The yeast *Kloeckera apiculate* isolated from citrus roots, effectively control the postharvest pathogens *Penicillium italicum* and *B. cinerea* on citrus and grapes, respectively (Long et al., 2005). As mentioned previously, the natural soil

habitat is a good and diverse source of microbial antagonists (Janisiewicz and Korsten, 2002). For example, the *Bacillus subtilis* strain B3, isolated from agricultural soil, was the first microbial antagonist strain studied extensively for the successful biocontrol of *Monilia fructicola*, an etiological agent of peach brown rot (Janisiewicz and Korsten, 2002). The success of this strain resulted in a strong interest in the control of postharvest diseases, based on natural antagonist.

Microbial antagonists have also been found in unique natural habitats. For example, the cold-tolerant yeast *Leucosporidium scottii* (strain At17), an isolate from the Antarctic soil, was identified as an efficient microbial antagonist to *P. expansum* and *B. cinerea* responsible for blue and grey mold of apples, respectively (Vero et al., 2013). Similarly, the marine yeast *Rhodosporidium paludigenum*, an osmotolerant yeast isolate from the East China Sea, inhibits *P. expansum* growth on pear fruits, while *Alternaria alternata* inhibits *P. expansum* growth on Chinese winter jujube (Wang et al., 2010). Compared to yeasts isolated from the fruit surface, marine yeasts typically have a greater osmotolerance ability and therefore may potentially be more suitable candidates for use under conditions with high abiotic stress (Hernández-Montiel *et al.*, 2010). A list of microbial antagonists isolated from different sources and used as

Table 1. List of microbial antagonists isolated from different sources and used for management of postharvest diseases.

Sources of isolation	Antagonistic microbes	References
Fruit surface		
Apple	*Candida sake*	Viñas et al. (1998)
Orange	*Candida saitoana*	El-Ghaouth et al. (1998)
Peach	*Pichia membranaefaciens*	Fan and Tian, (2000)
Grape	*Metschnikowia fructicola*	Kurtzman and Droby, (2001)
Apple	*Candida ciferrii*(283)	Vero et al. (2002)
Pome	*Pantoea agglomerans*	Nunes et al. (2002)
Apple	*Rhodotorula glutinis*	Qin et al. (2004)
Apple	*Cryptococcus laurentii*	Qin et al. (2004)
Fresh fruits and vegetables	Lactic acid bacteria	Trias et al. (2008)
Lemon	*Cystofilobasidium infirmominiatum*	Vero et al. (2011)
Plum	*Pantoea agglomerans*	Janisiewicz et al. (2013)
Plum	*Citrobacter freundii*	Janisiewicz et al. (2013)
Kumquat	*Paenibacillus brasilensis*	Tu et al. (2013)
Plum	*Aureobasidium pullulans Rhodotorula phylloplana*	Janisiewicz et al. (2014)
Banana	*Pantoea agglomerans Enterobacter* spp.	Khleekorn and Ongrueng (2014)
Grape	*Starmerella bacillaris*	Lemos et al. (2016)
Phyllosphere		
Tomato	*Rhodotorula glutinis*Y-44	Kalogiannis et al. (2006)
Citrus	*Pichia Spp Wickerhamomyces Spp*	Perez et al. (2016)
Mango	*Bacillus* spp.	Rungjindamai, (2016)
Citrus leaves/ flower	*Rhodotorula minuta, Candida azyma, Aureobasidium pullulans*	Ferraz et al. (2016)
Root		
Citrus root	*Kloeckera apiculata 34–9*	Long et al. (2005)
Soil		
Orchard soil	*Pichia caribbica*	Zhao et al. (2012)
Antarctic soil	*Leucosporidium scottii*	Vero et al. (2013)
Apple orchard soil	*Paenibacillus polymyxa* APEC128	Kim et al. (2016)

biocontrol agents for the management of diseases of harvested fruits are reported in Table 1.

3.2 Criteria for the selection of ideal microbial antagonist

An efficient and potential antagonistic microorganism should possess desirable attributes for use as postharvest biocontrol agent. The microbial antagonists for postharvest disease control should be genetically stable, control disease at low concentrations and compatible with other physical and chemical treatments (Sharma et al., 2009). The antagonist need inexpensive nutrition for growth, longer shelf-life, easy to distribute, resistant to general fungicides and non-virulent towards human health and host fruits (Nunes, 2012). Demand of an antagonist increases when it is effective against many fungal pathogens for variety of fruits, survives for longer time under adverse environmental conditions, unable to grow at 37°C and does not cause any infections in humans (Barkai-Golan, 2001; Liu et al., 2013). Similarity in the growth conditions for the antagonists and fungal pathogens results in better disease suppression (Janisiewicz and Korsten, 2002). The microbial antagonist must perform better in the conditions favorable for pathogen growth (Janisiewicz and Korsten, 2002). Further, the antagonists should survive, grow and multiply also in the environment favorable for the pathogen. Therefore, the microbial antagonists isolated from the same locale are appropriate for disease management (Manso and Nunes, 2011). The antagonists having better adaptability than the pathogens under given environmental conditions offer better pathogenic control.

High viable cell count of a microbial antagonist is another criterion for selection of antagonists as biocontrol agents for commercial uses (Janisiewicz, 1997). The effective concentration of antagonists to control postharvest fungal diseases vary from10^7 cfu ml^{-1} for *Pantoea agglomerans* (Nuneset al., 2012) to 1.9×10^9 cfu ml^{-1} for *Pseudomonas glathei* (Huang et al., 1995), whereas for the yeasts, it varies from 2×10^7 cfu ml^{-1} to 2×10^9 cfu ml^{-1} for *C. sake* (Viñas et al., 1998). Further, the cost-economic play important role in commercial production even when the identified antagonist fulfills all other desirable characteristics.

3.3 Mechanisms of actions of microbial antagonists

Several studies have demonstrated the antifungal potential of many microbial antagonists against postharvest fungal pathogens (Nunes, 2012; Gbadeyan et al., 2016; Wisniewski et al., 2016). Applications of the more efficient genomic-based technologies have further provided a deeper understandings of the microbial antagonist-host and pathogen interactions and their mechanisms of biocontrol actions. There are numerous possible mechanisms, operating in a tritrophic interaction system, to suppress pathogen infection, as shown in Figure 1. However, competition for nutrients and space, antibiosis through antibiotic production, mycoparasitism, production of cell wall lytic enzymes, and induction of host resistance are major biocontrol mechanisms displayed by antagonists (El-Ghaouth et al., 2004; Sharma et al., 2009; Di Francesco et al., 2016). Recent studies have elucidated the roles of biofilm formation, quorum sensing, alleviation of host oxidative damage and antifungal volatile compound production in suppressing the activity of postharvest fungal pathogens on fruits (Liu et al., 2013). Often, there is
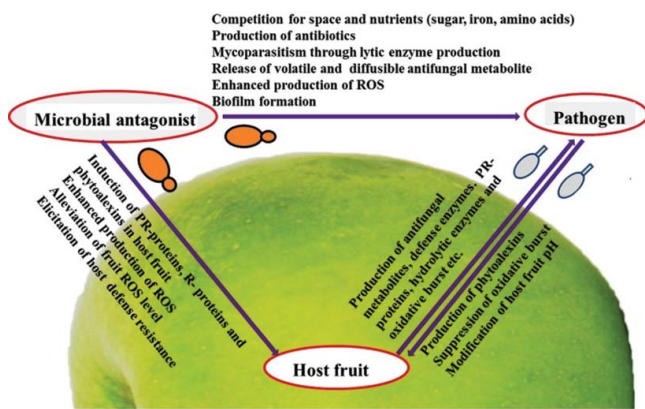
Figure 1. Schematic diagram representing the possible mechanisms of biocontrol actions implicated in tritrophic system, involving interaction between microbial antagonists, pathogen and host fruit.

more than one mechanism in successful postharvest biological control. The mechanisms of action of microbial antagonists are discussed below.

3.3.1 Competition for nutrients and space

An effective competition for nutrients such as carbohydrates, amino acids, vitamins and minerals as well as for oxygen and/ or space is vital to suppress postharvest pathogens of fruits (Spadaro et al, 2016). This mode of action is described in several biocontrol studies for antagonists such as *P. agglomerans* (Poppe et al., 2003), *Serratia plymuthica* (Meziane et al., 2006), *Aureobasidium pullulans* (Bencheqroun et al., 2007), *Metschnikowia pulcherrima* (Saravanakumar et al., 2008), *Debaryomyces hansenii* (Taqarort et al., 2008) and *Cryptococcus laurentii* (Liu et al., 2010).

As the major postharvest diseases are caused by fungi, the majority of antagonists are highly efficient biocontrol agents by successfully competing with fungi for nutritional resources (Janisiewicz et al., 2001; Zhang et al., 2010). Under nutrient starvation, the antagonists diminish the available nutrients in the wound site and make nutrients inaccessible for the pathogens to germinate, grow and infect. Poppe et al. (2003) observed that antagonist (*P. agglomerans* CPA-2) is capable of preventing conidia germination at low nutrient concentrations, whereas at higher concentrations, the prevention of germination was non-significant. The capability of antagonistic yeasts to attach with their pathogen hyphae also increases nutrient competition and thus obstructs the initiation process of the pathogenic infection (Talibi et al., 2014). In the case of competition for space, the growth rate of yeast antagonists is generally high, and the organisms form an extracellular polysaccharide matrix at the wound site (Andrews et al., 1994). Further, yeasts can use most of the carbohydrate and nitrogen sources for cell growth (Spadaro and Gullino, 2004; Spadaro et al., 2010). The analysis of the radio-labelled glucose distribution pattern among the antagonistic yeast *Sporobolomyces roseus* and an etiological agent of grey mold disease (*B. cinerea*) revealed strong sugar use by the antagonistic yeast, which ultimately blocked the conidial germination of the pathogen due to sugar deficiency (Spadaro and Gullino, 2004). Similarly, the important role of the competition for

sugars and nitrates was observed in the interactions of *Pichia guilliermondii* with *B. cinerea* on apples (Zhang et al., 2011; Spadaro and Droby, 2016) and *Colletotrichum* spp. on peppers (Chanchaichaovivat et al., 2008).

In fruit wounds, competition is extended to other essential nutrients such as oxygen, amino acids or vitamins when present at low concentrations. A significant decrease in the efficacy of a yeast strain of *A. pullulans*, an antagonistic against *P. expansum* was observed when high concentrations of amino acids were applied exogenously to apple wounds (Bencheqroun et al., 2007). This shows the important role of competition for nutrients, which represents major mechanism in biocontrol activity.

The colonisation by antagonists and the effective competition with pathogens in fruits wounds can also be influenced by other factors. Surface residing non-pathogenic natural microbiota of fruits can also intervene in nutrient and space competition by effective colonisation and toxic metabolite production (Galvez et al., 2010; Di Francesco et al., 2016). Further, the rapid colonisation of wound site also depends on the antagonist concentration and the host fruit species, as certain antagonists prefer certain nutrient types.

The spatial distribution of nutrients and their availability in fruit wounds can be assessed using a biosensor based on nutrient-responsive reporter genes and by encoding the Green Fluorescent Protein (GFP) at decisive times for the infection and colonisation by the pathogens. These fused reporter genes are particularly suitable to evaluate the expression of genes involved in nutrient and niche competition (Smith and Lindow, 2013; Spadaro and Droby, 2016). Yu and Lee (2015) identified the genes associated with nutrient competition in *Pseudomonas putida* (JBC17), an antagonist against *Penicillium digitatum* of the satsuma mandarin. Nutrient competition assays revealed that the inhibition of pathogen conidial germination was exerted by nutrient starvation. The authors recognised exopolyphosphatase (ppx) and Xaa-Pro aminopeptidase as potential factors responsible for nitrogen acquisition by reducing proteins and peptides under nutrient stress situations.

3.3.2 Competition for iron: The role of iron-chelating compounds

Among micronutrients, iron (Fe^{3+}) is necessary for the growth and virulence of pathogens; in this context, competition for iron plays an important role in the biological control of pathogenic fungi (Saravanakumar et al., 2008; Talibi et al., 2014). Iron is a biologically important micronutrient and a constituent of cytochrome, other heme or non-heme proteins and iron sulphur clusters (Fe/S); it also acts as a co-factor in various cellular enzymes. Under iron-limiting conditions, the antagonist synthesises low molecular weight (500–1,000 Da) compounds (siderophore) to competitively obtain ferric ion (Saraf et al., 2014). Siderophores are chelating compounds that form a tight and stable complex by binding with ferric ion and transport it into the cell. Based on chemical structural moieties, siderophores are classified either as catecholates produced only by bacteria or as hydroxymates produced by yeasts and bacteria (Saraf et al., 2014). Rhodotorulic acid produced by *Rhodotorula glutini* is a dihydroxamate-containing siderophore and assists

in improving its biocontrol efficacy against postharvest blue mold disease of apples caused by *P. expansum* (Calvente et al., 1999).

In the competition for iron, siderophores produced by microbial antagonists compete with pathogens for iron and thereby impede their growth, germination and pathogenesis. Among bacteria, the biocontrol ability of fluorescent *Pseudomonas* spp. is well recognized and associated with iron sequestration by the production of siderophores (Duijff et al., 1994). Similarly, the biocontrol ability of the bacterium *Rahnella aquatilis* against postharvest pathogens (*B. cinerea* and *P. expansum)* of apples is correlated with siderophores production (Calvo *et al.*, 2007). Yeast antagonists, namely *M. pulcherrima* and *M. fructicola* produce the siderophore pulcherrimin, which controls *B. cinerea, A. alternata* and *P. expansum* on apples (Saravanakumar et al., 2008). Iron depletion in the growth medium by *M. pulcherrima* resulted in the inhibition of mycelial growth and conidial germination of pathogen *B. cinerea, A. alternata* and *P. expansum*. The addition of iron at higher concentrations resulted in the restoration of the pathogen activity of *M. pulcherrima*. Furthermore, with iron sequestration in the medium, hyphal breakdown was observed around *M. pulcherrima* streaks, demonstrating the activation of a complex physiological process in the pathogen cell in response to iron starvation (Saravanakumar et al., 2008).

The biocontrol action of microbial antagonists through competition is possible when such antagonists can adapt better to various environmental conditions and effectively use limited nutrient resources (El-Ghaouth et al., 2004). Supplementing the limiting nutrient may considerably enhance antagonist growth and ensure biocontrol performance.

3.3.3 Antibiosis through antibiotic production

Antibiosis is the phenomenon where antagonists secrete chemical compounds that inhibit or kill potential pathogens in close proximity. Some antagonists suppress pathogen growth by producing antibiotics. Many bacterial genera, such as *Bacillus, Pseudomonas, Streptomyces, Burkholderia, Pantoea, Lysobacter* and *Enterobacter*, are predominantly involved in antibiotic production. Antibiotics produced by *Bacillus* and *Pseudomonas* are well known for their antifungal activity against postharvest fungal pathogens. The most common effective antifungal antibiotic compounds produced by bacteria are pyrrolnitrin by *S. plymuthica* and *Pseudomonas* spp. (Meziane et al., 2006; Weller 2007) lipopeptides of iturin by *Bacillus* spp. (Dimkic et al., 2013; Pretorius et al., 2015; Waewthongrak et al., 2015) and syringomycin by *P. syringae* (Grgurina et al., 2002). In addition, *Bacillus spp.* also synthesises antibacterial and antifungal metabolites such as gramicidin S (Cho et al., 2003), surfactin, bacillomycin and fengycin (Arrebola et al., 2010). Antibiotic compounds inhibit the growth and development of fungal pathogens via various mechanisms, including inhibition of cell wall synthesis, destruction and alteration of cell membrane structures and prevention of the formation of initiation complexes on the small sub-units of the ribosomes in protein synthesis (De Souza et al., 2003).

Antifungal antibiotic-producing bacterial strains are successfully deployed as postharvest biocontrol agents. Antibiotic iturin, produced by *B. subtilis* and *Pseudomonas cepacia* Burkh, inhibits the growth of fungal pathogens (Abano and Sam-Amoah, 2012). *Bacillus* strains with the ability to produce copious amounts of antibiotic compounds are used as antifungal agents for postharvest disease prevention in several fruits (Stein, 2005; Korstenet al., 2007; Yánez-Mendizábal et al., 2011). Similarly, pyrrolnitrin-producing *P. cepacia* were deployed for the control of *P. digitatum* in lemon and *B. cinerea* and *P. expansum* against pathogens of apples (Di Francesco et al., 2016). Also, the syringomycin-producing *P. syringae* was used successfully for the suppression of green mold of citrus and grey mold of apple (Sharma et al., 2009).

Although antibiotic producing microbial antagonists are used in postharvest disease control, the role of antibiotic-mediated antibiosis in some biocontrol systems has not been completely deciphered (Nunes et al., 2012). Therefore, more emphasis is placed on the use of the non-antibiotic-producing microbial antagonists to control postharvest fungal pathogens. This approach may have a wider acceptance and will also avoid fast emergence of pathogen resistance to these antimicrobial compounds (Singh and Sharma, 2009; Di Francesco et al., 2016).

3.3.4 Mycoparasitism through production of fungal cell wall lytic enzymes

Direct parasitism, mycoparasitism or hyperparasitism, is the ability of antagonistic microorganism to attach with the hyphae of fungal pathogens to produce extracellular cell wall lytic enzymes. Mycoparasitism of antagonist depends upon the sequential occurrence of the following events: come into close contact of fungal pathogens, mutual recognition by antagonist and pathogen, lytic enzymes secretion and, active growth of antagonist into the host (Spadaro and Gullino, 2004; Talibi et al., 2014). Parasitism causes either complete killing of fungal propagules or destruction and lysis of their structure. Wisniewski et al. (1991) reported mycoparasitism initially in the studies on biocontrol of *Botrytis cinera* by yeast antagonist *P. guilliermondii*. The authors demonstrated that lectin-like interaction resulted into firm attachment of antagonist cell to *B. cinerea*. Lysis of fungal cell wall also occurred due to the action of extracellular β-(1–3) glucanase enzyme secreted by the antagonistic yeast.

Fungal cell wall is composed of sugar compounds such as chitin and glucan in association with cell wall protein to provide mechanical strength and structural integrity. Chitin is a linear polymeric compound of β-1–4 linked subunits of acetylated amino sugar N-acetylglucosamine while glucan acts as a filling material and constitutes about 50–60% of the total cell wall (Spadaro and Droby, 2016). Chitin content in filamentous fungi cell wall is more than 20% (Seidl, 2008). Cell wall protein is present in the form of glycoprotein and represents 20–30% of the total cell wall. Disintegration of pathogenic fungal cell wall by the action of extracellular hydrolytic enzymes of antagonists, such as chitinases, chitosanases, glucanases, cellulase and/or protease, individually or in combination, contributes to biocontrol activity (Spadaro and Droby, 2016). The lytic enzymes also impede pathogen spores germination, elongate of germ-tube and destroy oospores (El-Tarabily, 2006). Figure 2 demonstrates action of different chitinolytic enzymes in the complete dissolution of chitin-containing cell wall of pathogenic fungi.

In consonance, many other biocontrol microbial agents produce extracellular cell wall lytic enzymes. Essghaier et al. (2009) correlated biocontrol action of halophilic bacteria *Bacillus* spp.
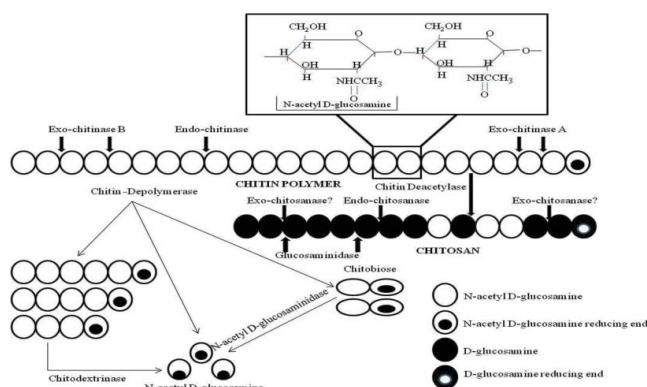
Figure 2. Schematic representations of chitinase and associated enzymes involved in breakdown of chitin polymer in nature by (Adapted after modification from Neeraja et al., 2010).

against *B. cinera* grey mold pathogen of strawberry with the ability to produce extracellular antifungal hydrolytic enzymes such as chitinase, b-1, 3-glucanase, cellulase and protease. Antifungal activity of *Bacillus* and *Pseudomonas* spp. was attributed to the extracellular chitinolytic activity (Yu et al., 2008). Banani et al. (2015) reported chitinase activity of antagonistic yeast *Metschnikowia fructicola* and demonstrated that chitinase gene MfChi was over induced in the presence of yeast *Monilinia fructicola* cell wall. An overexpressed MfChi chitinase in *Pichia pastoris* controlled the growth of *M. fructicola* and *Monilinia laxa* under *in vitro* and *in vivo* studies on peach fruits. Urbina *et al.* (2016) demonstrated the role of extracellular exo-β-1, 3-glucanase from yeast *C. oleophila* in biocontrol of *P. expansum* in apples. The authors observed that purified glucanase enzyme reduced conidial germination and inhibited growth of pathogen mycelia. Similarly, antifungal activity of alkaline serine protease, secreted by yeast-like fungus *A. pullulans*, is documented as mycoparasitism (Zhang et al., 2012). Enzymatic breakdown of fungal pathogens hyphae results in cellular deformities, including cytological damages, lysis and distortion in mycelia, altered cell membrane permeability and leakage of cytoplasmic content (Di Francesco et al., 2016). It may, therefore, be inferred that enzymatic dissolution of cell walls leads to the loss of fungal protoplasm and accountable for antagonistic activity (Kim and Chung, 2004).

3.3.5 Initiation of systemic resistance

Several studies have demonstrated that the application of microbial biocontrol agents to fruit surfaces induced systemic resistance against invading fungal pathogens (Janisiewicz et al., 2008; Romanazzi et al., 2016; Droby et al., 2016). Induction of resistance to biotic or abiotic stresses involves accumulation of structural barriers and elicitation of many biochemical and molecular defense responses in the host, including mitogen-activated protein kinase signaling (MAPK), reactive oxygen species generation (ROS), biosynthesis of terpenoid and phytoalexin via phenylpropanoid pathway, octadecanoic pathway, production of phytoalexins and PR-proteins, enhanced accumulation of phenolic compounds, lignification at the infection site and strengthening of host cell wall by formation of glycoproteins, lignin, callose, and other phenolic polymers (Shoresh et al., 2010; Lloyd et al., 2011).

Treatment of peaches with yeast *C. laurentii* and methyl jasmonic acid (MeJA) stimulated the activities of enzymes chitinase, b-1, 3-glucanase, phenylalanine ammonia-lyase (PAL) and peroxidase (POD) in comparison to the application of yeast or MeJA alone (Yao and Tian, 2005). The treatment reduced the diameter of disease lesions on fruit caused by *M. fructicola* and *P. expansum*. Onset of disease resistance against both pathogens paralleled closely with the increase in chitinase, b-1, 3-glucanase, PAL and POD activity.

Extraneous application of microbial antagonist results in differential expression of genes and proteins levels in the host and the antagonists both. DNA microarray analysis of cherry tomato in response to antagonistic yeast *C. laurentii* revealed the differential expression of genes levels in host tissue (Jiang et al., 2009). The genes responsible for signal transduction, metabolism, and stress response up-regulated whereas the genes responsible for energy metabolism and photosynthesis down-regulated. All genes expression changes increased the resistance of fruit against invading pathogen (Jiang et al., 2009). Application of yeast *Pichia membranifaciens* on peaches stimulated cellular proteins and antioxidant enzymes activity (Chan et al., 2007). Higher levels of the enzymes, such as catalase (CAT), glutathione peroxidase, methionine sulfoxide reductase peroxiredoxin, and polyphenol oxidase (PPO), protect the host tissues against oxidative damage by *P. expansum* pathogen. Further, *P. membranifaciens* enhanced the activity of pathogenesis-related proteins (PR), such as PR-9, PR-10, GTP-binding and heat shock proteins. Application of antagonistic yeast *R. paludigenum* on mandarins at pre-harvest stage induced defense response by increasing production of defense-related enzymes, including b-1, 3-glucanase, PAL, POD and PPO (Lu et al., 2013; Spadaro et al., 2016).

Although, correlation between induction of host defense and inhibition of pathogenic growth has not been completely established, molecular tools can be explored to identify different genes profile implicated in the antagonistic microbes–host–pathogen interactions in the induction of resistance in host.

3.3.6 Production of antifungal volatile compounds (VOCs)

Microbial antagonists produce several antifungal metabolites of which VOCs also play important role in inhibition of fungal pathogen growth (Mari et al., 2016). VOCs are low molecular weight lipophilic compounds mixture. Role of VOCs produced by fungi (Morath et al., 2012); yeast (Di Francesco et al., 2015); and bacteria (Zheng et al., 2013) have been reported to control postharvest disease of fruits.

VOCs produced by *Bacillus spp.* are well known for fungistatic activity against fungal pathogens. VOCs produced by *B. thuringiensis* and *B. pumilus* reduced about 88.5% anthracnose infections in mangos (Zheng et al., 2013). Similarly, VOCs of *B. subtilis* reduced the growth of *P. digitatum* by 30–70% during in-vitro trials (Leelasuphakul et al., 2008). Arrebola et al. (2010) evaluated VOCs produced by *B. amyloliquefaciens* and *B. subtilis* for antifungal activity against citrus *Penicillium* pathogen. Electron microscopic analysis of the pathogen hyphae exposed to the volatiles compounds revealed many morphological abnormalities, such as alteration of cell vacuolation, membrane permeability and swelling in the hyphae, which caused weak conidia germination and appressorial formation (Li et al., 2012).

Table 2. Representative modes of action involved in the tritrophic interactions (antagonistic microbe-fungal pathogen-host fruit) in postharvest biocontrol system.

Modes of action	Microbial antagonist(s)	Target pathogen(s)	Host fruit	References
Antibiotic production	*Bacillus subtilis* M4	*Botrytis cinera*	Apple	Ongena et al. (2005)
	Bacillus amyloliquefaciens PPCB004	*Alternaria citri, Colletotrichum gloeosporioides Penicillium crustosum*	Citrus	Arrebola et al. (2010)
	Wickerhamomyces anomalus	*Penicillium digitatum*	Citrus	Platania et al. (2012)
	Bacillus atrophaeus CAB-1	*B. cinera*	Citrus	Zhang et al. (2013)
	B. subtilis	*P. digitatum*	Citrus	Waewthongrak et al. (2015)
Lytic enzyme production	*Cryptococcus laurentii*	*Penicillium expansum*	Pear	Yu et al. (2008)
	Pichia guilliermondii	*Colletotrichum capsici*	Chilli	Chanchaichaovivat et al. (2008)
	Halophilic bacteria	*B. cinerea*	Strawberry	Essghaier et al.(2009)
	Rahnella aquatilis BNM	*B. cinerea*		Sansone et al. (2011)
	P. guilliermondii	*B. cinerea*	Apple	Zhang et al. (2011)
	Aureobasidium pullulans	*P. expansum, B. cinerea, Monilinia fructicola, Alternaria alternata*	Apple	Zhang et al. (2012); Banani et al. (2014)
	Gluconobacter cerinus	*B. cinerea*	Grapes	Guzzon et al. (2014)
	Metschnikowia fructicola	*Monilinia laxa, M. fructicola*	Peach	Banani et al. (2015)
	Candida oleophila	*P. expansum*	Apple	Urbina et al. (2016)
	Bacillus amyloliquefaciens	*B. cinera*	Pear	Qu et al. (2016)
	Paenibacillus polymyxa Bacillus subtilis	*Colletotrichum gloeosporioides Colletotrichum acutatum, Botryosphaeria dothidea.*	Apple	Kim et al. (2016)
	Rhodotorul aminuta, Candida azyma, Aureobasidium pullulans	*Geotrichumcitri aurantii.*	Citrus	Ferraz et al. (2016)
	C. oleophila	*P. expansum*	Apple	Urbina et al. (2016)
Induction of host defense	*Pichia membranaefaciens*	*Penicillium expansum*	Peach	Chan et al. (2007)
	Bacillus thuringiensis	*Guignardia citricarpa*	Citrus	Lucon et al. (2010)
	P. membranaefaciens	*Penicillium* spp	Citrus	Luo et al. (2012)
	Pichia caribbica	*Rhizopus stolonifer*	Peach	Xu et al. (2013)
	Pseudomonas fluorescens and *Bacillus subtilis*	*Lasiodiplodia theobromae*	Mango	Seethapathya et al. (2016)
Production of antifungal volatile compounds	*Muscodor albus*	*Penicillium italicum Geotrichum candidum*	Lemon	Mercier and Smilanick (2005)
	Streptomyces globisporus	*Penicillium italicum*	Citrus	Li et al. (2010)
	Candida intermedia	*Botrytis cinerea*	Strawberry	Huang et al. (2011)
	Bacillus pumilus and *Bacillus thuringiensis*	*C. gloeosporioides*	Mango	Zheng et al. (2013)
	Wickerhamomyces anomalus	*B. cinerea*	Grapes	Parafati et al. (2015)
Alleviation of oxidative damage of fruit host	*Pichia membranaefaciens Cryptococcus laurentii Candida guilliermondii Rhodotorula glutinis*	*Monilinia fructicola*	Peach	Xu et al. (2008)
Induction of ROS production in host	*Candida oleophila* (I-182) *Metschnikowia fructicola* (277)	N/A	Apple	Macarisin et al. (2010)

Biological control mechanism of antagonists also correlates with the ability to produce volatile antifungal metabolites. Fungal species, such as *Trichoderma harzianum, Fusarium oxysporum* and *A. pullulans*, produce volatile antifungal substances in low concentrations (Mari et al., 2012). Yeast antagonist *A. pullulans* produces VOCs, including 2-methyl-1-butanol, 3-methyl-1-butanol, phenethyl alcohol and 2-methyl-1-propanol, which showed fungistatic activity against *C. acutatum, B. cinerea* and *Penicillium* species (Di Francesco et al., 2014). Fungi having ability to produce VOCs may be used as bio-fumigant. Endophytic fungus *Muscodor albus*, isolated from *Cinnamomum zeylanicum* in a botanical garden of Honduras, is a typical example of volatile producing bio-fumigant fungi for control of postharvest decay (Strobel, 2011). Therefore, VOCs producing microbes have potential to suppress pathogenic fungi.

3.3.7 Other mechanisms

Some alternate mechanisms of microbial antagonists, such as reactive oxygen species (ROS) tolerance, alleviation of oxidative damage to host, biofilm formation and stimulation of ROS production, are also reported. Extraneous application of some antagonistic microorganisms to fruit surfaces and wounds alleviated ROS generated oxidative stress in the fruit tissue (Liu

et al., 2013). The strains have ability to tolerate high level of ROS generated in fruit.

Biofilm formation is another biocontrol mechanism to suppress growth of pathogens and metabolism. Yeast *Saccharomyces cerevisiae* (M25) secretes and forms extracellular biofilm as biocontrol agent against *P. expansum* spoilage in apples (Scherm et al., 2003). Actions involved in the tritrophic interactions system encompass antagonistic microbe, fungal pathogen and host fruit in postharvest biocontrol system are reported in Table 2.

4. Application methods of biocontrol agents

Identification and selection of promising antagonists are generally followed by the selection of the appropriate time and application method for the effective suppression of postharvest pathogens. In general, both pre-harvest and postharvest application approaches are practiced.

4.1 Pre-harvest application

Latent infections, associated with field infestation of fruits by pathogens, often become a major factor for the occurrence of spoilage during transportation and storage. Under these conditions, pre-harvest applications of antagonistic microbes are often

more useful to control postharvest spoilage (Ippolito et al., 2004). Field application of microbial antagonists can augment the biocontrol efficiency, as the antagonist has enough interaction time with the pathogens. This also allows the antagonist to pre-colonise on the fruit surface before the pathogens arrive, which occurs in latent infections and incipient infections through inflicted injuries during harvest (Ippolito and Nigro, 2000).

Although this strategy may not be viable on the commercial scale due to the poor endurance of antagonists under field conditions, it has some success in certain biocontrol studies. For example, field inoculation of the antagonistic yeast *Candida sake* CPA-1, 48 hours prior to harvest minimised the blue mold incidence caused by *P. expamsum* by 50% on injured apples during cold storage for 4 months (Teixidó et al., 1999). The application of the bacterial antagonist *P. agglomerans* at the pre-harvest stage efficiently protected oranges against *P. digitatum* pathogen during storage (Canamas et al., 2008). Similarly, field application of *Epicoccum nigrum* was successful in reducing fungal brown rot incidence in harvested peach fruits (Larena et al., 2005). The application of mixed cultures of antagonists is another strategy to improve the control of postharvest diseases. The combined application of the antagonistic yeast *C. sake* and the bacterium *Pseudomonas syringae* on apples and pears during the pre-harvest stage resulted in enhancement of their overall biocontrol activity against *P. expansum* during storage (Teixidó et al., 2010). However, to achieve higher levels of postharvest biocontrol through the pre-harvest

application of potential antagonists, such agents must be able to tolerate various environmental stresses in the field, including limited nutrient availability, direct ultraviolet irradiation, extreme temperatures, water stress and rapid climatic changes (Nunes et al., 2012). Genetic modifications and physiological improvements of the antagonists may enhance their tolerance.

4.2 Postharvest application

In this approach, the microbial antagonists are applied either as sprays or as dips in solution. In the suppression of storage pathogens, this approach was more successful in several biocontrol studies than pre-harvest application. High levels of control of postharvest grey mold disease in strawberries and *Alternaria* rot in lemons were achieved by postharvest application of fungal biocontrol agents, such as *Trichoderma viride, T. harzianum, Paecilomyces variotii*, and *Gliocladium roseum*, compared to pre-harvest application (Pratella and Mari, 1993). More often, postharvest application of potential antagonists results in a considerable reduction of fungal spoilage. Studies on postharvest biocontrol showed that postharvest, exogenous application of microbial bio-agents is an appropriate and reliable approach for the management of fungal diseases during postharvest handling.

Table 3. Microbial antagonist successfully deployed for biological control of postharvest diseases of fruits.

Microbial antagonists	Target pathogen(s)	Host fruit (s)	Reference
Bacterial antagonists			
Bacillus subtilis	*Botrytis cinerea*	Strawberry	Zhao et al. (2007)
	Penicillium digitatum	Citrus	Yanez Mendizabal et al. (2011)
	Rhizopus stolonifer	Tomato	Ma et al. (2015)
	Colletotrichum gloeosporioides, C. acutatum, Botryosphaeria dothidea	Apple	Kim et al. (2016)
B. atrophaeus	*C. acutatum, C. gloeosporioides*	Pepper	Han et al. (2015)
B. amyloliquefaciens	*P. italicum, P. digitatum*	Citrus	Hao et al. (2011)
Pseudomonas fluorescens	*Botrytis mali*	Apple	Mikani et al. (2008)
P. syringae	*Fusarium pallidoroseum, F. proliferatum*	Banana	Williamson et al. (2008)
Pantoea agglomerans	*P. digitatum, P. italicum*	Citrus	Torres et al. (2007)
	P. expansum	Apple	Morales et al. (2008)
Enterobacter cloacae	*Fusarium sambucinum*	Potato	Al-Mughrabi (2010)
Yeasts antagonists			
Candida oleophila	*Colletotrichum musae*	Banana	Lassois et al. (2008)
	Penicillium expansum, Botrytis cinerea	Apple	Liu et al. (2012b)
Metschnikowia fructicola	*P. expansum*	Apple	Liu et al. (2011)
	Penicillium digitatum	Grape fruit	Hershkovitz et al. (2013)
Pichia guilliermondii	*Colletotrichum capsici*	Chillies	Chanchaichaovivat et al. (2007)
	Rhizopus nigricans	Tomato	Zhao et al. (2008)
	B. cinerea	Apple, Kiwifruit	Sui and Liu (2014)
Candida sake	*P. expansum*	Apple	Morales et al. (2008)
	B. cinerea, P. expansum	Grape	Cañamás et al. (2011)
Rhodotorula mucilaginosa	*P. expansum*	Pear	Hu et al. (2015)
Debaryomyces hansenii	*Rhizopus stolonifer*	Peach	Mandal et al. (2007)
	P. digitatum	Citrus	Taqarort et al. (2008)
Cryptococcus laurentii	*B. cinerea*	Strawberry	Wei et al. (2014)
Fungal antagonist			
Trichoderma harzianum	*Botryodiplodia theobromae*	Rambutan	Sivakumar et al. (2001)
	Colletotrichum musae	Banana	Devi and Arumugam (2005)
	Botrytis cinerea	Grape	Batta (2007)
	Colleotrichum gloeosporioides	Rambutan	Marikar et al. (2008)
T. harzianum, Trichoderma koningii	*Lasiodiplodia theobromae, C. musae*	Banana	Sangeetha et al. (2009)
T. viride	*B. theobromae*	Mango	Kota et al. (2006)
T. virens	*Penicillium expansum*	Apple	Bordbar et al. (2010)
Verticillium lecanii	*P. digitatum*	Citrus	Benhamou (2004)

4.3 Antagonistic microorganisms deployed as postharvest biocontrol agents

As seen in Table 3, copious amounts of naturally occurring bacterial and fungal antagonists have been successfully deployed as biological control agents of many postharvest pathogens. Several distinct positive attributes in yeast make them suitable candidates for the biological control of diseases, such as their ability to grow and quickly colonise the fruit surface for a longer period, even under unfavourable environmental conditions (Janisiewicz and Korsten, 2002). They rapidly use the fruit surface nutrients, thereby reducing nutrient availability for pathogens (Richard and Prusky, 2002). Additionally, extracellular polysaccharides produced by yeasts enhance their survivability and block pathogen propagules. These organisms are least affected by the use of pesticides on harvested fruits. Successful control of many postharvest fruit diseases was achieved with antagonistic yeasts such as *Candida guilliermondii, Candida oleophila, C. sake, C. laurentii, Cryptococcus albidus* and *Debayromyces hansenii* (Tian et al., 2002; Zhang et al., 2007c; Lassois et al., 2008; Morales et al., 2008). Some yeast strains, including *Saccharomyces, Hansenula, Pichia* and *Kluyveromyces*, have an ecological advantage over their competitors due to the production of toxic extracellular protein (Magliani et al., 2008; Comitini et al., 2009).

Antagonistic bacteria, isolated from natural habitats produce various metabolites with potential antifungal and antibacterial capabilities (Lucon et al., 2010; Yanez-Mendizabal et al., 2011). During the last few years, several strains of *Bacillus, Burkholderia, Enterobacter* and *Pseudomonas* spp. have been studied and effectively used to reduce diseases caused by a variety of pathogens. Suppression of postharvest pathogenic growth has been achieved by the use of bacterial antagonist species such as *B. subtilis, B. thuringiensis, Burkolderia, Enterobacter cloacae* (*Pseudomonas*) *cepacia, P. agglomerans* and *S. plymuthica* (Lamsal et al., 2012; Han et al., 2015; Kim et al., 2016). However, only a few species of bacterial antagonists, such as *B. subtilis*, and *P. syringae* have been mass-produced, formulated and commercialized in the trade names Bio-Save 100 and Serenade, respectively.

In addition to yeasts and bacteria, some promising antagonistic fungal strains also potentially protect the harvested fruits from fungal pathogen attacks. Various species of the genus *Trichoderma* have received attention as postharvest biocontrol agents. *Trichoderma* fungi occur naturally in a variety of environments and can be easily isolated from soil, decaying wood and organic matter. The biocontrol activity of *Trichoderma* species is attributed to their ability compete for nutrients, secrete antifungal compounds, parasitize and activate systemic resistance in the host fruit (Whipps and Lumsden, 2001; Harman et al., 2004). They have been successfully used to control numerous postharvest pathogens of various fruit crops including mangos, grapes, pears, kiwifruits, strawberries and rambutans.

5. Improvement of biocontrol efficacy

Despite the ability of microbial antagonists to control postharvest spoilage, application of microbial biocontrol agents alone is usually not sufficient to achieve a consistently high level (> 95%) of disease control. Further, individual application of either physical methods, such as UV-C illumination and thermotherapy or chemical methods, such as chemicals and food additives Generally Regarded as Safe (GRAS), is not sufficient to achieve nearly 100% control of postharvest diseases (Palou, 2009). To overcome these limitations, the combination of biological control with physical and chemical control methods has been explored and adopted in an integrated approach of postharvest disease management. This approach has the advantage of using the synergistic effects of each method and thereby improves the overall performance and efficacy of biocontrol methods.

Chitosan (Poly-β-(1-4) N-acetyl-D-glucosamine) and its derivatives possess antifungal properties and the ability to elicit host defence responses against invading pathogens (Bautista-Baños et al., 2006). Based on these properties, chitosan has been suggested as an effective additive to improve the biocontrol performance of the antagonistic yeasts *Candida saitoana* and *C. laurentii* (Meng et al., 2010). The biocontrol efficacy of *R. glutinis* against *P. expansum* and *A. alternata* in sweet cherry fruit was enhanced by the combination with salicylic acid (SA) (Qin et al., 2003). At low concentrations, SA had only a slight effect on the growth of both antagonists and pathogens. However, the overall improvement in its performance was due to the induction of defence-related enzymes, including polyphenoloxidase, phenylalanine ammonia-lyase and β-1, 3-glucanase in host fruits rather than a fungicidal effect on the pathogens. The effectiveness of the antagonistic bacterium *P. agglomerans* in the control of postharvest green- and blue mold pathogens of lemon and other citrus fruits was considerably improved when applied in combination with either hot air (at 33°C for 6 h) or sodium bicarbonate (Usall et al., 2008). Other chemicals and methods, used alone or in combination with biocontrol agents, are inorganic salts and minerals (calcium chloride, ammonium molybdate, silicon etc.,), glucose, UV-C, heat treatments, organic salts and surfactants, ethylene inhibitors and modified atmosphere storage (Liu *et al.*, 2013; Wisniewski et al., 2016). These approaches directly inhibit pathogens, but have little effects on the viability of the biocontrol agents at the same concentration. Further, these microbial disease control agents, in combination with small doses of chemical fungicides, have also provided a similar level of disease control as obtained by the sole use of the same fungicide at a commercial dosage (Arras et al., 2002). The success of this integrated disease management approach has led to the development of commercial biocontrol products such as "Biocoat", containing the antagonist *C. saitoana* in combination with chitosan, or "Biocure", mainly containing *C. saitoana* and lysozyme (Wisniewski et al., 2007).

Food safety and quality are ensured by the implementation of a multiple hurdle approach to manage and control the growth of food-borne pathogens. Therefore, a similar model may be used in developing effective strategies for the prevention and control of postharvest fruit diseases. It may, therefore, be interfered that integrated approaches will be the key to success in developing safe and reliable alternatives for efficient postharvest disease management.

6. Development of effective formulations of biocontrol agents

Usually, a formulated biocontrol product consists of a microbial antagonist as an active ingredient, carrier material, and adjuvants in the form of nutrients and compounds to augment

the survival of the antagonist cells and to facilitate their protection from various environmental stresses (Droby et al., 2016). The formulation of biocontrol products should meet certain criteria in order to succeed commercially under a wide array of environmental conditions. These include: (a) improvement and augmentation of biocontrol efficacy on a commercial scale; (b) maintenance of viable cell populations in the formulation; (c) shelf-life extension of products by at least six months and (d) compatibility of formulated products with the existing other methods and application equipment.

Both dry and liquid products can be prepared, obtaining a higher biocontrol efficiency and maximum shelf life (Melin et al., 2006). Dry products are prepared in the form of dust, wettable powders or granules, while liquid formulations are prepared as emulsions of oils, water or combinations of both. Biocontrol products prepared in dry formulations have a longer storage time, a minimum contamination risk and are easier store, ship and distribute (Li and Tian, 2006). However, high cell mortality due to dehydration and rehydration processes is one of the disadvantages of dry formulations. Liquid formulation is therefore an alternative process. Abadias et al. (2003) have observed that isotonic liquid formulations of the antagonistic yeast *C. sake* achieved better results than the dry formulation in terms of maximum cell viability. They obtained a cell viability of 77% after storage of 7 months at 4°C, when C. *sake* was grown in a sorbitol-modified medium and preserved in an isotonic solution of trehalose. Using a similar approach, a liquid formulation of *Rhodotorula minuta* was prepared with the addition of glycerol and xanthan gum to decrease water activity and increase viscosity, respectively (Patiño-Vera et al., 2005). However, cell viability loss was observed after storage of six months at 4°C.

An exogenous inclusion of a protectant in both dry and liquid formulations is often necessary to ameliorate the impacts of a wide array of environmental stress conditions, encountered by biocontrol agents during large-scale commercial fermentation and formulation. Furthermore, the augmentation of stress tolerance, using this strategy, is also useful in improving both the cell viability and biocontrol efficacy of antagonistic strains. The exogenous inclusion of the protectant trehalose at a concentration of 5–10% in the freeze-dried formulation of two antagonistic biocontrol yeast strains *C. laurentii* and *R. glutinis* markedly increased their cell viability (Li et al., 2008). Similarly, the

amelioration of oxidative stress damage, generally encountered during the storage of liquid formulation, is trounced by the addition of antioxidants compounds such as L-ascorbic acid (Liu et al., 2009). This antioxidant not only increases the viable cell population of antagonist strains, but also positively enhances the effects of sugar protectants (trehalose and galactose) on cell viability.

7. Commercial application

The commercial success of biocontrol formulations, based on antagonistic microbes, depends upon the maximum and reliable level of target disease control. Therefore, efficacies of biocontrol agents need to be evaluated in pilot, semi-commercial and large-scale commercial studies in different packing conditions (Droby et al., 2009). Once successful in the above stages, the next step involves regulatory licensing and obtaining approval from the regulatory agencies. The regulatory approval of biocontrol formulations is generally based on their disease control efficacy and third-party evaluation for the safety of the formulated product.

Over the past few decades, many antagonistic microbes have been identified for the control of postharvest disease; however, only some have been formulated and commercialised. Some of the antagonistic microbe based biocontrol product formulations available on the market are given in Table 4. These products are registered for use against several different postharvest fungal pathogens on horticultural produce. Furthermore, it is economically sound to prepare and formulate such biocontrol products that are effectual against different fungal decay diseases. For example, Shemer, a formulation based on the yeast strain NRRL Y-27328 of *M. fructicola* has been successfully used to control spoilage caused by fungal genera such as *Aspergillus, Botrytis, Penicillium*, and *Rhizopus* (Blachinsky et al., 2007). This approach has also been used in the development of some successful postharvest biocontrol products.

8. Recent advances in the study of postharvest biocontrol systems

Advances in DNA and proteomics-based technologies in combination with bioinformatics, have provided new opportunities to deeply understand the possible interactions between the

Table 4. Commercially available antagonistic microbe-based biocontrol products for control of spoilage/diseases in harvested fruits/vegetables (adopted from Wisniewski et al., 2016 after modifications).

Biocontrol Products	Active Ingredient	Producing firm and/or Country	Host Fruit(s)	Target spoilage pathogens
Biocontrol products based on bacterial antagonists				
Biosave	*Pseudomonas syringae*	Jet Harvest Solutions USA	Pome, Citrus, Strawberry, Cherry, Potato	Penicillium, Botrytis, Mucor
Avogreen	*Bacillus subtilis*	South Africa	Avocado	Cercospora, Colletotrichum
Pantovital	*Pantoea agglomerans*	IRTA/ Sipcam-Inagra Spain	Citrus, Pome	Penicillium, Botrytis, Monilinia
Biocontrol products based on yeast antagonists				
Candifruit	*Candida sake*	IRTA/ Sipcam-Inagra, Spain	Pome	Penicillium, Botrytis, Rhizopus
Aspire	*Candida oleophila*	Ecogen, USA	Pome, Citrus, Stone fruit, Strawberry	Botrytis, Penicillium, Monilinia
Nexy	*Candida oleophila*	Lesaffre, Belgium	Pome	Botrytis, Penicillium
Yield Plus	*Cryptococcus albidus*	Lallem, South Africa	Pome, Citrus	Botrytis, Penicillium, Mucor
Boni Protect	*Aureobasisium pullulans*	Bio-ferm, Austria	Pome	Penicillium, Botrytis, Monilinia
Shemer	*Metschnikowia fructicola*	Bayer/Koppert, The Netherlands	Table grape, Pome, strawberry, Stone fruit, Sweet potato	Botrytis, Penicillium, Rhizopus, Aspergillus

microbial antagonist, pathogen and host at the molecular level (An et al., 2014). Moreover, developments and advancement in various "omics" technologies, including deep sequencing, metagenomics, comparative genomics, functional genomics, transcriptomics and proteomics, could be better exploited for detailed elucidation of the disease suppression mechanisms of biocontrol agents. Also, changes in the expression level of "biocontrol genes" during bulk production, its formulation and storage period, the physiological status of microbial biocontrol agents and the effects of various environmental stresses on its intracellular machinery can be determined using such advanced technologies (Herschkowitz et al., 2013). In addition to this, changes in the transcriptome and/or proteome level and differential up-regulated and down-regulated genes of host fruits, in response to the inoculation of microbial biocontrol agents, can also be measured. All these technologies have the potential to enhance understanding and knowledge of the microbial control of postharvest fungal pathogens.

The application of biocontrol agents generally activates fruit defence responses against pathogen and suppresses its energy metabolism and carbon assimilation processes. Grapefruit surface wounds treated with the antagonistic yeast *M. fructicola* have shown stimulated expression of the MAPK cascade and PRPs genes implicated in the signalling of defence responses, while several antioxidant genes for superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were down-regulated (Hershkovitz et al., 2012). Up-regulations of these genes in the presence of *M. fructicola* were responsible for the stimulation of induced resistance in the host and an improvement of the biocontrol action of antagonists against the pathogen *P. digitatum* in harvested grapefruit. Microarray analysis of orange fruit revealed that the application of the yeast biocontrol agent *K. apiculate* strain 34–9, against postharvest *Penicillium* mold induced as many as 801 differentially expressed genes (DEGs). In addition to the induced expression of defence-related genes, genes responsible for the metabolism of ethylene (ET), phenylalanine and jasmonic acid (JA) and the signaling of calcium and MAPK were also induced by yeast application. In contrast, down-regulated genes included monodehydroascorbate reductase, SOD, CAT, POD and genes for carotenoid biosynthesis (Liu et al., 2016).

This indicates how the expressions levels of different genes/proteins involved in the tripartite interactions of biocontrol agents, host fruit and fungal pathogen are regulated. These studies also demonstrate the dynamics of various postharvest biocontrol systems and provide information regarding the mode of actions of antagonistic microbes.

9. Conclusions and future prospects

The development of fungicide resistance in pathogens and the presence of toxic residues in fruits and vegetables are the main concerns in the use of synthetic fungicides in postharvest disease control. Therefore, eco-friendly technologies, with no or negligible dependence on synthetic fungicides, are prioritised. During the past few decades, considerable progress has been made towards biological and integrative approaches used in postharvest disease control. However, it is impractical to assume that the application of bio-fungicides alone will result

in a complete control of postharvest diseases under all conditions, as perfect conditions for the development of biocontrol agents are rare. Therefore, the biocontrol efficiency of microbial antagonists is comparatively lower than that of synthetic fungicides. Some bio-fungicides are already registered and commercialised for the management of postharvest fungal pathogens on a wide range of commodities. Therefore, microbe-mediated biological disease suppression should be perceived as a vital constituent of an integrated disease control strategy when the aim is a long-term reduction of fungicide use.

The use of bio-based fungicides is expected to gain impetus in the near future, along with their wider public acceptance as a constituent of an integrated postharvest diseases management approach. However, several challenges need to be addressed in order to develop a commercially successful, viable and economical microbial biocontrol product. The microbial antagonists displaying a broad spectrum of antifungal potential on different produce, their upgrading, basic understanding of postharvest biocontrol systems and environmental impacts need to be further explored. The development of cost-effective methods of mass multiplication and formulation of microbial antagonists at a commercial scale is also a major challenge.

Conflict of interest

The author(s) declare(s) that there is no conflict of interest.

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