Exploitation of Biocontrol Agents as an Alternative Strategy to Control Post Harvest Diseases of Fruits

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Abstract

There is an increasing concern about the environmental effects and safety of chemical pesticides and fungicides all over the world. Microbial biocontrol agents possess a number of important advantages over traditional chemical pesticides which make their commercial outlook particularly promising and can be commercially developed with relative ease to control post harvest diseases of fruits. Biological control of different plant diseases was focused primarily using bacteria or filamentous fungi and so, application of yeastsas biocontrol agents acts as a new trend against different pathogens. Various observations suggest thatcompetition for space and nutrients between biocontrol agents and pathogens, antibiosis, parasitism, induction of hostresistance and also resistance to oxidative stress are likely to be the main mechanisms of action. Products based on biocontrol agents have a share of 30% in total biopesticide market. There is still a need for research to develop suitable formulations of biopesticides from these microbial biocontrol agents. This review reveals that extensive ecological research is also required in order to achieve optimum utilisation of biological resources to manage various postharvest diseases of fruits.

Keywords: Fruits, mechanisms of action, microbial biocontrol agents, post harvest diseases

1. Introduction

Post harvest diseases of fruits pose a major challenge throughout the fruit growing areas of the world accounting to about 20–25% of the harvested produce during postharvest handling (El-Ghaouth et al., 2004; Droby, 2006; Zhu, 2006; Singh and Sharma, 2007) in developed countries and even more exasperating in the developing countries, where it often exceeds over 35% due to inadequate storage, processing and transportation facilities (Abano and Sam-Amoah, 2011). Aside from direct economic considerations, diseased produce poses a potential health risk. A number of fungal genera such as *Aspergillus, Penicillium, Alternaria* and *Fusarium* are known to produce mycotoxins under certain conditions. Losses due to postharvest disease are affected by a great number of factors including: commodity type, the postharvest environment (temperature, relative humidity, atmosphere composition, etc.), produce handling methods, postharvest hygiene, produce maturity and ripeness stage,cultivar susceptibility to postharvest diseases and treatments used for disease control.

Fungicides are commonly used to control these post harvest decays. However, though harvested fruits treated with fungicides retard post harvest diseases, there is a greater likelihood of direct human exposure to them. Synthetic chemicals are also discouraged due to their carcinogenicity, teratogenicity, high and acute residual toxicity, environmental pollution and their effects on food and side effects on humans (Lingk, 1991; Unnikrishnan and Nath, 2002). Besides these, phytoxicityand off odour effects of some fungicides have limited theiruse. Development of resistance to commonly usedfungicides within populations of post harvest pathogenshas also become a significant problem. For example, acquired resistance by *Penicillium italicum* and *Penicillium digitatum* to fungicides used for treatingcitrus fruits has been reported (Fogliata et al., 2001). This calls for a newparadigm shift from the use of synthetic fungicides to a safer and environmentally friendly alternative for reducing the postharvest decay in fruits and vegetables (Ragsdale and Sisler, 1994; Mari et al., 2007). In the last few years, biological control of postharvest diseases of fruits has been developed as a promising alternative to chemical control (Wisniewski and Wilson, 1992; Grahovac et al., 2014).

The objective of this review is to critically assess the use of biocontrol agents for controlling postharvest diseases of fruits, in order to reduce the use of chemical agents that may be harmful to humans and the environment. In the current review, a brief overview of research that has led to comprehensive understanding of various filamentous fungi, yeasts and bacteria which have been used for postharvest biological control of fruits is presented.

2. Mode of Action of Biocontrol Agents against Postharvest Pathogens

Knowledge of the mode of action is a key factor to achieve an efficient inhibition of pathogens in their hosts. The mode of action involved in the biocontrol process can permit establishment of optimum conditions for interaction between the pathogen and the biological control agent and is important in implementing a biological control strategy in a particular pathosystem (Cook, 1993; Handelsman and Stabb, 1996). Several mechanisms have been suggested to operate in postharvest biocontrol, including antibiosis, induced resistance, mycoparasitism, cell-wall degradation, competition for space and limited resources.

Several strains of antagonistic fungi use a single mechanism to inhibit the growth of pathogenic fungi, while some strains are reported to use multiple mechanisms. The most effective biological control agent studied antagonise a plant pathogen using multiple mechanisms utilising both antibiosis and induction of host resistance to suppress the disease-causing microorganisms, such as in *Pseudomonas* (Janisiewicz and Roitman, 1988; Junaid et al., 2013), it is reported that *Pseudomonas* produces antibiotic 2,4-diacetylphloroglucinol and also induces host defences (Iavicoli et al., 2003).

Antibiosis is a direct toxic effect on the pathogen by antibiotic substances released by the antagonist. *Trichoderma harzianum* and *Clonostachys rosea* (formerly *Gliocladium roseum*) control anthracnose of fruits through antibiosis (Zivkovic et al., 2010). Pyrrolnitrin can be the main mode of action of *Pseudomonascepacia* in controlling *Botrytis cinerea* and *Penicillium expansum* on apples and pears (Janisiewicz and Roitman, 1988). *Bacillus subtilis* may control *Monilinia fructicola* by the production of iturine (Pusey and Wilson, 1984), grey mould by the production of cyclolipopeptides like fengecins (Ongena et al., 2005). A yeast-like antagonist, *Aureobasidium pullulans*, controls anthracnose caused by *Colletotrichum acutatum* by antibiosis (1,3-glucanase and chitinase) and hyperparisitism (Hartati et al., 2015).

Competitive exclusion of the pathogen from sites of infection by better use ofnutrients and colonisation than the pathogen is also acommon mechanism thatcan accompany other mechanisms and is considered as the major modes of action by which microbial agents control pathogens causing postharvest decay ofpome fruits (Sharma et al., 2009). Competition for nutrients was suggested to playa role in the biocontrol of *Penicillium digitatum* by *Debaryomyces hansenii* (Droby et al., 1989) and of *Botrytis cinerea* by *Cryptococcus* sp. (Filonow et al., 1996). Pre-emptive exclusion of fungal infection sites by the antagonist was observedin *Candida oleophila* and *Cryptoccocus laurentii*, which control *Botrytiscinerea* (Roberts, 1990a; Mercier and Wilson, 1995).

Inhibition of plant pathogens by *Pantoea agglomerans* is dependent on thestrain and has been attributed to production of an acidic environment (Riggle and Klos, 1972; Beer et al., 1984), competition for nutrients (Goodman, 1967), productionof herbicolin or other antibiotics (Ishimaru et al., 1988; Vanneste et al., 1992), pre-emptive colonisation (Wilson et al., 1992; Kearnsand Hale, 1996), parasitism of the pathogen (Bryk et al., 1998) and induction of plantdefense responses (Slade and Tiffin, 1984).

Attachment alone or in combination with secretion of cell-wall degrading enzymes has been proposed as the viable mechanism operating in the biocontrol of *Botrytis cinerea* by *Pichia guilliermondii* (Wisniewski et al., 1991a). It is reported that *Pichia guilliermondii* and *Candida saitoana* cells have the ability to attach to the hyphae of *Botrytis cinerea* and cause degradation of the cell wall at the attachment sites (Wisniewski et al., 1991b; El-Ghaouth et al., 1998). The antagonistic activity of *Aureobasidium pullulans* against *Botrytis cinerea, Rhizopus stolonifer, Penicillium expansum* and *Aspergillus niger*was found to be the result of antibiosis in conjugation with attachment of microbial antagonist to the hyphae of pathogenic fungi (Castoria et al., 2001).

Several hyperparasites, especially fungi like *Pichia* and *Trichoderma*, interact directly and degrade the fungal cell or exert antagonism through antimicrobial compounds, develop hyperparasitism involving trophic growth of the biocontrol organism towards the pathogenic fungi, causes coiling, attack and dissolution of the cell wall and membrane of the pathogenic fungi by the activity of enzymes (Tewari, 1996), or directly attach to the pathogen cells, interfere with pathogen signals, or induce resistance in the plant host.

Some bacteria and fungi are able to induce defense responses in plants by producing either elicitors (e.g. cell-wall components) or messenger molecules (e.g. salicylic acid) (Spadaro and Gullino, 2004). Induction of host defence reactions was proposed to be the mechanism in the biocontrol of *Botrytis cinerea* by *Candida saitoana* (El-Ghaouth et al., 1998) and of *Penicillium digitatum* by *Verticillium lecanii* (Benhamou and Brodeur, 2000).

3. Biological Control Using Fungi

Considerable research effort has been devoted to identify yeasts and otherfungi which caneffectively control postharvest diseases of fruit, vegetables andgrains (Droby et al., 2002; Zhang et al., 2004; Sharma et al., 2009; Mishra et al., 2013; Sharma, 2014). Postharvest decay of fruits occurs either betweenflowering and fruit maturity or during harvesting, handling and storage. Preharvest infections remain latent upto fruit maturityand storage, such as infection of peaches, cherries, plums and apricots by *Monilinia* sp. The majority of postharvest pathogens infect the fruit throughwounds that occur during harvest or subsequent handling (Eckert and Ogawa,1988).

Most postharvest rots of several fruits could be reduced considerably by spraying with spores of antagonistic fungi at different stages of fruit development or by dipping the harvested fruits in their suspensions. Experiments haveshown that several antagonistic unicellular fungi have the ability to protect manyfruits from *Botrytis cinerea*, *Penicillium expansum*, *Monilinia fructicola* and *Rhizoctonia* rots (Agrios, 1997; Karabulut and Baykal, 2003). Once the antagonistic fungal cells come into contact with the fruit surface, they also occupy the wounds and affect the germination of pathogenic fungal spores mainly by niche exclusion and competition for nutrients (Liu et al., 2012).

Strains of *Candida guilliermondii* have been studied for the biological control of grey and blue moulds of apple (McLaughlin et al., 1990; McLaughlin, 1991). Control by *Candida guilliermondii* is directly related to the spore concentration of the pathogen andcell concentration of the antagonistic fungi.*Candida oleophila* was approved for postharvest decay control in citrusand apples under the trade name Aspire (Droby et al., 1998; Lahlaliet al., 2004; Wisniewski et al., 2007). It is used for the biological control of greymould caused by *Botrytis cinerea* (Mercier and Wilson, 1994), *Penicillium* rot caused by *Penicillium expansum* (Lahlali and Jajakli, 2009) and *Penicillium digitatum* (Lahlali et al., 2004). *Kloeckera apiculata* has been used as a biocontrol agent in controlling rots caused by *Penicilliumexpansum*, *Botrytis cinerea* (McLaughin et al., 1992; Long et al., 2005) and *Rhizopus* rot of peaches (McLaughlin et al., 1992; Qing and Shiping, 2000). Another species of *Candida*, namely *Candida sake* was approved for the control of *Penicillium expansum*, *Botrytis cinerea* and *Rhizopus nigricans* under the trade name Candifruit (Vinas et al., 1998; Janisiewicz, 2010).

Cryptococcus albidus has been found effective against *Mucor* rot caused by *Mucor piriformis* (Roberts, 1990b), blue mould caused by *Penicillium expansum* (Chand-Goyal and Spotts, 1996) and grey mould caused by *Botrytis cinerea* (Fan and Tian, 2001). It is approved under the trade name Yield Plus in South Africa (Mari et al., 2014). Another species of *Cryptococcus*, namely *Cryptococcus laurentii*, have been studied for the postharvest biological control of grey mould rot of apples (Roberts, 1990a), *Mucor* rot of pears (Roberts, 1990b), grey and blue mould rot of pears (Zhang et al., 2003), *Rhizopus* rot of strawberries andpeaches (Zheng et al., 2004; Zhang et al., 2007), as well as postharvest diseases ofother fruits such as strawberries, kiwi fruits and table grapes (Lima et al., 1998). According to Zhang et al. (2007), *Cryptococcus laurentii* is effective in the controlof a wide range of pathogens and can be used in combination with cold storage to enhance disease control. Another yeast strain, *Leucosporidium scottii,*has been found effective against blue mould and grey mould of apple caused by *Penicillium expansum* and *Botrytis cinerea*, respectively (Vero et al., 2013). *Metschnikowia pulcherrima* has been reported to occur commonly on appleand in apple cider and is known to controlvarious postharvest decays on pome fruits and grapes (Janisiewicz et al., 2001; Spadaro et al., 2002). An anotherstrain, *Metschnikowia fructicola*, is effective against rots caused by *Botrytis*sp., *Penicillium* sp.,*Rhizopus*sp., and *Aspergillus* sp. It is marketed in Israelunder the trade name 'Shemer' (Liu et al., 2011a).

A fungal antagonist, *Pichia membranifaciens,* isolated from wounds of peach fruits, was evaluated for its biocontrol capability against *Rhizopus stolonifer*, *Monilinia fructicola* and *Penicillium expansum* (Chan and Tian, 2005). *Rhodotorula glutinis* was found effective against apple fruit decay caused by *Penicillium expansum* and *Botrytiscinerea* (Zhang et al., 2009). It has been reported by many researchers that a mixtureof different fungal antagonists used in combination proved more effective incontrolling postharvest rots of many fruits than any antagonist applied alone (Calvo et al., 2003; Janisiewicz et al., 2008).

Calvo et al. (2003) reported that themixture of *Rhodotorula glutinis* and *Cryptococcus albidus* was more effectiveagainst grey mould of apples. Janisiewicz et al. (2008) reported that the mixtureof antagonists *Metschnikowia pulcherrima* and *Cryptococcus laurentii*,originallyisolated from apples, exhibit better biocontrol against blue mould of applethan either antagonist applied alone. Many other yeasts, viz. *Clonostachys rosea*,*Candida saitoana*, *Cystofilobasidium infirmominiatum*, *Rhodosporidium paludigenum*, *Pichia caribbica*, *P. fermentans*, *P. guilliermondii* and *P. membranifaciens* have been found effective against various postharvest rot causingpathogens of fruits (El-Ghaouth et al., 2003; Chan et al., 2007; Liu et al., 2011b; Fioriet al., 2012; Wang et al., 2010a; Xu et al., 2013) (Table 1).

Trichoderma is among the most common saprotrophic fungi. Many *Trichoderma*strains have been identified as having potential applications in biological control, being effective against a wide range of plant pathogenic fungi (including wood-rotfungi) or fungus-like organisms: *Armillaria*, *Botrytis*, *Colletotrichum*, *Dematophora*, *Endothia*, *Fulvia*, *Fusarium*, *Chondrostereum*, *Fusicladium*, *Macrophomina*,*Monilia*, *Nectria*, *Phoma*, *Phytophthora*, *Plasmopara*, *Pseudoperonospora*, *Pythium*, *Rhizoctonia*, *Sclerotinia*, *Sclerotium*, *Venturia* and *Verticillium* (Sawantet al., 1995; Agrios, 1997; Monte, 2001, Batta, 2004; Wani et al., 2009; Mishra et al., 2013; Motlagh and Samimi, 2013). Many recent studies have demonstrated the effect of various *Trichoderma* species on postharvest rot diseases caused by many fungal pathogens (Odebode, 2006; Patale and Mukadam, 2011; Hafez et al., 2013). *Trichoderma harzianum* is used to control the fungal diseases caused by *Alternaria alternata*, *Penicillium expansum* (blue mould on apples),*Botrytis cinerea*(grey mould on apples), damping-off diseases caused by *Pythium* species and *Rhizoctonia* sp. (Agrios, 1997; Batta,

Table 1: Fungal antagonists against post harvest diseases of fruits

Fungal antagonist	Disease	References
Candida guilliermondii	Blue mould (*Penicillium expansum*)	McLaughlin et al. (1990)
	Grey mould (*Botrytis cinerea*)	McLaughlin et al. (1992)
Candida oleophila	Penicillium rot (*Penicillium expansum*)	El-Neshawy and Wilson (1997)
	Grey mould (*Botrytis cinerea*)	Mercier and Wilson (1994)
Penicillium roqueforti and Penicillium viridicatum	Black rot disease (*Aspergillus niger*)	Khokhar et al. (2013)
Metschnikowia fructicola	Apple rot (*Penicillium expansum*)	Liu et al. (2011a)
Metschnikowia pulcher-rima	Blue mould (*Penicillium expansum*)	Spadaro et al. (2002), Janisiewicz et al. (2001)
	Grey mould (*Botrytis cinerea*)	Spadaro et al. (2002)
Epicoccum nigrum	Brown rot of stone fruits (*Monilinia laxa*)	Madrigal et al. (1994), Foschi et al. (1995)
Trichoderma atroviride	*Phomopsis* sp.	Das et al. (2014)
Trichoderma hamatum	Fungal diseases (*Phytophthora palmivora, Rhizoctonia solani, Fusarium* spp., *Sclerotium rolfsii, Pythium* sp.)	Ha (2010), Ngullie et al. (2010)
Trichoderma harzianum	Grey mould (Botrytis cinerea)	Batta (2003)
	Blue mould (Penicillium expansum)	Batta (2004)
Trichoderma koningii	Alternaria diseases (Alternaria alternata)	Odebode (2006), Shaikh and Nasreen (2013)
Trichoderma pseudokon-ingii	Brown rot (Monilinia laxa)	Tronsmo and Raa (1977)
Trichoderma viride	Fruit rots (Colletotrichum gloeosporioides)	Ngullie et al. (2010), Jagtap et al. (2013)
Cystofilobasidium infirmominiatum	Penicillium rot of apple (Penicillium expansum)	Liu et al. (2011b)
Leucosporidium scottii	Blue mould of apple (Penicillium expansum)	Vero et al. (2013)
Pichia caribbica	Rhizopus rot of peach (Rhizopus stolonifer)	Xu et al. (2013)
Pichia fermentans	Apple and peach decay (Monilinia fructicolaand Botrytis cinerea)	Fiori et al. (2012)
Pichia guilliermondii	Grey mould (Botrytis cinerea)	Wisniewski et al. (1991a)
Pichia membranifaciens	Penicillium expansum (peach)	Chan et al. (2007)
	Apple fruit decay (Penicillium expansum,Monilinia fructicola, Rhizopus stolonifer)	Chan and Tian (2005)
Rhodosporidium paludi-genum	Pear fruit decay (Alternaria alternata,Penicillium expansum)	Wang et al. (2010a)

2003; Biswas, 1999; Harman and Kubicek,1998; Dutta and Das, 1999; Omarjee et al., 2001).

Other strains of *Trichoderma*, namely*T. pseudokoningii*, *T. koningii*, *T. hamatum*, *T. gamsii*, *T. atroviride*, *T. virens* and*T. viride* are also used as biological control agents to suppress the growth of variouspathogenic fungi (Ngullie et al., 2010; Jagtap et al., 2013; Shaikh and Nasreen, 2013). Several commercial biocontrol products and theirformulations have been developed and approved, e.g. Trichodermil, Bio-tricho, Supresivit, Eco-77, Trichodex (*Trichoderma harzianum*), Trichdermax EC, Ecohope, Quality WG, Trichotech (*T. asperellum*), Trichospray, Trichopel, Trichodry, Vinevax (*T. atroviride*), Remedier WP (*T. gamsii*), Biocure F, Bio-shield, Binab T (*T. viride*), BW240 G, BW240 WP, G-41 technical (*T. virens*), Floragard (*T. hamatum*) (Kabaluket al., 2010; Bettiol et al., 2012; Woo et al., 2014).

4. Biological Control Using Bacteria

Several bacteria have been identified to play an important role as biological control agents in controlling diseases caused by many plant pathogenic fungi (Frances et al., 2006; Pal andGarderner, 2006; Sreevidya and Gopalakrishnan, 2012; Mishra et al., 2013). Among differentbacteria used as biological control agents, an isolate of *Burkholderia cepacia*provided biological control of blue mould and grey mould of Golden Delicious apples (Janisiewicz and Roitman, 1988).

A saprophytic strain of *Pseudomonas syringae*marketed under trade name BiosaveTM, provided biological control against greymould, blue mould and *Mucor* rot on pear and apple (Janisiewicz and Marchi, 1992; Jeffers and Wright, 1994; Mari et al., 2014). On pears it was reported to be the most effectivepostharvest treatment against various diseases in an integrated managementprogramme (Sugar, 2006). Another species of *Pseudomonas*, namely *Pseudomonasfluorescens*, has been reported to control grey mould caused by *Botrytis* sp. (Mikaniet al., 2008). Strains of *Pantoea agglomerans* were reported to be effective againstrots caused by *Botrytis cinerea*, *Rhizopus stolonifer*, *Penicillium expansum*,*Penicillium digitatum*and *Penicillium italicum* (Nunes et al., 2001; Teixido et al., 2001; Frances et al., 2006; Kotan et al., 2009; Trias et al., 2010).

Bacillus subtilis applied to wounded apples reduced fruit rot caused by *Botrytis cinerea*, *Alternariaalternata*, *Penicillium expansum* and *P. malicorticis* (Wang etal., 2010b). It has been reported that the postharvest brown rot of stone fruits can also be controlled by the application of *Bacillus subtilis* and *Pseudomonas* sp. (Pusey and Wilson, 1984; Smilanick et al., 1993). *Bacillus pumilus* and *Bacillusamyloliquefaciens* were reported to control grey mould in pears and tomatoes causedby *Botrytis cinerea* (Mari et al., 1996). Another species of *Bacillus*, namely *Bacillus licheniformis*, has been reported to control grey mould caused by *Botrytis mali* (Jamalizadeh et al., 2008). *Rahnella aquatilis* has been studied as a possiblebiocontrol agent against plant pathogenic fungi, viz., *Penicillium expansum*, *Botrytis cinerea* and *Alternaria alternata*, which produce postharvest spoilage in applefruits (Nunes et al., 2001; Calvo et al., 2007) (Table 2).

Table 2: Bacterial antagonist against post harvest diseases of fruits

Bacterial antagonist	Disease	References
Bacillus amyloliquefaciens	Grey mould (*Botrytis cinerea*)	Mari et al. (1996)
Bacillus licheniformis	Grey mould (*Botrytis mali*)	Jamalizadeh et al. (2008)
Bacillus pumilus	Grey mould (*Botrytis cinerea*)	Mari et al. (1996)
Bacillus subtilis	Brown rot (*Monilinia* sp.)	Pusey et al. (1986)
	Apple fruit rot (*Botrytis cinerea, Penicillium expansum*)	Leibinger et al. (1997)
	Apple ring rot (*Botryosphaeria berengeriana*)	Liu et al. (2009)
	Alternaria diseases (*Alternaria alternata*)	Wang et al. (2010b)
Burkholderia cepacia	Blue mould (*Penicillium expansum*), Mucor rot (*Mucor piriformis*)	Janisiewicz & Roitman (1988)
Burkholderia gladioli	Phytopathogenic fungi (*Botrytis cinerea, Penicillium* sp., *Aspergillus flavus*, *Aspergillus niger*, Phyto- phthora cactorum)	Elshafie et al. (2012)
Enterobacter cloacae	Fusarium dry rot (*Fusarium sambucinum*)	Al-Mughrabi (2010)
Pantoea agglomerans	Penicillium rot (*Penicillium expansum*)	Nunes et al. (2001), Teixido et al. (2001), Frances et al. (2006)
	Brown rot (*Monilinia laxa*)	Bonaterra et al. (2003)
Pantoea vagans	Disease of apple and pears (*Erwinia amylovora*)	Smits et al. (2010)
Pseudomonas fluorescens	Grey mould (*Botrytis* spp.)	Mikani et al. (2008)
Pseudomonas syringae	Blue mould (*Penicillium expansum*)	Janisiewicz (1987)
	Grey mould (*Botrytis cinerea*)	Zhou et al. (2001)
	Mucor rot (*Mucor piriformis*)	Janisiewicz & Marchi (1992), Jeffers & Wright (1994)
Rahnella aquatilis	Penicillium rot (*Penicillium expansum*)	Calvo et al. (2007)

5. Conclusion

Pesticide residues in fresh fruits and vegetables have been and will continue to be one of the main concerns of regulatory agencies and consumers. Therefore, reducing or eliminating the pre and postharvest use of synthetic chemical fungicides by developing alternative management strategies remains a high research priority. This review article has provided a brief overview on the use of biocontrol agents as a viable alternative to synthetic, chemical fungicides. The use of biocontrol agents is expected to gain momentum in the coming years and become more widely accepted as a component of an integrated approach to manage post harvest diseases. It is important to carry out more research studies on less known aspects of biological control including development of novel formulations from microbial agents reported by several researchers, their impact on the environment, and mass production to make new biocontrol products effective, stable, safer and cost effective. The approach would undoubtly encourage environmentally friendly products to reach the market and would lead us towards a sustainable agricultural system in the future.

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