BIOLOGICAL CONTROL OF POSTHARVEST DISEASES OF FRUITS AND VEGETABLES

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Keywords: antibiosis, bacteria, biocontrol agents, biofungicide, biomass, competition, formulation, fruits, fungi, integrated disease management, parasitism, plant pathogens, postharvest, preharvest, resistance, vegetables, yeast

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Summary

Biological control using antagonists has emerged as one of the most promising alternatives to chemicals to control postharvest diseases. Since the 1990s, several biocontrol agents (BCAs) have been widely investigated against different pathogens and fruit crops. Many biocontrol mechanisms have been suggested to operate on fruit including competition, biofilm formation, production of diffusible and volatile antibiotics, parasitism, induction of host resistance, through oxidative stress mechanisms of induction and tolerance. Molecular techniques are useful tools in the characterization of the microorganisms and enhancement of their biocontrol capabilities, through genetic engineering. The biomass production process and the development of appropriate stabilization and formulation are key issues to extend the shelf life of the

biocontrol product and to develop a commercial biofungicide. The enhancement of the biocontrol capability can be achieved through the manipulation of the postharvest environment, but also by modifying the physiological or genetic characteristics of the antagonists. Several studies were carried out to extend the use of the postharvest biofungicides, by applying antagonist mixtures, by using BCAs also in preharvest, or by integrating them with chemicals(fungicides, GRAS substances, natural compounds, inducers of resistance) and physical (thermotherapy and UV irradiation) means of protection. The essential steps bringing to the commercial development of BCAs and some key examples of commercial biofungicides will be considered.

1. Introduction

Fruits and vegetables (F&V) are an important part of the human diet, because they supply essential nutrients such as vitamins, minerals, and they are important to human health and well-being, for their contents in antioxidants and anticancer substances. An increasing awareness by consumers that diet and health are linked resulted in a greater consumption of F&V. At the same time, consumers are also more concerned about the safety of the F&V they eat, and they ask for food free from pesticide residues, toxins and pathogens.

2. Postharvest Diseases

Losses due to pests and diseases on F&V in field and during storage, transit, and commercialization steps, before reaching the consumer, are not easily assessed, but can result in 25% of the total production in industrialized countries. In developing countries damages are often higher, exceeding 50%, because of the lack of adequate storage structures.

Infection by fungi and bacteria may occur during the growing season, at harvest time, during handling, storage, transport and marketing, or even after purchase by the consumer. Disease development may be divided into two stages: infection, followed by the manifestation of symptoms.

The high water content of plant products, such as F&V, is one of the features that makes them more susceptible to pathogen attack, since they are in orchard. Another factor favorable to pathogenic fungi, particularly to the necrotrophic ones, is the presence during storage on the plant organs of wounds, often produced during harvest and transport of fruit, which represent an ideal way of access for microorganisms. Entry via wounds or natural openings (such as stomata, lenticels or hydathodes) is typical of many bacteria and fungi. Certain species of fungi, however, are capable of direct penetration of the intact cuticle, the waxy outermost layer possessed by leaves, stems and fruits. Breach of this barrier is often facilitated by a special procedure following germination of the mould spore on the plant surface; the fungus produces a swelling (appressorium) from the underside of which a thin strand grows through the cuticle and into or between the plant cells. Penetration is achieved by mechanical pressure and, more importantly, by an array of enzymes specific to the fungus involved. The plant tissue has several lines of defense. If physical injury has been sustained, an active process of wound-healing may ensue, during which corky cells are formed as a means

of protection. If a fungus or bacterium gains entry, its growth may be inhibited by plant substances which are either present or else produced in response to injury or infection. The water content or the pH of the plant cells may be too high or low to permit infection, but some of these factors change with time and, if the microorganism remains viable, then invasion or complete colonization may eventually take place.

The ecology and the etiology of the targeted pathogen must be understood in developing a control strategy. Pathogens that tolerate environmental stress often have few competitors, since few species can exist under such conditions. For example, the opportunistic pathogen *Botrytis cinerea* may be a poor competitor in comparison to *Penicillium* spp., which often produces secondary metabolites that inhibit competitors. Stress-tolerant and competitive species would therefore require control strategies different from those of species which depend upon physical adaptations to limited environmental resources or carrying-capacity environment, and are more stable and permanent members of the community.

Many fruits are resistant to fungal attack when unripe; the infection process is halted almost as soon as it has begun, but the fungus remains alive, entering a quiescent or latent phase. Latent contamination involves fungal spores on the surface which fail to germinate until the host reaches maturity or senescence. Quiescent infections, however, are macroscopically visible although mycelial development is arrested after infection and resumes only as host reaches maturity and/or senescence. Some postharvest rots result from preharvest latent infections, especially in tropical and subtropical regions where environmental conditions in the field are particularly conducive to fruit infection. Controlling rots resulting from preharvest latent infections with postharvest treatments is difficult. Nevertheless, successful control of latent infections by postharvest applications has been reported.

3. Postharvest Disease Management

Any postharvest decay management program needs to begin with preharvest practices that promote a healthy crop, reduce conducive environments for pathogen infection and disease development, and minimize the amount of the pathogen that may infect or contaminate the crop before harvest.

Preharvest practices such as the use of resistant cultivars, irrigation practices that minimize wetness duration, balanced nitrogen fertilization, canopy management (pruning), insect and weed control, and the use of fungicides may reduce the amount of fruit decay before and after harvest and reduce inoculum levels of the target pathogens.

Similarly to preharvest disease management in the field, postharvest decay control practices should also be considered as part of an integrated pest management (IPM) strategy to control pathogens. Postharvest handling practices should focus on maintaining a healthy physiology of the produce and on minimizing losses from decay. F&V with an active metabolism show considerable resistance to microbial infection and decay, whereas stressed or senescent F&V are prone to disease. In addition, activity of decay microorganisms depends on the presence of conducive environmental conditions. Any environment that slows microbial activity and maintains fruit quality will reduce

the amount of decay. Physical methods that maintain the vitality of the crop include temperature management and modification of the atmospheres using reduced oxygen and elevated carbon dioxide.

Synthetic fungicides, when admitted, are the primary means to control postharvest diseases. Properly applied treatments prevent or impede the development of pathogens and are generally economical. However, several reasons, such as the growing public concern over the human health conditions and the environmental pollution associated with pesticide usage in orchards, the development of fungicide resistant strains of postharvest pathogens, and the lack of reregistration of some of the most effective fungicides have encouraged the search of alternative approaches.

4. Biological Control

Biological control (BC) is the use of microorganisms to reduce the effects of noxious organisms, such as pathogens, and favor beneficial organisms, such as crops, or crop products. BC well fits with the concept of sustainable agriculture, because it mostly exploits natural cycles with zero or reduced environmental impact. Among the biological strategies adoptable in postharvest, the induction of resistance in the fruit, the use of plant or animal products with a fungicidal activity, and, above all, the application of antagonistic microorganisms can be considered. BC using antagonists has emerged as one of the most promising alternatives, either alone or as part of an integrated pest management to reduce pesticide use.

Since the 1990s, several biocontrol agents (BCAs) have been exploited and widely investigated against different postharvest fungal pathogens (*Alternaria, Botrytis, Colletotrichum, Monilinia, Penicillium, Rhizopus* spp.) on different host species. Most of the research has been conducted in Europe (mainly Belgium, Italy, Spain and Sweden), the United States, Israel, South Africa and China. The expansion of this research began with the publication of the report by Pusey and Wilson in 1984 on the successful control of brown rot of peach caused by *Monilinia fructicola* after harvest by using a strain of *Bacillus subtilis* isolated from soil. Postharvest application attempts were made because the field application of this bacterium to peach trees from bloom to harvest failed to control this disease. Results from pilot tests on the control of brown rot of peach, conducted in commercial packinghouses, were encouraging but this antagonist was never commercialized because it was a producer of antibiotics. The consideration that the application of such bacterial antagonists on the fruit was not commercially acceptable, brought to switch the interest on antagonists using modes of action different from antibiosis.

Wilson and Wisniewski in 1994 indicated the characteristics of an ideal antagonist: genetic stability, efficacy at low concentrations and against a wide range of pathogens on various fruit products, simple nutritional requests, survival in adverse environmental conditions, growth on cheap substrates in fermenters, lack of pathogenicity for the host plant and lack of production of metabolites potentially toxic for humans, resistance to the most frequently used pesticides, compatibility with other chemical and physical treatments (Table 1).

Genetically stable
Effective at low concentrations
Effective against a wide range of pathogen species
Effective on various host species
Simple in its nutritional requirements
Able to grow in cheap substrates
Able to be formulated with a long shelf life
Easy to be applied and distributed
Resistant to pesticides used in field and during storage
Compatible with other chemical and physical treatments
Compatible with commercial processing procedures
Able to survive in adverse environmental conditions
Not pathogenic for the host plant
Not toxic for humans
Not able to grow at 37°C

Table 1. Main characteristics of an ideal antagonistic microorganism for the control of postharvest pathogens of fruits and vegetables

The discovery of bacterial and yeast antagonists effective against various postharvest diseases of pome fruits among the resident microflora of apple and pear provided a new source of antagonists. The next milestone was the registration by the United States Environmental Protection Agency (EPA) and commercialization of the first two BCAs in 1995: a yeast, *Candida oleophila*, and a saprophytic strain of *Pseudomonas syringae*.

5. The Postharvest Environment

The postharvest environment represents a particular sector for the development of BC. Wounds made during harvesting and fruit handling can be protected from wound invading pathogens with a single postharvest application of the antagonist directly to wounds, using existing delivery systems (drenches, sprayers, dips). Once harvested, fruits are placed in cold storage for various periods of time ranging from a few days to months, depending on the commodity.

The short period between harvesting and placing fruit in storage, from less than a day to a few days, requires rapid antagonist action. Once fruit is placed in cold storage, metabolic rates of the host and associated microflora will decline depending on the temperature regime selected. The search for antagonists to control postharvest wound invading pathogens should be narrowed to rapid colonizers of the wound site that can still be metabolically active at low storage temperatures.

Peculiar difficulties are present in the control of postharvest diseases: the disease control level required is extremely high (also 95-98%); the nutritional safety imposes special care to the direct use of living microorganisms on food products; the potential market to employ a biofungicide expressly developed for postharvest use is relatively small.

On the other side, the possibilities of success for postharvest biological means can be numerous. The storage conditions partially controlled, such as temperature and humidity, can switch the host-pathogen-antagonist equilibrium towards the antagonist and the laboratory trials and results have a higher possibility to be transferred into practice. Furthermore, biotic interference is minimal so antagonists encounter minimal competition from indigenous microorganisms. Consequently, BC of postharvest diseases tends to be more consistent than BC under field conditions, and the occasional variation in performance usually can be traced to nonstandard procedures or conditions. The application site of the antagonist, which is the fruit, is limited, permitting an increase of the BCA efficacy and avoiding the presence of some interfering factors. Finally, the high value of fruit can justify a treatment with a product relatively expensive, whereas under field conditions this usage might not be cost effective.

6. Isolation of Antagonists

The first step in developing BCAs is the isolation and screening process which will largely influence its efficacy and ultimately its success under commercial conditions. The isolation procedure of potential antagonists depends on the characteristics of the pathogen infection.

To control postharvest diseases, investigators usually isolated naturally occurring microorganisms from F&V just before harvesting or during storage. The fruit surface is an excellent source of naturally occurring antagonists against postharvest fruit decay. Searching for antagonists on healthy fruits in the orchard and storage, resulted in the isolation of many ecologically fit bacterial and yeast antagonists effective against postharvest decays.

Isolation of the antagonists can be improved by using fruit from unmanaged or organic orchards, where natural populations have not been disturbed by chemical usage, and the pool of potential antagonists is greater than in a chemically managed conventional orchard. A variety of enrichment procedures have been used that favor isolation of microorganisms growing efficiently on the substrate, which occurs at the infection site (wound) that must be protected. An elegant and fast method of antagonist isolation was adopted by Wilson and colleagues in 1993. They applied rinsing waters from tomatoes and apples directly on wounds inoculated with the pathogen (*Botrytis cinerea*) and isolated antagonists from wounds which did not exhibit any symptom. This strategy allows for the rapid selection of a number of potential antagonists for the control of postharvest diseases of fruit with a minimal expenditure of time and expense and has been used in many postharvest BC programs throughout the world.

A shortcoming of this strategy is that it favors the selection of antagonists that are generally fast growers with the ability to colonize a specific niche rich in nutrients, that mainly exhibit protective rather than curative activity, and appear to have little effect on latent infections. Present screening methods also favor the selection of organisms whose primary mechanism of action is nutrient competition. A direct consequence of the type of screening procedures currently in use is the observation that several research programs in postharvest BC worldwide have independently identified and selected antagonists from a narrow range of species (Table 2 and Table 3).

Yeast or fungal species	**Pathogens controlled**	**Host species**
Acremonium breve	*Botrytis cinerea*	apple
Aureobasidium pullulans	*B. cinerea, Monilinia fructicola, Monilinia laxa, Penicillium expansum, Rhizopus stolonifer*	apple, grapes, peach, strawberry, sweet cherry
Candida ciferrii	*P. expansum*	apple
Candida ernobii	*Diplodia natalensis*	citrus
Candida membranifaciens	*Colletotrichum gloeosporioides*	mango
Candida oleophila	*B. cinerea, C. gloeosporioides, Colletotrichum musae,Penicillium digitatum, P. expansum, Penicillium italicum, R. stolonifer*	apple, banana, cherry, citrus, papaya, peach, pear, strawberry, tomato
Candida saitoana	*B. cinerea, P. digitatum, P. expansum*	apple, citrus
Candida sake	*B. cinerea, P. digitatum, P. expansum, Rhizopusnigricans*	apple, citrus, kiwifruit, pear
Cryptococcus albidus	*B. cinerea, Mucor piriformis, P. expansum*	apple, pear
Cryptococcus flavus	*M. piriformis*	pear
Cryptococcus humiculus	*B. cinerea*	apple
Cryptococcus infirmominiatus (Cystofilobasidium infirmominiatum)	*B. cinerea, M. fructicola,P. expansum,*	apple, cherry, pear
Cryptococcuslaurentii	*Alternaria alternata, B. cinerea, Geotrichumcitri-aurantii, Glomerellacingulata, M. fructicola, M. piriformis,P. expansum, R. stolonifer*	apple, cherry, citrus, jujube, peach, pear, strawberry, tomato
Cryptococcus magnus	*M. fructicola*	peach
Debaryomyces hansenii	*Geotrichum candidum, P. digitatum, P. italicum, R. stolonifer*	citrus, peach
Filobasidium floriforme	*B. cinerea*	apple
Hanseniaspora uvarum	*B. cinerea*	grapes
Kloeckera apiculata	*B. cinerea, P. digitatum, P. italicum*	cherry, citrus
Leucosporidium scotti	*P. expansum*	apple
Metschnikowia andauensis	*B. cinerea, P. digitatum, P. expansum, P. italicum, R. stolonifer*	apple, mandarin, orange, pear
Metschnikowia fructicola	*B. cinerea, P. digitatum, P. expansum, R. stolonifer*	carrot, cherry, citrus, grapes, strawberry, sweet potato
Metschnikowia gruessii	*B. cinerea*	strawberry
Metschnikowia pulcherrima	*Alternaria* sp., *B. cinerea, Colletotrichum acutatum, Monilia* sp., *P. expansum*	apple, cherry tomato, grapefruit, kiwifruit, peach, strawberry, table grapes
Muscodor albus	*B. cinerea, C. acutatum, Colletotrichum coccodes, Fusarium sambucinum, G. candidum, Helminthosporium solani, M. fructicola, Pectobacterium atrosepticum, P. expansum, Rhizopus* spp.	apple, peach, potato
Oxyporus latemarginatus	*B. cinerea*	apple
Pichia anomala	*B. cinerea, P. digitatum, P. expansum, P. italicum*	apple, citrus
Pichia angusta	*B. cinerea, M. fructicola*	apple
Pichia guilliermondii	*A.alternata, B. cinerea, Colletotrichum capsici,M. fructicola, P. digitatum, P. expansum, P. italicum, R. nigricans, R. stolonifer*	apple, chilly, citrus, grapefruit, grapes, nectarine, peach, strawberry, tomato

Pichia membranaefaciens	*C. acutatum, M. fructicola, P. expansum, Rhizopus* sp.	cherry, loquat, nectarine, peach
Pseudozyma fusiformata	*M. laxa*	peach
Rhodosporidiumpaludigenum	*A. alternata,B. cinerea, Geotrichumcitri-aurantii*	citrus, jujube, tomato
Rhodotorula glutinis	*A. alternata, B. cinerea, M. fructicola, P. expansum, R. stolonifer*	apple, cherry, jujube, peach, pear, strawberry
Rhodotorula mucilaginosa	*B. cinerea, P. expansum*	apple
Sporidiobolus pararoseus	*M. fructicola*	peach
Sporobolomyces roseus	*B. cinerea*	apple
Trichoderma harzianum	*B. cinerea, C. gloeosporioides, C. musae, Gliocephalotrichum microchlamydosporum, Lasidiodiplodia theobromae*	banana, grapes, kiwifruit, pear, rambutan, strawberry
Trichoderma viride	*B. cinerea, L. theobromae, P. digitatum*	citrus, mango, strawberry
Trichosporon pullulans	*A. alternata, B. cinerea*	cherry

Table 2. Main yeast or fungal species with antagonistic properties against postharvest pathogens of fruits and vegetables studied since the end of the 1980s

Bacterial species	**Pathogens controlled**	**Host species**
Bacillus amyloliquefaciens	*Botrytis cinerea, Colletotrichum gloeosporioides, Colletotrichum musae, Geotrichum candidum, Lasidiodiplodia theobromae, Penicilllium digitatum, Penicillium expansum, Penicillium italicum, Phomopsis* sp., *Rhizopus stolonifer*	banana, citrus, papaya, peach
Bacillus subtilis	*Alternaria alternata, Alternaria citri, Botryosphaeria berengeriana, B. cinerea, Cercospora purpurea, Colletotrichum gloeosporioides, C. musae, G. candidum, L. theobromae, Monilinia fructicola, Monilinia laxa, P. digitatum, P.expansum, P. italicum, Phomopsis citri, Pseudocercospora musae*	Apple, apricot, avocado, banana, cherry, citrus, litchi, nectarine, peach, pear, plum, strawberry
Bacillus licheniformis	*C. gloeosporioides, Dothiorella gregaria*	mango
Bacillus pumilus	*B. cinerea*	pear
Burkholderia cepacia	*Colletotrichum musae*	banana
Burkholderia gladioli	*P. digitatum, P. expansum*	apple, citrus (lemon, orange)
Burkholderia glathei	*P. digitatum*	citrus
Brevundimunas diminuta	*C. gloeosporioides*	mango
Enterobacter aerogenes	*A. alternata*	cherry
Enterobactercloacae	*R. stolonifer*	peach
Pantoeaagglomerans	*P. digitatum, P. expansum, P. italicum, R. stolonifer*	apple, citrus, pear
Pseudomonasaeruginosa	*Erwinia carotovora*	cabbage
Pseudomonascepacia	*B. cinerea, M. fructicola, Mucor piriformis, P. digitatum, P. expansum*	apple, nectarine, orange, peach, pear
Pseudomonas corrugata	*M. fructicola*	nectarine, peach
Pseudomonas fluorescens	*B. cinerea*	apple
Pseudomonas putida	*E. carotovora*	potato
Pseudomonas syringae	*B. cinerea, G. candidum, M. fructicola, M. piriformis, P. digitatum, P. expansum, P. italicum*	apple, cherry, citrus, peach, pear, potato, sweet potato

Rahnella aquatilis	*B. cinerea, P. expansum*	apple
Stenotrophomonas maltophilia	*C. gloeosporioides*	mango

Table 3: Main bacterial species with antagonistic properties against postharvest pathogens of fruits and vegetables studied since the end of the 1980s

Other enrichment procedures include isolation from natural cracks on the fruit surface; agar plates containing apple juice that were seeded with fruit washings; fruit wounds treated with fruit washings and incubated for several days; freshly made wounds on apples in the orchard that were exposed to colonization by fruit-associated microbiota from one to four weeks before harvest; and from an apple juice culture resulting from seeding diluted apple juice with the orchard-colonized wounds and repeated reinoculation to fresh apple juice.

The fructoplane has provided the most abundant and most desirable source for isolating antagonists against postharvest fruit pathogens. However, the antagonists may also come from other closely related or unrelated sources. The phylloplane has also been a good source of antagonists, as it may share part of the resident microflora of fruits as well as contain other microorganisms dislodged from the fruit. Screening collections of yeast or starter cultures used in the food industry may also yield effective antagonists. Soil also maybe an abundant and diverse source of antagonists.

Since the method of screening will have a major impact on the type and properties of the antagonist that are identified, it is important to evaluate the consequence of the methods for screening that are presently being utilized and appraise whether or not they can be improved.

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24. Fleet G.H. (2003). Yeasts in fruit and fruit products, in *Yeasts in food*, (ed. T. Boekhout, V. Robert), pp. 267-287. Hamburg: Behr's Verlag. [The chapter presents the different yeast genera colonizing the fruits and fruit derived products.]

25. Fravel D.R., Rhodes D.J., Larkin R.P. (1999). Production and commercialization of biocontrol products, in *Integrated pest and disease management in greenhouse crops*, (ed. R. Albajes, M.L. Gullino, J. van Lenteren, Y. Elad), pp. 365-376. Dordrecht: Kluwer Academic Publishers. [Interesting chapter about the current status of production, stabilization, formulation and commercialization of biopesticides.]

26. Friel D., Pessoa N.M.G, Vandenbol M., Jijakli M.J. (2007). Separate and combined disruptions of two exo-β-1,3-glucanase genes decrease the efficiency of Pichia anomala (strain K) biocontrol against Botrytis cinerea on apple. *Molecular Plant-Microbe Interactions* 20, 371-379.[Two exo-β-1,3-glucanase-encoding genes PAEXG1 and PAEXG2 were separately and sequentially disrupted in P. anomala. The resulting mutant strains showed a significantly reduced efficiency of biocontrol of B. cinerea on apple.]

27. Giobbe S., Marceddu S., Scherm B., Zara G., Mazzarello V.L., Budroni M., Migheli Q. (2007). The strange case of a biofilm-forming strain of Pichia fermentans, which controls Monilinia brown rot on apple but is pathogenic on peach fruit. *FEMS Yeast Research* 7, 1389-1398. [Pseudohyphal growth of a yeast plays a major role in governing the potential pathogenicity, emphasizing the importance of a thorough risk assessment for the safe use of any new BCA.]

28. Grevesse C., Lepoivre P., Jijakli M.H. (2003). Characterization of the exo-glucanase encoding gene PaEXG2 and study of its role in the biocontrol activity of Pichia anomala strain K. *Phytopathology* 93, 1145-1152. [An exo-glucanase gene was isolated and disrupted in P. anomala. The strain showed inferior BC activity and colonization of wounds of apple.]

29. Guetsky R., Shtienberg D., Elad Y., Fischer E., Dinoor A. (2002). Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathology* 92, 976-985. [The BC of greymould could be enhanced by applying a combination of two BCAs with different mechanism of action, such as competition and secretion of antibiotics.]

30. Gullino M.L., Migheli Q., Mezzalama M. (1995). Risk analysis in the release of biological control agents: antagonistic Fusarium oxysporum as a case study. *Plant Disease* 79, 1193-1201. [A paper to be used as a model to establish the risk assessment in the release of new BCAs.]

31. Hofstein R., Friedlender B., Chalutz E., Droby S. (1994). Large scale production and pilot testing of biocontrol agents of postharvest diseases, in *Biological Control of Postharvest Diseases – Theory and Practice*, (ed. C.L. Wilson, M. Wisniewski), pp.89-100. Boca Raton: CRC Press Inc. [The chapter presents the mass production, the quality control, the pilot testing and the formulation of BCAs for postharvest disease control.]

32. Ippolito A., Nigro F. (2000). Impact of preharvest application of biological control agents on postharvest diseases of fresh fruits and vegetables. *Crop Protection* 19, 715-723. [Interesting review about the fiel application of BCAs to control postharvest diseases.]

33. Janisiewicz W.J., Korsten L. (2002). Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* 40, 411-441. [Interesting review on biological control of postharvest diseases, published on a prestigious journal.]

34. Janisiewicz W.J., Tworkoski T.J., Kurtzman C.P. (2001). Biocontrol potential of Metschnikowia pulcherrima strains against blue mold of apple. *Phytopathology* 91, 1098-1108. [Eight strains of M. pulcherrima were characterized phenotypically, genetically, and for their BC potential against blue mould on apples.]

35 Janisiewicz W.J. (2010). Quo vadis of biological control of postharvest diseases, in *Post-harvest Pathology. Plant Pathology in the 21st Century*, Vol. 2.,(ed. D. Prusky, M.L. Gullino), pp. 137-148. Dordrecht: Springer. [A review that presents the emerging new areas of postharvest BC, including new strategies to develop BCAs to control of latent infections.]

36. Janisiewicz W.J., Bastos Pereira I., Almeida M.S., Roberts D.P., Wisniewski M., Kurtenbach E. (2008). Improved biocontrol of fruit decay fungi with Pichia pastoris recombinant strains expressing Psd1 antifungal peptide. *Postharvest Biology and Technology* 47, 218-225. [The expression in Pichia pastoris of antimicrobials is able to improve the biocontrol potential of postharvest pathogens on apple.]

37. Jiang F., Chen J., Miao Y., Krupinska K., Zheng X. (2009). Identification of differentially expressed genes from cherry tomato fruit (Lycopersicon esculentum) after application of the biological control yeast Cryptococcus laurentii. *Postharvest Biology and Technology* 53, 131-137. [A forward subtractive suppression hybridization (SSH) cDNA library was used to identify a number of transcripts encoding proteins which are up-regulated after the application of a BC yeast to tomato fruit.]

38. Jiang, F., Zheng X., Chen J. (2009). Microarray analysis of gene expression profile induced by the biocontrol yeast Cryptococcus laurentii in cherry tomato fruit. *Gene* 430, 12-16. [A microarray analysis was performed in tomato to obtain an overall view on transcript modification during the fruit response to the biocontrol yeast Cryptococcus laurentii.]

39. Jones R.W., Prusky D. (2002). Expression of an antifungal peptide in Saccharomyces: a new approach for biological control of the postharvest disease caused by Colletotrichum coccodes. *Phytopathology* 92, 33-37. [The expression of cecropin A, an antifungal peptide, in yeast represents a new approach for the BC of postharvest diseases.]

40. Kader A. (2002). *Postharvest Technology of Horticultural Crops*, 3rd Ed., pp.535. Davis: University of California Agriculture & Natural Resources. [A syllabus about the postharvest biology and technology of horticultural crops.]

41. Kurtzman C.P., Droby S. (2001). Metschnikowia fructicola, a new ascosporic yeast effective for biocontrol of postharvest fruit rots. *Systematic and Applied Microbiology* 24, 395-399. [The first paper about the new ascosporic species of M. fructicola, used as active ingredient for the development of the commercial product Shemer™.]

42. Larralde-Corona C.P., del Socorro Ramírez-González M., Pérez-Sánchez G., Oliva-Hernández A.A., Narváez-Zapata J.A. (2011). Identification of differentially expressed genes in the citrus epiphytic-yeast Pichia guilliermondii during interaction with Penicillium digitatum. Biological Control 57, 208-214. [Antagonist gene expression was evaluated by using differential expressed sequence tags (ESTs) obtained by suppression subtractive hybridization (SSH) and differential display (DD).]

43. Lee S.O., Kim H.Y., Choi G.J., Lee H.B., Jang K.S., Choi Y.H., Kim J. (2009). Mycofumigation with Oxyporus latemarginatus EF069 for control of postharvest apple decay and Rhizoctonia root rot on moth orchid. *Journal of Applied Microbiology* 106, 1213-1219. [Oxyporus latemarginatus produced antifungal volatile compounds and could be used as a biofumigant for the control of postharvest diseases.]

44. Li B.Q., Tian S.P. (2006). Effect of trehalose on stress tolerance and biocontrol efficacy of Cryptococcus laurentii. *Journal of Applied Microbiology* 100, 854-861. [The content of trehalose in C. laurentii was increased by culturing the yeast in trehalose-containing medium. Survival of the yeast was increased as internal trehalose accumulation after freeze drying.]

45. Li B.Q., Tian S.P. (2007). Effect of intracellular trehalose in Cryptococcus laurentii and exogenous lyoprotectants on its viability and biocontrol efficacy on Penicillium expansum in apple fruit. *Letters in Applied Microbiology* 44, 437-442. [Increasing intracellular trehalose content of a BCA and adding exogenous protectants could improve its viability and BC efficacy after freeze drying.]

46. Liu J., Wisniewski M., Droby S., Tian S., Hershkovitz V., Tworkoski T. (2011). Effect of heat shock treatment on stress tolerance and biocontrol efficacy of Metschnikowia fructicola. *FEMS Microbiology Ecology* 76, 145-155. [The effect of high temperature and oxidative stress on the cell viability of a yeast antagonist was determined: heat-shock-treated yeast cells showed less accumulation of reactive oxygen species, transcription of a trehalose-6-phosphate synthase gene was upregulated and trehalose content increased.]

47. Liu J., Wisniewski M., Droby S., Vero S., Tian S., Hershkovitz V. (2011). Glycine betaine improves oxidative stress tolerance and biocontrol efficacy of the antagonistic yeast Cystofilobasidium infirmominiatum. *International Journal of Food Microbiology* 146, 76-83. [The effect of H2O2-induced oxidative stress on the viability of a yeast antagonist as well as the effect of exogenous glycine betaine on yeast viability under oxidative stress were determined.]

48. Macarisin D., Droby S., Bauchan G., Wisniewski M. (2010). Superoxide anion and hydrogen peroxide in the yeast antagonist-fruit interaction: A new role for reactive oxygen species in postharvest biocontrol? *Postharvest Biology and Technology* 58, 194-202. [BCAs may induce a transient production of ROS in a fruit which triggers defense-related oxidative responses to postharvest pathogens.]

49. Massart S., Jijakli H.M. (2007). Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: A review. *Journal of Microbiological Methods* 69, 229-241. [A good overview about the possible uses of molecular technologies to unravel the mechanisms of action used by biocontrol microorganisms.]

50. Mercier J., Jiménez J.I. (2004). Control of fungal decay of apples and peaches by the biofumigant fungus Muscodor albus. *Postharvest Biology and Technology* 31, 1.8. [Use of M. albus for the mycofumigation of apples and peaches to control the postharvest pathogens]

51. Mercier J., Smilanick J.L. (2005). Control of green mold and sour rot of stored lemon by biofumigation with Muscodor albus. *Biological Control* 32, 401-407. [Biofumigation of citrus fruit with the antifungal VOCs produced by M. albus to control postharvest diseases.]

52. Mounir R., Durieux A., Bodo E., Allard C., Simon J.-P., Achbani E.-H., El-Jaafari S., Douira A., Jijakli M.H. (2007). Production, formulation and antagonistic activity of the biocontrol like-yeast Aureobasidium pullulans against Penicillium expansum. *Biotechnology Letters* 29, 553-559. [A. pullulans was grown in a fed-batch fermentor and its cells were fluidized bed driedbefor being used as biofungicide to control P. expansum on apple.]

53. Mousdale D.M., Melville J.C., Fischer M. (1999). Optimization of fermentation process by quantitative analysis: from analytical biochemistry to chemical engineering, in *Fermentation Microbiology and Biotechnology*, (ed. E.M.T. El-Mansi, C.F.A. Bryce), pp. 147-178. London: Taylor and Francis. [It describes the process of fermentation optimization, by choosing the right carbon and nitrogen sources, and inorganic components, and by measuring growth and biomass profiles.]

54. Nantawanit N., Chanchaichaovivat A., Pinijpan B., Ruenwongsa P. (2010). Induction of defense response against Colletotrichum capsici in chili fruit by the yeast Pichia guilliermondii strain R13. *Biological Control* 52, 145-152.[Pretreatment of chili with a BC yeast significantly reduced disease incidence, by enhancing the activities of PAL, CHI, GLu and the accumulation of the phytoalexin capsidiol.]

55. Nunes C., Bajji M., Stepien V., Manso T., Torres R., Usall J., Jijakli M.H. (2008). Development and application of a SCAR marker to monitor and quantify populations of the postharvest biocontrol agent Pantoea agglomerans CPA-2. *Postharvest Biology and Technology* 47, 422-428. [A system to monitor the population dynamics of a BCA on the fruit surface, based on a semiselective medium and a PCR with SCAR primers, was developed.]

56. Prusky D., Gullino M.L. (2010). *Post-harvest Pathology. Plant Pathology in the 21st Century*, Vol. 2, pp. 211. Dordrecht: Springer. [The second volume coming out from the 9th International Congress of Plant Pathology is dedicated to Post-harvest pathology and has several chapters focusing on biological control.]

57. Pujol M., De Clercq D., Cognet S., Lepoivre P., Jijakli M.H. (2004). Monitoring system for the biocontrol agent Pichia anomala strain K using quantitative competitive PCR-ELOSA. *Plant Pathology* 53, 103-109. [An innovative system based on a PCR-ELOSA to monito the BCA P. anomala.]

58. Reddy K.R.N., Spadaro D., Gullino M.L., Garibaldi A. (2011). Potential of two Metschnikowia pulcherrima (yeast) strains for in vitro biodegradation of patulin. *Journal of Food Protection* 74, 154-156. [BCAs not only can control the development of postharvest pathogens but also they can degrade the mycotoxins they are able to produce.]

59. Saravanakumar D., Ciavorella A., Spadaro D., Garibaldi A., Gullino M.L. (2008). Metschnikowia pulcherrima strain MACH1 outcompetes Botrytis cinerea, Alternaria alternata and Penicillium expansum in apples through iron depletion. *Postharvest Biology and Technology* 49, 121-128. [M. pulcherrima can to produce a red pigment, called pulcherrimin, able to subtract ferric ions from the substrate and to inhibit the pathogen development.]

60. Saravanakumar D., Spadaro D., Garibaldi A., Gullino M.L. (2009). Detection of enzymatic activity and partial sequence of a chitinase in Metschnikowia pulcherrima strain MACH1 used as postharvest biocontrol agent. *European Journal of Plant Pathology* 123, 183-193. [The chitinase activity of a M. pulcherrima was detected and the chitinase gene was cloned and partially characterized.]

61. Schena L., Finetti Sialer M., Gallitelli D. (2002). Molecular detection of strain L47 of Aureobasidium pullulans, a biocontrol agent of postharvest diseases. *Plant Disease* 86, 54-60. [Scorpion primers were designed, starting from SCAR primers obtained from a RAPD amplicon, to monitor by real time PCR the BCA A. pullulans]

62. Segal E., Yehuda H., Droby S., Wisniewski M., Goldway M. (2002). Cloning and analysis of CoEXGII, a secreted 1,3-beta-glucanase of the yeast biocontrol agent Candida olephila. *Yeast* 19, 1171-1182. [The 1,3-beta-glucanase gene of a yeast BCA was cloned and characterized.]

63. Sharma R.R., Singh D., Singh R. (2009). Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biological Control* 50, 205-221. [A review about the BC of postharvest diseases, with special emphasis on the integration with other physical and chemical protection means.]

64. Snowdon A.L. (1990). *A color atlas of post-harvest diseases and disorders of fruits and vegetables. General Introduction and Fruits*, Vol. 1.,pp. 302. Boca Raton: CRC Press. [Essential book to know the occurrence, biology, epidemiology and control strategies of postharvest diseases of fruits.]

65. Spadaro D., Ciavorella A., Lopez G., Garibaldi A., Gullino M.L. (2010). Effect of protectants and initial cell concentration on viability of freeze-dried cells of Metschnikowia pulcherrima. *Canadian*

Journal of Microbiology 56, 809-815. [The effect of protectant type, protectant concentration, initial cell concentration, cell age on the freeze drying process of a BCA and on its BC efficacy.]

66. Spadaro D., Ciavorella A., Zhang D., Garibaldi A., Gullino M.L. (2010). Effect of culture media and pH on the biomass production and biocontrol efficacy of a Metschnikowia pulcherrima strain to be used as a biofungicide for postharvest disease control. *Canadian Journal of Microbiology* 56, 128-137. [The optimization of the biomass production process of a BCA in a 5-l fermenter and its effect on the BC efficacy.]

67. Spadaro D., Garibaldi A., Gullino M.L. (2004). Control of Botrytis cinerea and Penicillium expansum on apple combining a biocontrol agent with hot water dipping and acibenzolar-S-methyl, baking soda, or ethanol application. *Postharvest Biology and Technology* 33, 141-151.[Example of integration of a BCA with a physical practice – hot water treatment – and inducers of resistance or GRAS compounds.]

68. Spadaro D., Gullino M.L. (2004).State of art and future perspectives of biological control of postharvest fruit diseases. *International Journal of Food Microbiology* 91, 185-194. [A state of art about BC of postharvest diseases of fruits, focusing on the mechanisms of action involved.]

69. Spadaro D., Gullino M.L. (2010).Opportunities and constraints in the development of antagonistic yeasts for the control of postharvest diseases of fruit. *Stewart Postharvest Reviews* 3(2), 1-8. [The review focuses on the main achievements and on the research needs in the field of BCAs against postharvest pathogens of fruits.]

70. Spadaro D., Sabetta W., Acquadro A., Portis E., Garibaldi A., Gullino M.L. (2008). Efficacy and genetic diversity of Metschnikowia pulcherrima strains isolated from different food matrices against postharvest diseases in apple. *Microbiological Research* 163, 523-530. [Use of AFLP to characterize the genetic diversity and to relate it to the biocontrol capability of different strains of M. pulcherrima.]

71. Spadaro D., Vola R., Piano S., Gullino M.L. (2002).Mechanisms of action, efficacy and possibility of integration with chemicals of four isolates of the yeast Metschnikowia pulcherrima active against postharvest pathogens on apples. *Postharvest Biology and Technology* 24, 123-134. [Four strains of M. pulcherrima were studied for their mechanism of action, mainly based on competition for nutrients, and for their tolerance to fungicides.]

72. Spotts R.A., Wallis K.M., Serdani M., O'Gorman D.T., Sholberg P.L.(2009). Real time polymerase chain reaction for rapid and quantitative determination of Cystofilobasidium infirmominiatum on the surfaces of apple, pear, and sweet cherry fruit. *Postharvest Biology and Technology* 51, 227-231. [A real time PCR protocol was developed to monitor the population of a yeast BCA on the surface of fruits.]

73. Teixidó N., Cañamás T.P., Abadias M., Usall J., Solsona C., Casals C., Viñas I. (2006). Improving low water activity and desiccation tolerance of the biocontrol agent Pantoea agglomerans CPA-2 by osmotic treatments. *Journal of Applied Microbiology* 101, 927-937. [NaCl treatments are very appropriate for improving P. agglomeranslow aw tolerance obtaining high production levels and maintaining biocontrolefficacy.]

74. Torres R., Teixidó N., Usall J., Abadias M., Mir N., Larrigaudiere C., Viñas I. (2011). Anti-oxidant activity of oranges after infection with the pathogen Penicillium digitatum or treatment with the biocontrol agent Pantoea agglomerans CPA-2. *Biological Control* 57, 103-109. [P. digitatum suppresses H2O2 production as well as SOD and CAT activities in orange tissue as a response to infection process. In contrast, the BCA triggers H2O2 production and both enzymatic activities as a mechanism to prevent pathogen infections.]

75. Touré Y., Ongena M., Jacques P., Guiro A., Thonart P. (2004). Role of lipopeptides produced by Bacillus subtilis GA1 in the reduction of greymould disease caused by Botryitis cinerea on apple. *Journal of Applied Microbiology* 96, 1151-1160. [B. subtilis endospores inoculated on apple pulp can readily germinate allowing significant cell populations to establish and efficient in vivo synthesis of lipopeptides which could be related to greymould reduction.]

76. Usall J., Teixidó N., Abadias M., Torres R., Cañamas T., Viñas I. (2010). Improving formulation of biocontrol agents manipulating production process, in *Post-harvest Pathology. Plant Pathology in the 21st Century*, Vol. 2,(ed. D. Prusky, M.L. Gullino), pp. 149-169. Dordrecht: Springer. [A review about the improvement of BCA liquid and dry formulations during the production process, by exploiting thermal and osmotic stress.]

77. Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., Zabeau M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acid Research* 23, 4407-4414. [The first paper to present the AFLP technique, useful to characterize populations of microorganisms.]

78. Wang Y., Wang P., Xia J., Yu T., Lou B., Wang J., Zheng X.D. (2010). Effect of water activity on stress tolerance and biocontrol activity in antagonistic yeast Rhodosporidium paludigenum. *International Journal of Food Microbiology* 143, 103-108. [Salt-adapted R. paludigenum showed better viability than not adapted cells after being frozen, which may be related to the accumulation of intracellular trehalose.]

79. Wilson C.L., Wisniewski M.E. (1994).*Biological Control of Postharvest Diseases. Theory and Practice*, pp. 187. Boca Raton: CRC Press. [The first book published on biological control of postharvest diseases, and the most complete on the topic, with different points of view taken into consideration. Some technologies are nowadays out-of-date.]

80. Wraight S.P., Jackson M.A., de Kock S.L. (2001). Production, stabilization and formulation of fungal biocontrol agents, in *Fungi as Biocontrol Agents*, (ed. T.M. Butt, C. Jackson, N. Magan), pp. 253-287. Wallingford: CAB International. [Interesting review over production and downstreaming technologies, it is a chapter of a book dedicated to antagonistic fungi.]

81. Xu X.B., Tian S.P. (2008). Reducing oxidative stress in sweet cherry fruit by Pichia membranaefaciens: A possible mode of action against Penicillium expansum. *Journal of Applied Microbiology* 105, 1170-1177. [Induction of antioxidant defence response, including CAT and GPX enzymes, may be an important mechanism of antagonistic yeast in mitigating pathogen-induced oxidative stress in fruit and controlling disease.]

82. Xu X., Qin G., Tian S.P.(2008). Effect of microbial biocontrol agents on alleviating oxidative damage of peach fruit subjected to fungal pathogen. *International Journal of Food Microbiology* 126, 153-158. [Yeast BCA treatments may be related to alleviating protein carbonylation and mitigating pathogen-induced oxidative damage.]

83. Yan R., Ding D., Guan W., Hou J., Li M. (2008). Control of greymould rot of loquat with chitinase expressed in Pichia pastoris. *Crop Protection* 27, 1312-1317. [A rice chitinase was expressed in P. pastoris, purified and investigated for its antifungal activity against greymould of loquat.]

84. Yehuda H., Droby S., Bar-Shimon M., Wisniewski M., Goldway M. (2003). The effect of under- and overexpressed CoEXG1-endoded exoglucanase secreted by Candida oleophila on the biocontrol of Penicillium digitatum. *Yeast* 20, 771-780. [The gene CoEXG1 encoding for a secreted exo-β-1,3-glucanase was characterized in C. olephila by producing knockout and overexpressing transformants.]

85. Yu T., Zhang H., Li X., Zheng X. (2008). Biocontrol of Botrytis cinerea in apple fruit by Cryptococcus laurentii and indole-3-acetic acid. *Biological Control* 46, 171-177. [The combined treatment with C. laurentii and IAA was effective to reduce the gray mold rot in apple wounds and stimulated the activities of superoxide dismutase, catalase and peroxidase.]

86. Yu T., Wang L., Yin Y., Feng F., Zheng X. (2008). Suppression of postharvest blue mould of apple fruit by Cryptococcus laurentii and N6-benzyladenine. *Journal of the Science of Food and Agriculture* 88, 1266-1271. [The combination of C. laurentii with a cytokinin was more effective in suppressing Penicillium expansum infection in apple fruit and resulted in stimulation of superoxide dismutase activity but in inhibition of the increase in peroxidase activity.]

87. Zhang D., Lopez G., Spadaro D., Garibaldi A., Gullino M.L. (2010). Efficacy of yeast antagonists used individually or in combination with hot water dipping for control of postharvest brown rot in peaches. *Journal of Plant Diseases and Protection* 117, 226–232. [Yeast BCAs can be combined to hot water dipping to improve postharvest BC of Monilinia spp. on stone fruit.]

88. Zhang D., Spadaro D., Garibaldi A., Gullino M.L. (2010).Modes of action of the antagonist Aureobasidium pullulans PL5 against postharvest pathogens of fruit: production of hydrolytic enzymes and competition for nutrients. *Biological Control* 54, 172-180. [A strain of A. pullulans effective against different postharvest pathogens of apple, plum and peach was characterized for its mechanism of action and showed to produce β-1,3-glucanase, exo-chitinase and endo-chitinase.]

89. Zhang D., Spadaro D., Garibaldi A., Gullino M.L. (2010).Screening and efficacy evaluation of three antagonists against postharvest brown rot of peaches. *Postharvest Biology and Technology* 55, 174-181.

[Three BCAs, belonging to the species Metschnikowia sp., Aureobasidium pullulans and Pseudozyma fusiformata have been selected for their efficacy against brown rot of peaches.]

90. Zhang D., Spadaro D., Garibaldi A., Gullino M.L. (2011).Potential biocontrol activity of a strain of Pichia guilliermondii against greymould of apples and its possible modes of action. *Biological Control* 57, 193-201. [Different mechanisms of action can be used by yeast BCAs, such as competition for nutrients, release of hydrolytic enzymes and induction of host resistance, to control postharvest diseases.]

91. Zhang H., Ma L., Turner M., Xu H., Dong Y., Jiang S. (2009). Methyl jasmonate enhances biocontrol efficacy of Rhodotorula glutinis to postharvest blue mold decay of pears. *Food Chemistry* 117, 621-626. [The combination of MeJA and R. glutinis was an effectiveapproach to reduce the disease incidence and lesion diameter of blue mould decay of pears.]

92. Zhang H., Ma L., Jiang S., Lin H., Zhang X., Ge L., Xu Z. (2010). Enhancement of biocontrol efficacy of Rhodotorula glutinis by salicyclic acid against gray mold spoilage of strawberries. *International Journal of Food Microbiology* 141, 122-125. [The combination of R. glutinis and SA was an effective treatment in controlling the natural diseases of strawberries.]

Biographical Sketch

Davide Spadaro, assistant professor at the University of Torino, DiVAPRA – Plant Pathology, since 2005.
Research fields:
- Biological control of plant pathogens, particularly postharvest pathogens.
- Postharvest storage and disease control of fruits and vegetables.
- Occurrence, prevention and control of mycotoxins in different food matrices.
- Molecular diagnostics for the identification of phytopathogenic fungi and bacteria.

Professor at the University of Torino of:
"Plant pathology and biotechnologies applied to crop protection" and "Plant pathology and crop protection" of the MS in Plant Biotechnologies
"Phytopathological Biotechnologies" of the BS in Biotechnology curriculum Plant Biotechnology
Since 2004, Professor at the Venice International University on topics related to Sustainable Agriculture.
Supervised around 50 students in the preparation of their BS or MS thesis.
Member of the Teaching Board and Tutor of the Ph.D. in Biological Sciences and Applied Biotechnologies Sciences of the Ph.D. School in Natural Sciences and Innovative Technologies, University of Torino.
Member of the Scientific Board of the University Master in Landscape and Green Areas Planning, University of Torino.
Member of the Management Board and Scientific Secretary of Agroinnova – Centre of Competence for the Innovation in the Agroenvironmental Sector.
Member of the Editorial Board of the Journal of Hill Agriculture.
Member of ISHS (International Society for Horticultural Science), ISMPMI (International Society for Molecular Plant-Microbe Interactions), SIPAV (Italian Society of Plant Pathology), IAFP (International Association of Food Protection),
ISM (International Society of Mycotoxicology).
Author and coauthor of around 270 publications in English and in Italian, 56 of them on journals with impact factor.
Author of 3 national patents and 1 international patent.
2003-2005 Post-Doc at University of Turin.
2003 Ph.D. in Agricultural, Forestry and Agro-food Sciences – University of Turin.
2000 M.S. in Biotechnology curriculum Plant Agricultural Biotechnology - University of Turin.
2010 Research period (1 month) at the Dept. Food Science and Technology, Thammasat University of Bangkok (Thailand).
2009 Research period (6 months) at the Institute of Molecular Plant Sciences, University of Edinburgh (United Kingdom).
2004 Research period (2 months) at the Postharvest Unit of the IRTA – University of Lleida (Spain).
2002 Education period (3 months) at the Institute of Plant Disease – University of Bonn (Germany).
2000-2004 Editor-in-chief of the monthly "Informatore fitopatologico", IlSole-24Ore Group, Bologna, Italy.