Unraveling the mechanisms used by antagonistic yeast to control postharvest pathogens on fruit.

Unraveling the Mechanisms Used by Antagonistic Yeast to Control Postharvest Pathogens on Fruit

Davide Spadaro
DISAFA and AGROINNOVA
University of Torino
Grugliasco
Italy

Samir Droby
Department of Postharvest Science
ARO, The Volcani Center
Bet Dagan
Israel

Keywords: biofungicide, biological control, fruit, omics, rots, yeast.

Abstract

Biological control using microbial antagonists is one of the most promising alternatives for reducing fungicide use during the postharvest life of fruits. To date, there are hundreds of reports about using yeast antagonists to biologically control postharvest diseases. Very few of these antagonists, however, have reached the commercial development stage and launched as commercial products. In most cases, there are inherit problems in the biocontrol systems related to poor performance and inconsistency under commercial conditions. Among the reasons for these shortcoming is the lack of understanding of mechanisms of actions of these BCAs. A deep understanding of the mode of action is essential to develop appropriate formulation and methods of application, and to obtain registration. In recent years, there has been a phenomenal advancement in the use of molecular techniques contributing to the development of innovative tools for improving knowledge on the antagonistic mechanisms of BCAs. In particular, the omics techniques are providing a powerful tool to dissect the complex interactions between the antagonist, the pathogen, the fruit host, the natural microflora, and the environmental conditions.

INTRODUCTION

Postharvest losses due to pests and diseases on fruit during harvest, storage, transit, and commercialisation steps can reach 25% of the total production in industrialised countries. In developing countries, however, postharvest losses are often higher, exceeding 50%, because of the lack of adequate postharvest handling systems and storage structures. While several approaches were suggested for managing postharvest decay, chemical control of postharvest diseases, applied in orchard or after harvesting, is still the most widely used method. Increasing concerns, however, regarding residues of fungicides in the fruit, development of resistant biotypes of the pathogens, as well as risks associated with their continued use have prompted the search for safe and effective alternative strategies. Among these strategies, biological control based on naturally occurring microorganisms, has been the most studied. Different products reached advanced stages of development and commercialization. Among the antagonistic microorganisms used as BCAs against postharvest pathogens, a relatively high number of yeasts was reported and this is related to their features that make them effective as BCAs on fresh agricultural commodities as well as other foods (Spadaro et al., 2008). Yeasts are tolerant to extreme environmental conditions prevailing before and after harvest (low and high temperatures, desiccation, wide range of relative humidity, low oxygen levels, pH fluctuations, UV radiation). Furthermore, yeasts are uniquely adapted to the fruit micro-

environment, can grow rapidly on inexpensive substrates in fermenters, and are therefore easy to produce in large quantities. In addition, they do not produce allergenic spores or mycotoxins, in contrast to filamentous fungi, and they have simple nutritional requirements that enable them to colonize dry surfaces for long periods of time.

Elucidating the tri-trophic interactions in the biocontrol system

To successfully inhibit infection and development of the pathogen, several possible mechanisms operate in a tritrophic host-pathogen-antagonist interaction system, including antibiosis, mycoparasitism, production of lytic enzymes, induced resistance, biofilm formation, and competition for limiting nutrients and space. Often, more than one mechanism is involved. Furthermore, the role of BCAs in modulating the oxidative state of the wound is essential.

Advanced microbiological, microscopic, biochemical and molecular techniques are currently available and can be utilized effectively to improve our knowledge about mechanisms of action of microbial antagonists (Liu et al., 2013). The availability of more efficient DNA-based and proteomics technologies, along with bioinformatics, has provided new opportunities and tools to gain deeper and more accurate insights about the interactions already indicated. Developments in deep sequencing, transcriptomics, MS-MS proteomics, metagenomics, comparative and functional genomics can be utilized to determine changes in the physiological status of BCAs, and the effect of environmental stress on its intracellular machinery.

Competition for nutrients and space

Competition for nutrients and space has been considered the primary mode of action of yeast antagonistic microorganisms against postharvest fungal pathogens. Competition can be an effective biocontrol mechanism when the antagonist is present in sufficient amounts at the right time and location, and it is able to use limited resources more efficiently than the pathogen. Yeast can successfully compete with the pathogen at the wound site or *in vitro* for limiting nutritional factors, inhibiting its growth, but often leaving it alive. In the competition for space, yeasts usually have the advantage of rapid growth and formation of an extracellular polysaccharide capsule that can promote adhesion to fruit surface forming biofilms covering the entire wound area.

Competition for nutrients was demonstrated for *Pichia guilliermondii* against *P. digitatum* co-cultivated on synthetic medium (Droby et al., 1989). Yeast can satisfactorily use a wide range of carbohydrates, which include disaccharides and monosaccharides, and nitrogen sources (Spadaro et al., 2010). Competition for sugars and nitrates plays a key role in the interactions of *P. guilliermondii* with *B. cinerea* on apple. Recently, Kwasiborski *et al.* (2014) reported that during the exponential growth phase of *Pichia anomala*, and in presence of *B. cinerea*, the pentose phosphate pathway seems to be enhanced and would provide the needed nucleic acids and energy for wound colonization by the antagonist.

Iron is essential for fungal growth and pathogenesis, and competition for iron is believed to play a significant role in the biocontrol of postharvest pathogens. Siderophores are designed to form tight and stable complexes with ferric iron. Yeasts produce hydroxamate-type compounds, while bacteria produce hydroxamate as well as catecholate siderophores. Rhodotorulic acid is a dihydroxamate-containing siderophore produced by *Rhodotorula glutinis*, essential to improve the control of blue mold caused by *P. expansum* in apples. *Metschnikowia pulcherrima* and *Mestschnikowia fructicola* are

able to produce the red pigment pulcherrimin, formed non-enzymatically from pulcherriminic acid and ferric ions, which is involved in the control of *B. cinerea*, *Alternaria alternata* and *P. expansum* on apple (Saravanakumar et al., 2008). Iron depletion by the *M. pulcherrima* in the medium inhibited the mycelial growth and conidial germination of *B. cinerea*, *A. alternata*, and *P. expansum*. When iron was added at higher concentrations, the pathogen inhibition activity of *M. pulcherrima* disappeared and the colonies turned brown red. Furthermore, hyphae cracked when entering the pigmented zones around *M. pulcherrima* streaks, demonstrating that iron starvation elicits complex physiological changes in the fungal cells. Also *Metschnikowia fructicola* is able to produce pulcherrimin and to inhibit the growth of both *B. cinerea* and *Penicillium digitatum in vitro*.

Determining the role, the components and the factors involved in competition for nutrients and space in the biocontrol system is crucial for enhancing biocontrol efficacy of the antagonist. This information would be useful during upscale production and formulation. The supplementation of a limiting factor or essential nutrient for improved growth of the BCA may significantly contribute to its consistent performance against the pathogen on wide range of fruits and vegetables.

Biofilm formation and quorum sensing

To successfully colonize intact and injured fruit surfaces, the antagonist should have the ability to use specific features facilitating its adherence, colonization, and multiplication. In most cases this feature is associated with the formation of a biofilm, where micro colonies are enclosed in a hydrated matrix of microbe produced proteins, nucleic acids, and polysaccharides. In *Candida albicans* two families of adhesin genes showed to play a critical role in host cell recognition, adhesion, invasion, and biofilm formation. Aromatic alcohols exert different effects on morphogenesis in *S. cerevisiae* and *C. albicans*. Two quorum sensing (QS) regulatory molecules, tyrosol and farnesol, coordinating phenotype switching (yeast-to-hypha and *vice versa*), have been identified in *C. albicans*.

Little is known about the role of biofilms in the biocontrol activity of yeast antagonists used to manage postharvest diseases and the mechanisms involved in their formation. Experiments carried out on *Saccharomyces cerevisiae,* capable of forming a biofilm in liquid culture, demonstrated its effectiveness against some postharvest pathogens. The biocontrol activity of a biofilm-forming *S. cerevisiae* strain is tightly related to the morphological phase of cell harvesting after growing in liquid culture. Only yeast cells collected from the biofilm phase are effective in limiting pathogen growth, apparently being able to colonize more efficiently the inner surface of artificial wounds. In this relation, the ability to form biofilms and filamentous growth are often correlated (Ianiri et al., 2013). Interestingly, a strain of *Pichia fermentans*, which controls brown rot on apple fruit, showed to become a destructive pathogen when applied to peach fruit. On apple surfaces and within apple wounds, the antagonist retained its yeast-like shape whereas colonization of peach fruit tissue was always characterized by a transition from budding growth to pseudohyphal growth, suggesting that pseudohyphal growth could play a major role in governing the potential pathogenicity of *P. fermentans* on peaches (Giobbe et al., 2007). Phenylethanol, as a QS molecule, can induce *Kloeckera apiculata* to adhere and form biofilm on citrus fruit and embed in an extracellular matrix, by creating a mechanical barrier interposed between the wound surface and the pathogen (Pu et al., 2014).

We suggest that understanding the mechanisms of biofilm formation as well as the environmental cues regulating morphogenetic transformations in postharvest BCAs will lead to the selection of more effective antagonists and new methods of optimizing their activity.

Antimicrobial compounds

Certain yeast strains with a killer phenotype produce extracellular protein toxins designated as killer toxins or killer proteins, which are lethal to sensitive microbial cells belonging to either the same or a different species. Producers of these toxins are able to kill each other, but are immune to killer toxins of their own class. The most studied examples are the toxins K1, K2 and K28 of *Saccharomyces cerevisiae.* The killer toxins confer an ecological advantage to yeast cells over their competitors. Most killer toxins are stable and active at pH values ranging from 3 to 5.5, typical of wounded or injured fruits, and they are protease-sensitive and heat labile. *Pichia membranifaciens* can produce two killer toxins (PMKT and PMKT2) that are active against spoilage yeast and fungi (Santos et al., 2009). Though there is diversity in the mode of action of killer toxins, several killer toxins (K1, PMKT) seem to be membrane pore forming-related toxins.

Among the antibiotic metabolites, the most thoroughly studied example is farnesol from *C. albicans*, which can inhibit various bacteria and fungi. Another antifungal volatile substance, 2-phenylethanol, was isolated from *Kloeckera apiculata* and demonstrated to have antimicrobial activity against *P. italicum*. *A. pullulans* may produce aureobasidin A, a cyclic depsipeptide, with antifungal and antibiotic properties, particularly against *Botrytis* spp., *Monilinia* spp., and *Penicillium* spp. (Liu et al., 2007). Aureobasidin A is able to block the activity of inositol phosphorylceramide synthase, an essential enzyme for fungal sphingolipid biosynthesis.

The main concern, related to the use of antifungal and antibiotic compounds in food products, is the development of human pathogens resistant to these compounds and the possible development of resistance in fruit pathogens. Particular care should be taken in using BCAs producing antimicrobials on fruit, though antibiotic producers may be able to control also wound infections established before antagonist application.

Many fungi are known to produce low concentrations of volatile antifungal substances (Mari et al., 2012). Fungi capable of producing antifungal volatile organic compounds (VOCs) have the potential of being used as biofumigants and to be potential new BCAs for controlling postharvest diseases. The production of VOCs, including 2-phenethyl alcohol, could play an essential role in the antagonistic activity of *A. pullulans* against fruit postharvest pathogens (Di Francesco et al., 2015). *Candida intermedia* and *Sporodiobolus pararoseus* were able to suppress conidial germination and mycelial growth of *B. cinerea* and control grey mould of strawberry due to the release of a variety of VOCs, including 2-nonanone and 2-ethyl-1-hexanol (Huang et al., 2012).

VOC-producing microorganisms open new possibilities to control microbial decays in the agro-food chain, as biofumigation does not require physical contact with the product or commodity to be treated. Volatile compounds are ideal antimicrobials because their spectrum of activity extends from proximal interaction through water diffusion to greater distances via air diffusion.

Parasitism and release of cell wall hydrolases

Parasitism occurs when the antagonist attack the pathogen, resulting in a direct destruction or lysis of fungal propagules and structures. Wisniewski et al. (1991)

observed a strong adhesion *in vitro* of *P. guilliermondii* antagonist cells to *B. cinerea* mycelium, perhaps due to a lectin like interaction. Such adhesion was blocked by exposure to compounds able to alter the protein integrity and the respiration process.

In the fungal cell walls, chitin, serve as a structural backbone, is arranged in regularly ordered layers, and β-1,3-glucan as a filling material is arranged in an amorphous manner. In addition, proteins represent approximately 20–30% of the cell wall. Breakdown of fungal cell wall requires the participation of different enzymes, especially β-1,3-glucanase and chitinase, but also proteases. Glucanases, chitinases and proteases can be directly or indirectly involved in the mechanism of several yeast antagonists of postharvest pathogens.

Due to the assumed potential role of exo-β-1,3-glucanase in biocontrol systems, glucanase genes have been cloned and characterized from different yeast BCAs, including *C. oleophila*, *P. anomala,* and *P. guilliermondii* (Zhang et al., 2011). The contribution of exo-ß-1,3-glucanase to the biocontrol activity of *C. oleophila* was investigated by generating *CoEXG1*-knockouts and double-*CoEXG1* transformants: the control activity of the transformants against *P. digitatum* on kumquat fruit did not differ, however, from that of the wild-type strain (Yehuda et al., 2003). Different results were obtained when two exo-ß-1,3-glucanase genes of *P. anomala* – *PaEXG1* and *PaEXG2* – were separately and sequentially disrupted (Friel et al., 2007). The resulting mutant strains showed a significantly reduced efficiency of grey mould control when applied to wounded apple fruit, suggesting that exo-ß-1,3-glucanases play a role in antagonism.

A significant number of investigations were performed on chitinases produced by antagonistic yeast. Extracellular chitinase enzymes produced by strains of *M. pulcherrima* showed an inhibitory effect against *B. cinerea*. *Metschnikowia fructicola* exhibited chitinase activity and the chitinase gene MfChi was highly induced in the presence of *Monilinia fructicola* cell wall, suggesting a possible primary role of MfChi chitinase in the antagonistic activity of the yeast (Banani et al., 2015). The MfChi chitinase overexpressed in *Pichia pastoris* significantly controlled *Monilinia fructicol*a and *Monilinia laxa in vitro* and on peaches.

A. pullulans in apple and peach wounds releases extracellular glucanases, chitinases and proteases, that presumed to play a role in the antagonistic activity. An alkaline serine protease gene (ALP5) was cloned from *A. pullulans* and expressed in *Escherichia coli* (Zhang et al., 2012) and in *Pichia pastoris* (Banani et al., 2014). When the efficacy of ALP5 was evaluated against postharvest pathogens on apples, the protease was more efficient in controlling *Monilinia fructicola, B. cinerea* than *P. expansum* and *A. alternata*. However, the extent of the activity was dependent on the enzyme concentration and the length of fruit storage.

Induction of resistance

Yeast antagonists have the capability to interact with the host tissue, particularly the wounds, increasing the cicatrisation processes. These antagonists are much more effective when applied before pathogen inoculation. Yeast cells could induce resistance processes in fruit skin through elicitors that are either secreted or component of their cell wall.

Induction of several biochemical and molecular defence responses following the application of yeast BCAs to fruit surfaces have already been demonstrated in the past. In apple wounds, *A. pullulans* caused transient increases in β-1,3-glucanase, chitinase, and peroxidase activities. These increases started 24 h after treatment and reached maximum

levels at 48 h and 96 h (Ippolito et al., 2000). Application of *C. oleophila* to surface wounds or to intact grapefruit elicited systemic resistance against *P. digitatum* (Droby et al., 2002). The induction of pathogen resistance in fruit was pronounced already 24 h after elicitation; it was distance, yeast cells concentration, and time dependent, and it was restricted to the peel tissue closely surrounding the yeast application site. The induction of pathogen resistance required viable yeast cells at concentrations of 10^8 to 10^9 cells/ml. Non-viable autoclaved or boiled yeast cells or lower yeast concentrations were ineffective in enhancing fruit disease resistance. Application of *C. oleophila* cell suspensions to grapefruit peel tissue increased ethylene biosynthesis, phenylalanine ammonia lyase activity, phytoalexin accumulation, and increased chitinase and endo-β-1,3-glucanase protein levels. Using a proteomic approach, Chan et al. (2007) demonstrated that application of the yeast antagonist *P. membranifaciens* on peach fruits induced various proteins in fruit tissue including antioxidant proteins, such as glutathione peroxidase, catalase and peroxiredoxin, methionine sulfoxide reductase, polyphenol oxidase that are related to the repair of oxidative damage and to protect the tissue against oxidative damage and responsible for diseases resistance. In addition, *P. membranifaciens* increased activities of PR-9, PR-10, GTP-binding, and heat shock proteins.

Although all the results about induction of resistance responses in the host tissue following antagonist treatment are correlative, direct evidence for the ability of induced substances in fruit tissue to inhibit pathogen infection and development has not yet been established.

The role of oxidative stress

The production of ROS in plants is an initial response to microorganisms, both pathogenic and non-pathogenic. In the case of a non-compatible host–parasite interaction, an initial moderate increase in the production of ROS usually precedes a stronger oxidative burst, while in a compatible interaction no further increase in the level of reactive radicals in host tissue is observed.

Oxidative burst at the injury site following the colonization of antagonist cells was suggested to have a role in the mechanism of action of antagonistic yeasts and possibly involved in signalling pathways resulting in activation of fruit resistance systems. To play this role, antagonist cells must be able to tolerate alleviated levels of oxidative stress. In this regard, Castoria et al. (2003) were the first to report that postharvest biocontrol fitness of the yeast antagonists *C. laurentii* LS-28 and *Rhodotorula glutinis* was correlated with their ability to tolerate relatively high levels of ROS. These findings highlighted the role of oxidative stress in biocontrol systems and its possible direct and indirect effects either on the fruit tissue or on the antagonist cells at intercellular and intracellular level. Macarasin et al. (2010) demonstrated that yeast antagonists used to control postharvest diseases have the ability to produce relatively high amounts of super oxide anions. Interestingly, in this work, yeast applied to surface wounds of fruits produced higher amounts of super oxide anions than yeast grown *in vitro* in artificial media.

Superoxide anion production on the intact fruit surface could also serve as a QS signal to trigger aggregation into a biofilm which would increase yeast attachment and improve survival on the fruit surface by providing a microenvironment resistant to environmental stress. While the role of O_2^- in yeast cell multiplication, intercellular communication, or as an adaptive response to an unstable environment remains to be elucidated, results clearly show that, when yeasts sense host tissue, they are able to

produce and apparently tolerate high levels of $O2^{-}$, regardless of the availability of nutrients.

The potential of Omics to study antagonist-pathogen-host interactions

With the availability of high-throughput sequencing technologies, global changes in gene expression both in host tissue and antagonist cells have been reported. New omic tools can be used to evaluate the global effect of the application of BCAs on the transcriptome and/or proteome of fruit.

To obtain an overview on transcript modification during the interaction of cherry tomato fruit with *C. laurentii*, a microarray analysis was performed (Jiang et al., 2009). The results showed up-regulated genes in BCA-treated tomato fruit included genes involved in metabolism, signal transduction, and stress response. Conversely, genes related to energy metabolism and photosynthesis were generally down-regulated. BCA treatment induces fruit resistance response and it suppresses energy metabolism and photosynthesis. In grapefruit surface wounds treated with *Metschnikowia fructicola* cells, there was significant expression of PRPs genes and MAPK cascade genes involved in defence signalling, and down-regulation in antioxidant genes, like peroxidase, superoxide dismutase and catalase. The genes up-regulated by *Metschnikowia fructicola* in grapefruit were consistent with an induced resistance response and it was suggested that the induced response played a role in the efficacy of *Metschnikowia fructicola* against postharvest pathogens like *P. digitatum* (Hershkovitz et al., 2012). Hershkovitz et al. (2013) conducted a transcriptomic analysis, using RNA-Seq, to examine changes in gene expression in *Metschnikowia fructicola* when it was exposed to citrus tissues and the postharvest pathogen *P. digitatum*. Genes related to transmembrane, multidrug transport, and amino acid metabolism were induced in the yeast-pathogen interaction, while expression of genes involved in oxidative stress, iron homeostasis, zinc homeostasis, and lipid metabolism were induced in the yeast-fruit interaction.

Collectively, these reports indicate that different gene/protein profiles are involved in different antagonistic yeast–host–pathogen interactions, demonstrating the dynamics of different biocontrol system and how “omic” technologies can provide insights into the modes of action of antagonistic yeast. The above reported studies were the first to report molecular changes at the biocontrol system. It is expected however, that many more results will be reported in the near future about interactions between antagonistic yeast, host tissue, the pathogen, and also the epiphytic microflora.

CONCLUSIONS

To date, there are hundreds of reports about using of yeast antagonists to biologically control postharvest diseases. Very few of these antagonists, however, have reached the commercial development stage and launched as commercial products. In most cases, there are inherit problems in the biocontrol systems related to poor performance and inconsistency under commercial conditions. Among the reasons for these shortcoming is the lack of understanding of mechanisms of actions of these BCAs. It is apparent that the performance of yeast BCA is the result of complex interactions taking place between all the components of the biocontrol system (plant host, the antagonist, the pathogen, and resident microflora). Although these interactions have been the subject of research for over thirty years, our understanding is still incomplete. This because of the difficulties associated with the study of complex interactions and the lack of appropriate research tools and technologies.

In recent years there has been a phenomenal advancement in the use of molecular techniques contributing to the development of innovative tools for improving knowledge on the antagonistic mechanisms of BCAs. In particular, the omics techniques, including genomics, transcriptomics, proteomics, metagenomics, and metabolomics are providing a powerful tool to dissect the complex interactions between the antagonist, the pathogen, the fruit host, the natural microflora, and the environmental conditions. Induced resistance has been suggested to be one of the mechanisms of action of postharvest BCAs. However, information about elicitors/effectors of the antagonist involved and our ability to genetically and physiologically manipulate them is still lacking. Fundamental knowledge on the physiology, genetic traits, and molecular basis of colonization, survival and differentiation of BCAs on plant tissue is needed.

From a commercial point of view, complex modes of action make antagonistic performance and efficacy more dependent on production, formulation, packing, application, and storage. A deep understanding of the mode of action is essential to develop appropriate formulation and methods of application, and to obtain registration.

Literature cited

Banani, H., Spadaro, D., Zhang, D., Matic, S., Garibaldi, A. and Gullino, M.L. 2014. Biocontrol activity of an alkaline serine protease from *Aureobasidium pullulans* expressed in *Pichia pastoris* against four postharvest pathogens on apple. Int. J. Food Microbiol. 182-183:1-8.

Banani, H., Spadaro, D., Zhang, D., Matic, S., Garibaldi, A. and Gullino, M.L. 2015. Postharvest application of a novel chitinase cloned from *Metschnikowia fructicola* and overexpressed in *Pichia pastoris* to control brown rot of peaches. Int. J. Food Microbiol. 199:54-61.

Castoria, R., Caputo, L., De Curtis, F. and De Cicco, V. 2003. Resistance of postharvest biocontrol yeasts to oxidative stress: A possible new mechanism of action. Phytopathology 93:564-572.

Chan, Z., Qin, G., Xu, X., Li, B. and Tian, S. 2007. Proteome approach to characterize proteins induced by antagonist yeast and salicylic acid in peach fruit. J. Proteome Res. 6:1677-1678.

Di Francesco, A., Ugolini, L., Lazzeri, L. and Mari, M. 2015. Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. Biol. Control 81:8-14.

Droby, S., Chalutz, E., Wilson, C.L. and Wisniewski, M.E. 1989. Characterization of the biocontrol activity of *Debaryomices hansenii* in the control of *Penicillium digitatum* on grapefruit. Can. J. Microbiol. 35:794-800.

Droby, S., Vinokur, V., Weiss, B., Cohen, L., Daus, A., Goldschmidt, E.E., et al. 2002. Induction of Resistance to *Penicillium digitatum* in Grapefruit by the Yeast Biocontrol Agent *Candida oleophila.* Phytopathology 92:393-399.

Friel, D., Pessoa, N.M.G, Vandenbol, M. and 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. Mol. Plant-Microbe Interact. 20:371-379.

Giobbe, S., Marceddu, S., Scherm, B., Zara, G., Mazzarello, V.L., Budroni, M., et al. 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 Res. 7:1389-1398.

Hershkovitz, V., Ben-Dayan, C., Raphael, G., Pasmanik-Chor, M., Liu, J., Belausov, E., et al. 2012. Global changes in gene expression of grapefruit peel tissue in response to the yeast biocontrol agent *Metschnikowia fructicola.* Mol. Plant Pathol. 13:338–349.

Hershkovitz, V., Sela, N., Taha-Salaime, L., Liu, J., Rafael, G., Kessler, C., et al. 2013. De-novo assemble and characterization of the transcriptome of *Metschnikowia fructicola* reveals differences in gene expression following interaction with *Penicillium digitatum* and grapefruit peel. BMC Genomics 14:168.

Huang, R., Che, H.J., Zhang, J., Yang, L., Jiang, D.H. and Li, G.Q. 2012. Evaluation of *Sporidiobolus pararoseus* strain YCXT3 as biocontrol agent of *Botrytis cinerea* on post-harvest strawberry fruits. Biol. Control 62:53-63.

Ianiri, G., Idnurm, A., Wright, S.A.I., Duran-Patron, R., Mannina, L., Ferracane, R., et al. 2013. Searching for genes responsible for patulin degradation in a biological control yeast provides insights into the basis for resistance to this mycotoxin. Appl. Environ. Microbiol. 79:3101-3115.

Ippolito, A., El Ghaouth, A., Wilson, C.L. and Wisniewski, M. 2000. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. Postharvest Biol. Technol. 19:265-272.

Jiang, F., Zheng, X. and Chen, J. 2009. Microarray analysis of gene expression profile induced by the biocontrol yeast *Cryptococcus laurentii* in cherry tomato fruit. Gene 430:12-16.

Kwasiborski, A., Bajji, M., Renaut, J., Delaplace, P. and Jijakli, H. 2014. Identification of metabolic pathways expressed by *Pichia anomala* Kh6 in the presence of the pathogen *Botrytis cinerea* on apple: New possible targets for biocontrol improvement. PLoS ONE 9 (3):e91434. doi:10.1371/journal.pone.0091434

Liu, J., Sui, Y., Wisniewski, M., Droby, S. and Liu, Y. 2013. Review: Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. Int. J. Food Microbiol. 167:153–160.

Liu, X., Wang, J., Gou, P., Mao, C., Zhu, Z.-R. and Li, H. 2007. *In vitro* inhibition of postharvest pathogens of fruit and control of gray mold of strawberry and green mold of citrus by aureobasidin A. Int. J. Food Microbiol. 119:223-229.

Macarisin, D., Droby, S., Bauchan, G. and 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 Biol. Technol. 58:194-202.

Mari, M., Martini, C., Spadoni, A., Rouissi, W. and Bertolini, P. 2012. Biocontrol of apple postharvest decay of *Aureobsidium pullulans.* Postharvest Biol. Technol. 73:56-62.

Pu, L., Jingfan, F., Kai, C., Chao-an, L. and Yunjiang, C. 2014. Phenylethanol promotes adhesion and biofilm formation of the antagonistic yeast *Kloeckera apiculata* for the control of blue mold on citrus. FEMS Yeast Res. 14:536–546.

Santos, A., San Mauro, M., Bravo, E. and Marquina, D. 2009. PMKT2, a new killer toxin from *Pichia membranifaciens*, and its promising biotechnological properties for control of the spoilage yeast *Brettanomyces bruxellensis*. Microbiol. 155:624–634.

Saravanakumar, D., Ciavorella, A., Spadaro, D., Garibaldi, A. and Gullino, M.L. 2008. *Metschnikowia pulcherrima* strain MACH1 outcompetes *Botrytis cinerea*, *Alternaria alternata* and *Penicillium expansum* in apples through iron depletion. Postharvest Biol. Technol. 49:121-128.

Spadaro, D., Sabetta, W., Acquadro, A., Portis, E., Garibaldi, A. and Gullino, M.L. 2008. Use of AFLP for differentiation of *Metschnikowia pulcherrima* strains for postharvest disease biological control. Microbiol. Res. 163:523-530.

Spadaro, D., Ciavorella, A., Zhang, D., Garibaldi, A. and 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. Can. J. Microbiol. 56:128–137.

Wisniewski, M.E., Biles, C., Droby, S., McLaughlin, R., Wilson, C.L. and Chalutz, E. 1991. Mode of action of the postharvest biocontrol yeast, *Pichia guilliermondii*. I. Characterization of attachment to *Botrytis cinerea*. Physiol. Mol. Plant Pathol. 39:245-258.

Yehuda, H., Droby, S., Bar-Shimon, M., Wisniewski, M. and Goldway, M. 2003. The effect of under- and overexpressed *CoEXG1*-encoded exoglucanase secreted by *Candida oleophila* on the biocontrol of *Penicillium digitatum*. Yeast, 20:771-780.

Zhang, D., Spadaro, D., Valente, S., Garibaldi, A. and Gullino, M.L. 2011. Cloning, characterization and expression of an exo-1,3-beta-glucanase gene from the antagonistic yeast, *Pichia guilliermondii* strain M8 against grey mold on apples. Biol. Control 59:284-293.

Zhang, D. Spadaro, D., Valente, S., Garibaldi, A. and Gullino, M.L. 2012. Cloning, characterization, expression and antifungal activity of an alkaline serine protease of *Aureobasidium pullulans* PL5 involved in the biological control of postharvest pathogens. Int. J. Food Microbiol. 153:453-464.