Contents lists available at ScienceDirect

Postharvest Biology and Technology

Postharvest biocontrol ability of *Pseudomonas synxantha* against *Monilinia fructicola* and *Monilinia fructigena* on stone fruit

Dalia Aiello[a], Cristina Restuccia[a], Emilio Stefani[b], Alessandro Vitale[a,*], Gabriella Cirvilleri[a]

[a] *Department of Agriculture, Food and Environment, University of Catania, via S. Sofia 100, 95123, Catania, Italy*
[b] *Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, 42122, Reggio Emilia, Italy*

ARTICLE INFO

ABSTRACT

The biocontrol properties of the endophyte *Pseudomonas synxantha* DLS65 were tested *in vitro* and *in vivo* against *Monilinia fructicola* and *Monilinia fructigena*, causal agents of postharvest brown rot of stone fruit. *P. synxantha* cells significantly reduced the mycelial growth of both pathogens on Potato Dextrose Agar (PDA), and strongly inhibited the *Monilinia fructicola* growth on Peach Extract Agar (PEA). Cell-free culture filtrates inhibited the pathogens on PDA and PEA to lesser extent. The production of volatile organic compounds (VOCs), with *in vitro* inhibitory effects on mycelial growth, was also observed. *P. synxantha* significantly reduced brown rot incidence and severity on peach fruit artificially inoculated with *M. fructicola* after 5 d at 25 °C. Moreover, *P. synxantha* more significantly reduced incidence and severity after 10 d at 10 °C and after 20 d in cold storage at 0 °C in comparison to control fruit, even if its activity was never comparable to that of the synthetic fungicide Scholar® (fludioxonil). Similarly, *P. synxantha* exhibited an excellent antagonistic activity against *M. fructigena* on fruit at 10 and at 0 °C, and a weak biocontrol activity at 25 °C. Competition for nutrients and space, production of diffusible toxic metabolites and VOCs may play a role in the antagonism of *P. synxantha* toward *M. fructicola* and *M. fructigena*, especially at the lowest temperatures of storage. For that reason, this strain of *P. synxantha* could be suggested as active ingredient for the setting up of bioformulates against *Monilinia* species representing a limiting factor for stone fruit production.

1. Introduction

Peach and nectarine represent important crops widely cultivated in Europe with 259.286 ha of area harvested and a production of 4,373,494 tons in 2016 (FAOSTAT, 2016). Approximately 33% of this production is mainly concentrated in Italy, one among the most important country in European area. Unfortunately, the cultivation of stone fruit species including peach (*Prunus armeniaca*) is heavily threatened by brown rot disease caused by *Monilinia* species. In detail, three species are considered 'key' postharvest pathogens for stone fruit i.e. *Monilinia fructicola* (Winter) Honey, *Monilinia fructigena* (Aderhold and Ruhland) and *Monilinia laxa* (Aderhold and Rulhland) Honey. These fungal species infect blooms, twigs and fruit in the field, but prevalent damages occur in the postharvest stage during storage, shipping and marketing inducing up to 80% yield losses under favorable conditions to disease development (Usall et al., 2015).

Up to recent days, fungicide applications are needed in orchards but different factors i.e. the arising of fungicide resistance and relative in field breakdown efficacy, latent infections, and the fungal capacity to

develop at low temperatures could strongly limit their performances (Miessner and Stammler, 2010). Moreover, the recent EuropeanDirective on "Sustainable Use of Pesticide" encouraged developing of alternative control methods (Lopez-Reyes et al., 2013; Sisquella et al., 2014). Therefore, numerous scientific papers and world-wide programs focused on isolation of biological control agents (BCAs) effective against several postharvest fungal pathogens, including species belonging to *Botrytis, Monilinia* and *Penicillium* genera (Mari et al., 2012; Panebianco et al., 2015; Parafati et al., 2015; Parafati et al., 2017; Platania et al., 2012; Restuccia et al., 2006; Scuderi et al., 2009; Zhang et al., 2010a, b) on numerous foodstuffs. For example, many bacteria have also shown a good biocontrol potential, including *Rahnella aqualitis* (Calvo et al., 2007), *Bacillus amyloliquefaciens* (Calvo et al., 2017), *Pseudomonas cepacia* (Janisiewicz and Roitman, 1988), *P. syringae* (Cirvilleri et al., 2005; Panebianco et al., 2015), *Burkholderia gladioli* (Scuderi et al., 2009), and *P. fluorescens* (Wallace et al., 2017). In detail, the history of biological control in postharvest began when Pusey and Wilson (1984) successfully proposed a bacterium recovered from soil to manage brown rot on stone fruit caused by *Monilinia fructicola*. Since

---

* Corresponding author at: Department of Agriculture, Food and Environment, University of Catania, via S. Sofia 100, 95123, Catania, Italy.
 E-mail address: alevital@unict.it (A. Vitale).

then, more papers were produced about potential use of alternatives to control postharvest diseases of stone fruit although only few of them are currently applied under commercial conditions (Usall et al., 2015).

Currently, no bioformulate containing BCAs is registered for *Monilinia* postharvest decays of peach whereas only an active ingredient (a.i.) - fludioxonil (Scholar®) - is used in Italy for managing postharvest *Monilinia* decay. This molecule is a derivative of pyrrolnitrin, an antibiotic initially isolated from *Pseudomonas* species with high antagonistic activity versus postharvest fungal decays (Arima et al., 1964; Janisiewicz and Roitman, 1988). *Pseudomonas* species are widely distributed in the rhizosphere, and are able to promote the plant growth and to counteract plant diseases. Some of these antagonistic bacterial species have been often isolated from natural environment in which they compete with pathogenic epiphytic microorganisms (Tontou et al., 2016a, b). Several characteristics of *Pseudomonas* spp. can explain the dominance and persistence of this genus in a large range of environments, such as frequent introduction into the food production environment and its ability to survive cleaning and disinfection and to grow even with low levels of nutrients and at low temperatures (Moore et al., 2006). Among *Pseudomonas* species, *P. synxantha* produces a bioactive compound, a long chain aliphatic hydrocarbon with a terminal double bond and intermediate electronegative atom with activity similar to bio-surfactant molecule, which is effective against several strains of Mycobacteria (Mukherjee et al., 2014). In addition, *P. synxantha* strain DLS65, isolated from vascular tissues of *Actinidia* spp. plants, showed strong *in vitro* inhibition activity against Gram + and Gram -phytopathogenic bacteria (Tontou et al., 2016a). Therefore the aims of the present study were i) to determine antagonistic activity of DLS65 strain against *Monilia fructicola* and *Monilia fructigena in vitro* and ii) to evaluate the potential biocontrol activity of brown rot caused by these pathogens in postharvest on peach fruit by comparison with a standard fungicide.

2. Materials and methods

2.1. Pseudomonas strain

P. synxantha DLS65, isolated from kiwi tissues in the region of Emilia Romagna, Italy (Tontou et al., 2016a), and genotipically characterized (Tontou et al., 2016b), was maintained in 15% glycerol at –80 °C and subcultured on Nutrient Agar medium (NA, Oxoid, Basingstoke, UK) at 25 ± 1 °C.

2.2. Pathogens

Two strains of *Monilinia* spp., i.e. *M. fructicola* and *M. fructigena* were employed for this study. *Monilinia fructicola* was isolated from rotten peach fruit in South Italy (Sicily), preliminarily identified and maintained in collection belonging to Dipartimento di Agricoltura, Alimentazione e Ambiente (Di3A, University of Catania) while *M. fructigena* (strain number 2138) was provided by Bank of Plant Pathogens (Environmental Protection Institute in Poznan, Poland) (Grzegorczyk et al., 2017). To maintain their virulence both pathogenic strains were routinely recovered from inoculated peach fruit, and the monoconidial isolates of both fungi were used in *in vitro* and *in vivo* experiments. Fungal strains were stored on Potato Dextrose Agar (PDA, Oxoid) slants at 4 °C.

2.3. In vitro antagonistic activity of P. synxantha cells

P. synxantha DLS65 was tested for its inhibitory activity against *M. fructicola* and *M. fructigena* on PDA and on Peach Extract Agar (PEA) (100 g L^{-1} of peach pulp extract; 20 g L^{-1} of agar). Fungi to be tested were grown on PDA for 10 d at 25 °C. An aliquot (1 mL) of *P. synxantha* DLS65 suspension, obtained from 2-day-old culture on NA (approximately 1×10^9 CFU mL^{-1} on phosphate buffer), was added to 14 mL of fluid PDA/PEA media maintained at 45 °C. After mixing and agar solidification, an agar plug (5 mm diameter) from actively growing margins of *M. fructicola* and *M. fructigena* colonies was placed at the center of PDA/PEA plates 1 h (co-inoculation) and 24 h (differed inoculation) after DLS65 application. The control samples consisted of PDA/PEA Petri plates with only fungal inoculums. After incubation at 25 °C for 6 d, the diameter of the fungal mycelium was measured. Each experiment was conducted twice and included four replicates per treatment.

2.4. In vitro inhibitory activity of P. synxantha cell-free culture filtrates

P. synxantha DLS65 culture filtrates were prepared as reported by Cirvilleri et al. (2005) partially modified. *P. synxantha* DLS65 was grown overnight in NA and 1 mL of bacterial suspension (approximately 1×10^9 CFU mL^{-1}) was added to 100 mL of Nutrient Broth (NB, Oxoid). After 2 d of incubation at 25 °C in still culture and centrifugation ($9000 \times g$ for 20 min) of bacterial suspensions, the supernatant was passed through a 0.22 μm Millipore filter (Millipore, Billerica, MA, USA) to obtain cell-free culture filtrates. Aliquots (1 mL) of culture filtrates were added to 14 mL of fluid PDA/PEA maintained at 45 °C. After mixing and agar solidification, agar plugs of *M. fructicola* and *M. fructigena* were placed at the center of plates 1 h (co-inoculation) and 24 h (differed inoculation) after DLS65 culture filtrate application. After incubation at 25 °C for 6 d, the diameter of the fungal mycelium was measured. Each experiment was conducted twice and included four replicates per treatment.

2.5. In vitro effect evaluation of volatile organic compounds

To test the activity of volatile organic compounds (VOCs) produced by bacterial strain in reducing mycelial growth of both fungi a dual culture technique was employed according to recent studies (Grzegorczyk et al., 2017; Parafati et al., 2017). Amounts of 100 μL of bacterial suspension (1×10^9 CFU mL^{-1}) were cultured on PDA plates and incubated at 25 °C for 48 h. Subsequently, agar plugs (5-mm in diameter) from active growing mycelium of *M. fructicola* and *M. fructigena* were put on the center of plates. The PDA dishes with pathogens were individually covered face to face under plates containing 48 h bacterial strain cultures. The control consisted of bacteria-unseeded PDA plates. The two plates were wrapped together with Parafilm around the edges to prevent air leakage, and incubated at 25 °C. Diameter of the fungal mycelium and radial growth reduction was calculated after 6 d of incubation as previously described. All experiments were repeated twice and included four replicates per treatment.

2.6. In vivo biocontrol activity of P. synxantha DLS65

To assess the *in vivo* efficacy of *P. synxantha* DLS65, experiments hereinafter referred as experiment I were carried out, and the method described by Panebianco et al. (2015) and Grzegorczyk et al. (2017) with modifications was used. Peach fruit [*Prunus persica* (L.) Batsch.] 'Leonforte' (Sicilian variety awarded by the European Community with label "Protected Geographical Indication") used in this study were recovered from commercial orchard in Sicily (Italy), and they have not received any in field (pre-harvest) fungicide treatment. Homogenous and healthy fruit were selected and randomly assigned to different treatments. Prior to treatment and inoculation, peach fruit were washed, disinfected and wounded as it was recently reported by Grzegorczyk et al. (2017). Bacterial strain was grown in NB medium for 48 h at 25 °C. Resulting suspension was centrifuged at 9000 g for 10 min, suspended in phosphate buffer and concentration was adjusted to 10^8 CFU mL^{-1}. Each wound was inoculated with 20 μl of *P. synxantha* DLS65 and allowed to dry. Fruit were put onto perforated aluminum support and placed into plastic trays containing wet paper towels. The plastic containers were put into plastic bags, hermetically sealed to avoid air dispersal, and incubated at 25 °C. Following 24 h, treated

wounded sites were inoculated with mycelia disks (5-mm in diameter) of active growing mycelium of *M. fructicola* and *M. fructigena*. Fruit were then incubated for 5 and 10 d at 25 °C and 10 °C, respectively. Chemical control was represented by fruit treated with a suspension containing 130 mL/100 L of fludioxonil (Scholar®, Syngenta; 23.0% a.i.). Peach fruit treated with sterile distilled water (SDW) and only with fungal inocula were included as controls. Three replicates of 9 peach fruit were used for each treatment (27 fruit/treatment). The experiments were performed twice. Data concerning disease incidence (DI) (percentage of decayed fruit) and disease severity (DS) (diameter of infected area around the wounds) were calculated as the average of each replicate. Data concerning the disease incidence (DI) were transformed into arcsine square root values to normalize the distribution before performing the analysis of variance.

2.7. Effect of low storage temperature on biocontrol efficacy

To determine the effect of low storage temperatures on biocontrol efficacy, experiments hereinafter referred as experiments II were carried out. To this aim, the artificially wounded peach fruit were treated with 20 μL of 1×10^8 CFU mL^{-1} of *P. synxantha* DLS65. After 24 h, mycelial disks (5-mm square plugs) of actively growing fungal mycelium of *M. fructicola* and *M. fructigena* were inoculated as described above. Chemical control was represented by fruit treated with a suspension containing 130 mL/100 L of Scholar. Fruit treated with SDW and only with fungal inocula were included as control. Treated fruit were stored at 0 °C for 20 d followed by 4 d at 25 °C. The incidence and severity of disease was calculated as previously described. Three replicates of 9 peach fruit were used for each treatment (27 fruit/treatment). The experiment was performed twice.

2.8. Statistical analysis

Data from *in vitro* and *in vivo* experiments were analyzed separately by using the factorial ANOVA module of Statistica 10 software package (StatSoft Inc, Tulsa, OK, USA) to determine significant differences among the tested treatments *in vitro* and *in vivo* performances against *M. fructicola* and *M. fructigena*. Initial analyses were conducted by calculating *F* and *P* values associated for all experiments to evaluate any significant treatment × species, treatment × temperature, treatment × species × temperature interactions. Thus, in all repeated experiments, arithmetic means of mycelial growth, disease incidence (DI) and disease severity (DS) were calculated, averaging the values determined for the single replicates of each treatment. Percentage data concerning DI were previously transformed using the arcsine transformation (sin^{-1} square root x). Post-hoc comparisons among different treatments were achieved by means of the Fisher's least significant difference test.

3. Results

3.1. In vitro antagonistic activity of P. synxantha cells

P. synxantha DLS65 was able to inhibit the mycelial growth of *M. fructicola* and *M. fructigena* with variable efficacy in a substrate-dependent manner (Table 1; Figs. 1 and 2). A complete fungal growth inhibition (100%) of both pathogens was obtained on PDA, both in co-inoculation and in differed-inoculation treatments. Otherwise, on PEA medium the growth of *M. fructicola* and *M. fructigena* was partially reduced, with *M. fructicola* inhibited more efficiently (49.8% fungal growth reduction) than *M. fructigena* (24.9% fungal growth reduction) in differed inoculation treatment (*significant data*). The worst results were obtained on PEA in co-inoculation treatments, with 42.5% and 0% of fungal growth reduction, respectively, for *M. fructicola* and *M. fructigena* (Figs. 1 and 2).

Table 1

In vitro inhibition activity of *P. synxantha* DLS65 cells on mycelial growth of *Monilinia fructicola* and *M. fructigena* after 6 d at 25 °C on PDA and PEA media.

| | Mycelial diameter (mm) | | | |
| | *Monilinia fructicola*[a] | | *Monilinia fructigena*[a] | |
Treatment (h)[b]	PDA	PEA	PDA	PEA
P. synxantha (1 h)	0 b	54.6 b	0 b	43.7 a
P. synxantha (24 h)	0 b	47.7 b	0 b	30.8 b
Control	72.7 a	95 a	64.2 a	41 a

[a] Each value represents the mean of 4 replicates, each formed by 3 Petri dishes. Data followed by different letters within each column are significantly different according to Fisher's least significant difference test (α = 0.05).

[b] 1 h = antagonist applied simultaneously to pathogen (co-inoculation); 24 h = antagonist applied 24 h prior to pathogen (differed inoculation).

3.2. In vitro inhibitory activity of P. synxantha cell-free culture filtrates

Cell free culture filtrates of *P. synxantha* showed variable inhibition activity against *M. fructicola* and *M. fructigena* (Table 2). The effectiveness of culture filtrates was significantly higher when they were simultaneously applied (co-inoculation) with *M. fructicola* (21.8% growth reduction in PDA; 26.7% growth reduction in PEA), whereas reduction data were significantly lower (15.7% growth reduction in PDA) or similar to the control (20.1% growth reduction in PEA) when they were applied 24 h prior to pathogens (differed inoculation). Culture filtrates inhibited less efficiently *M. fructigena,* both on PDA and PEA, in co- and differed inoculation, with reduction data similar to the control. Overall, *P. synxantha* culture filtrate never showed higher inhibitory activity if compared with bacterial cells.

3.3. In vitro production of volatile organic compounds (VOCs)

As shown in Table 3, there were no treatment × fungal species interactions (*F* = 2.3, *P* = 0.17), thus indicating that VOCs produced by *P. synxantha* showed similar performances against both targeted species. For this reason, the data referring to *M. fructicola* and *M. fructigena* were combined and analyzed together. In detail, VOCs were always able to reduce significantly the mycelial growth of *M. fructicola* and *M. fructigena* if compared with relative controls (Table 3). Averagely, these data show a lesser activity of VOCs than those of bacterial cells.

3.4. In vivo biocontrol activity of P. synxantha DLS65

Significant effects were detected in experiment I for two interactions (treatment × temperature and treatment × species × temperature on disease severity) (Table 4).

For this reason, the trials were analyzed separately for targeted species and temperature and post-hoc comparisons among different treatments were achieved by means of the Fisher's least significant difference test (Table 5).

Treatments with *P. synxantha* DLS65 reduced the incidence and severity of brown rot in wounded fruit of peaches inoculated with *M. fructicola* and *M. fructigena* and stored at 25 °C and at 10 °C for 5 d and for 10 d, respectively (Table 5; Figs. 3 and 4).

After storage at 25 °C for 5 d, *P. synxantha* significantly reduced the disease incidence and severity caused by *M. fructicola* on peach fruit by 50% and 76% respectively, whereas it was less effective against *M. fructigena* reducing significantly only severity by 70%.

Following storage at 10 °C, *P. synxantha* showed the best performances being always able to significantly reduce disease incidence and severity caused by both pathogens. Moreover, the antagonist was more effective against *M. fructicola* (68% DI reduction; 92% DS reduction) than against *M. fructigena* (55% DI reduction; 90% DS reduction).
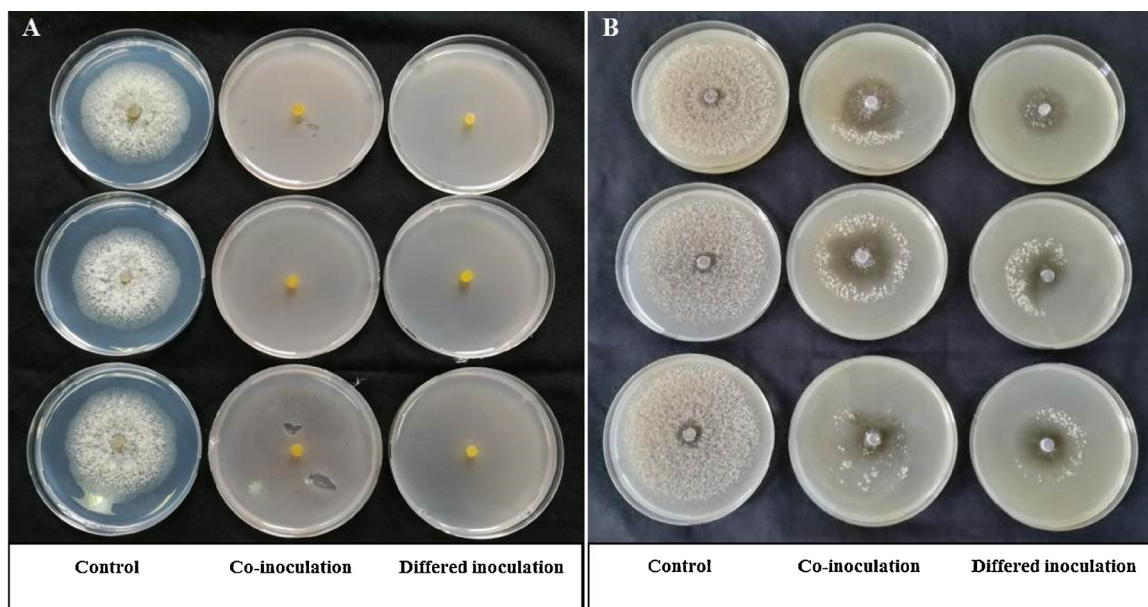
Fig. 1. Reduction of mycelial growth diameter of *Monilinia fructicola* induced by *Pseudomonas synxantha* on potato dextrose agar (A) and peach extract agar (B), respectively in the tested treatments.

However, the fungicide (Scholar) applications always showed the best performances in reducing (100%) incidence and severity of brown decay caused by *M. fructicola* and *M. fructigena* at two different temperatures.

3.5. Effect of low storage temperature on biocontrol efficacy

Since the interactions (treatment × species) detected in experiment II (0 °C for 20 d followed by 25 °C for 4 d) were not significant (Table 4), the data obtained for each pathogen were combined (Table 6). As clearly shown in this table, a good efficacy was detected with antagonist application at 0 °C for 20 d followed by a 4-day storage period at 25 °C, since *P. synxantha* significantly reduced disease incidence and severity caused by both fungi by about 63.9% and 78.5%, respectively. Also under these conditions (0 °C) fludioxonil application showed the

best performances in reducing decay amount (*significant data*; Fig. 5).

4. Discussion

A possible and safe alternative to manage *Monilinia* fruit decays is biological control using antagonistic microorganisms often isolated from food sources and from natural environment in which they compete with pathogenic epiphytic microorganisms (Grzegorczyk et al., 2017; Tontou et al., 2016a). Also bacterial endophytes, which provide important benefits to plants, might be deserved for developing innovative and sustainable biocontrol strategies (Compant et al., 2005). To this regard, the present study evaluated the antagonistic activity of *P. synxantha* DLS65, isolated from vascular tissues of *Actinidia* spp. plants, against *M. fructigena* and *M. fructicola*.

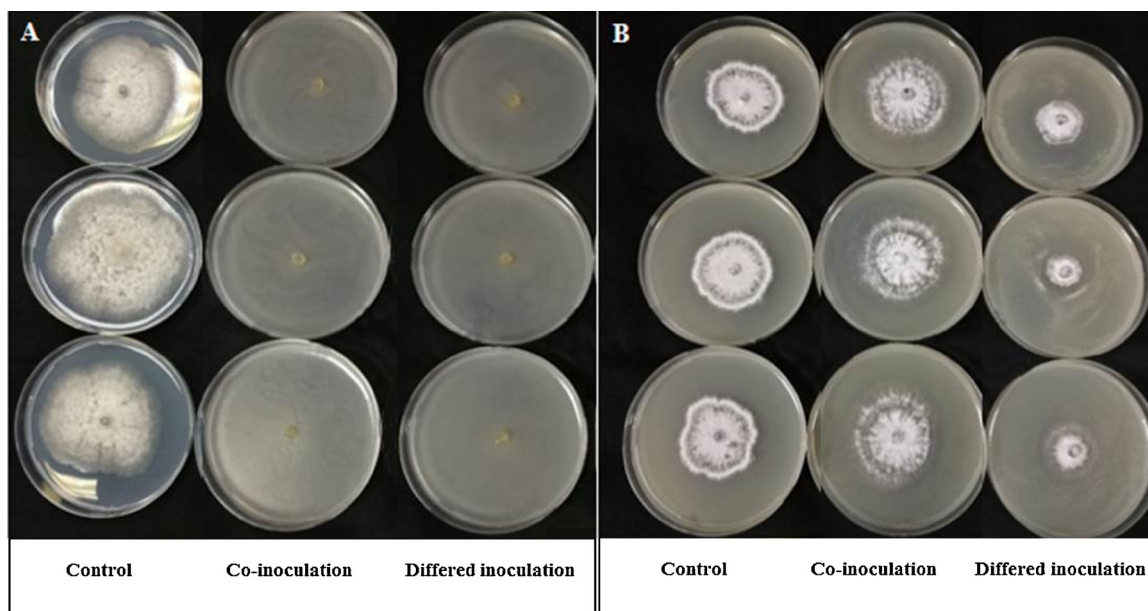In *in vitro* experiments, *P. synxantha* strain totally inhibited mycelial

Fig. 2. Reduction of mycelial growth diameter of *Monilinia fructigena* induced by *Pseudomonas synxantha* on potato dextrose agar (A) and peach extract agar (B), respectively in the tested treatments.

Table 2

In vitro inhibition activity of *P. synxantha* DLS65 cell-free culture filtrates on mycelial growth of *M. fructicola* and *M. fructigena* after 6 d at 25 °C on PDA and PEA media.

	Mycelial diameter (mm)			
	Monilinia fructicola[a]		*Monilinia fructigena*[a]	
Treatment (h) [b]	PDA	PEA	PDA	PEA
P. synxantha (1 h)	59.2 c	68.7 b	55.9 b	41.9 ns
P. synxantha (24 h)	63.8 b	74.1 b	63.8 a	42.7 ns
Control	75.7 a	93.7 a	65.1 a	42.4 ns

[a] Each value represents the mean of 4 replicates, each formed by 3 Petri dishes. Data followed by different letters within each column are significantly different according to Fisher's least significant difference test ($\alpha = 0.05$).

[b] 1 h = antagonist applied simultaneously to pathogen (co-inoculation); 24 h = antagonist applied 24 h prior to pathogen (differed inoculation).

Table 3

In vitro inhibition activity of VOCs produced by *P. synxantha* DLS65 and treatment × target pathogen interactions on mycelial growth of *Monilinia fructicola* and *Monilinia fructigena* on PDA medium.

	ANOVA effects[a]	
Source of variation	*F*	*P* value
Treatment	77.8	0.000022 **
Targeted species	22.9	0.001377 *
Trt × species	2.3	0.17 ns
	Average mycelial diameter of *Monilinia* spp. (mm)[b]	
P. synxantha	60.7b	
Control	72.6a	

[a] *F* test of fixed effects and *P* value associated to *F*; *, ** = significant at $0.001 < p < 0.01$ and $p < 0.001$, respectively; ns = not significant.

[b] Each value represents the mean of 4 replicates, each formed by 3 Petri dishes with fungal pathogen. Data followed by different letters within each column are significantly different according to Fisher's least significant difference test ($\alpha = 0.05$).

Table 5

Post-hoc analyses of treatment effects on disease incidence (DI) and severity (DS) of fruit decays caused by target *Monilinia fructicola* and *Monilinia fructigena* in the experiment I at T = 25 °C and at T = 10 °C.

	Monilinia fructicola[a]		*Monilinia fructigena*[a]	
Treatment[a]	DI (%)	DS (mm)	DI (%)	DS (mm)
Temperature = 25 °C				
Fludioxonil	0.0 c	0.0 c	0.0 b	0.0 c
P. synxantha	50.0 b	13.9 b	88.9 a	14.3 b
Control	100 a	57.1 a	100 a	47.5 a
Temperature = 10 °C				
Fludioxonil	0 c	0 c	0 c	0 b
P. synxantha	27.8 b	2.5 b	44.43 b	4.4 b
Control	88.87 a	31.2 a	100 a	43.5 a

[a] Each value represents the mean of 3 replicates, each constituted by 9 fruit. Data followed by different letters within each column are significantly different according to Fisher's least significant difference test ($\alpha = 0.05$).

compared to filtrates and VOCs clearly suggested that *P. synxantha* acts, besides by antibiosis and VOCs, also by other mechanisms involving the presence of living cells (e.g. competition for the space and nutrients, etc.).

Comprehensively, *P. synxantha in vivo* applications effectively managed *Monilinia* peach decays under all storage temperatures. However, the best performances of *P. synxantha* in controlling brown rot were recorded at the lowest storage temperatures considered. At 10 °C, *P. synxantha* had a slightly higher activity against *M. fructicola* than against *M. fructigena*, whereas at 0 °C antagonistic bacterium performances were similar toward both phytopathogenic fungi. Otherwise, at 25 °C storage conditions, *P. synxantha* applications revealed the least performances in managing Monilinia rots and in one case the bacterium failed in reducing the number of peach infections caused by *M. fructigena*. *P. synxantha* is a fluorescent bacterium placed within *Pseudomonas fluorescens* group (Wechter et al., 2002). Past researches demonstrated the efficacy of this bacterium as a biological control agent against mycobacteria (Mukherjee et al., 2014), and its activity as nematicidal agent (Wechter et al., 2002). Accordingly, our data showed for the first time the antagonistic effects of living cells, diffusible compounds (cell-free culture filtrates) and volatile organic compounds (VOCs) of *P. synxantha* against the postharvest fungal pathogens *M. fructicola* and *M. fructigena*, although a certain activity against *Botrytis cinerea* was preliminarily reported on lettuce (Card, 2005 - PhD Thesis).

Several strains of *Pseudomonas* genus have been studied as potential BCAs against a wide range of fungal pathogens and for their production of anti-fungal compounds (Lugtenberg and Kamilova, 2009; Rojas-Solís et al., 2018; Sang and Kim, 2014; Smilanick et al., 1993). Some of them are able to produce hydrolytic enzymes, such as proteases, cellulases, chitinase and ß-glucosidase, as well as hydrogen cyanide (HCN), pyoluteorin, phenazines, siderophores, cyclic lipopeptides, 2,4-diacetylphloroglucinol (DAPG), and pyrrolnitrin (Calderón et al., 2015;
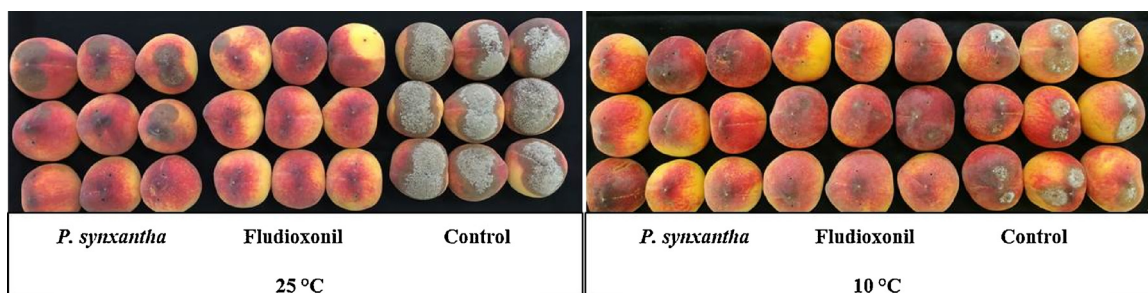
growth of *M. fructicola* e *M. fructigena* both in co- and differed inoculation treatments on standard medium. Also on a more acidic medium resembling the fruit tissue composition, the strain significantly inhibited both pathogens when applied 24 h before, with the higher activity detected against *M. fructicola*. Unlikely, Grzegorczyk et al. (2017) reported that *M. fructigena* is more efficiently inhibited by killer yeasts in acidic medium, whereas *M. fructicola* was more efficiently inhibited in media at pH 6.0.

Diversely to living cells, the greatest inhibition by culture filtrates was averagely observed in co-inoculation trials, and *M. fructicola* was more efficiently inhibited by culture filtrates if compared to *M. fructigena*. The lower activity showed by bacterial filtrates after 24 h could be probably due to a reduction of activity of produced antifungal compounds over time. The best performances showed by bacterial cells

Table 4

In vivo effects of treatments and treatments × target species interactions on disease incidence and severity of fruit decays caused by target *Monilinia fructicola* and *Monilinia fructigena* at T = 25 °C and at T = 10 °C in the experiment I and at T = 0 °C in experiment II, respectively.

Experiment	Source of variation	Disease incidence (%)[a]			Disease severity (cm)[a]		
		df	*F*	*P* value	df	*F*	*P value*
I	Treatment	2	134.8411	< 0.001	2	417.5533	< 0.001
I	Trt × species	2	1.7572	0.194002 ns	2	0.0948	0.909926 ns
I	Trt × temper.	2	3.6616	0.040941 ns	2	10.9754	0.000412
I	Trt × sp. × temp.	2	2.1893	0.133853 ns	2	6.8830	0.004338
II	Treatment	2	97.4335	< 0.001	2	309.9505	< 0.001
II	Trt × species	2	1.5116	0.25973 ns	2	3.4139	0.067033 ns

[a] *F* test of fixed effects, df = degrees of freedom, and *P* value associated to *F*; ns = not significant data.

Fig. 3. Compared effects of *P. synxantha* and fludioxonil applications in controlling *M. fructicola* decay on peach fruit at storage temperatures of 25 and 10 °C, respectively.
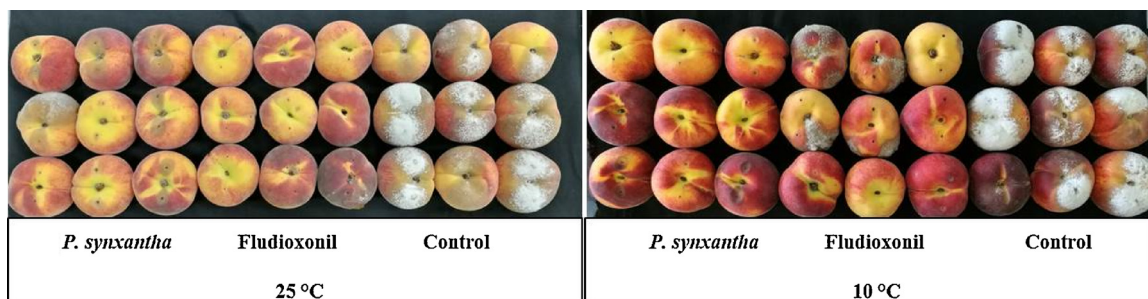

Fig. 4. Compared effects of *P. synxantha* and fludioxonil applications in controlling *M. fructigena* decay on peach fruit at storage temperatures of 25 and 10 °C, respectively.

Table 6
Post-hoc analyses of treatment effects on disease incidence (DI) and severity (DS) of fruit decays caused by target *Monilinia fructicola* and *Monilinia fructigena* in the experiment II at T = 0 °C.

Monilinia spp.[a]		
Treatment[a]	DI (%)	DS (mm)
Fludioxonil	2.78 c	1.39 c
P. synxantha	36.1 b	13.39 b
Control	100 a	62.28 a

[a] Each value represents the mean of 3 replicates, each constituted by 9 fruit. Data followed by different letters within each column are significantly different according to Fisher's least significant difference test (α = 0.05).

Raaijmakers et al., 2010). In addition, *Pseudomonas* spp. are able to induce systemic resistance in plants, and to promote plant growth by excreting phytohormones (e.g. indole-3-acetic acid, IAA) and by emitting VOCs (de Vleesschauwer et al., 2008; Lo Cantore et al., 2015; Raza et al., 2016; Rojas-Solís et al., 2018). Previous studies were performed to understand the molecular basis of the biocontrol properties of *P. synxantha*; in detail an acyl-homoserine lactone acylase gene (pvdQ), a glucose-6-phosphate dehydrogenase gene (zwf) and an mbtH-like gene were found to be involved directly or indirectly in Non Ribosomal Peptides (NRPs) synthesis (Tontou et al., 2016b). These authors suggested that a molecule with antibiotic properties, produced by NRP synthetases (NRPSs), contributes to the antagonistic activity of this bacterium. Non Ribosomal Peptides (NRPs) have antibiotic properties and simultaneously they induce plant defence (Rosenblueth and Martinez-Romero, 2006; Compant et al., 2010). Moreover, Mukherjee et al. (2014) identified a bioactive compound produced by *P. synxantha*, i.e. a long chain aliphatic hydrocarbon with a terminal double bond and intermediate electronegative atom with activity similar to bio-surfactant molecule.

About side effects of *P. synxantha* application, our data preliminarily showed no effects for peach fruit; moreover, Tontou et al. (*data not published*) demonstrated that this bacterium is an endophyte able to colonise, move and persist inside the vascular tissue of kiwifruit plants without side effects on this host. Related to human and animal effects of this bactetirum, the available data showed that both *P. synxantha* and its metabolites could induce the hemolysis of human blood and no effects for growth and survival of prawns (Van Hai et al., 2009; Mukherjee et al., 2014).
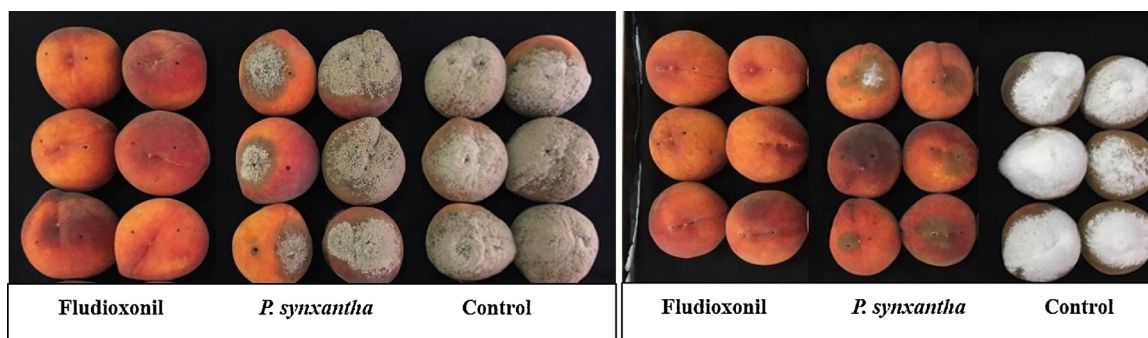

Fig. 5. Compared effects *P. synxantha* and fludioxonil applications in controlling *M. fructicola* (on the left) and *M. fructigena* (on the right) decays on peach fruit at storage temperature 0 °C.

However, further studies should be performed to evaluate the specific compounds involved in antagonistic activity and the important implications for human and environment.

5. Conclusions

Even if the findings of the present study underlined that fludioxonil is the most effective against Monilinia decays, it is clear that *P. synxantha* could represent a promising alternative for a sustainable management of brown rots of peach caused by *M. fructicola* and *M. fructigena*. Although additional studies should be performed prior to promote and develop a new commercial bio-formulate for large scale applications, the good efficacy of *P. synxantha* in preventative treatments against *M. fructicola* and *M. fructigena*, together with their ability to maintain or increase its antifungal activity at cool temperatures, could make this antagonist suitable for postharvest application in peach production.

Acknowledgments

References

Arima, K., Imanaka, H., Kousaka, M., Fukuta, A., Tamura, G., 1964. Pyrrolnitrin, a new antibiotic substance, produced by Pseudomonas. Agric. Biol. Chem. 28, 575–576. https://doi.org/10.1080/00021369.1964.10858275.

Calderón, C.E., Ramos, C., de Vicente, A., Cazorla, F.M., 2015. Comparative genomic analysis of *Pseudomonas chlororaphis* PCL1606 reveals new insight into antifungal compounds involved in biocontrol. Mol. Plant Microbe Interact. 28, 249–260. https://doi.org/10.1094/MPMI-10-14-0326-FI.

Calvo, J., Calvente, V., de Orellano, M.E., Benuzzi, D., Sanz de Tosetti, M.I., 2007. Biological control of postharvest spoilage caused by *Penicillium expansum* and *Botrytis cinerea* in apple by using the bacterium *Rahnella aquatilis*. Int. J. Food Microbiol. 113, 251–257. https://doi.org/10.1016/j.ijfoodmicro.2006.07.003.

Calvo, H., Marco, P., Blanco, D., Oria, R., Venturini, M.E., 2017. Potential of a new strain of *Bacillus amyloliquefaciens* BUZ-14 as a biocontrol agent of postharvest fruit diseases. Food Microbiol. 63, 101–110. https://doi.org/10.1016/j.fm.2016.11.004.

Card, S.D., 2005. Biological Control of *Botrytis cinerea* in Lettuce & Strawberry Crops. PhD Thesis. Lincoln University, Canterbury, New Zealand.

Cirvilleri, G., Bonaccorsi, A., Scuderi, G., Scortichini, M., 2005. Potential biological control activity and genetic diversity of *Pseudomonas syringae* pv. *syringae* strains. J. Phytopathol. 153, 654–666. https://doi.org/10.1111/j.1439-0434.2005.01033.x.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., Ait Barka, E., 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl. Environ. Microbiol. 71, 1685–1693. https://doi.org/10.1128/AEM.71.4.1685-1693.2005.

Compant, S., Clément, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42, 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024.

de Vleesschauwer, D., Djavaheri, M., Bakker, P.A.H.M., Höfte, M., 2008. *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. Plant Physiol. 148, 1996–2012. https://doi.org/10.1104/pp.108.127878.

Grzegorczyk, M., Żarowska, B., Restuccia, C., Cirvilleri, G., 2017. Postharvest biocontrol ability of killer yeasts against *Monilinia fructigena* and *Monilinia fructicola* on stone fruit. Food Microbiol. 61, 93101. https://doi.org/10.1016/j.fm.2016.09.005.

FAOSTAT, 2016. (Food Agriculture Organization of the United Nations). http://www.fao.org/faostat/en/#data/QC.

Janisiewicz, W.J., Roitman, J., 1988. Biological control of blue mold and gray mold on apple and pear with Pseudomonas cepacia. Phytopathology 78, 1697–1700. https://doi.org/10.1094/Phyto-78-1697.

Lo Cantore, P., Giorgio, A., Iacobellis, N.S., 2015. Bioactivity of volatile organic compounds produced by Pseudomonas tolaasii. Front. Microbiol. 6, 1082. https://doi.org/10.3389/fmicb.2015.01082.

Lopez-Reyes, J.G., Spadaro, D., Prelle, A., Garibaldi, A., Gullino, M.L., 2013. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. J. Food Prot. 4, 560–735. https://doi.org/10.4315/0362-028X.JFP-12-342.

Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. Annu. Rev. Microbiol. 63, 541–556. https://doi.org/10.1146/annurev.micro.62.081307.162918.

Mari, M., Martini, C., Guidarelli, M., Neri, F., 2012. Postharvest biocontrol of *Monilinia laxa, Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. Biol. Control 60, 132–140. https://doi.org/10.1016/j.biocontrol.2011.10.013.

Miessner, S., Stammler, G., 2010. *Monilinia laxa, M. fructigena* and *M. fructicola*: risk estimation of resistance to QoI fungicides and identification of species with cytochrome b gene sequences. J. Plant. Dis. Prot. 117, 162–167. https://doi.org/10.1007/BF03356354.

Moore, E.R.B., Tindall, B.J., Dos Santos, V.A.P.M., Pieper, D.H., Ramos, J.L., Palleroni, N.J., 2006. NonMedical: Pseudomonas. In: In: Dworking, M., Falkow, S., Rosenberg, E., Schleifer, K.-H., Stackebrandt, E. (Eds.), The Prokaryotes Volume 6: Proteobacteria 6. Gamma Subclass Springer, Verlag New York, pp. 646–703. https://doi.org/10.1007/0-387-30746-X_21. 2006.

Mukherjee, K., Mandal, S., Mukhopadhyay, B., Mandal, N.C., Sil, A.K., 2014. Bioactive compound from *Pseudomonas synxantha* inhibits the growth of mycobacteria. Microbiol. Res. 169, 794–802. https://doi.org/10.1016/j.micres.2013.12.005.

Panebianco, S., Vitale, A., Polizzi, G., Scala, F., Cirvilleri, G., 2015. Enhanced control of postharvest citrus fruit decay by means of the combined use of compatible biocontrol agents. Biol. Control 84, 19–27. https://doi.org/10.1016/j.biocontrol.2015.02.001.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2015. Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing postharvest bunch rot of table grape. Food Microbiol. 47, 85–92. https://doi.org/10.1016/j.fm.2014.11.013.

Parafati, L., Vitale, A., Restuccia, C., Cirvilleri, G., 2017. Performance evaluation of volatile organic compounds by antagonistic yeasts on immobilized hydrogel spheres against gray, green and blue postharvest decays. Food Microbiol. 63, 191–198. https://doi.org/10.1016/j.fm.2016.11.021.

Platania, C., Restuccia, C., Muccilli, S., Cirvilleri, G., 2012. Efficacy of killer yeast in the biological control of *Penicillium digitatum* on Tarocco orange fruits (*Citrus sinensis*). Food Microbiol. 30, 219–225. https://doi.org/10.1016/j.fm.2011.12.010.

Pusey, P.L., Wilson, C.L., 1984. Postharvest biological control of stone fruit brown rot by Bacillus subtilis. Plant Dis. 68, 753–756. https://doi.org/10.1094/PD-69-753.

Raaijmakers, J.M., de Bruijn, I., Nybroe, O., Ongena, M., 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. FEMS Microbiol. Rev. 34, 1037–1062. https://doi.org/10.1111/j.1574-6976.2010.00221.x.

Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q., 2016. Response of tomato wilt pathogen Ralstonia solanacearum to the volatile organic compounds produced by a biocontrol strain Bacillus amyloliquefaciens SQR-9. Sci. Rep. 6, 24856. https://doi.org/10.1038/srep24856.

Restuccia, C., Giusino, F., Licciardello, F., Randazzo, C., Caggia, C., Muratore, G., 2006. Biological control of peach fungal pathogens by commercial products and indigenous yeasts. J. Food Prot. 69, 2465–2470. https://doi.org/10.4315/0362-028X-69.10.2465.

Rojas-Solís, D., Zetter-Salmón, E., Contreras-Pérez, M., Rocha-Granados, M.D.C., Macías-Rodríguez, L., Santoyo, G., 2018. *Pseudomonas stutzeri* E25 and *Stenotrophomonas maltophilia* CR71 endophytes produce antifungal volatile organic compounds and exhibit additive plant growth-promoting effects. Biocatal. Agric. Biotechnol. 13, 46–52. https://doi.org/10.1016/j.bcab.2017.11.007.

Rosenblueth, M., Martinez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. Mol. Plant-Microbe Interact. 19, 827–837. https://doi.org/10.1094/MPMI-19-0827.

Sang, M.K., Kim, K.D., 2014. Biocontrol activity and root colonization by *Pseudomonas corrugata* strains CCR04 and CCR80 against Phytophthora blight of pepper. Biocontrol 59, 437–448. https://doi.org/10.1007/s10526-014-9584-9.

Scuderi, G., Bonaccorsi, A., Panebianco, S., Vitale, A., Polizzi, G., Cirvilleri, G., 2009. Some strains of *Burkholderia gladioli* are potential candidates for postharvest biocontrol of fungal rots in citrus and apple fruits. J. Plant Pathol. 91, 207–213. https://doi.org/10.4454/jpp.v91i1.645.

Sisquella, M., Vinas, I., Picouet, P., Torres, R., Usall, J., 2014. Effect of host and *Monilinia* spp. variables on the efficacy of radio frequency treatment on peaches. Postharv. Biol. Technol. 87, 6–12. https://doi.org/10.1016/j.postharvbio.2013.07.042.

Smilanick, J.L., Denis-Arrue, R., Bosch, J.R., Gonzalez, A.R., Henson, D., Janisiewicz, W.J., 1993. Control of postharvest brown rot of nectarines and peaches by Pseudomonas species. Crop Prot. 12, 513–520. https://doi.org/10.1016/0261-2194(93)90092-W.

Tontou, R., Giovanardi, D., Ferrari, M., Stefani, E., 2016a. Isolation of bacterial endophytes from *Actinidia chinensis* and preliminary studies on their possible use as antagonists against *Pseudomonas syringae* pv. *actinidiae*. J. Berry Res. 6, 395–406. https://doi.org/10.3233/JBR-160118.

Tontou, R., Gaggia, F., Baffoni, L., Devescovi, G., Venturi, V., Giovanardi, D., Stefani, E., 2016b. Molecular characterisation of an endophyte showing a strong antagonistic activity against *Pseudomonas syringae* pv. *actinidiae*. Plant Soil 405, 97–106. https://doi.org/10.1007/s11104-015-2624-0.

Usall, J., Casals, C., Sisquella, M., Palou, L., De Cal, A., 2015. Alternative technologies to control postharvest diseases of stone fruits. Stewart Postharv. Rev. 11, 1–6. https://doi.org/10.2212/spr.2015.4.2.

Van Hai, N., Buller, N., Fotedar, R., 2009. Effects of probiotics (*Pseudomonas synxantha* and *Pseudomonas aeruginosa*) on the growth, survival and immune parameters of juvenile western king prawns (*Penaeus latisulcatus* Kishinouye, 1896). Aquac. Res. 40, 590–602. https://doi.org/10.1111/j.1365-2109.2008.02135.x.

Wechter, W.P., Begum, D., Presting, G., Kim, J.J., Wing, R.A., Kluepfel, D.A., 2002. Physical mapping, BAC-End sequence analysis, and marker tagging of the soilborne nematicidal bacterium, *Pseudomonas synxantha* BG33R. OMICS 6, 11–21. https://doi.org/10.1089/15362310252780807.

Wallace, R.L., Hirkala, D.L., Nelson, L.M., 2017. Postharvest biological control of blue mold of apple by Pseudomonas fluorescens during commercial storage and potential modes of action. Food Microbiol. 133, 1–11. https://doi.org/10.1016/j.postharvbio.2017.07.003.

Zhang, D., Spadaro, D., Garibaldi, A., Gullino, M.L., 2010a. Selection and evaluation of new antagonists for their efficacy against postharvest brown rot of peach. Postharv. Biol. Technol. 55, 174–181. https://doi.org/10.1016/j.postharvbio.2009.09.007.

Zhang, D., Spadaro, D., Garibaldi, A., Gullino, M.L., 2010b. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. Biol. Control 54, 172–180. https://doi.org/10.1016/j.biocontrol.2010.05.003.