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ORIGINAL PAPER

Antifungal activity of avocado rhizobacteria against *Fusarium euwallaceae* and *Graphium* spp., associated with *Euwallacea* spp. nr. *fornicatus,* and *Phytophthora cinnamomi*

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Abstract Plant rhizobacteria have been successfully used as biocontrol agents against fungal phytopathogens. However, their potential to control two important avocado diseases, namely Fusarium dieback (FD) and Phytophthora root rot (PRR), has been poorly studied. FD is an emerging disease triggered by fungi associated with two ambrosia beetle species (*Euwallacea fornicatus* species complex), while PRR is caused by *Phytophthora cinnamomi,* a soil-borne oomycete. In the present work, the antifungal activity of bacteria isolated from avocado rhizosphere was tested in dual culture assays against *Fusarium euwallaceae*, *Graphium euwallaceae* and *Graphium* sp., causal agents of FD, and against *P. cinnamomi*. In 2015, rhizosphere soil samples of FD infested and non-infested avocado trees were collected from a commercial avocado orchard in Escondido, California. In an initial screening, 72 of the 168 assessed bacterial isolates reduced mycelial growth of *F. euwallaceae* by up to 46%. Eight bacterial isolates showing inhibition percentages larger than 40% were then selected for further antagonism assays against the other fungal pathogens. Five bacterial isolates, determined by 16S rDNA sequencing to belong to the *Bacillus subtilis/Bacillus amyloliquefaciens* species complex, successfully inhibited the mycelial growth of both *Graphium* species by up to 30%. The same isolates and an additional isolate identified as *Bacillus mycoides,* inhibited the growth of *P. cinnamomi* by up to 25%. This is the first report of avocado rhizobacteria with antifungal activity against pathogens responsible for FD and PRR in avocado.

Keywords Fusarium dieback · Phytophthora root rot · Biocontrol · *Bacillus* · Bacterial lipopeptides

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Introduction

Mexico is the world largest producer of avocados (*Persea americana* Mill.) with approximately 65% of the global production, followed by the United States of America (USA) with 23%, of which 85% is from California (AGMRC 2014; FAOSTAT 2015; Dunlap et al. 2017). Despite the economic importance of avocado production for these two neighboring countries, the productivity of avocado orchards has been hampered by fast-spreading diseases that are threatening avocado production in both countries. Those diseases include Fusarium dieback (FD), a new disease of avocado vectored by invasive shot hole borers (*Euwallacea* spp. nr. *fornicatus*) in California, and avocado root rot caused by *Phytophthora cinnamomi* Rands (PRR).

FD is caused by a complex of fungi including *Fusarium euwallaceae* Freeman, Mendel, Aoki & O'Donnell and *Graphium euwallaceae* Twizeyimana, Lynch & Eskalen, which form a symbiotic relationship with the invasive ambrosia beetle *Euwallacea* sp. nr. *fornicatus*, also known as Polyphagous Shot Hole Borer (PSHB) (Lynch et al. 2016). This new pest disease complex was first discovered in Los Angeles in 2012 (Eskalen et al. 2012). Another closely related *Euwallacea* species, the Kuroshio Shot Hole Borer (KSHB), was found in 2013 throughout Orange and San Diego Counties, very close to the Mexican border. More recently, KSHB was detected in Tijuana, Mexico (Garcia-Ávila et al. 2016). FD has a very wide host range and has been reported to pose a globally significant threat to natural forests, urban landscapes and fruit crops, particularly avocado (O'Donnell et al. 2016). The colonization by the fungi of the galleries burrowed by the beetle precedes a fungal invasion of vascular tissues, which blocks nutrient transport to higher parts of the tree leading to wilting, branch dieback, and in severe cases, tree mortality (Eskalen et al. 2013).

Phytophthora cinnamomi, on the other hand, is one of the most devastating plant pathogens worldwide. This soil-borne pathogen affects more than 3000 plant species (Pagliaccia et al. 2013) and is especially devastating in crop monocultures. It also causes root rot disease on avocado, which is estimated to affect about 70% of avocado orchards and to cause annual losses of 40 million US dollars in California alone (Toerien 2007). Following the root rot, defoliation and branch dieback occur, usually leading to tree mortality within 1–2 years (Coffey 1987).

Different management strategies have been implemented to control or mitigate the negative effects of FD and PRR in avocado orchards. Since the use of agrochemicals in commercial orchards is often restricted due to the inherent risks posed by their residues, more efforts should be placed in finding some other effective and environmental friendly management strategies such as biological control (Umeda et al. 2016). Biocontrol strategies using naturally occurring beneficial bacteria have been recently explored to control laurel wilt and FD (Dunlap et al. 2017). These authors used an in-house collection of Bacillaceae strains to test for antifungal activity, and reported that three *Paenibacillus* species and one *Bacillus* species caused antagonism in vitro against *F. euwallaceae*. In other studies, different strains of *Bacillus*, *Pseudomonas* and *Streptomyces* have shown some level of antagonistic activity against *P. cinnamomi* (You et al. 1996; Cazorla et al. 2007; Vida et al. 2017). Bacterial isolates belonging to the genus *Pseudomonas* have also been reported to produce antifungal substances and to be able to inhibit the mycelial growth of *P. cinnamomi* in dual cultures (Stirling et al. 1992; Vida et al. 2017), while *Actinobacteria* or *Bacillus* species have been associated with *P. cinnamomi* suppressiveness (You et al. 1996; Yin et al. 2004).

Microorganisms associated with the rhizosphere are of particular importance in the search for successful biological agents since they secrete a wide range of substances that could act in the suppression of pathogens (Yang et al. 2001; Bais et al. 2006; Compant et al. 2010). Recently, rhizobacteria such as *Bacillus subtilis* (Ehrenberg) Cohn and *Serratia plymuthica* (Lehmann & Neumann) Breed et al. were proved effective to inhibit the growth of the pathogenic fungi *Moniliophtora perniciosa* (Stahel) Aime & Phillips-Mora and *Rhizoctonia solani* J.G. Kuhn respectively, through the emission of diffusible and volatile compounds (Chaves-López et al. 2015; Neupane et al. 2015). The objective of this study was therefore to identify bacterial strains in avocado rhizospheres with antagonistic activity against *F. euwallaceae*, *G. euwallaceae*, and *Graphium* sp., three fungal pathogens responsible for FD that are affecting avocado orchards in California and threatening avocado production in Mexico. Furthermore,

since these beneficial bacteria were recovered from the rhizosphere of avocado trees, their antifungal effects were also evaluated against the avocado root rot agent *P. cinnamomi*.

Materials and methods

Isolation of bacteria associated with avocado rhizospheres

Rhizosphere soil samples were collected in December 2015 from an avocado orchard located in Escondido, San Diego County, California, where the majority of the trees were infested with both PRR and FD. Five non symptomatic avocado trees and five avocado trees presenting symptoms of FD were selected. Four soil and root samples were taken per tree, approximately 50 cm away from the trunk and at a depth of 5–10 cm, where most of the feeder roots of avocado grow, and subsequently mixed to obtain one bulk sample of rhizosphere soil per tree. The hand shovel used for sample collection was disinfected between each tree with 70% ethanol. Samples were transported in a cooler and immediately processed upon arrival at the laboratory at UC Riverside. Loose soil was removed from the roots, and the remaining soil, which was strongly adhered to the roots, was recovered as rhizosphere soil. Solutions were subsequently prepared from 1 g rhizosphere soil and 99 ml distilled water, and homogenized by shaking vigorously. Dilutions of 1:10 and 1:100 were then streaked onto Petri dishes with Luria–Bertani agar (Difco), in triplicate. Plates were incubated at room temperature and isolates were taken from the plates as they grew and subcultured in nutrient agar until pure cultures were obtained.

In vitro antagonism assays against *F. euwallaceae*, *Graphium* spp. and *P. cinnamomi*

The bacterial isolates that were obtained from the rhizosphere of healthy and infected avocado trees were first screened for in vitro antagonism against *F. euwallaceae*. To prepare the dual cultures for the antagonism assays, bacterial isolates were re-streaked onto nutrient agar plates (nutrient broth (Difco) and granulated agar (Fisher)) and incubated at 25 °C for 48 h. An isolate of *F. euwallaceae* (strain UCR4511 provided by the Eskalen Lab.) was incubated on Potato Dextrose Agar (PDA; Difco Laboratories) medium at 25 °C for 5 days prior dual plating.

One agar plug of 5 mM of diameter was taken from the border of the fungus mycelial growth with a sterile cork borer and placed on the center of a PDA plate. Bacterial isolates were taken from a single colony with a toothpick and inoculated at a 2-cm distance from the mycelial plug (Fig. 1). Three different bacterial isolates were tested per plate. Additionally, mock inoculation with a sterile toothpick was used as a control on each experimental plate. The antagonism assays were carried out in triplicate. Dual culture plates were incubated at 25 °C and after 5 days, the mycelium radial growth was measured towards the bacterial and control treatments. The percentage of inhibition of mycelial growth was calculated using the formula reported by Idris et al. (2007): % inhibition = [(R-r)/R] × 100, where R is the radius of fungal growth from the center of the plate towards the control treatment, and r is the radius of fungal growth towards the bacterial treatment.

Eight bacterial isolates were then selected from those isolates showing high inhibition of *F. euwallaceae* mycelial growth (inhibition percentage higher

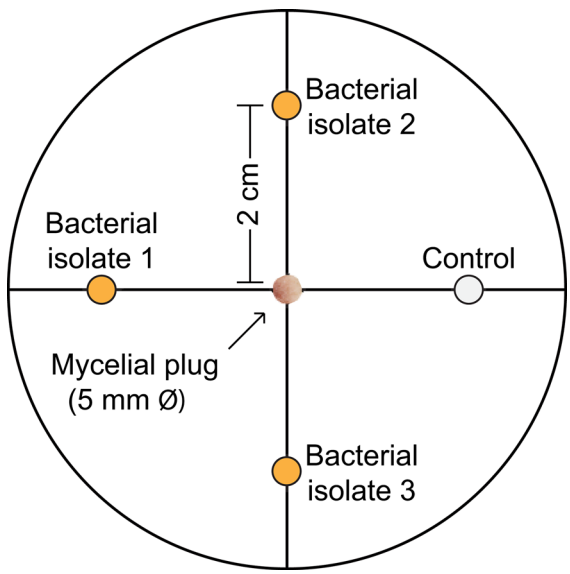

Fig. 1 Schematic design of the dual culture antagonism assays. Bacterial isolates were inoculated with a toothpick at a 2-cm distance of the central mycelial plug. Three different bacterial isolates were tested per plate. Additionally, a sterilized toothpick mark was used as a control. The antagonism assays were carried out in triplicate

than 40%), to be further evaluated for antagonism against other fungal pathogens of avocado. The selected bacterial isolates were tested against *G. euwallaceae* (fungal symbiont of PSHB), *Graphium* sp. (fungal symbiont of KSHB) and *P. cinnamomi* (strain UCR3458, provided by the Eskalen Lab.), following the same procedure as the in vitro antagonism assays against *F. euwallaceae*. The incubation time used to grow the fungal culture prior to set up the antagonism assays varied depending on the fungal species (11 days for *Graphium* species, 3 days for *P. cinnamomi*). Antagonism assays against *Graphium* spp. were carried out using five replicates whilst 10 replicates were used for *P. cinnamomi*.

Molecular identification of antagonistic bacterial isolates

DNA was extracted from each bacterial isolate showing in vitro antagonism against *F. euwallaceae* following the method proposed by Bollet et al. (1991). Briefly, the bacterial pellet was washed with 1 ml TE buffer and resuspended in 100 µl TE. The lysis step was carried out by adding 50 µl of 10% SDS and incubating the sample at 65 °C for 30 min. After centrifuging and removing the supernatants, the remaining pellets were heated for 2 × 1 min in a microwave and resuspended in 200 µl TE. An equal volume of chloroform—isoamyl alcohol—phenol (24:1:25) was then added and samples were shaken for 15 min, after which the aqueous phase was recovered by a 20 min centrifugation step and precipitated in ethanol. DNA integrity was verified by electrophoresis. Unsuccessful DNA extractions were repeated using DNeasy® Blood and Tissue kit (Qiagen, Germany) following the manufacturer's instructions.

The 16S rRNA region was amplified by PCR using universal primers 27F (5′- AGAGTTTGATCMTGG CTCAG-3′) and 1492R (5′- TACGGYTACCTTGT TACGACTT-3′), in 50 µl reactions containing 25–150 ng of template DNA, 1X of Taq buffer, 200 µM of each dNTP, 1.25 mM of $MgCl_2$, 0.4 µM of forward and reverse primers, and 0.5U of Taq DNA polymerase (Qiagen, Germany). Reactions were performed in a SureCycler 8800 thermal cycler (Agilent, California, USA) under the following conditions: initial denaturation at 95 °C for 4 min; 30 cycles of denaturation at 95 °C for 45 s, annealing at 53 °C for 45 s and extension at 72 °C for 2 min; and a final extension step at 72 °C for 5 min. Amplified DNA products were visually checked on an electrophoresis gel and purified using QiaQuick® Purification kit (Qiagen, Germany), according to the manufacturer's instructions. Purified DNA amplicons were then sent to Macrogen Inc. for sequencing. Sequences were deposited in GenBank (accession numbers MF377554 to MF377573).

Data analysis

Statistical analyses were carried out with R version 3.4.1. Means, standard deviation, and standard error values were calculated using the PLYR package (Wickham 2011). Fungal growth data was analyzed using a multiple linear regression model, with fungal species and bacterial isolates as independent factors. A contrast matrix was generated in order to compare all treatments to the control and a Post-hoc analysis was subsequently implemented by using the "multcomp" package (Holthorn et al. 2008) in R with link function glht (general linear hypothesis testing).

Sequences were manually checked in BioEdit 7.2.5. (Hall 1999). An alignment was constructed in MEGA 7 (Kumar et al. 2016), using the multiple alignment program MUSCLE with the edited sequences and their best matches in GenBank nucleotide database (www.ncbi.nlm.nih.gov). The resulting alignment was manually edited. Sequences with 99% of identity were grouped in operational taxonomic units (OTUs) using the rdp pipeline (Cole et al. 2009). A Maximum-Likehood tree was constructed, using a Kimura two parameter model with uniform rates, and a Bootstrap method with 1000 replicates.

Results

Antifungal activity against *Fusarium euwallaceae*

In total, 168 bacterial isolates from rhizospheres of avocado trees were tested in dual cultures against *F. euwallaceae*. The mycelial radial growth of *F. euwallaceae* was reduced by 72 bacterial isolates, with inhibition percentages ranging from 15 to 46% (Online Resource 1). These 72 antagonistic bacterial isolates were grouped into 9 morphotypes based on Gram-staining results and microscopic characteristics

such as cellular shape and size and presence of endospores. Up to 3 bacterial isolates per morphotype (20 isolates in total) were then selected for sequencing.

The inhibition percentages of mycelial growth of *F. euwallaceae* caused by the 20 sequenced bacterial isolates are shown in Fig. 2. All isolates belonged to the bacterial genus *Bacillus* and were clustered into two OTUs: OTU 1 was represented by sequences phylogenetically similar to *Bacillus amyloliquefaciens* Priest et al. and *B. subtilis*, whilst OTU 2 was represented by close relatives of *Bacillus mycoides* Flugge and *B. thuringiensis* Berliner (Table 1). A phylogeny of the 20 sequenced bacterial isolates with antagonism against *F. euwallaceae* is included in Online Resource 2.

Antifungal activity against *Graphium* spp. and *P. cinnamomi*

Eight bacterial isolates with inhibition percentage higher than 40% were randomly selected from the isolates that were showing high antagonistic activity against *F. euwallaceae*, to be tested for antagonism against *G. euwallaceae*, *Graphium* sp. and *P. cinnamomi*. The selected isolates were isolates: INECOL-4720, INECOL-4740, INECOL-4742, INECOL-4743, INECOL-5920, INECOL-5922, INECOL-5924, and INECOL-5927. Isolates INECOL-4742, INECOL-4743, INECOL-5922, INECOL-5924, and INECOL-5927, phylogenetically related with *B. amyloliquefaciens*, significantly reduced the mycelial radial growth of both *Graphium* species. In particular, bacterial isolate INECOL-5922 exhibited the greatest inhibition (30.2%) against *G. euwallaceae* (Fig. 3), whilst isolate INECOL-4742 inhibited the growth of *Graphium* sp. by 27% (Fig. 4). The mycelial radial growth of *P. cinnamomi* was significantly reduced by bacterial isolates INECOL-4740, INECOL-4742, INECOL-4743, INECOL-5922, INECOL-5924 and INECOL-5927, with isolate INECOL-5924 showing the greatest inhibition (25.5%, Fig. 5). Interestingly, the growth of *P. cinnamomi* seems to be promoted by bacterial isolates INECOL-4720 and INECOL-5920, although not significantly. Five isolates were able to inhibit the mycelial growth of all four avocado fungal pathogens: isolates INECOL-4742, INECOL-4743, INECOL-5922, INECOL-5924, and INECOL-5927 (Table 2; Fig. 6).

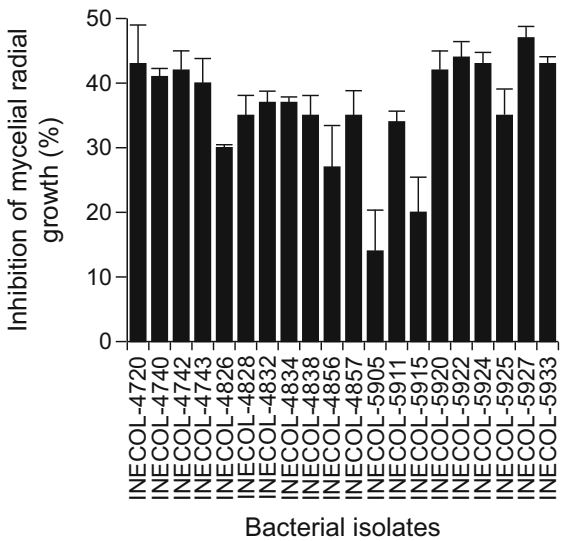

Fig. 2 Inhibition percentage of mycelial radial growth of *Fusarium euwallaceae* grown in dual cultures with antagonistic bacterial isolates. Values represent the average of 3 replicates. Bars represent standard errors (s.e.). All isolates significantly inhibited mycelial radial growth in comparison with a control (Post-hoc analysis implemented using the "multcomp" package (Holthorn et al. 2008) in R, with link function glht (general linear hypothesis testing), $P \leq 0.05$)

Discussion

The use of chemical pesticides in agriculture has allowed the reduction of crop losses due to microbial phytopathogens, but is associated with environmental pollution, emergence of resistant pathogens and human health hazards (Prabhukarthikeyan et al. 2017). In order to counteract the negative effects of agrochemicals and provide an alternative solution to problems caused by pathogenic microorganisms, several reports have recommended the exploitation of beneficial rhizobacteria as biocontrol agents (Abdallah et al. 2016; Tokpah et al. 2016; Egamberdieva et al. 2017). Identifying rhizobacteria with antifungal properties constitutes the first step for the development of formulations that could biologically control FD or PRR. Such biological fungicides may include beneficial bacterial consortia in the form of concentrated powder, or oil-based or polymer-based products (Shaikh and Sayyed 2015), which could be sprayed directly onto the soil or directly injected into the stem of avocado trees, as shown by Na (2016).

Table 1 Sequenced bacterial isolates showing antagonism against *F. euwallaceae* and their closest matches based on the NCBI database "16S ribosomal RNA sequences (Bacteria and Archaea)". Isolates in bold case were subsequently tested against *Graphium* spp. and *P. cinnamomi*

Bacterial isolate	GenBank Accession number	NCBI best match		Identity (%)
		Taxonomy	Accession number	
OTU 1				
INECOL-4740	MF377572	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-4742	MF377571	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-4743	MF377570	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-4826	MF377569	*Bacillus subtilis*	NR_113265.1	100
INECOL-4832	MF377567	*Bacillus subtilis*	NR_113265.1	99
INECOL-4834	MF377566	*Bacillus subtilis*	NR_113265.1	99
INECOL-4838	MF377565	*Bacillus tequilensis*	NR_104919.1	99
INECOL-4857	MF377563	*Bacillus subtilis*	NR_113265.1	99
INECOL-5905	MF377562	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5911	MF377561	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5915	MF377560	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5920	MF377559	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5922	MF377558	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5924	MF377557	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5927	MF377555	*Bacillus amyloliquefaciens* subsp. *plantarum*	NR_075005.1	99
INECOL-5933	MF377554	*Bacillus amyloliquefaciens*	NR_117946.1	99
OTU 2				
INECOL-4720	MF377573	*Bacillus mycoides*	NR_113990.1	100
INECOL-4828	MF377568	*Bacillus thuringiensis*	NR_112780.1	99
INECOL-4856	MF377564	*Bacillus thuringiensis*	NR_112780.1	99
INECOL-5925	MF377556	*Bacillus mycoides*	NR_113990.1	100

In this study, all sequenced avocado rhizobacteria showing significant inhibition of the mycelial growth of *F. euwallaceae* belonged to the genus *Bacillus*. Recently, Dunlap et al. (2017) reported that *Bacillus velezensis* Ruiz-García et al. and several *Paenibacillus* species, isolated from human feces and honey bee larvae, presented antagonistic activity against *F. euwallaceae*. In another study, strains of *B. subtilis*, isolated from California sycamore (*Platanus racemosa* Nutt.) and avocado wood samples, significantly inhibited the growth of *F. euwallaceae* (Na 2016). *Bacillus subtilis* endophytic strains, isolated from avocado roots, were also found to reduce *P. cinnamomi* mycelial growth by up to 28% (Hakizimana et al. 2011). Interestingly, in our study, the bacterial isolates which presented antagonistic activity against all fungal pathogens also belonged to the *B. subtilis* species complex. Within the *B. subtilis* species complex, representatives of the subgroup *B. amyloliquefaciens* subsp. *plantarum* are known to be plant-associated strains with plant-growth promoting and antifungal activities and are therefore widely used as biofertilizer and biocontrol agents in agriculture (Dunlap et al. 2017; Fan et al. 2017). Our results corroborate these findings, and constitute the first report of avocado rhizobacteria with antifungal properties against FD and PRR causal agents.

Different mechanisms of fungal growth inhibition are reported for species of the genus *Bacillus.* Several studies indicate that, for members of the *B. subtilis* species complex, the antagonism is related to the secretion of antibiotic lipopeptides. Cawoy et al. (2015) showed that, in dual culture tests, *B. subtilis/B. amyloliquefaciens* secreted lipopeptides such as iturin and fengycin, which inhibited the growth of *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen.

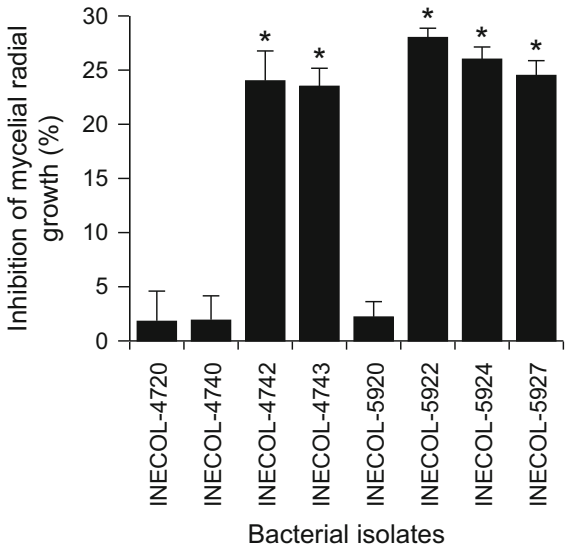

Fig. 3 Inhibition percentage of mycelial radial growth of *Graphium euwallaceae* grown in dual cultures with antagonistic bacterial isolates. Values represent the average of 5 replicates. Bars represent standard errors (s.e.). * indicates significant inhibition of mycelial growth in comparison with a control (Post-hoc analysis implemented using the "multcomp" package (Holthorn et al. 2008) in R, with link function glht (general linear hypothesis testing), $P \leq 0.05$)

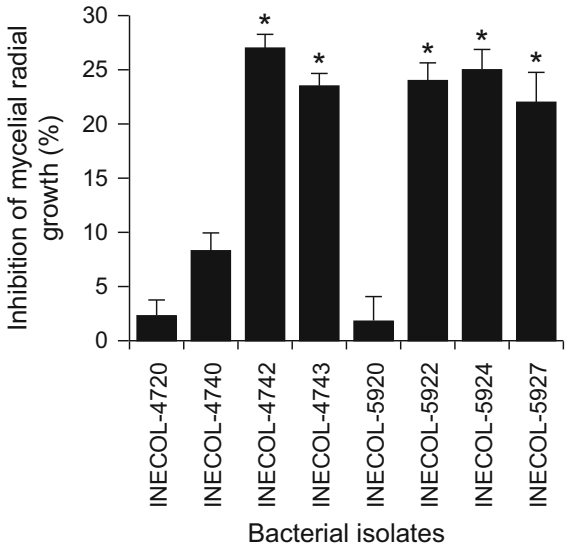

Fig. 4 Inhibition percentage of mycelial radial growth of *Graphium* sp. grown in dual cultures with antagonistic bacterial isolates. Values represent the average of 5 replicates. Bars represent standard errors (s.e.). * indicates significant inhibition of mycelial growth in comparison with a control (Post-hoc analysis implemented using the "multcomp" package (Holthorn et al. 2008) in R, with link function glht (general linear hypothesis testing), $P \leq 0.05$)

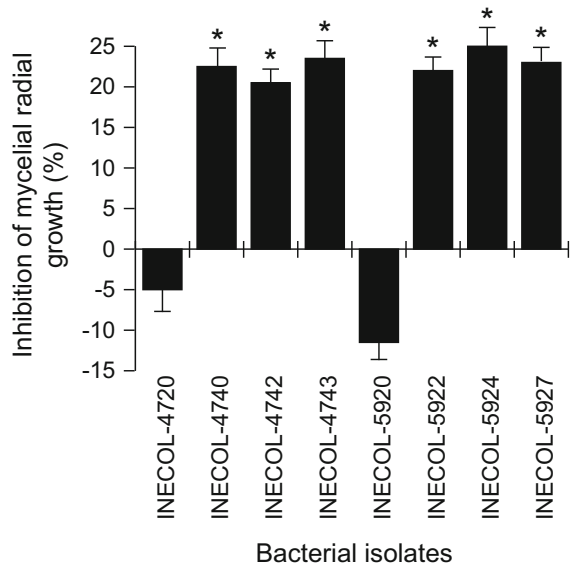

Fig. 5 Inhibition percentage of mycelial radial growth of *Phytophthora cinnamomi* grown in dual cultures with antagonistic bacterial isolates. Values represent the average of 10 replicates. Bars represent standard errors (s.e.). * indicates significant inhibition of mycelial growth in comparison with a control (Post-hoc analysis implemented using the "multcomp" package (Holthorn et al. 2008) in R, with link function glht (general linear hypothesis testing), $P \leq 0.05$). Bacterial isolates INECOL-4720 and INECOL-5920 stimulated the mycelial growth of *P. cinnamomi,* although not significantly

Cazorla et al. (2007) also reported that *B. subtilis* strains, isolated from avocado rhizosphere, inhibited the avocado pathogens *Rosellinia necatrix* Berl. ex Prill. and the tomato pathogen *F. oxysporum* f.sp. *radicis-lycopersici* Jarvis & Shoemaker through iturin and fengycin secretion. Moreover, the authors concluded that other compounds, such as hydrolytic enzymes, were also likely to act as antifungal molecules. The antifungal activity of bacterial lipopeptides was confirmed by Mnif et al. (2015) in an in vitro assay, using an extract of lipopeptides produced by *B. subtilis* SPB1. The authors observed that the bacterial extract generated mycelial lysis, polynucleation, spore destruction and inhibition of mycelial growth in *Fusarium solani*, which is phylogenetically closely related to *F. euwallaceae* (O'Donnell et al. 2015). The inhibition zone observed in some of our dual culture assays may therefore be due to the secretion of diffusible lipopeptide compounds by the tested bacterial isolates. Further studies thus need to be performed to confirm the identity of the antifungal

Table 2 Antagonistic activity of rhizosphere bacterial isolates against four fungal pathogens of avocado

Bacterial isolates	*F. euwallaceae*	*G. euwallaceae*	*Graphium* sp.	*P. cinnamomi*
INECOL-4720	+	−	−	−
INECOL-4740	+	−	−	+
INECOL-4742	+	+	+	+
INECOL-4743	+	+	+	+
INECOL-5920	+	−	−	−
INECOL-5922	+	+	+	+
INECOL-5924	+	+	+	+
INECOL-5927	+	+	+	+

+ represents bacterial isolates which inhibited fungal growth; − represents bacterial isolates with no effect on fungal growth

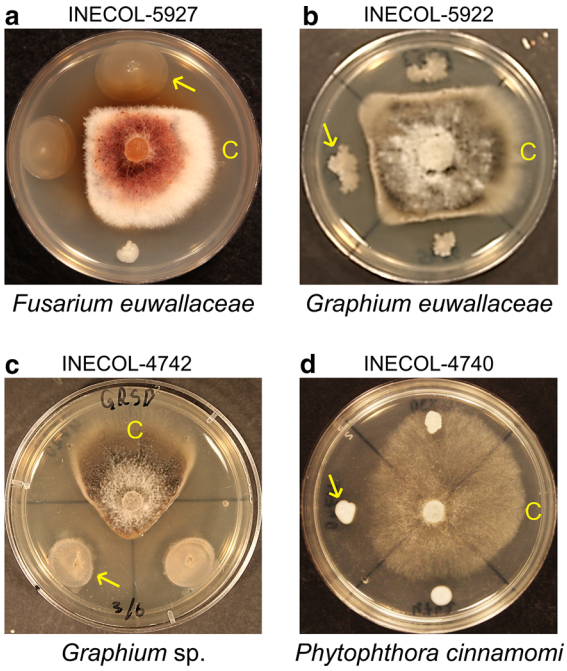

Fig. 6 Dual culture assays to evaluate the antagonism of **a** isolate INECOL-5927 against *F. euwallaceae*; **b** isolate INECOL-5922 against *G. euwallaceae*; **c** isolate INECOL-4742 against *Graphium* sp.; and **d** isolate INECOL-4740 against *P. cinnamomi*. Isolates are marked by yellow arrows. *C* control

diffusible compounds that were involved in the inhibition. The variety of antifungal compounds secreted by *B. subtilis/B. amyloliquefaciens* may also explain the fact that different bacterial strains, although belonging to the same OTU, differed in their capacity to inhibit fungal pathogens. Moreover, *B. subtilis* and *B. amyloliquefaciens* are also known to emit volatiles with antifungal properties (Fiddaman and Rossall 1993; Yuan et al. 2012). The inhibitory effect of volatiles emitted by the avocado rhizobacteria that were isolated in this study also needs to be tested, in order to assess the full potential of these bacterial strains to control avocado fungal pathogens. *Bacillus* species are considered as good candidates to develop biopesticide formulations due to their ability to produce a wide range of antibiotics and antifungal volatile compounds and to form heat- and UV-resistant spores (Ji et al. 2013). The effectiveness of the five *Bacillus* isolates with strong antifungal activity in vitro against *F. euwallaceae*, *G. euwallaceae*, *Graphium* sp., responsible for FD, and *P. cinnamomi* responsible for PRR in avocado, should also be evaluated in vivo, to confirm their potential use as biocontrol agents of these important avocado diseases.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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