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Research Article

Assessment of microbial antagonistic activity and Quorum Sensing Signal Molecule (Cyclopeptides-DKPs and N-Acyl Homoserine Lactones) detection in bacterial strains obtained from avocado thrips (Thysanoptera: Thripidae)

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ABSTRACT

The control of avocado pests and diseases heavily relies on the use of several types of pesticides, some of which are strictly monitored or not internationally accepted. New sources of bioactive molecules produced by phytopathogen-inhibiting microorganisms offer an excellent alternative for the control of pests and diseases. This study explores the potential antagonistic action against phytopathogenic microorganisms, using bacterial strains obtained from avocado thrips. In addition, we detected and identified quorum sensing (QS) signaling molecules that are related to virulence factors and antibiotic production. The results showed that *Bacillus, Pantoea*, and *Serratia* strains exhibited antagonism against five fungal phytopathogens. Additionally, some bacteria also produce specific signaling molecules like N-3-(oxododecanoyl)-l-homoserine lactone (OdDHL), N-(3-oxo)-hexanoyl l-HL (OHHL), 4-hydroxy-2-heptylquinoline (HHQ) or 2-heptyl-3,4-dihydroxyquinoline (PQS, Pseudomonas quinolone signal), cyclo(L-Phe-l-Pro), and cyclo(L-Pro-l-Tyr, which might give them antimicrobial properties. This research explores the biotechnological potential of these bacteria in fighting the diseases affecting avocados in Colombia.

1. Introduction

Avocado production worldwide has increased to meet the demands of the fine food and cosmetics industries [1]. In Colombia, avocado production accounts for approximately 5.5 % of total worldwide production. Due to its tropical location, this crop is susceptible to infestations by pests and diseases, which can greatly impact production by reducing yields and fruit quality, increasing production costs, and requiring the use of potentially harmful agricultural supplies such as glyphosate, imidacloprid, benomyl, paraquat, 2,4-D, permethrin, and copper sulphate [2–5].

Avocado crops are susceptible to various pests and diseases, with thrips being particularly significant due to their phytophagous nature. Thrips can inflict damage during the early stages of fruit development, causing small injuries that manifest as protrusions on the fruit's surface and lead to color loss. The impact becomes more pronounced as the fruit ripens. Furthermore, avocado crops face serious threats from diseases such as root rot, attributed to *P. cinnamomi*, and anthracnose, caused by *Colletotrichum* spp. These diseases not only cause damage to the fruit but can also affect the tree, resulting in significant losses for farmers [6,7].

Although the use of agrochemicals remains the most common practice of biocontrol in avocados and most crops worldwide, the adverse environmental effects caused by synthetic pesticides, along with the need to adopt sustainable approaches to crop management, have prompted research efforts prioritizing the exploration of alternative methods. These approaches aim to mitigate environmental impacts while ensuring the well-being of various crops. A focal point of these studies involves the development of new plant protection strategies that use biological agents known for their low environmental impact, environmental friendliness, and safety [8,9]. Research into bioactive inhibitory substances produced by microorganisms has gained prominence. These substances have proven effective as a viable method for the

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biological control of pests and diseases [7].

The exploration of microbial communities in symbiosis with their hosts represents a novel perspective in biotechnological research, offering a promising reservoir of microorganisms that has garnered increased attention in recent years. Insects, as one of the most diverse groups of animals, exhibit remarkable adaptability, providing an ideal environment to host a microbial community with diverse capacities. The interactions among insects, plants, and their associated microbial communities present a rich landscape for exploration. Comprehensive knowledge of insect-associated microbiota not only expands our understanding of ecological dynamics but also opens opportunities for developing innovative strategies in pest and disease control. Moreover, these microbial communities within the insect microbiota possess the potential to produce compounds with biotechnological interest as antimicrobial molecules able to control fungi and bacteria [10–12].

Microbial communities use chemical communication and signaling mechanisms to interact with each other. These signaling systems may also play a dual role by regulating the expression of phenotypic genes, leading to biofilm formation, synthesis of virulence factors, production of enzymes, and generation of secondary metabolites with antimicrobial activities. One of these signaling systems in bacteria is known as quorum sensing (QS). QS signaling molecules constitute a complex environmental system that is regulated based on the density dynamics of the bacterial population. Thus, the detection and identification of signaling molecules offer an initial view of the QS signaling mechanisms used by bacteria and their potential application in biocontrol processes against phytopathogens [13–15].

The presence of pests and the development of diseases in different crops is one of the main limitations in the acceptability of their products in addition to generating significant losses for farmers; therefore, it is necessary to screen new sources of antimicrobial agents with potential uses in the protection of crops against phytopathogens. The microbial communities associated with pest insects represent an underexplored domain with limited information. The present work was proposed to study the cultivable microbiota of *Frankliniella* spp. and *Scirtothrips hansoni*, pest insects, evaluating their potential antagonistic capacity and the detection of QS signaling molecules. The results obtained in this research contribute to the development of biotechnological applications for the control of agriculturally significant phytopathogenic microorganisms to reduce the use of fertilizers and chemical pesticides to sustainably improve plant growth and health.

2. Material and methods

2.1. Bacteria selection and culture conditions

In this study, we selected 10 bacteria (Table 1) from 42 isolates of wild populations of thrips species *Frankliniella* spp. and *Scirtothrips hansoni* collected in eastern Antioquia, Colombia; identified taxonomically and molecularly by Cano-Calle et al., [16].

Table 1
Bacteria selected and isolated from the microbiota of wild populations of *Frankliniella* spp. and *Scirtothrips hansoni* for bioactivity assays.

Bacteria strains	
Bacterial isolate code (Accession number_GenBank)	**Strain**
Gram-positive	
Isolate T7F1(3) (NR_074540.1)	*Bacillus cereus*
Isolate T7F4(1) (NR_114,581.1)	*Bacillus thuringensis*
Isolate T7F4(3) (NR_112,637.1)	*Bacillus safensis*
Isolate T9H4 (NR_116,022.1)	*Bacillus amyloliquefaciens*
Isolate T7H3(1) (NR_116,240.1	*Bacillus velezensis*
Gram-negative	
Isolate T8H6 (EU029105)	*Pantoea cypripedii*
Isolate T6H1 (NR_122,057.1)	*Serratia liquefaciens*
Isolate T3H1(1) (NR_041978.1)	*Pantoea agglomerans*
Isolate T9H2(7) (NR_104,936.1)	*Moraxella osloensis*
Isolate T9H2 (NR_157,757.1)	*Sphingomonas olei*

The selection of the ten bacterial strains was based on their identification as the most abundant groups within the microbiota of *Frankliniella* spp. and *Scirtothrips hansoni* [16]. Additionally, some of these strains have been reported in several scientific articles for their antimicrobial activity and biomolecule production, while others were chosen for their potential to be explored in these applications.

The phytopathogenic strains *Colletotrichum, Fusarium,* and *Phytophthora* sp., belonged to the group Biotecnología vegetal, *Cylindrocladium* sp., and *Xanthomonas axonopodis*, to the Laboratorio de Sanidad Vegetal; *Ralstonia* sp., to Laboratorio de Prospección y Diseño de Biomoléculas from the Universidad Nacional de Colombia, Medellín campus. Fungal strains were maintained on potato dextrose agar (PDA) plates at 25 °C, the bacterial strains on LB and Nutritive medium, the phytopathogenic bacteria *X. axonopodis* was maintained in nutrient agar medium (Merck KGaA, Darmstadt, Germany), and *Ralstonia* sp. in Casamino acid peptone glucose (CPG) medium.

2.2. In vitro screening for antagonism

2.2.1. In vitro antagonistic activity against phytopathogenic fungi by dual cultures

To identify bacterial antagonist candidates, all strains were assessed for their ability to inhibit the growth of five phytopathogens. Their antifungal activity was evaluated against phytopathogenic fungi using the dual culture technique on PDA media, as described by Hameeda et al. [17]. The conventional streaking method was modified by streaking the bacterium in a circle using the lid of a small petri dish around the agar plug. Plates with only fungus and no bacterial culture were used as negative controls. The plates were incubated at 28 °C for 72 h. The percent inhibition of the fungus was calculated using the following formula:

$$I = [(C - T) / C]x100.$$

where I is the percent inhibition of mycelial growth, C is the radial growth of fungus in the control plate (mm), and T is the radial growth of fungus on the treatment with the bacterium (mm).

2.2.2. In vitro antagonistic activity against phytopathogenic bacteria by disc diffusion and cell-free extracts

2.2.2.1. Antibacterial activity by disc diffusion. Their antibacterial activity was tested using the technique described by Kheirandish and Harighi [18]; briefly, 300 µL of the phytopathogen culture suspension (approximately 10^8 CFU/mL) was poured into the plates. A paper disc (approximately 10 mm in diameter) was immersed in each isolated suspension of bacterial culture, spectrophotometrically adjusted to a concentration of approximately 10^8 CFU/mL (as measured by absorbance at 600 nm), and then placed on the pathogen-inoculated plates. Sterile water was spotted in the plates used as a control. The plates were incubated at 28 °C for 48 to 72 h, and the presence of inhibition halos was considered a positive test result.

2.2.2.2. Antibacterial activity of cell-free extracts

2.2.2.2.1. Preparation of cell-free supernatants. The bacterial strains that showed antagonistic activity against phytopathogenic bacteria were used to evaluate the antibacterial activity of their extracts against these and the other bacteria. The selected cultures were reactivated by seeding them in nutrient broth until they reached an optical density measurement (OD) at a wavelength of 600 nm (OD600) of 1 (exponential growth). The supernatants were recovered by centrifugation at 10,000 × *g* for 10 min at 4 °C, followed by filtration using 0.22 µm Millipore membranes [36].

2.2.2.2.2. Effect of supernatants on the growth of X. axonopodis and Ralstonia sp. The supernatants obtained were used for microdilution tests in 96-well plates (from Nest Scientific) against strains of *X. axonopodis* and *Ralstonia* sp. The final volume inoculated in each well was 150 μL, distributed as follows: 50 μL of each supernatant, 50 μL of the medium, and 50 μL of the phytopathogenic bacteria. In the negative control well, 50 μL of phytopathogenic bacteria (at a concentration of 5 × 10^8 CFU/mL) and 100 μL of the corresponding broth were added. In the positive control well, 50 μL of antibiotic (tetracycline at a concentration of 50 mg/L), 50 μL of the medium, and 50 μL of the phytopathogenic bacteria were added. Five replications of supernatants of each isolate for each treatment were evaluated, and the experiment was performed equally to the positive control (Tetracycline 50mg/L). Additionally, the remaining wells were used to control that the supernatants were cell-free, for each one three wells were inoculated. The plate was incubated at 29 °C in a MultiSkan Sky spectrometer (Thermo Scientific™) for 48 h. Absorbance readings at 600 nm were taken every 1.5 h using the SkanIt RE 6.1 program (Thermo Scientific™).

2.2.2.3. Detection of quorum sensing (QS) molecules in gram-negative bacteria

2.2.2.3.1. Preparation of bacterial cultures. All the isolated 5 Gram-negative strains were grown in LB medium at 37 °C overnight and sub-cultured in LB agar. A single colony was inoculated and incubated overnight at 37 °C at 200 rpm until the cell culture reached an optical density of 1.0 (OD600). Next, 0.25 mL of each culture was transferred to a 250 mL Erlenmeyer flask containing 25 mL of LB broth and incubated overnight at 37 °C with continuous stirring at 200 rpm [19,20].

2.2.2.3.2. Extraction of AHL and AHQ molecules from cells and supernatant. AHL and AHQ extraction was performed following the method previously described by Fletcher and co-workers [19]. After culture preparation, 10 ml of each culture was separated by centrifugation at 10,000 × *g* for 10 mins at 4 °C. Supernatants were filter-sterilized using a Nalgene syringe filtration system (with a 0.2 μm pore size, Thermo Scientific, NY, USA) and transferred to a 50 mL falcon tube.

The supernatant was combined with a 1:1 vol of acidified ethyl acetate (0.01 % acetic acid). and the sample was vortexed for 30 s to mix two phases (organic phase/aqueous phase). The mixture was then transferred to a separating funnel, previously washed with acetone, and it was left undisturbed until two phases were separated. Next, the organic phase was transferred into another 50 mL falcon tube [19]. The extraction process was repeated twice or more until a total volume of 30 mL was obtained. The organic phase was dried by rotary evaporation, and to recover the extract, the round-bottom flask was washed three times with 0.5 mL of methanol, resulting in a total recovery of 1.5 mL of extract. This extract was then concentrated using a DNA concentrator at 45 °C until completely dry and stored at −20 °C.

2.2.2.3.3. Standard molecules and biosensor strains. The synthetic molecules AHL and AHQ, N-3-Oxo-Dodecanoyl-l-Homoserine Lactone (OdDHL), N-3-Oxo-Hexanoyl-l-Homoserine Lactone (OHHL), 2-Heptyl-3-Hydroxy-4-Quinolone (PQS), and 2-Heptyl-4-Hydroxyquinoline (HHQ), along with the biosensor strains (pSB401, pSB1142, and pqsA-Lux) (Table 2), were kindly provided by Dr. Miguel Cámara and Stephen Hebbs from the Bioscience School of Nottingham University, UK; and conserved as previously described by Fletcher et al., 2007 [19].

2.2.2.3.4. Preparation of TLC plates and running of samples. The extracts were resuspended in 100 μl of methanol and tested for QS activity, spotting 5 μL of each extract and syntetic molecules on 60 F254 reversed-phase TLC plates (20 cm × 20 cm TLC aluminum plates, Millipore, Germany) with a dichloromethane:methanol 95:5 (v/v) mobile phase. Once dried, the migration patterns (Rf) visualized through UV and green light and compared with each other and with control QS AHL molecules. The Rf migration value was calculated according to the formula Rf=*X*/Y, where (X): is the distance from the origin of the sample to the center of the detected molecule's spot, and (Y) is the distance traveled by the solvent front from the origin to the end of the silica gel plate. TLC plates were overlaid with 100 mL of soft LB agar (at a concentration of 0.65 % agar) inoculated with the biosensor and incubated at 37 °C 6 to 10 h. Bioluminescence was visualized in a dark room and captured with X-ray autoradiography plates [21].

Table 2
List of synthetic QS molecules and biosensors used in TLC detection bioassays.

Syntetic Molecules				
Name	**Synonyms**	**Molecular Formula**	**KEGG ID**	**Isotopic Mass**
N-3-Oxo-Dodecanoil-l-Homoserin Lactona	OdDHL	$C_{16}H_{27}NO_4$	C11840	298,2012
N-3-Oxo-hexanoil-l-Homoserin Lactona	OHHL	$C_{10}H_{15}NO_4$	C21198	214,1074
2-heptil-3-hidroxi-4-quinolona	PQS	$C_{16}H_{21}NO_2$	C11848	260,1651
2-heptil-4-hidroxiquinolina	HHQ	$C_{16}H_{21}NO$	C20643	244,1715

Biosensor Strains				
Biosensor	**Strain**	**Molecule detected**	**Signal**	**Reporter**
pSB401	*E. coli*	OdDHL	Bioluminescense	LuxCDABE
pSB1142	*E. coli*	OHHL		LuxCDABE
pqsA-Lux	*P. aeruginosa*	PQS and HHQ		pqsABCDE

2.2.2.3.5. Ultrahigh-performance liquid chromatography and high-resolution mass spectrometry analysis. The extract from the most bioactive bacteria was fractioned in a UHPLC system, and the samples were injected in 1 μL volumes and then separated by reversed-phase chromatography using a Thermo® Ultimate 3000 system and a Reprosil-Pur Basic C18 (DR Maisch brand) column. The separation was performed, maintaining a temperature of 40 °C and using buffer A: 100 % Water Milli-Q with 0.1 % formic acid and B: 100 % acetonitrile with 0.1 % formic acid. The solvent gradient was an isocratic flow initially maintained with 5 % B for 3 min and then changed to 20 % B in one minute. The gradient ran from 20 % to 95 % B in 18 min. Condition B was maintained for 3 min and returned to the initial condition in 3 more minutes, for a total time of 32 min. High-resolution mass spectra were recorded on an Impact II ESI-QTOF from Bruker® in MRM mode based on the ions ($M + H$) 200.1281, 214.1073, 298.1073, 244.1695, and 260.1650, which correspond to HHL, OHHL, OdDHL, HHQ, and PQS. The mass spectrometer conditions were as follows: dry heater: 200 °C, nebulizer: 29 psi, dry gas: 8 L/min, capillary: 4500 V, charging voltage: 2000 V, and fragmentor: 700 Vp. The spectra in MS/MS mode were collected using the following parameters: MS range (full scan): 100–1200 *m/z*. Auto MS/MS in the range of 50–1200 *m/z*, collected at a rate of 5 spectra/s with a maximum of 3 precursors selected and fragmented using a collision energy of 10 eV

Using the Data Analysis and Target Analysis software from Bruker®, the spectra were processed in search of the exact masses of the standards and possible adducts with sodium and methanol. Additionally, the complete report of the compound spectra was analyzed using the MetFrag platform, which compares spectra, fragmentation patterns, and molecular weights against various databases, primarily KEGG (Kyoto Encyclopedia of Genes and Genomes) and PubChem.

2.3. Statistical analysis

The differences among treatments in each experiment were compared using one-way analysis of variance (ANOVA) followed by Tukey's test through R software. In all cases, the threshold significance was 5 %. Assumptions of normality and equality of variances of data were previously tested using Shapiro–Wilk and Levene tests, respectively.

3. Results

3.1. In vitro antimicrobial activity

To investigate whether the isolates had an antagonistic effect against fungal phytopathogen agents, all bacterial strains selected in this study were tested by in vitro inhibition assays. Among the 10 isolates assessed, *Serratia liquefaciens* T6H1, *Pantoea agglomerans* T3H1(1), *Bacillus amyloliquefaciens* T9H4, and *Bacillus velezensis* T7H3(1) showed significant antagonistic activity. The antifungal activity of *Bacillus* species in the dual-plate assays effectively inhibited the growth of all target fungi. However, the inhibition percentages of the gram-negative strains varied. *Pantoea agglomerans* T3H1 [1] was found to inhibit the four phytopathogenic fungi with a higher percentage compared to *Serratia liquefaciens* T6H1, except for *C. gloeosporioides*, which showed a lower inhibition percentage (20.9 ± 3.8 %). Additionally, there were no significant differences between the treatments against *Fusarium* sp. F4. (Fig. 1)

Fig. 2 shows that for each phytopathogenic fungus, the three cultures had the highest percentage of inhibition. Overall, it is clear from the growth of the fungi that, in most cases, there is no direct contact between the two microorganisms, suggesting that the bacterial inoculum acts as a physical barrier.

The detection methodology using sensi-disc for the confrontation of the isolated bacterial strains against the phytopathogenic bacteria *X. axonopodis* and *Ralstonia* sp. aimed to perform an initial screening to identify which of these cultures have potential antibacterial activity. However, no inhibition halos were observed in the assay (Supplementary Table 1). As a result, the two gram-negative bacteria that displayed antifungal activity and five gram-positive strains were further evaluated using a plate assay. Despite none of the supernatants showing any inhibitory activity against *Ralstonia* sp., treatment 1 (Tto 1), corresponding to T9H4 (*Bacillus amyloliquefaciens*), had an inhibitory effect on *X. axonopodis*, limiting its growth, similar to the control with a tetracycline antibiotic (Fig. 3).

3.2. Detection of AHL and AHQ molecules by specific biosensors

The QS signaling molecule extraction process in the supernatant and TLC analysis of the five analyzed samples allowed the detection of some AHL and AHQ molecules through bioluminescence production (Table 3). N-3-(oxododecanoyl)-l-homoserine (OdDHL) was detected in all five supernatants evaluated, with similar migration patterns in X-ray autoradiography plates and similar Rf values compared to the positive control (Rf= 0.765), as shown in Table 3. The *Pantoea agglomerans* and *Sphingomonas olei* samples showed a similar migration pattern with the AHL N-3-(oxohexanoyl)-l-homoserine (OHHL) molecule, and the Rf values were close to those reported in the positive control pSB401 (Rf= 0.665).

The detection of quinolones 2-heptyl-3-hydroxy-4-quinolone (PQS) and 2-heptyl-4-hydroxyquinoline (HHQ) was either null or weak, respectively. *Pantoea cypripedii* showed a weak signal in the detection of HHQ; however, the migration pattern and Rf value did not match (Table 3), suggesting that there could be another molecule of a similar nature, considering that the biosensor was able to respond with a positive signal. The separation of molecules by TLC provides a visual relationship of the molecules produced by bacteria evaluated against the synthetic molecules (positive control). However, AHL and/or AHQ require additional analysis to confirm the identity of the molecule.

2.3. Ultrahigh-performance liquid chromatography and high-resolution mass spectrometry analysis

The standardization process of the synthetic molecules through UHPLC/MS to obtain the fragmentation patterns and their corresponding masses showed that traces of the OdDHL and HHQ molecules were detected with intensity values in the peaks below 500 in the extracts of *Pantoea cypripedii* (Fig. 4); however, in the next run, these traces were not detected. Nevertheless, the spectrometry analysis allowed the detection of two different molecules in the five gram-negative bacterial extracts, which belong to the group of 2,5-Diketopiperazines, also known as cyclopeptides (Supplementary Table 2). Protonated ions were

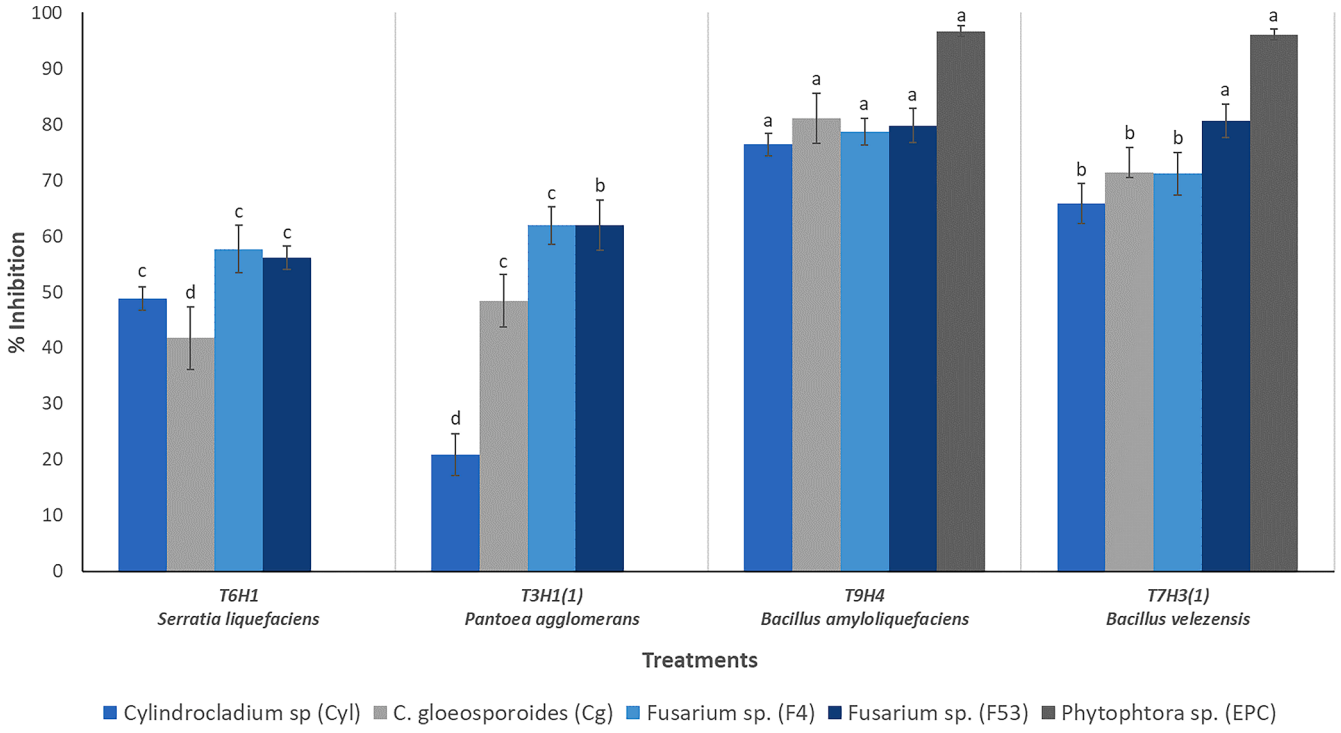

Fig. 1. Screening of antagonistic bacterial strains isolated from trips of avocado for the ability to inhibit the growth of diverse fungal phytopathogens in dual plate assays. Different letters indicate statistically significant differences ($p < 0.05$).

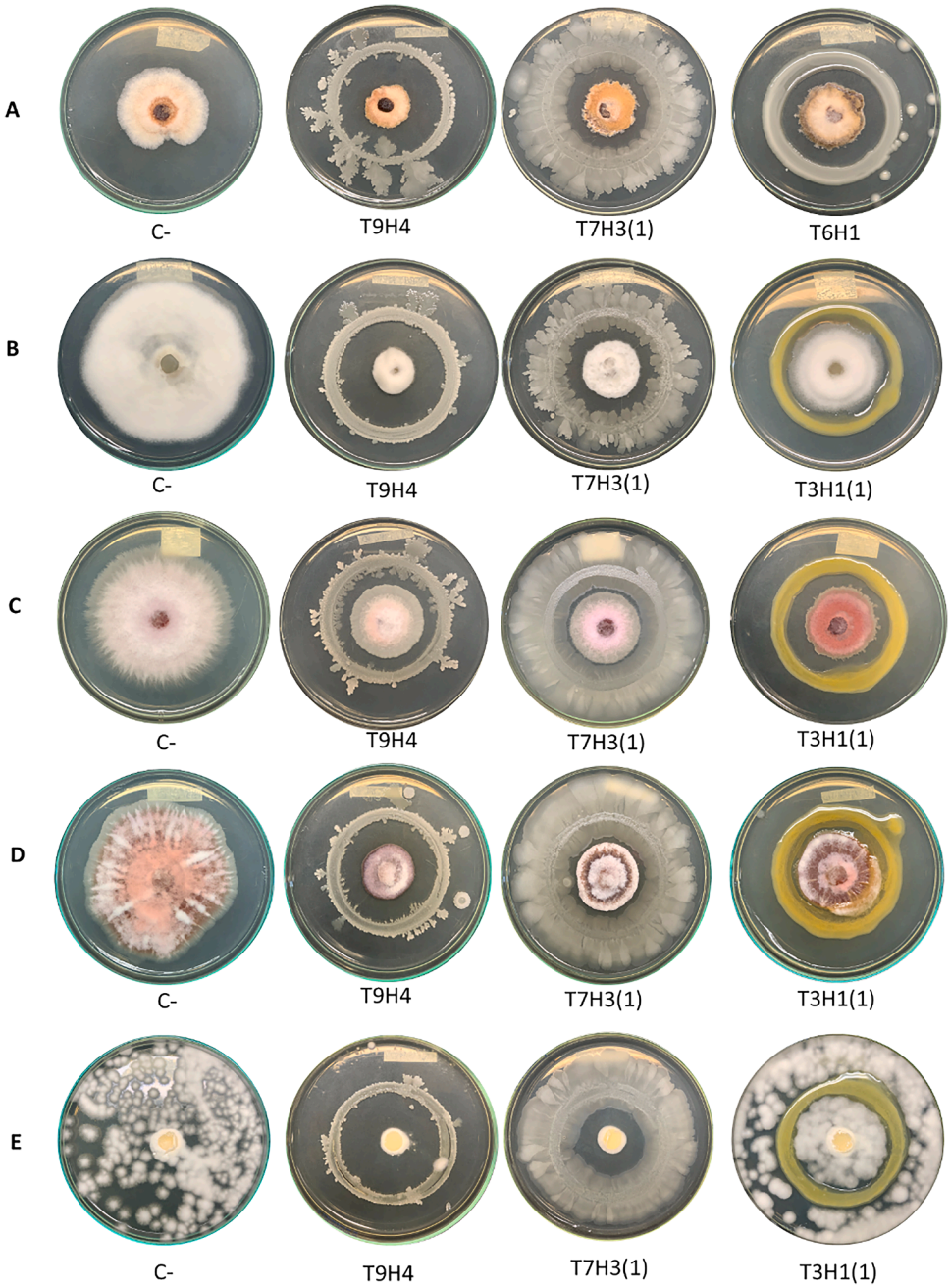

Fig. 2. Dual cultures in PDA medium. (A) corresponds to *Cylindrocladium* sp., (B) *C. gloeosporoides*, (C and D) *Fusarium* sp. F4 and F53, respectively, (E) *Phytophthora* sp.; (C-) Negative control: phytopathogen without treatment.

identified in the mass spectra of cyclo(L-Phe-l-Pro) ($C_{14}H_{16}N_2O_2$) and cyclo(L-Pro-l-Tyr) ($C_{14}H_{16}N_2O_3$), and their masses were fragmented (Figs. 5 and 6).

The fragmentation pattern of cyclo(L-Phe-l-Pro) in the extracts showed seven of eight main fragments, among the largest peak detected (Fig. 4). It was possible to observe other smaller ones that correspond to some isotopic variants, sharing up to 19 fragments, according to reports in databases (KEGG and PubMed) the intensity of the ion corresponding to 200 usually has low detection levels, the same fragment that represents the missing one.

The cyclopeptide cyclo(L-Pro-l-Tyr), also known as maculosin, was also detected in all the samples, sharing coincidences in all the fragments. For example, for the T6H1 sample (Fig. 5), 8 main peaks were detected, and together with the isotopic variants, 20 shared fragments were obtained.

4. Discussion

The different genera of bacteria associated with insect guts are closely related to the rhizosphere, phyllosphere, and soil bacteria, showing some beneficial effects, such as antagonistic activity against pathogenic bacteria and fungi [21]. Therefore, bacteria related to insect pests could represent a useful source of biocontrol systems for different phytopathogens. Bacteria have a wide variety of antagonistic mechanisms. Six mechanisms have been described, and each bacterium can use at least two of them: 1) competition for nutrients and space [8,22,23]; 2) production of siderophores [8,24]; 3) production of lytic enzymes [8, 25]; 4) biofilm formation and quorum sensing [8]; 5) antibiosis through the production of antibiotics, antifungal molecules and volatile organic compounds (VOCs) [8,26–28]; and finally, 6) induction of resistance in the host [8,9,29].

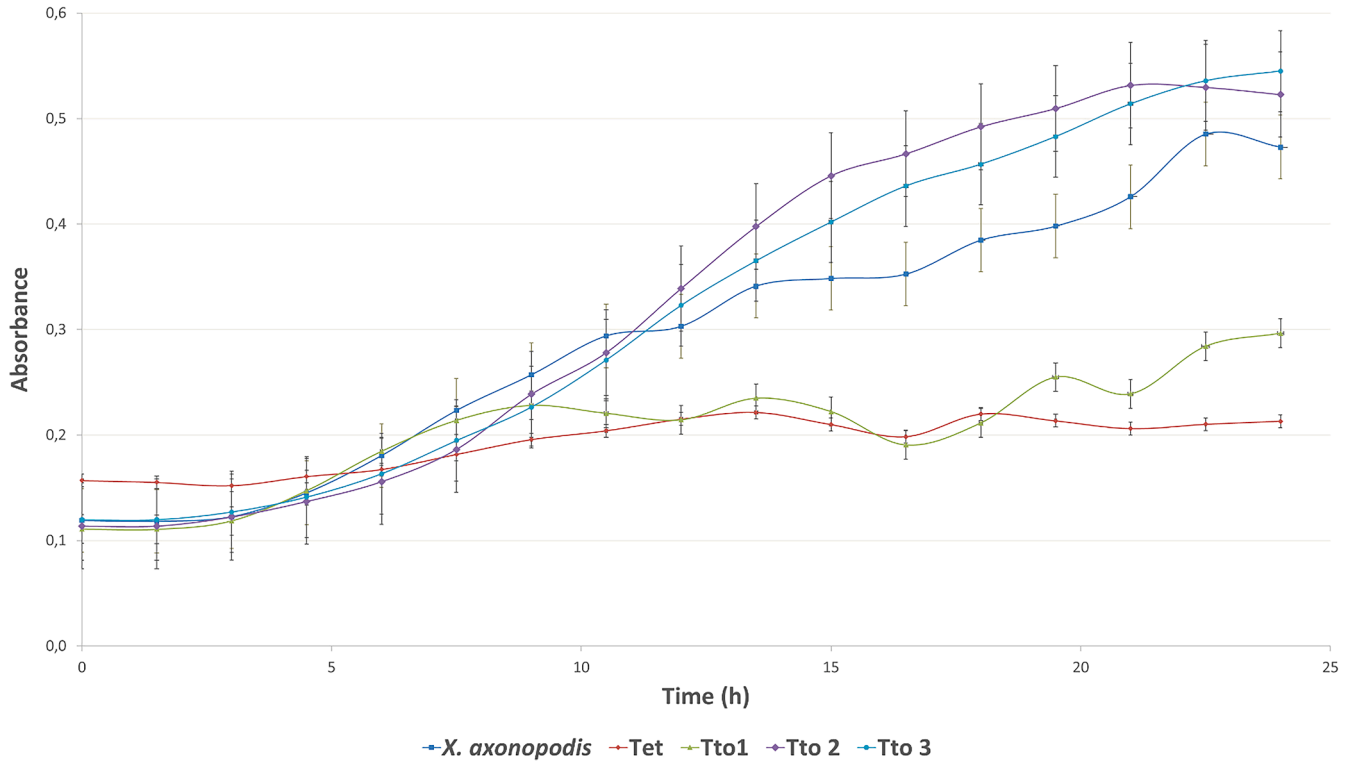

Fig. 3. Effect of bacterial supernatants on the growth of *Xanthomonas axonopodis*. Tto1: *Bacillus amyloliquefaciens* T9H4, Tto2: *Pantoea agglomerans* T3H1(1), Tto 3: *Pantoea cypripedii* T8H6, Tet: (C+) Tetracycline.

Table 3
Molecules detected by TLC bioassay.

Code	Microorganism	Biosensor			
		psB1142	psB401	pqsA-lux	
		odDHL Value (Rf)	OHHL Value (Rf)	PQS* Value (Rf)	HHQ Value (Rf)
T8H6	*Pantoea cypripedii*	+ (0.715)	–	–	±0,294
T6H1	*Serratia liquefaciens*	+ (0.713)	–	–	–
T9H2 (7)	*Moraxella osloensis*	+(0.681)	–	–	–
T3H1 (1)	*Pantoe agglomerans*	+ (0.685)	+ (0,675)	–	–
T9H2	*Sphingomonas olei*	+ (0.693)	+ (0.636)	–	–

* Control detection was weak. (+) Presence, (-) Absence, (±) Weak signal.

The in vitro prescreening test of dual cultures allowed us to select four strains, T9H4, T7H3(1), T6H1, and T3H1(1), with notable antifungal activity against *C. gleosporoides, Cylindrocladium* sp., and *Fusarium* sp. The *Bacillus* genus has been reported as an efficient suppressor of various important pathogenic fungi and bacteria. Several bioactive secondary metabolites have been isolated from this genus [30–33].

Bacillus sp. strains tend to mainly use competition for nutrients and space as a mechanism of antagonism, as reported by Kong et al. [34] in their study with *B. megaterium*; however, some metabolites, such as antibiotics and antifungal peptides, were produced by a strain of *B. amyloliquefaciens* against seven phytopathogenic fungi, identified as fengycin, surfactin, and iturin A, showing the iturin A the most significant antifungal activity [30,32].

These metabolites, in addition to iturin, have been previously reported as being produced by the species *B. velezensis*, along with VOCs, siderophores, and inducers of systemic resistance in plants [35]. A study by Lim et al. [36] reported the production of two diffusible compounds by *B. velezensis*: Bacilomycin L and Fengicin A. The study also found that volatile compounds such as dimethyl sulfoxide, 1-butanol, and 3-hydroxy-2-butanone (acetoin) inhibited the growth of *Phytophthora* sp. and *Colletotrichum* sp. by 31 % and 21 %, respectively.

The high percentages of inhibition of the strain T9H4 *B. amyloliquefaciens* against the four evaluated phytopathogenic fungi were characterized by a growth behavior in which direct contact with the fungus was not strictly necessary for inhibition. This could be attributed to the production of antifungal metabolites diffused in the medium, affecting the normal development of their cellular structures [37].

Although the activity of *Bacillus* sp. species against *R. solanacearum* has been reported in previous studies, none of the evaluated strains in the present study exhibited such activity. It has been observed that the antagonistic mechanisms used by these bacteria for the biological control of phytopathogenic bacteria are mainly indirect, such as competition for iron, induction of resistance, and promotion of growth in the host plant [38,39]. In a study by Li et al. [40], a significant in vivo inhibitory activity of a strain of the species *B. amyloliquefaciens* against *X. axonopodis* and its effect on the development of the "bacterial blight" disease was reported. The role of surfactin was highlighted by the multiple beneficial effects on plants, including facilitating the adhesion of biological control agents (BCAs) to plant surfaces, activating the plant resistance system, and inhibiting the formation of biofilms of phytopathogenic organisms. Considering the previous studies and the results of the present study, it cannot be ruled out that the evaluated strains of *Bacillus* sp. may have potential as biological control agents (BCAs) for the management of bacterial diseases in plants. Therefore, further in vivo evaluations are needed to detect other types of control mechanisms, as previously reported.

The *Pantoea* genus has previously been reported for its wide application as a biological control agent in the postharvest of various fruits [41]. This genus is considered ubiquitous in the environment, and it is

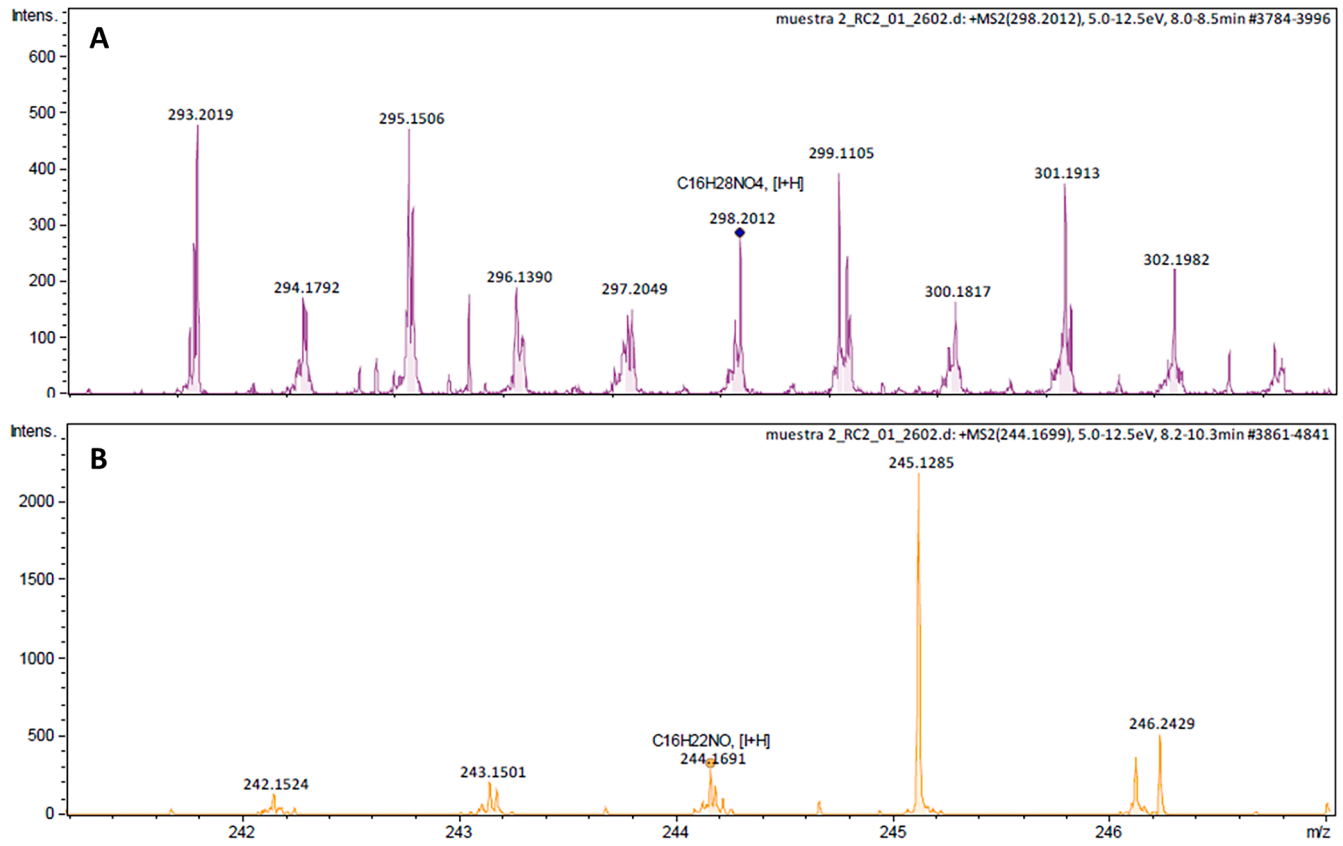

Fig. 4. Mass spectra consistent with standard AHL and AHQ molecules obtained by UHPLC-MRM-MS on sample T8H6. (A) Detection odDHL, [$M + H$] = 298.2012. (B) Detection HHQ, [$M + H$] =244.1691.

frequently found associated with plants [42]. *P. agglomerans* strains show the ability to produce VOCs [43,44]; the study conducted by Dagher et al. [43] found that these VOCs inhibited 34.5 % of the phytopathogenic fungus *Macrophomia phaseolina* in soybeans. However, the percentages of inhibition by antibiosis were 89 %, and in dual cultures at 24 and 48 h, the percentages of inhibition were 43 % and 62 %, respectively. The study also highlighted that the application of this antagonist in the soil reduced the population of the phytopathogen, resulting in a significant decrease in disease in the host plant.

The genus *Serratia* sp. is recognized for its virulence factors, such as the production of chitinases, antifungal and antibacterial activity [45, 46] for example a strain of *Serratia quinivorans* showed some antagonistic interactions with fungal phytopathogens [47]; however, the species *S. liquefaciens* is mainly associated with clinical infections and food contamination and has some benefits, such as bioremediation processes and the promotion of plant growth [48]. According to the literature reviewed, no specific reports were found on the antifungal or antibacterial activity of the species *S. liquefaciens*, except for a study by Kalbe et al. [46], who detected antifungal activities against two phytopathogenic fungi through the production of chitinolytic enzymes.

This study could be part of the early reports of antifungal activity by this bacterium. Under the conditions of the present study, this gram-negative bacterium managed to reach percentages of inhibition from 20 % to 62 % in phytopathogenic fungi. It could be hypothesized that the bacteria, having a higher growth rate over time, can use the nutrients available, exhibiting the competition for nutrients and space mechanism that this species could use; however, to confirm this, specific tests must be carried out [49,50].

The results obtained through the utilization of these bacterial species position them as potential candidates for biological control agents (BCAs) in the management of phytopathogens. However, it is crucial to assess whether these bacteria possess the necessary characteristics for practical application [8,51,52]. The demonstration of high inhibition percentages provides an opportunity for additional tests to identify specific antagonistic mechanisms and metabolic pathways in these strains, as well as strategies to enhance their efficacy. Molecular analysis, including the complete genome sequencing of these strains, would enable the identification of specific genes responsible to produce antimicrobial metabolites and the synthetic and regulatory pathways involved in their synthesis.

Understanding the interactions between isolated bacteria and selected phytopathogenic fungi is relevant. Thissera et al. [44] described how, through cocultures, it is possible to activate groups of biosynthetic genes. These microbial cocultures have proven to be a powerful method for replicating interactions among microbial communities in wild conditions, influencing the production of secondary metabolites that may go undetected in axenic cultures. This approach could be complemented by transcriptomic analyses, revealing the response systems of phytopathogens to antimicrobial agents and how they mediate the gene expression of the biological control agent [53,54]. Integrating genomic and transcriptomic data could identify essential genes with changes in expression, providing strategies to intervene and enhance antimicrobial activity.

The main objective of consolidating the available tools is to develop a crop-applicable product that aligns with current agricultural requirements. Although the isolation and purification of the detected bioactive metabolites could be achieved through a scale-up process in a bioreactor [7], understanding the biosynthesis processes of these metabolites is presented as a crucial step for their enhancement.

Studies, mostly focused on *Bacillus* species, have identified metabolites such as bacillomycin, fengycin, and iturin. In the case of *Pantoea agglomerans*, the detection of pulicatina homologs has been reported [44]. These metabolites share the characteristic of biosynthesizing through the nonribosomal peptide (SNPR) [55,56]. This type of

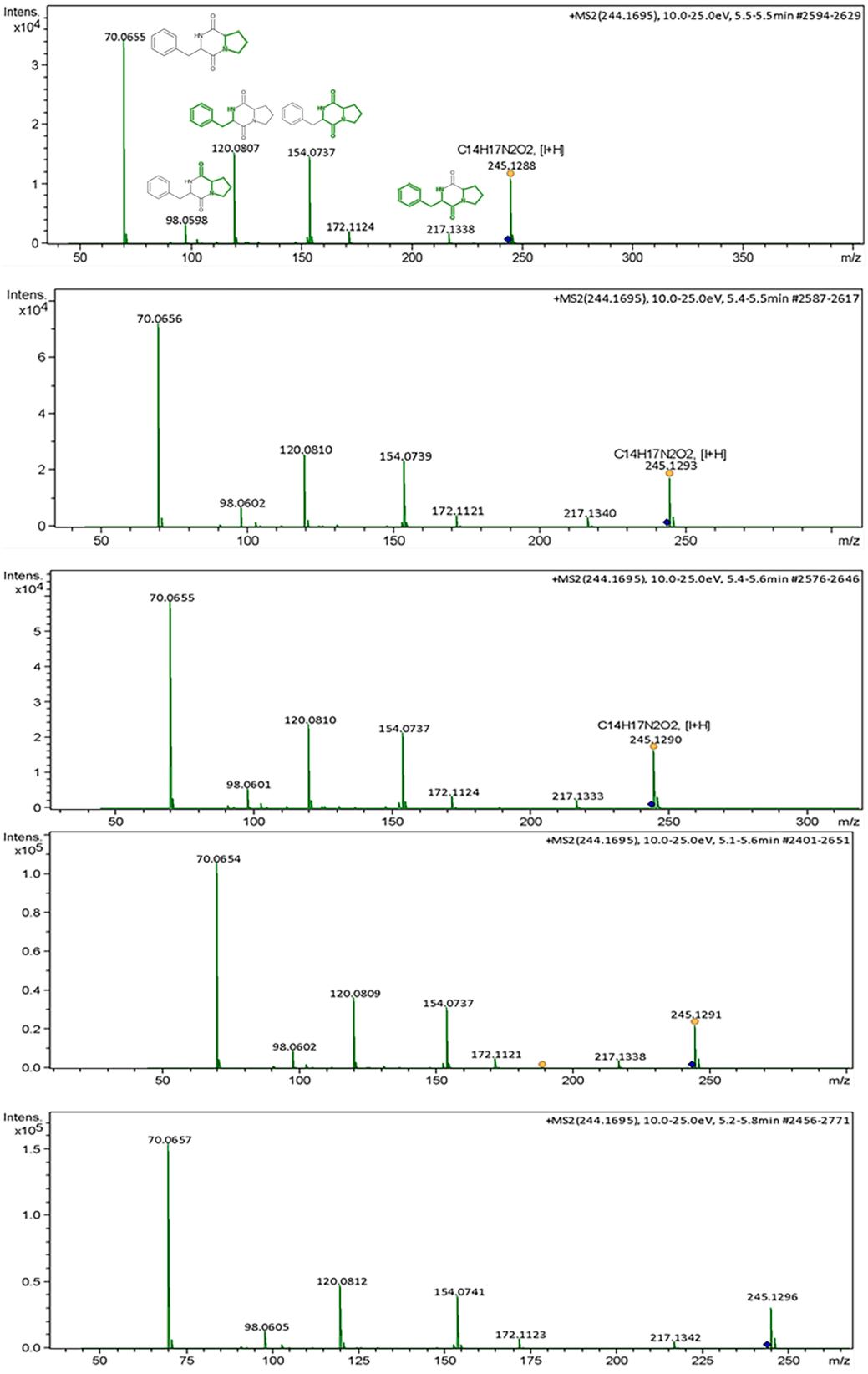

Fig. 5. Mass spectra corresponding to the cyclo(L-Phe-l-Pro) molecule were obtained in the bacterial extracts of the gram-negative strains through UHPLC/MS. A) T3H1(1), $[M + H] = 245.1288$, B) T8H6, $[M + H] = 245.1293$, C) T9H2(7), $[M + H] = 245.1290$, D) T9H2, $[M + H] = 245.1291$, E) T6H1, $[M + H] = 245.1296$.

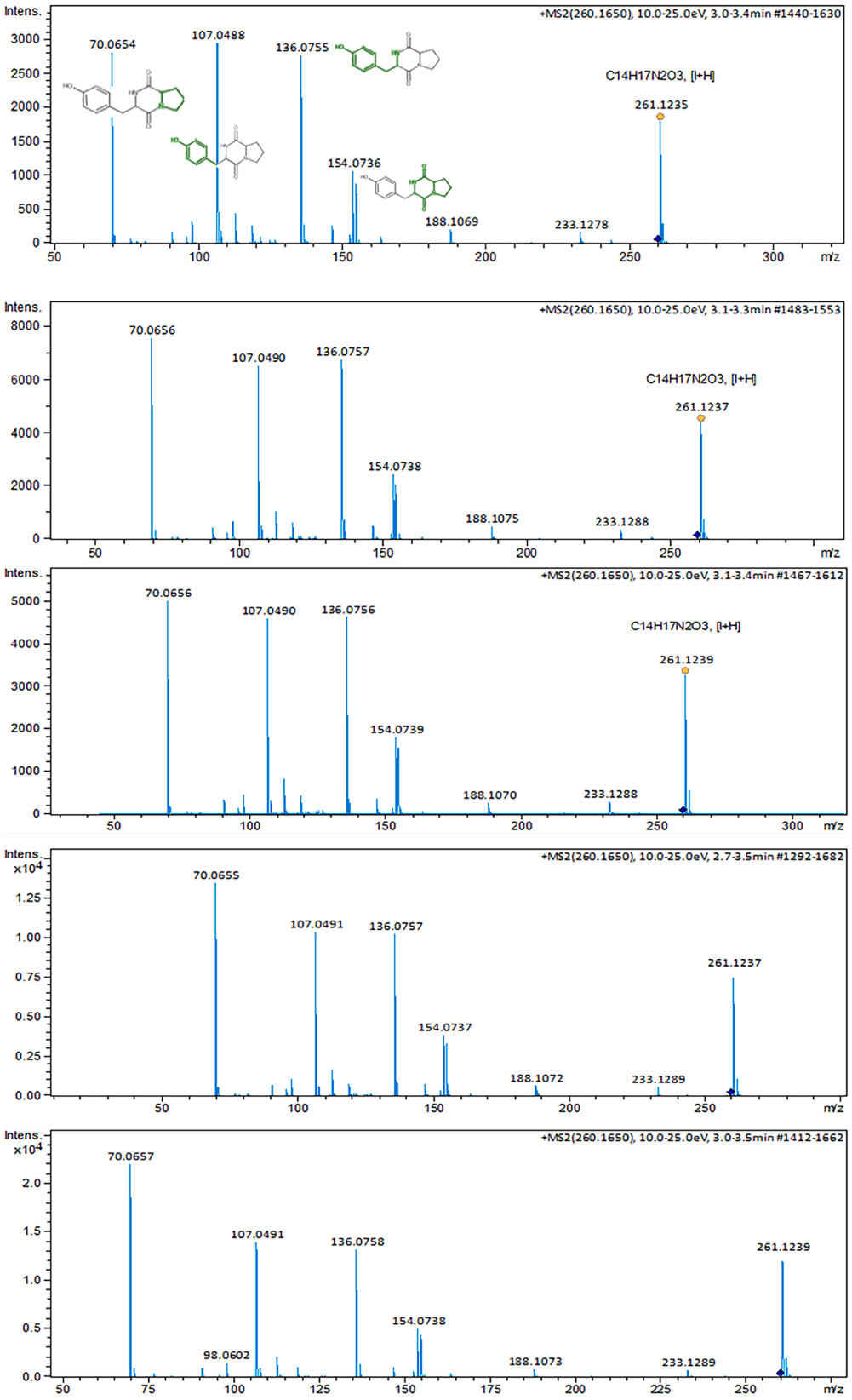

Fig. 6. Mass spectra corresponding to the cyclo(L-Pro-l-Tyr) molecule were obtained in bacterial extracts of gram-negative strains through UHPLC/MS. A) T3H1(1), $[M + H] = 261.1235$, B) T8H6, $[M + H] = 261.1237$, C) T9H2(7), $[M + H] = 261.1239$, D) T9H2, $[M + H]] = 261.1237$, E) T6H1, $[M + H] = 261.1239$.

metabolite consists of cyclic lipopeptides, and their biosynthetic process is of great interest due to the wide variety of monomers; this, in turn, has allowed the creation of synthetic products derived from those obtained from natural sources [57]. Considering the exceptional properties of these lipopeptides, research has intensified toward identifying new biosynthetic models of these peptides in genomic sequences and projecting them toward the production of unnatural peptides on a larger scale [58].

Several related gene systems are regulated by quorum sensing detection [59,60]. For that reason, it is important to evaluate the ability to produce Quorum Sensing signaling molecules and identify them. The quorum sensing phenomenon occurs when the density of the cell population increases and specific signal molecules reach a concentration threshold, further inducing the signal and resulting in a process of gene regulation that affects the behavior of the population [13,61,62]. For the most part, these changes have been associated with gene regulatory mechanisms for biofilm formation, enzyme and antimicrobial production, synthesis of virulence factors, motility, and sporulation, among others [13–15,63]. These capabilities can be used to exert antagonistic activity, producing metabolites that can interfere with or interrupt the development of other organisms [44,64].

Through the quorum sensing (QS) signaling system, bacteria can give rise to various types of molecules, such as N-acyl homoserine lactones (AHLs), peptides, furanones, AHQs, and DKPs. These molecules may represent only a small percentage of all those that may exist [19,65], making their identification and characterization necessary. The AHL and AHQ molecules have been detected by thin-layer chromatography (TLC) techniques, which, when combined with the use of a biosensor, allow for the detection of the response by bioluminescence in the presence of these molecules. This technique has been widely used in various QS studies [64,66,67]. However, an incorrect separation of some signaling molecules can occur, resulting in false-negatives and positives. The biosensors used can respond to signals other than the specific one and vary in intensity, which is not indicative that a sample has a higher concentration than another. This could explain what was obtained through the detection of bioluminescence in the control molecules of PQS and HHQ, where the observed signal was weak and could be attributed to the response of the biosensor [19,65,66].

Although bioassays allow us to rapidly screen Quorum Sensing (QS) signaling molecules, precise and sensitive methods are needed that provide clear information on the structure and identity of the molecules detected. For this reason, the analysis was complemented with UHPLC/MS tests [65–68]. The peaks initially obtained for the AHL and AHQ molecules had intensities below 1000, low values that would indicate the presence of traces, making it difficult to characterize the molecule, a problem that has been previously reported by other researchers. Ortori et al. [65] found that the peaks for AHQ molecules were very low, and one way to correct this type of result was to increase the concentration of the extract to be analyzed and modify the characteristics of the column. In this case, a modification of the protocol was made to increase the signal of the AHL and AHQ molecules in the samples, again using the supernatant obtained in the culture preparation step and adjusting the conditions to maintain the same proportions, with the aim that if these molecules were present in the medium, they would be induced; however, the result was the same weak or no signal from the molecules.

The weak signals detected for the N-acyl homoserine lactone (AHL) molecules in the samples analyzed could be due to various factors, such as chemical degradation, metabolic degradation, or enzymatic inactivation of these molecules [69,70]. However, more studies should be performed to confirm whether these factors affect the results of the samples analyzed. The detection of cyclopeptides could also explain the difficulty in detecting AHL molecules.

It has been reported that these molecules may exhibit agonistic and/or antagonistic activity in QS systems in gram-negative bacteria. One of the first studies to describe these activities was conducted by Holden et al. [71], who purified and identified three DKPs (Cycle (Val-Δ-Ala), Cycle (L-Phe-l-Pro), and Cycle (L-Tyr-l-Pro)) from cell-free supernatants of gram-negative bacteria. Their findings demonstrated that these compounds are able to activate lux-based AHL biosensors and promote "swarm" formation in S. *liquefaciens*. Furthermore, the study explored the competition between AHL molecules and DKPs, revealing that all three cyclopeptides antagonize OHHL, suggesting competition for the LuxR binding site. These DKPs were also able to activate various biosensors and even other pathogens [60,72].

It is not yet possible to establish the function and mechanism of these molecules in bacterial communication processes. However, the production of these molecules by some bacterial genera has already been reported previously. For example, *Sphingomonas* sp. isolated from marine mud produces a cycle (L-Pro-l-Phe), which functions as a QS signaling molecule that further promotes gellan gum production compared to control groups [73]. In a second study, carried out with the *P. agglomerans* bacterium using the coculture technique, it was discovered that it suppresses the production of secondary metabolites in the phytopathogenic fungus *Penicillium citrinum*, resulting in the formation of new metabolites, some with antifungal activity, siderophores and QS signaling molecules such as DKPs and HHQ [44].

The proline base in the composition of these DKPs gives these molecules some antibacterial and antifungal activities. Other studies have detected both cyclopeptides Cyclo (L-Phe-l-Pro) and Cyclo (L-Tyr-l-Pro) and even some new cyclopeptides in the cell-free supernatants of different bacterial species [74–76]. These reports allow us to suggest that the production of these cyclopeptides, in addition to having a role in QS communication processes, could influence the antifungal activity detected in gram-negative strains of *P. agglomerans* and *S. liquefaciens*, and their production could be another mechanism of antagonism [9]. Considering that the source of these molecules can be gram-negative or gram-positive bacteria, this allows us to think about the future development of new research. This could include isolating and purifying these molecules from the bacteria studied, including strains of *Bacillus* sp., using in vitro tests to evaluate their antimicrobial activity, carrying out an in-depth chemical characterization, and determining their mechanisms of action.

Diketopiperazines (DKPs) have been reported to exhibit a range of biological activities, including antifungal and antibacterial properties, which highlights their importance in quorum sensing (QS) signaling systems. Their isolation from various bacterial sources makes them promising candidates for further study regarding the mechanisms by which DKPs function. This initial exploration aimed to detect and identify QS signaling molecules and discovered several compounds that differed from initial expectations. Understanding the effects of these compounds is crucial, especially as they are products of bacterial activity from strains isolated from thrips. Therefore, the initial focus should be on identifying the role QS signaling systems play in bacterial communication to ensure that no unintended aspects of these interactions are altered.

5. Conclusion

This study demonstrates the potential of microbial communities associated with pest insects, particularly *Frankliniella* spp. and *Scirtothrips hansoni*, as a promising source of antimicrobial agents. By screening this cultivable microbiota, we identified significant antifungal properties in selected bacterial strains, notably from *Bacillus* and *Pantoea* strains, suggesting them potential candidates as biological control agents against phytopathogenic fungi. Further in vivo evaluations are essential to confirm the efficacy and elucidate the mechanisms used for their antimicrobial activity. The identification of diverse signaling molecules, including diketopiperazines (DKPs), emphasizes their complexity and role for quorum sensing systems and microbial interactions, highlighting the need for further investigation. These findings invite for future research into the mechanisms of these interactions to support the development of biotechnological applications aimed at

reducing reliance on chemical pesticides.

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Field study permissions

The following information was supplied relating to field study approvals (Permisomarco de recolección de especímenes silvestres, resolución 0255, 14/03/2014 (art.3)): The collection of the larvae and genetic access was provided by ANLA (Autoridad Nacional De Licencias Ambientales to Universidad Nacional de Colombia.

CRediT authorship contribution statement

A.N. Pereira-Bazurdo: Writing – review & editing, Writing – original draft, Visualization, Methodology. **G.E. Cadavid-Restrepo:** Writing – review & editing, Investigation, Formal analysis, Conceptualization. **R. E. Arango-Isaza:** Writing – review & editing, Methodology, Investigation. **C.X. Moreno-Herrera:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

Medellín, august 22nd, 2024Editorial Board
Biotechnology Reports
Manuscript submitted to consideration in the original research papers category, it is related with **"Assessment of microbial antagonistic activity and Quorum Sensing Signal Molecule (Cyclopeptides-DKPs and N-Acyl Homoserine Lactones) detection in Bacterial strains obtained from avocado thrips (Thysanoptera: Thripidae)"**. This manuscript has not been published, and it is not waiting for a decision in any other journal. All the authors of this paper agree with the whole content of this manuscript and declare that they have no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.btre.2024.e00866.

Data availability

No data was used for the research described in the article.

References

[1] ICA, Manejo Fitosanitario Del Cultivo Del Aguacate Hass (Persea Americana Mill), Medidas Para La Temporada Invernal, 2012.
[2] P. Arévalo, F. Quintero, L. Correa, Survey of Thrips (Insecta: thysanoptera) in flower crops at three localities of the municipality of medellín, antioquia (Colombia), Revista Colombiana de Entomología 29 (2) (2003) 169–175.
[3] F.J. Byrne, E.C. Humeres, A.A. Urena, M.S. Hoddle, J.G. Morse, Field evaluation of systemic imidacloprid for the management of avocado thrips and avocado lace bug in California avocado groves, Pest Manage. Sci. 66 (10) (2010) 1129–1136, https://doi.org/10.1002/ps.1991.
[4] A. Retana, O. García, M. Cantu, C Carvajal, Thrips (Thysanoptera) of Avocado (Persea americana) in Nayarit, Mexico, Revista Colombiana de Entomología 36 (1) (2010) 47–51, https://doi.org/10.25100/socolen.v36i1.9118.
[5] A. Merlo-Reyes, C. Baduel, C. Duwig, M.I. Ramírez, Risk assessment of pesticides used in the eastern avocado belt of michoacan, Mexico: a survey and water monitoring approach, Sci. Total Environ. 916 (2024) 170288.
[6] Tamayo Molano, Pablo Julian, Enfermedades Del Aguacate, Revista Politécnica (4) (2007) 51–70.
[7] S.D. Granada, S. Ramírez-Restrepo, L. López-Luján, C.A. Peláez-Jaramillo, J. C. Bedoya-Pérez, Screening of a biological control bacterium to fight avocado diseases: from agroecosystem to bioreactor, Biocatal. Agric. Biotechnol. 14 (2018) 109–115, https://doi.org/10.1016/j.bcab.2018.02.005.
[8] S. Carmona-Hernandez, J.J. Reyes-Pérez, R.G. Chiquito-Contreras, G. Rincon-Enriquez, C.R. Cerdan-Cabrera, L.G. Hernandez-Montiel, Biocontrol of postharvest fruit fungal diseases by bacterial antagonists: a review, Agronomy 9 (3) (2019), https://doi.org/10.3390/agronomy9030121.
[9] N. El-Wakeil, M. Saleh, M. Abu-hashim, Cottage industry of biocontrol agents and their applications. Cottage Industry of Biocontrol Agents and Their Applications, 2020, https://doi.org/10.1007/978-3-030-33161-0.
[10] L.G. Almeida, L, A.B. Moraes, J.R. Trigo, C. Omoto, F.L. Cônsoli, D Anspaugh, The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: a potential source for biotechnological exploitation, PLoS One 12 (2017) 3, https://doi.org/10.1371/journal.pone.0174754.
[11] A. Malacrinò, Meta-omics tools in the world of insect-microorganism interactions, Biology 7 (4) (2018) 50, https://doi.org/10.3390/biology7040050.
[12] A.N. Hernández-Lauzardo, S. Bautista-Baños, M.G. Velázquez-del Valle, A. Hernández-Rodríguez, Uso de microorganismos antagonistas en el control de enfermedades postcosecha en frutos, Revista mexicana de fitopatología 25 (1) (2007) 66–74.
[13] F. Boyen, V. Eeckhaut, F. Van Immerseel, F. Pasmans, R. Ducatelle, F. Haesebrouck, Quorum sensing in veterinary pathogens: mechanisms, clinical importance and future perspectives, Vet. Microbiol. 135 (3–4) (2009) 187–195, https://doi.org/10.1016/j.vetmic.2008.12.025.
[14] S.R. Kim, K.M. Yeon, Quorum sensing as language of chemical signals, in: Comprehensive Analytical Chemistry, 81, Elsevier, 2018, pp. 57–94, https://doi.org/10.1016/bs.coac.2018.03.010.
[15] G.A. March Rosselló, J.M. Eiros Bouza, Quorum sensing en bacterias y levaduras, Med. Clin. (Barc) 141 (8) (2013) 353–357, https://doi.org/10.1016/j.medcli.2013.02.031.
[16] D. Cano-Calle, L.M. Montoya-Porras, S. Ochoa-Giraldo, H. Junca, E. Garcia-Bonilla, C. Saldamando-Benjumea, C.X. Moreno-Herrera, R.E. Arango-Isaza, Thrips microbiome study in commercial avocado (Persea americana Mill.) from northwest colombian andes (Antioquia, Colombia) shows the presence of wolbachia, Ehrlichia, Enterobacter. Diversity 14 (7) (2022) 540, https://doi.org/10.3390/d14070540.
[17] B. Hameeda, O.P. Rupela, Gopal Reddy, K. Satyavani, Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of pearl millet (Pennisetum Glaucum L.), Biol. Fertil. Soils 43 (2) (2006) 221–227, https://doi.org/10.1007/s00374-006-0098-1.
[18] Zeinab Kheirandish, Behrouz Harighi, Evaluation of bacterial antagonists of ralstonia solanacearum, causal agent of bacterial wilt of potato, Biol. Control 86 (2015) 14–19.
[19] M.P. Fletcher, S.P. Diggle, M. Cámara, P. Williams, Biosensor-based assays for PQS, HHQ and related 2-alkyl-4-quinolone quorum sensing signal molecules, Nat. Protoc. 2 (5) (2007) 1254–1262, https://doi.org/10.1038/nprot.2007.158.
[20] T. Kawaguchi, P.C. Yung, R.S. Norman, A.W. Decho, Rapid Screening ofQuorum-SensingSignal N-AcylHomoserine Lactones byan In Vitro Cell-FreeAssay, Appl. Environ. Microb. 74 (12) (2008) 3667–3671, https://doi.org/10.1128/AEM.02869-07.
[21] P. Indiragandhi, R. Anandham, M. Madhaiyan, T.M. Sa, Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback moth Plutella xylostella (Lepidoptera: plutellidae), Curr. Microbiol 56 (2008) 327–333, https://doi.org/10.1007/s00284-007-9086-4.
[22] M. Ghoul, S. Mitri, The ecology and evolution of microbial competition, Trends Microbiol. 24 (2016) 833–845.
[23.] A. Di Francesco, M. Mari, Biological control of postharvest diseases by microbial antagonists: how many mechanisms of action? Eur. J. Plant Pathol. 145 (2016) 711–717.
[24] J. Zeng, T. Xu, L. Cao, C. Tong, X. Zhang, D. Luo, S. Han, P. Pang, W. Fu, J. Yan, et al., The role of iron competition in the antagonistic action of the rice endophyte Streptomyces sporocinereus OsiSh-2 against the pathogen Magnaporthe oryzae, Microb. Ecol. 76 (2018) 1021–1029.
[25] S. Shivakumar, A.N. Karmali, C. Ruhimbana, Partial purification, characterization, and kinetic studies of a low-molecular-weight, alkali-tolerant chitinase enzyme

Bacillus subtilis JN032305, a potential biocontrol strain, Prep. Biochem. Biotechnol. 44 (2014) 1–37.
[26] L. Amaíz, R. Vargas, L. Medina, N. Izzeddin, O. Valbuena, Evaluación del efecto antagonista de un consorcio bacteriano sobre Rhizoctonia solani Kühn en cultivos de arroz, Revista Latinoamericana de Biotecnología Ambiental y Algal 6 (1) (2015) 1–12, https://doi.org/10.7603/s40682-015-0002-5.
[27] C. Layton, E. Maldonado, L. Monroy, L.C. Corrales, L.C. Sánchez, Bacillus spp.; perspectiva de su efecto biocontrolador mediante antibiosis en cultivos afectados por fitopatógenos, Cienc. Bioméd. (2011).
[28] Cabra Teresa, Cristian Alonso Rodriguez, Claudia Patricia Villota, Capacidad Antagónica y Quitinolítica de Microorganismos Aislados de Residuos de Higuerilla (Ricinus Communis), Biotecnología en el Sector Agropecuario y Agroindustrial 12 (1) (2014) 56–61.
[29] S.A. Bahadou, A. Ouijja, A. Karfach, A. Tahiri, R. Lahlali, New potential bacterial antagonists for the biocontrol of fire blight disease (Erwinia amylovora) in Morocco, Microb. Pathog. 117 (2018) 7–15.
[30] E. Arrebola, R. Jacobs, L. Korsten, Iturin A is the principal inhibitor in the biocontrol activity of Bacillus amyloliquefaciens PPCB004 against postharvest fungal pathogens, J. Appl. Microbiol. 108 (2) (2010) 386–395, https://doi.org/10.1111/j.1365-2672.2009.04438.x.
[31] F.M. Cazorla, D. Romero, A. Pérez-García, B.J.J. Lugtenberg, A.D. Vicente, G. Bloemberg, Isolation and characterization of antagonistic Bacillus subtilis strains from the avocado rhizoplane displaying biocontrol activity, J. Appl. Microbiol. 103 (5) (2007) 1950–1959, https://doi.org/10.1111/j.1365-2672.2007.03433.x.
[32] X. Chen, Y. Zhang, X. Fu, Y. Li, Q. Wang, Isolation and characterization of Bacillus amyloliquefaciens PG12 for the biological control of apple ring rot, Postharvest Biol. Technol. 115 (2016) 113–121, https://doi.org/10.1016/j.postharvbio.2015.12.021.
[33] P. Mohammadi, E. Tozlu, R. Kotan, M. Şenol Kotan, Potential of some bacteria for biological control of postharvest citrus green mould caused by Penicillium digitatum, Plant Protect. Sci. 53 (3) (2017) 134–143, https://doi.org/10.17221/55/2016-PPS.
[34] P. Mohammadi, E. Tozlu, R. Kotan, M. Şenol Kotan, Potential of some bacteria for biological control of postharvest citrus green mould caused by Penicillium digitatum, Plant Protect. Sci. 53 (3) (2017) 134–143, https://doi.org/10.17221/55/2016-PPS.
[35] M.F. Rabbee, M. Sarafat Ali, J. Choi, B.S. Hwang, S.C. Jeong, K.hyun Baek, Bacillus velezensis: a valuable member of bioactive molecules within plant microbiomes, Molecules 24 (6) (2019) 1–13, https://doi.org/10.3390/molecules24061046.
[36] S.M. Lim, M.Y. Yoon, G.J. Choi, Y.H. Choi, K.S. Jang, T.S. Shin, J.C. Kim, Diffusible and volatile antifungal compounds produced by an antagonistic Bacillus velezensis G341 against various phytopathogenic fungi, Plant Pathol. J. 33 (5) (2017) 488–498, https://doi.org/10.5423/PPJ.OA.04.2017.0073.
[37] S. Kadaikunnan, T.S. Rejiniemon, J.M. Khaled, N.S. Alharbi, R. Mothana, In-vitro antibacterial, antifungal, antioxidant and functional properties of Bacillus amyloliquefaciens, Annals of Clinical Microbiol. Antimicrobials 14 (2015) 1–11.
[38] Wei Yang, et al., Evaluation of biological control agents against ralstonia wilt on ginger, Biol. Control 62 (3) (2012) 144–151, https://doi.org/10.1016/j.biocontrol.2012.05.001.
[39] D. Singh, D.K. Yadav, G. Chaudhary, V.S. Rana, R.K. Sharma, Potential of Bacillus amyloliquefaciens for biocontrol of bacterial wilt of tomato incited by Ralstonia solanacearum, J. Plant Pathol. Microbiol. 7 (327) (2016) 1–6.
[40] S.Bin Li, M. Fang, R.C. Zhou, J Huang, Characterization and evaluation of the endophyte Bacillus B014 as a potential biocontrol agent for the control of Xanthomonas axonopodis pv. dieffenbachiae - Induced blight of Anthurium, Biol. Control 63 (1) (2012) 9–16, https://doi.org/10.1016/j.biocontrol.2012.06.002.
[41] C. Nunes, J. Usall, N. Teixidó, E. Fons, I. Viñas, Post-harvest biological control by Pantoea agglomerans (CPA-2) on Golden Delicious apples, J. Appl. Microbiol. 92 (2) (2002) 247–255, https://doi.org/10.1046/j.1365-2672.2002.01524.x.
[42] Y. Feng, D. Shen, W. Song, Rice endophyte Pantoea agglomerans YS19 promotes host plant growth and affects allocations of host photosynthates, J. Appl. Microbiol. 100 (5) (2006) 938–945, https://doi.org/10.1111/j.1365-2672.2006.02843.x.
[43] F. Dagher, S. Olishevska, V. Philion, J. Zheng, E. Déziel, Development of a novel biological control agent targeting the phytopathogen Erwinia amylovora, Heliyon 6 (10) (2020), https://doi.org/10.1016/j.heliyon.2020.e05222.
[44] B. Thissera, H.A. Alhadrami, M.H.A. Hassan, H.M. Hassan, F.A. Behery, M. Bawazeer, M.E. Rateb, Induction of cryptic antifungal pulicatin derivatives from Pantoea agglomerans by microbial co-culture, Biomolecules 10 (2) (2020), https://doi.org/10.3390/biom10020268.
[45] A. Ortiz, E. Sansinenea, The possibility of using Serratia isolates for the production of biopreparations in the protection of plants against diseases and pests, Arch. Microbiol. 205 (8) (2023) 288.
[46] C. Kalbe, P. Marten, G. Berg, Strains of the genus Serratia as beneficial rhizobacteria of oilseed rape with antifungal properties, Microbiol. Res. 151 (4) (1996) 433–439, https://doi.org/10.1016/S0944-5013(96)80014-0.
[47] D. Chlebek, V. Grebtsova, A. Piński, J. Żur-Pińska, K. Hupert-Kocurek, Genetic determinants of antagonistic interactions and the response of new endophytic strain serratia quinivorans KP32 to fungal phytopathogens, Int. J. Mol. Sci. 23 (24) (2022) 15561.
[48] M.A. El-Esawi, I.A. Alaraidh, A.A. Alsahli, S.M. Alzahrani, H.M. Ali, A.A. Alayafi, M. Ahmad, Serratia liquefaciens KM4 improves salt stress tolerance in maize by regulating redox potential, ion homeostasis, leaf gas exchange and stress-related gene expression, Int. J. Mol. Sci. 19 (11) (2018) 3310, https://doi.org/10.3390/ijms19113310.
[49] L. Poppe, S. Vanhoutte, M. Höfte, Modes of action of Pantoea agglomerans CPA-2, an antagonist of postharvest pathogens on fruits, Eur. J. Plant Pathol. 109 (2003) 963–973.
[50] S.M. Yu, Y.H. Lee, Genes involved in nutrient competition by Pseudomonas putida JBC17 to suppress green mold in postharvest satsuma mandarin, J. Basic Microbiol. 55 (2015) 898–906.
[51] A. Bonaterra, E. Badosa, N. Daranas, J. Francés, G. Roselló, E. Montesinos, Bacteria as biological control agents of plant diseases, Microorganisms 10 (9) (2022) 1759, https://doi.org/10.3390/microorganisms10091759. Aug 31PMID: 36144361; PMCID: PMC9502092.
[52] J.A. Stenberg, I. Sundh, P.G. Becher, C. Björkman, M. Dubey, P.A. Egan, M. Viketoft, When is it biological control? A framework of definitions, mechanisms, and classifications, J. Pest Sci. 94 (3) (2021) 665–676.
[53] S. Xu, M. Li, Z. Hu, Y. Shao, J. Ying, H. Zhang, The potential use of fungal co-culture strategy for discovery of new secondary metabolites, Microorganisms 11 (2) (2023) 464, https://doi.org/10.3390/microorganisms11020464. Feb 12PMID: 36838429; PMCID: PMC9965835.
[54] X. Li, H. Xu, Y. Li, S. Liao, Y. Liu, Exploring diverse bioactive secondary metabolites from marine microorganisms using co-culture strategy, Molecules 28 (17) (2023) 6371, https://doi.org/10.3390/molecules28176371. Aug 31PMID: 37687200; PMCID: PMC10489945.
[55] Z. Lin, R.R. Antemano, R.W. Hughen, M.D.B. Tianero, O. Peraud, M.G. Haygood, E. W. Schmidt, Pulicatins A– E, neuroactive thiazoline metabolites from cone snail-associated bacteria, J. Nat. Prod. 73 (11) (2010) 1922–1926, https://doi.org/10.1021/np100588c.
[56] N.L. López, J.B. Heredia, C.S.M. Hernández, R.I. Rodrígueza, M.Á.A. Escalante, R. S.G. Estrada, Biosíntesis inducida de fengicina y surfactina en una cepa de Bacillus amyloliquefaciens con actividad oomiceticida sobre zoosporas de Phytophthora capsica, Revista argentina de microbiología 54 (3) (2022) 91–100, https://doi.org/10.1016/j.ram.2022.03.002.
[57] M.J. Calcott, D.F. Ackerley, Genetic manipulation of non-ribosomal peptide synthetases to generate novel bioactive peptide products, Biotechnol. Lett 36 (2014) 2407–2416, https://doi.org/10.1007/s10529-014-1642-y.
[58] S.L. Wenski, S. Thiengmag, E.J. Helfrich, Complex peptide natural products: biosynthetic principles, challenges and opportunities for pathway engineering, Synthetic and Systems Biotechnol. 7 (1) (2022) 631–647, https://doi.org/10.1016/j.synbio.2022.01.007.
[59] C. Cha, P. Gao, Y.C. Chen, P.D. Shaw, S.K. Farrand, Production of acyl-homoserine lactone quorum-sensing signals by gram-negative plant-associated bacteria, Mol. Plant-Microbe Interact. 11 (11) (1998) 1119–1129, https://doi.org/10.1094/MPMI.1998.11.11.1119.
[60] D.K. Park, K.E. Lee, C.H. Baek, I.H. Kim, J.H. Kwon, W.K. Lee, K.S. Kim, Cyclo(Phe-Pro) modulates the expression of ompU in Vibrio spp, J. Bacteriol. 188 (6) (2006) 2214–2221, https://doi.org/10.1128/JB.188.6.2214-2221.2006.
[61] S. Horinouchi, K. Ueda, J. Nakayama, T. Ikeda, Cell-to-cell communications among microorganisms, Comprehens. Natural Products II: Chem. Biol. 4 (2010) 283–337, https://doi.org/10.1016/b978-008045382-8.00098-8.
[62] J. Zhao, C. Quan, L. Jin, M Chen, Production, detection and application perspectives of quorum sensing autoinducer-2 in bacteria, J. Biotechnol. 268 (2018) 53–60, https://doi.org/10.1016/j.jbiotec.2018.01.009.
[63] Teresa R De Kievit, Barbara H Iglewski, Bacterial quorum sensing in pathogenic relationships, Infect. Immun. 68 (9) (2000) 4839–4849, https://doi.org/10.1128/iai.68.9.4839-4849.2000.
[64] A. Leyton, H. Urrutia, J.M. Vidal, M. de la Fuente, M. Alarcón, G. Aroca, K. Sossa, Actividad inhibitoria del sobrenadante de la bacteria Antartica Pseudomonas sp. M19B en la formación de biopeliculas de Flavobacterium psychrophilum 19749, Revista de Biologia Marina y Oceanografia 50 (2) (2015) 375–381, https://doi.org/10.4067/S0718-19572015000300165.
[65] C.A. Ortori, J.-F. Dubern, S.R. Chhabra, M. Cámara, K. Hardie, P. Williams, D. A. Barrett, Simultaneous quantitative profiling of N-acyl-L-homoserine lactone and 2-alkyl-4 (1H)-quinolone families of quorum-sensing signaling molecules using LC-MS/MS, Anal. Bioanal.Chem. 399 (2) (2011) 839–850, https://doi.org/10.1007/s00216-010-4341-0.
[66] A.M. Pomini, W.L. Araújo, A.J. Marsaioli, Structural elucidation and biological activity of acyl-homoserine lactones from the phytopathogen Pantoea ananatis Serrano 1928, J. Chem. Ecol. 32 (2006) 1769–1778, https://doi.org/10.1007/s10886-006-9108-x.
[67] R.J. Vivero-Gomez, G.B. Mesa, J. Higuita-Castro, S.M. Robledo, C.X. Moreno-Herrera, G. Cadavid-Restrepo, Detection of quorum sensing signal molecules, particularly N-Acyl Homoserine Lactones, 2-Alky-4-Quinolones, and diketopiperazines. gram-negative bacteria isolated from insect vector of leishmaniasis, Front. Trop. Dis. 2 (2021) 760228, https://doi.org/10.3389/fitd.2021.760228.
[68] C.A. Ortori, N. Halliday, M. Cámara, P. Williams, D.A. Barrett, LC-MS/MS quantitative analysis of quorum sensing signal molecules. ***Pseudomonas Methods and Protocols***, Springer, 2014, pp. 255–270.
[69] J. Huang, Y. Shi, G. Zeng, Y. Gu, G. Chen, L. Shi, J. Zhou, Acyl-homoserine lactone-based quorum sensing and quorum quenching hold promise to determine the performance of biological wastewater treatments: an overview, Chemosphere 157 (2016) 137–151, https://doi.org/10.1016/j.chemosphere.2016.05.032.
[70] V.C. Kalia, H.J. Purohit, Quenching the quorum sensing system: potential antibacterial drug targets, Crit. Rev. Microbiol. 37 (2) (2011) 121–140, https://doi.org/10.3109/1040841X.2010.532479.
[71] M.T.G. Holden, S.R. Chhabra, R. De Nys, P. Stead, N.J. Bainton, P.J. Hill, P. Williams, Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from Pseudomonas aeruginosa and other Gram-negative bacteria,

Mol. Microbiol. 33 (6) (1999) 1254–1266, https://doi.org/10.1046/j.1365-2958.1999.01577.x.
[72] G. Degrassi, C. Aguilar, M. Bosco, S. Zahariev, S. Pongor, V. Venturi, Plant growth-promoting Pseudomonas putida WCS358 produces and secretes four cyclic dipeptides: cross-talk with quorum sensing bacterial sensors, Curr. Microbiol. 45 (4) (2002) 250–254, https://doi.org/10.1007/s00284-002-3704-y.
[73] H. Zhu, S.wei Sun, H. Li, A. Chang, Y.chen Liu, J. Qian, Y.ling Shen, Significantly improved production of Welan gum by Sphingomonas sp. WG through a novel quorum-sensing-interfering dipeptide cyclo(L-Pro-L-Phe), Int. J. Biol. Macromol. 126 (2019) 118–122.
[74] E. Sansinenea, F. Salazar, J. Jiménez, Á. Mendoza, A. Ortiz, Diketopiperazines derivatives isolated from Bacillus thuringiensis and Bacillus endophyticus, establishment of their configuration by X-ray and their synthesis, Tetrahedron Lett. 57 (24) (2016) 2604–2607, https://doi.org/10.1016/j.tetlet.2016.04.117.
[75] K. Ström, J. Sjögren, A. Broberg, J. Schnürer, Lactobacillus plantarum MiLAB 393 produces the antifungal cyclic dipeptides cyclo(L-Phe-L-Pro) and cyclo(L-Phe-trans-4-OH-L-Pro) and 3-phenyllactic acid, Appl. Environ. Microbiol. 68 (9) (2002) 4322–4327, https://doi.org/10.1128/AEM.68.9.4322-4327.2002.
[76] K. Yonezawa, K. Yamada, I. Kouno, New diketopiperazine derivatives isolated from sea urchin-derived Bacillus sp, Chem. Pharm. Bull. 59 (1) (2011) 106–108, https://doi.org/10.1248/cpb.59.106.