Archives of Microbiology (2021) 203:1383–1397
https://doi.org/10.1007/s00203-020-02136-y

ORIGINAL PAPER

Volatile organic compounds profile synthesized and released by endophytes of tomato (*Solanum lycopersici* L.) and their antagonistic role

Silvina M. Y. López[1,2] · **Graciela Noemi Pastorino**[3] · **Pedro Alberto Balatti**[1,2,3]

Received: 2 October 2020 / Revised: 12 November 2020 / Accepted: 19 November 2020 / Published online: 2 January 2021

Abstract

The endophytic microbiome uses mechanisms such as the secretion of diffusible antibiotic molecules, synthesis and release of volatile organic compounds, and/or toxins to protect plants. The aim of this research was to study the volatile organic compounds (VOCs) profile as well as the diffusible secondary metabolites produced and released by endophytic bacteria isolated from tomato plants that in in-vitro assays prevented growth of pathogenic fungi. Bacteria belonging to seven genera (*Acinetobacter*, *Arthrobacter*, *Bacillus*, *Microbacterium*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas*) were isolated from different tissues of tomato plants with and without symptoms of Gray leaf spot, a disease provoked by *Stemphylium lycopersici*. In vitro, antagonistic assays were performed and the effect of volatile and soluble compounds released by endophytic bacteria on the growth of pathogenic fungi was determined. The VOCs synthesized by the endophytes were extracted, identified and quantified. These isolates representatives of seven bacterial genera inhibited the growth of three fungal pathogens of tomato *S. lycopersici*, *Alternaria alternata* and *Corynespora cassiicola*, which was related to the synthesis of soluble compounds as well as VOCs. Endophytes synthesize and release different VOCs, probably due to the different type of interaction that each bacterium establishes with the fungus, presenting a range of fungal growth inhibition.

Keywords Endophytic bacteria · Vocs · Biocontrol · Fungal pathogens · Tomato · *S. lycopersici*

Introduction

Plant organs host each a specific endophytic bacterial community in terms of diversity and composition (Liu et al. 2017) that is dynamic and is either harmless or beneficial to their host (Ludwig-Müller 2015; Rosenblueth and Martínez-Romero 2006; Liu et al. 2017). Several activities of endophytes are under the control of quorum sensing that is based on the density of bacteria population that might be achieved within tissues. This response changes gene expression, and in this way, saprophytes might become pathogenic (Snelders et al. 2018; Abisado et al. 2018). However, endophytic saprophytic microorganisms also might colonize an ecological niche hosting phytopathogens. If this relationship between endophytes and pathogens is antagonistic, quite often it might lead to disease control. In this sense, those endophytic populations of beneficial microbes protect plants in three alternative ways, the direct antagonistic activity of pathogens, by outcompeting them or by stimulating host plant defenses (Ludwig-Müller 2015; Rosenblueth and Martínez-Romero 2006; Le Cocq et al. 2017; Snelders et al. 2018; Abisado et al. 2018; Ab Rahman et al. 2018).

Tomato is one of the most important horticultural crops worldwide, it's production area comprises around 4,76 Mha and generates a production of approximately 164 Mt (Rodríguez-Ortega et al. 2019). In Argentina, the main areas cultivated with tomatoes are located in the provinces of Corrientes and Buenos Aires. In the latter one, production is carried out mostly in greenhouses (Franco et al. 2017; Rodríguez-Ortega et al. 2019; Medina et al. 2019), where

Communicated by Erko Stackebrandt.

✉ Silvina M. Y. López
smyld03@hotmail.com

[1] Centro de Investigaciones en Fitopatología (CIDEFI), La Plata, Argentina

[2] Comisión de Investigaciones Científicas de La Provincia de Buenos Aires (CICBA), La Plata, Argentina

[3] Cátedra de Microbiología Agrícola, Universidad Nacional de La Plata (UNLP), Calle 60 y 119, La Plata, C. P. 1900, Buenos Aires, Argentina

relative humidity, as well as temperature, are high, favoring the development of diseases (Medina et al. 2019; Kakoti et al. 2020). At this moment, management of tomato production includes the use of chemicals like fungicides and insecticides, which not only increased the cost of production, but also raises the risk for workers and also has an impact on the product itself in terms of health safety. In addition to this, the use of agrochemicals frequently leads pathogens as well as insects to develop resistance. Therefore, farmers need to have alternative strategies to manage diseases as well as pests. Biological control, which is the use of biological tools to manage pathogens and pests, is highly potent since soils, as well as plants and insects, are an incommensurable source of microorganisms with capacities to promote plant growth and/or keep plants healthy (Bader et al. 2020).

The endophytic microbiome uses different alternative mechanisms to protect plants, such as secretion of diffusible antibiotic molecules, synthesis and release of volatile organic compounds, and/or toxins, and synthesis of cell wall degrading enzymes such as chitinase, β-1,3-glucanase, beta-xylosidase, pectin methylesterase and many more (Rahman et al. 2018). Microorganisms living endophytically also synthesize and release a large number of secondary metabolites that play many different physicochemical and biological roles. Soluble secondary metabolites are highly polar compounds and because of this, they are soluble in water, acting at short distances and usually they have stronger biological activity than toxins or antibiotics. But endophytes also synthesize volatile compounds (VOCs), which are used by the plant for self-defense against pathogens (Nair and Padmavathy 2014; Tyc et al. 2017), since they often trigger the synthesis of antimicrobial compounds. Compared with other secondary metabolites like enzymes, antibiotics and toxins, volatiles are small low molecular mass (100–500 Daltons) compounds of up to C20 that have a high vapor pressure, low boiling point and a lipophilic moiety. All this explains their ability to be converted in vapors and also to diffuse through both water and air (Romoli et al. 2014; Schmidt et al. 2015).

Volatiles synthesized and released by a wide range of bacteria and fungi as well as the chemical structure of ~ 1000 volatiles have already been described (Effmert et al. 2012; Lemfack et al. 2014). They are most often alkenes, alcohols, ketones, terpenes, benzenoids, pyrazines, acids and esters (Piechulla and Degenhardt 2014) that are known for their inhibitory activity upon fungal spore germination. Furthermore, exposure to bacterial volatiles changes fungal morphology, enzyme activity and gene expression (Tyc et al. 2017). Although the mode of action of antifungal volatiles remains to be studied in detail, their hydrophobicity likely enables them to interact with the lipid component of the cell membrane increasing its permeability. More recently, Kim et al. (2013) and Molina-Santiago et al. (2014) demonstrated that bacterial volatiles induced changes in gene expression, affected motility, biofilm development as well as quorum sensing of fungi, and as a consequence, they have an impact on fungal development and virulence (Romoli et al. 2014). It appears that VOCs act synergically with bacterial soluble compounds. Among other things, Claeson et al. (2007) proposed that they are waste material released by the detoxification system of microorganisms (Claeson et al. 2007). In summary, microbial volatiles play two major roles within plants microbial communities: they modify microorganisms' behavior, population dynamic and gene expression and antagonize plant pathogens. However, the main role volatiles synthetized by bacteria play in nature remains to be identified.

Researchers are interested in developing the use of microorganisms or their secondary metabolites to manage plant diseases because they might not pose a threat to human health and most probably are harmless to the environment (Dukare et al. 2019; Collinge et al. 2019; Thomashow et al. 2019; Köhl et al. 2019). In previous work, while studying isolated bacteria living within plants of tomato, we isolated and identified nine bacteria living endophytically that in-vitro controlled plant pathogens (López et al 2020). These antagonistic bacteria synthesized secondary metabolites and while some were diffusible others were volatile compounds. The aims of this study were to study and analyze the VOCs profile as well as that of diffusible secondary metabolites that are synthesized and released by *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Microbacterium*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas* isolated from tomato plants grown in culture media and the effect of the identified products on three fungal plant pathogens.

Materials and methods

Bacterial and fungal material

Bacteria living within seeds, roots, shoots, leaves and fruits of tomato plants of cultivar Elpida F1 (Enza Zaden) growing in farmers greenhouses, were isolated by culturing them on three different commercial culture media (TSA, Nutritive agar and King B—BritaniaLab S.A.) (López et al. 2018, 2020). The microorganism's identity was preliminary determined using the 1.5 kb sequence coding for the 16S rRNA (López et al. 2020). The *16S rRNA* gene sequences were have deposited in the GenBank database under accession numbers MH915620-MH915655 (Table 1) and were reported in Lopez et al. (2020). Sequence analysis and alignment were performed using Basic Local Alignment Search Tool of National Center for Biotechnology Information (BLAST-NCBI), limited to sequences from type material. The results are described in Table 1 as percent identity (%) at the species level. Aliquots of pure cultures of all these

Table 1 Species of bacteria isolated from tissues of healthy and diseased tomato plants based only on the *16S rRNA*

Isolate	Species—Accession number Percent identity	Plant tissue of origin
As	*Pseudomonas kribbensis*, MH915620.1 Percent identity:99.73%	Healthy leaves
Bs	*Pseudomonas chengduensis*, MH915623.1 Percent identity:99.93%	
Cs	*Pseudomonas kilonensis*, MH915625.1 Percent identity:99.46%	
Es	*Pseudomonas kribbensis*, MH915628.1 Percent identity:99.73%	
Fs	*Serratia liquefaciens*, MH915630.1 Percent identity:99.00%	Healthy roots
Gs	*Pseudomonas kribbensis*, MH915631.1 Percent identity:99.40%	
Hs	*Serratia liquefaciens*, MH915633.1 Percent identity:99.60%	
Is	*Microbacterium paraoxydans*, MH915634.1 Percent identity:99.80%	
Js	*Pseudomonas wadenswilerensis*, MH915635.1 Percent identity:99.00%	
Ks	*Pseudomonas kilonensis*, MH915637.1 Percent identity:97.65%	
Ls	*Stenotrophomonas maltophilia*, MH915639.1 Percent identity:99.67%	
Ms	*Serratia liquefaciens*, MH915640.1 Percent identity:99.73%	
Ns	*Curtobacterium pusillum*, MH915642.1 Percent identity:99.86%	Healthy shoots
Os	*Microbacterium paraoxydans*, MH915644.1 Percent identity:99.53%	
Qs	*Pantoea eucalypti*, MH915646.1 Percent identity:93.82%	
Rs	*Stenotrophomonas maltophilia*, MH915647.1 Percent identity:99.40%	
Ss	*Pseudomonas oryzihabitans*, MH915648.1 Percent identity:99.67%	
Ts	*Pseudomonas plecoglossicida*, MH915649.1 Percent identity:99.67%	
Us	*Stenotrophomonas maltophilia*, MH915650.1 Percent identity:99.34%	
Vs	*Terribacillus saccharophilus*, MH915651.1 Percent identity: 97.54%	Healthy fruits
Ws	*Phyllobacterium ifriqiyense*, MH915652.1 Percent identity:99.50%	
Xs	*Acinetobacter mesopotamicus*, MH915653.1 Percent identity:99.53%	
Ys	*Staphylococcus pasteuri*, MH915654.1 Percent identity:99.60%	
Zs	*Staphylococcus pasteuri*, MH915655.1 Percent identity:99.26%	

Table 1 (continued)

Isolate	Species—Accession number Percent identity	Plant tissue of origin
Ae	*Arthrobacter ureafaciens*, MH915621.1 Percent identity:98.65%	Symptomatic leaves
Be	*Curtobacterium pusillum*, MH915622.1 Percent identity:99.80%	
Ce	*Curtobacterium pusillum*, MH915624.1 Percent identity:99.46%	
De	*Curtobacterium pusillum*, MH915626.1 Percent identity:99.80%	
Ee	*Microbacterium paraoxydans*, MH915627.1 Percent identity:98.92%	
Fe	*Pantoea vagans*, MH915629.1 Percent identity:99.07%	
He	*Pseudomonas mediterranea*, MH915632.1 Percent identity:99.13%	Symptomatic roots
Ke	*Pantoea dispersa*, MH915636.1 Percent identity:97.74%	Symptomatic shoots
Le	*Arthrobacter phenanthrenivorans*, MH915638.1 Percent identity:98.19%	
Ne	*Acidovorax wautersii*, MH915641.1 Percent identity:96.51%	
Oe	*Acinetobacter oryzae*, MH915643.1 Percent identity:97.93%	Symptomatic fruits
Pe	*Pectobacterium aroidearum*, MH915645.1 Percent identity:98.80%	

Each isolate sequence access number and the plant tissue from where they were isolated are described. The whole collection of organisms obtained is described in López et al. (2020)

organisms were supplemented with 10% glycerol, were stored at −70 °C and were introduced in the CIDEFI bacterial collection under numbers CIDEFI TEB 22–57.

Bacterial isolates were cultured on nutritive agar or TYB media as required by the different antagonism tests performed. The inhibitory effect of bacterial cultures was evaluated against three fungal pathogens of tomato *Alternaria alternata*, *Corynespora cassiicola* and *Stemphylium lycopersici* (strains CIDEFI 209, CIDEFI 235, CIDEFI 234, respectively) that belong to the CIDEFI collection of fungi.

In vitro assays of bacteria–fungal pathogens antagonism

Bacterial slants used to inoculate plates in experiments were grown on nutritive agar media at 28 °C for 5 days. Pathogens were cultured on Glucose potato agar-APG (BritaniaLab S.A.) at 25 °C in the darkness for 7 days. In vitro antagonistic assays aimed at testing the inhibitory effect of each bacteria on pathogen growth consisted in making bacterial striae on nutritive agar plates in such a way that plates were divided in three sections, in each of them a 5 mm mycelial plug cut from the edge of 7-day-old cultures of each fungal pathogen was placed at the centre of each of the three sections of the plate (López et al. 2018). Then, plates were incubated at 25 °C for 5 days in the darkness and the inhibitory activity was evaluated based on the inhibition of fungal growth. A positive response was the visible zone of inhibition around the fungus.

Quantitative evaluation of bacterial antagonism due to volatile compounds

Inhibitory activity of the cell-free supernatant of endophytic bacteria against fungal pathogens

Nine bacteria isolated from tomato, *Bacillus* sp., *Arthrobacter* sp., *Microbacterium* sp., *Pantoea* sp., *Pseudomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp. and two control strains, *Bacillus subtilis* strain Er/S and *Bacillus* sp. isolate E7 that in preliminary experiments showed a high capacity to prevent fungal growth were cultured in liquid nutrient broth in a rotary shaker at 180 rev min^{-1} at 28 °C in the darkness for 48 h. Cell-free cultured supernatants were obtained by centrifugation at 6000 g for 20 min and filtered through 0.45 µm and 0.22 µm organic filter membranes (©GVS Filter Technology). The antimicrobial activity of culture filtrates was evaluated by testing their capacity to inhibit the growth

of fungal pathogens in vitro. To do this, extracts were supplemented to agar plates (1.5% w/v agar) containing nutrient agar to make a final concentration of 1%, 10% and 20% of the supernatant extract (v/v). Fungi were inoculated on the plates by putting a 5 mm mycelial plug in the centre of the plates that were incubated for 5 days at 25 °C, and then fungal growth was measured. The inhibitory activity was calculated with the formula: Inhibition (%) = [(Growth in control—Growth in treatment)/Growth in control] × 100 (Baysal et al. 2013; López et al. 2018).

Effect of volatiles from endophytic bacteria against fungal pathogens

A bioassay was performed in sealed Petri dishes as described by Baysal et al. (2013), with some modifications. Briefly, 300 µL of bacterial cultures were spread onto a sterile plate containing TYB medium (BritaniaLab S.A.). Five millimeters fungal mycelial plugs were then placed at the centre of another plate containing PDA (López et al. 2018). Then, plates containing mycelial plugs were inverted and placed on top of the plates containing bacterial cultures and immediately were sealed with three layers of parafilm and were incubated at 25 °C, until the fungal mycelium of the controls extended throughout 3/4 of the plate. Controls were mounted with un-inoculated TYB plates. The diameter (mm) of the fungal colony was measured. The results were expressed as Inhibition [%] which was calculated with the formula mentioned above.

Data analysis and interpretation

Three independent experiments were conducted where similar results were obtained, and which were considered as independent replicates. The data were analyzed using a one-way analysis of variance (ANOVA), followed by a comparison of multiple treatment levels, using the Tukey test. All statistical analyses were performed using Infostat (version 1.0, UNC, Cordoba, Argentina).

A Biplot analysis was used to analyze the correlation of organisms, compounds as well as pathogens that were inhibited by these within the same graph, leading this to the association of compounds and microorganisms that inhibited fungal growth the most (Balzarini et al. 2008).

Volatile organic compounds (VOCs): Extraction, identification and quantification

Bacteria were grown on TYB medium (BritaniaLab S.A) and were incubated in a rotary shaker at 150 rev. min^{-1} at 28 °C in the dark for 5 days when a concentration of 1×10^4 CFU/mL was reached. A10 µL aliquot of each culture was placed in a glass headspace vial (10 mL) filled with 3.5 mL of TYB medium, which was sealed with a silicone septum cap. Vials were incubated at 25 °C in the dark for 5 days.

The VOCs profiles of the bacteria were analyzed by gas chromatography-mass spectrometry (GC–MS) using a HP CGC 6890/MS Agilent 5975C VL gas chromatograph-mass spectrometer equipped with a ZB-5HT Inferno fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm, Phenomenex, Inc). A solid-phase microextraction fibre coated with 65 µm polydimethylsiloxane/divinylbenzene was used to extract VOCs from the standstill vials, which were incubated for an hour at 30 °C. After injection, the compounds were desorbed for 5 min in a splitless injector at 250 °C. The oven temperature was held at 40 °C for 2 min, then raised to 200 °C at 10 °C min^{-1} and 250 °C at 15 min^{-1}, then the temperature was held for 5 min. Helium was the carrier gas flowing at 1 mL min^{-1}. Compounds were identified by matching their mass spectra using the NIST Mass Spectra Search Program with NIST05 and Adams (Identification of Essential Oil Components by Gas chromatography/ Mass spectrometry, 4th Edition) libraries and using Kovalts index (KI) in reference to n-alkanes. The background of TYB media un-inoculated was analyzed as a control.

Results

Endophytic bacteria, culture supernatant and VOCS effect on fungal pathogens growth in in-vitro assays

The biocontrol capacity of 24 bacterial isolates living endophytically in healthy plants and 12 isolated from diseased plants was evaluated in Petri dishes where they were co-cultured with fungal pathogens like *A. alternata*, *C. cassiicola*, and *S. lycopersici*. While all bacteria inhibited fungal growth, only isolates belonging to the genera *Stenotrophomonas* (two isolates), *Acinetobacter, Arthrobacter* (two isolates), *Microbacterium, Pantoea* and *Pseudomonas* (two isolates) provoked a growth reduction of fungal plant pathogens larger than 25% of control cultures (Fig. 1). To understand and explain the biocontrol ability of these bacteria, cultures of them were studied further regarding the production of both diffusible as well as volatile organic compounds. Regarding this, *Bacillus* sp. (isolate E7) and *Bacillus subtilis* (isolate Er/S) were included in the study as controls, based on their already proven antagonistic capacity (López et al. 2018).

Cell-free supernatants collected from cultures of *Bacillus* sp., *Bacillus subtilis*, *Stenotrophomonas* sp. (two isolates)., *Acinetobacter* sp., *Arthrobacter* sp. (two isolates), *Microbacterium* sp., *Pantoea* sp., *Pseudomonas* sp. (two isolates) did not affect fungal growth, except for the supernatant of *Pantoea* that at a 20% concentration, inhibited

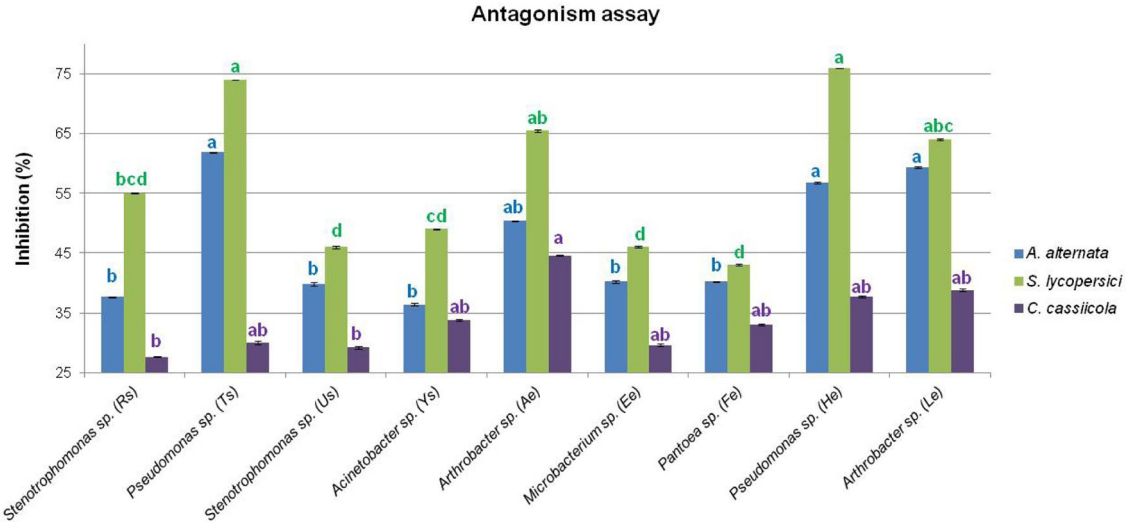

Fig. 1 Growth inhibition of *Alternaria alternata* (blue bars), *Corynespora cassiicola* (green bars) and *Stemphylium lycopersici* (violet bars), three fungal pathogens of plants by each of the bacterial isolates. Values are means of three independent biological replicates and error bars represents the standard deviation; letters in common on the bars are not significantly different according to the Tukey test at $p \leq 0.05$

the growth of *A. alternata*, *C. cassiicola*, and *S. lycopersici* in a 45%, 22%, and 1%, respectively. *Bacillus* sp. E7 and *Bacillus subtilis* Er/S were included as controls because they both inhibited the growth of *Alternaria*, *Stemphylium*, and *Corynespora* by 80, 75 and 27%, and by 70, 72 and 27%, respectively (López et al. 2018).

Because the supernatants of bacteria hardly affected growth of fungal pathogens we evaluated if the ability of these bacteria to inhibit fungal growth relied on the synthesis and release of volatile compounds. We found that all tested isolates released antifungal volatile compounds (VOCs) that inhibited the growth of *A. alternata*, *C. cassiicola* and *S. lycopersici* 144 h after plates were inoculated (Fig. 2). In

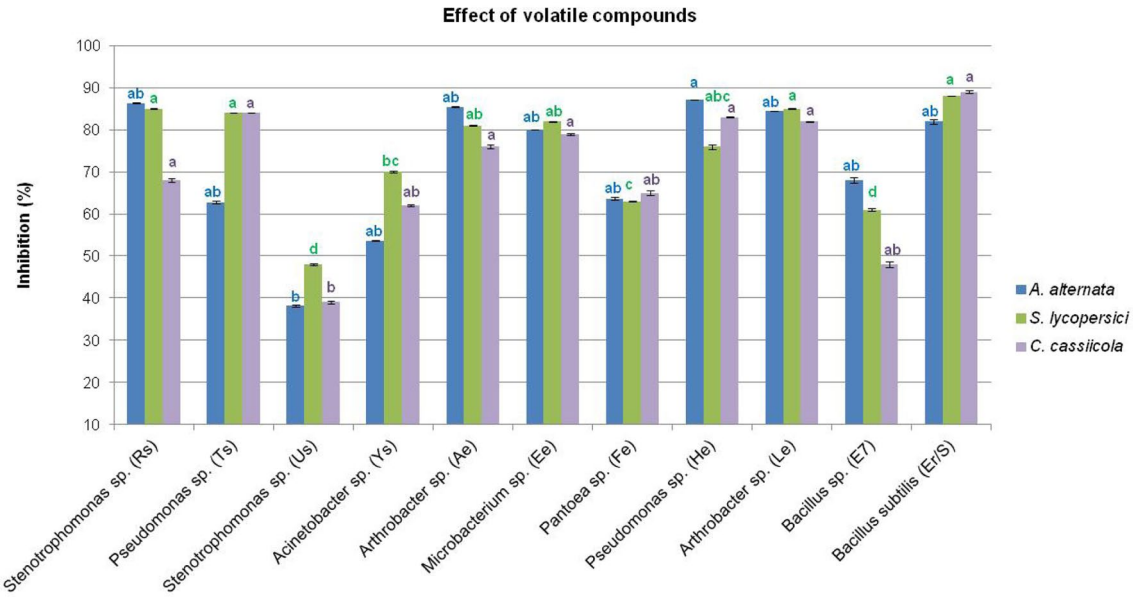

Fig. 2 Volatile Organic Compounds (VOCs) produced by each bacterial isolate effect on the growth of the fungal pathogens: *Alternaria alternata* (blue bars), *Corynespora cassiicola* (green bars) and *Stemphylium lycopersici* (violet bars). Values are means of three independent biological replicates and error bars represents the standard deviation; letters in common on the bars are not significantly different according to the Tukey test at $p \leq 0.05$

line with previous results, control strains *Bacillus* sp. E7 and *Bacillus subtilis* Er/S inhibited the growth of *A. alternata* by 68 and 82%, *C. cassiicola* by 61 and 82%, and *S. lycopersici* by 48 and 89%, respectively (López et al. 2018).

Considering the results described above it became important to analyze the volatilome of each bacterium that proved to inhibit fungal pathogens growth. Those VOCs present within the profiles of the nine bacterial isolates *Bacillus* sp. (E7) (control), *Bacillus subtilis* (Er/S) (control), *Stenotrophomonas* sp.*(Us)*, *Acinetobacter* sp. (Ys), *Arthrobacter* sp.(Ae), *Microbacterium* sp. (Ee), *Pantoea* sp. (Fe), *Pseudomonas* sp. (He) and *Arthrobacter* sp. (Le) and their relative amount are presented in Fig. 3 and in Table 2.

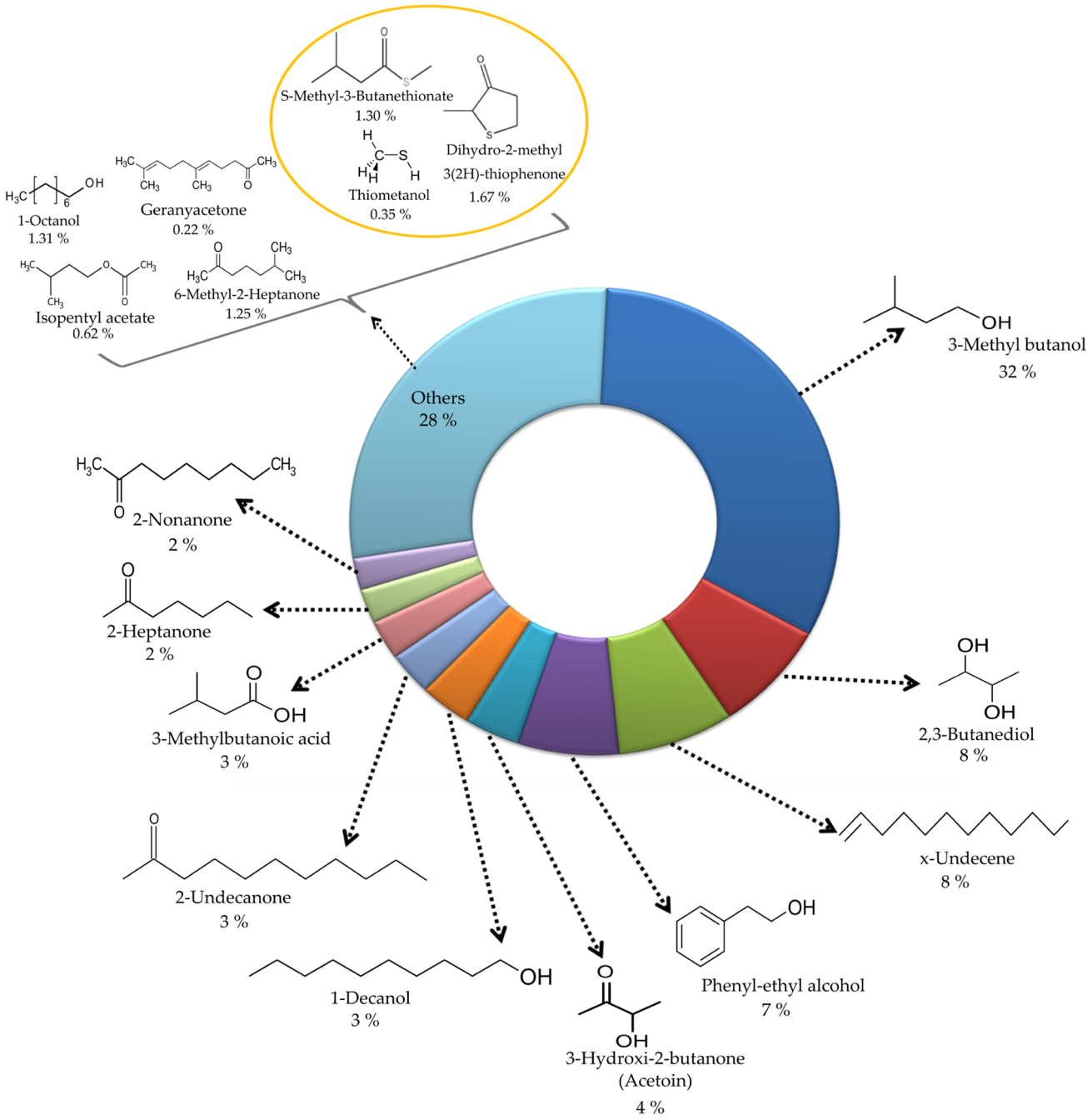

Fig. 3 Chemical structures of the compounds found within the volatilome of the nine bacterial isolates that inhibited growth of fungal pathogens. The analysis was performed with a GC–MS, a sample was withdrawn from vials containing cultures of the bacteria. The figure details the chemical structures of the VOCs mostly represented in these profiles

Table 2 Volatilome profiles of cultures of bacteria isolated from tissues of tomato plants analyzed by GC–MS

Isolate	Alcohols	Ketones	Esters, saturated and unsaturated Carbohydrates, Acids
Bacillus sp. E7	2,3-butanediol (KI = 790, 39.10%)	3-hydroxi-2-butanone (Acetoin) (KI = 721, 16.84%)	Ac. 2-methyl butanoic (KI = 879, 8.70%)
	3-methyl butanol (KI = 741.50, 7.16%)	3(2H)-thiophenone, dihydro-2-methyl (KI = 989, 11.96%)	Ac. 3-methyl butanoic (KI = 865, 8.36%)
			Ac. Isobutanoic (KI = 775, 4.33%)
Bacillus sp. Er/S	2,3-butanediol (KI = 785, 19.38%)	3-hydroxi-2-butanone (Acetoin) (KI = 719, 13.48%)	Ac. 3-methyl butanoic (KI = 868, 8.06%)
	3-methyl butanol (KI = 741.50, 3.41%)	2-undecanone (KI = 1294, 7.28%)	Ac. 2-methyl butanoic (KI = 879, 4.55%)
		2-tridecanone (KI = 1497, 7.20%)	
		2-heptanone (KI = 890, 7.12%)	
Stenotrophomonas sp. Us	3-methyl butanol (KI = 741.50, 22.91%)	6-Methyl-2-Heptanone (KI = 958, 7.66%)	S-methyl-3-methyl butanothionate (KI = 943, 8.97%)
	2-methyl butanol (KI = 745, 8.38%)	x-methyl-2-nonanone PM = 156 ($C_{10}H_{20}O$) (KI = 1159, 6.47%)	Ac. 3-methyl butanoic (KI = 860, 2.05%)
	phenyl ethyl alcohol (KI = 1119, 3.89%)	2-nonanone (KI = 1094, 3.88%)	
	thiometanol (KI = 621, 1.63%)	2-undecanone (KI = 1295, 2.65%)	
	2-heptanol (KI = 904,1.62%)	5-methyl-2-heptanone (KI = 968, 2.61%)	
Acinetobacter sp. Ys	3-methyl butanol (KI = 741.50, 52.41%)	x-methyl-2-undecanone PM = 184 ($C_{12}H_{24}O$) (KI = 1367, 7.25%; KI = 1360, 2.09%)	Ac. 3-methyl butanoic (KI = 852, 2.90%)
	phenyl ethyl alcohol (KI = 1118, 12.52%)	x-methyl-2-nonanone PM = 156 ($C_{10}H_{20}O$) (KI = 1164, 2.60%)	Ac. 2-methyl butanoic (KI = 864, 1.08%)
	2-octen-1-ol (KI = 1069, 1.97%)	5-methyl-2-heptanone (KI = 967, 1.87%)	
Arthrobacter sp. Ae	3-methyl butanol (KI = 741.50, 67.55%)	2-heptanone (KI = 894, 3.96%)	nonano (KI = 900, 1.52%)
	phenyl ethyl alcohol (KI = 1118, 22.72%)	2-butanone (KI = 657, 2.51%)	
Microbacterium sp. Ee	1-decanol (KI = 1274, 28.27%)	2-undecanone (KI = 1295, 5.70%)	CH unsaturated C14:1—PM = 196 (KI = 1362, 3.16%)
	1-octanol (KI = 1072, 11.24%)	2-nonanone (KI = 1092, 3.92%)	CH unsaturated C15:1—PM = 210 (KI = 1457, 2.24%)
	1-nonanol (KI = 1172, 4.54%)	x-methyl-2-tridecanone PM = 212 ($C_{14}H_{28}O$) (KI = 1570, 3.43%)	1-pentadecen (KI = 1493, 1.61%)
	3-methyl butanol (KI = 741.50, 2.42%)	2-tridecanone (KI = 1497, 2.10%)	
	9-decen-1-ol (KI = 1264, 1.99%)	x-methyl-2-undecanone PM = 184 ($C_{12}H_{24}O$) (KI = 1365, 1.93%)	
Pantoea sp. Fe	3-methyl butanol (KI = 741.50, 70.46%)	3-hydroxi-2-butanone (Acetoin) (KI = 720, 1.15%)	Ac. octanoic (KI = 1172, 1.09%)
	2,3-butanediol (KI = 793, 7.84%)	2-nonanone (KI = 1092, 1.11%)	Ac. decanoic (KI = 1366, 0.85%)
	phenyl ethyl alcohol (KI = 1117, 4.63%)		
	2-methyl butanol (KI = 745, 2.60%)		
	2-nonanol (KI = 1101, 2.09%)		

Table 2 (continued)

Isolate	Alcohols	Ketones	Esters, saturated and unsaturated Carbohydrates, Acids
Pseudomonas sp. He	3-methyl butanol (KI = 741.50, 5.63%)	2-undecanone (KI = 1295, 7.50%)	x-undecene (KI = 1093, 66.10%)
		x-tridecen-2-one (KI = 1474, 2.90%)	Ac. 3-methyl butanoic (KI = 868, 5.08%)
Arthrobacter sp. Le	3-methyl butanol (KI = 741.50, 45.26%)	2-nonanone (KI = 1093, 7.11%)	isopentyl acetate (KI = 879, 3.82%)
	phenyl ethyl alcohol (KI = 1118, 11.56%)	2-heptanone (KI = 893, 3.94%)	S-methyl-3-methyl butanothionate (KI = 942, 1.77%)
	2-nonanol (KI = 1101, 5.30%)	x-methyl-2-nonanone PM = 156 ($C_{10}H_{20}O$) (KI = 1164, 3.40%)	
	2-heptanol (KI = 902, 3.45%)	geranylacetone (KI = 1456, 1.87%)	

Cultures were grown on TYB media within caramel glass vials incubated as described in materials and methods

KI (Kovalts index) and relative abundance [%] of the compounds are detailed in parentheses. Compounds presented in bold letters only were detected in cultures of some isolates

Volatile organic compounds (VOCs): Extraction, identification and quantification

The volatilome of all the isolates presented profiles whose most abundant group was alcohols. Only *Bacillus subtilis* (Er/S) (Control) and a *Pseudomonas* isolate (He) synthetized more ketones and esters compounds, acids, as well as saturated and unsaturated carbohydrates, than the other ones. Although we failed to identify all the VOCs, the unidentified fraction represented a small fraction that ranged between 0.4 and 13.5% (Table 2).

In Fig. 3, the chemical structure of the volatilome compounds of the nine bacterial isolates that inhibited growth of fungal pathogens are presented. The VOC spectra showed that while some compounds were isolate specific, others are synthesized and released by several antagonists. *Bacillus* sp. (E7) and *Bacillus subtilis* (Er/S) that were included in the experiments as controls presented similar VOC profiles. Each category like alcohols, ketones, and others were represented by the same type of compounds, though they differed in their relative abundance. In Table 2, it can be seen that the overabundant alcohols were 2,3-butanediol and 3-methyl butanol, the main ketone Acetoin, and two acids, 2-methyl and 3-methyl butanoic were the most abundant ones among esters, saturated and unsaturated carbohydrates and acids. The *Pseudomonas* sp. isolate He, synthesized esters, acids and saturated and unsaturated carbohydrates with a relative abundance of 74.91%, being the main compound x-undecene that represented 66%. The rest of the isolates including *Stenotrophomonas, Acinetobacter, Arthrobacter* (two isolates), *Microbacterium* and *Pantoea*, presented profiles whose most abundant compound category was that of alcohols and, within them, 3-methyl butanol was the one produced the most, except for *Microbacterium* sp. that synthesized mostly alcohol, 1-decanol.

Within bacterial VOCs, some compounds contained sulfur-like methanethiol, dihydro-2-methyl-3(2H)-thiophenone and S-methyl-3-methyl butanothionate that were synthesized by *Stenotrophomonas* isolate (Us), *Bacillus* sp. isolate (E7) and *Stenotrophomonas* (Us) and *Arthrobacter* isolate (Le), respectively.

Also, the analysis of the profiles showed that among all the VOCs 16 were specifically synthesized by some isolates (Table 2). Within the category of "Alcohols" thiometanol, 2-octen-1-ol and three alcohols 1-decanol, 1-octanol and 9-decen-1-ol were synthesized by *Stenotrophomonas* (Us), *Acinetobacter* (Ys) and *Microbacterium* (Ee), respectively. Also, within ketones some were specific, like dihydro-2-methyl-3(2H)-thiophenone and 6-methyl-2-heptanone that were synthesized by *Bacillus* sp. (E7) (control), and *Stenotrophomonas* isolate (Us), respectively. x-tridecen-2- one was synthesized by *Pseudomonas* (He), 2-butanone was synthesized by *Arthrobacter* strain Ae and geranylacetone was synthesized by *Arthrobacter* strain Le. Within synthesized esters, carbohydrates, and acids, also some of them were specifically produced by certain isolates. 1-pentadecene, octanoic and decanoic acids, x-undecene as well as nonano and isopentyl acetate were synthesized by *Microbacterium* (Ee), *Pantoea* (Fe), *Pseudomonas* isolate (He) and both *Arthrobacter* isolates (Ae and Le), respectively.

Relationship between the production of volatile compounds and the antagonistic activity of endophytic bacteria

The VOCs synthesized by any of the following isolates like *Bacillus subtilis* (Er/S), *Arthrobacter* sp. (Ae), *Microbacterium* sp. (Ee), *Pseudomonas* sp. (He), and *Arthrobacter* sp. (Le) inhibited the growth of the three plant pathogens within a range of 75–85% (Fig. 2), which was much higher than when they were co-cultured with other bacteria. In addition, it should be highlighted the evident difference in sensibility of plant pathogens to VOCs, being *Stemphylium lycopersici* more sensible than *Alternaria alternata* and the latter one also more sensible than *Corynespora cassiicola*. Such differences also were observed when pathogens solely were exposed to bacterial VOCs and also when they were co-cultured in the same plates, which confirmed the different susceptibility of plant pathogens to the inhibitory activity of VOCs that might be crucial to develop biocontrol strategies.

The Biplot analysis presented in Fig. 4 shows that all bacteria located on the left side of the red line that is *Arthrobacter* Ae and Le, *Bacillus* Er/S, *Microbacterium* Ee and *Pseudomonas* He inhibited fungal growth at higher levels, which was related with the volatile compounds they synthetized that were x-undecene, 1-decanol, 1-octanol, 2-nonanol, 2-heptanol, phenyl–ethyl alcohol, 3-methyl-butanol, 2-nonanone, geranylacetone, 2-butanone, 2-heptanone, x-methyl-tridecanone, 2-undecanone,x-methyl-2-undecanone and isopentyl acetate. The other bacteria *Stenotrophomonas* Us, *Bacillus* E7, *Pantoea* Fe and *Acinetobacter* Ys were less efficient to inhibit growth and synthetized a different array of volatile compounds, among these some are known for their ability to promote plant growth. In a way this is supporting the findings shown on Fig. 2.

Discussion

Phytobiomes consist of plants, their environment, and their associated communities of organisms whose interactions have profound effects on soil, plant and agroecosystem health (Kerdraon et al. 2019). Knowledge regarding the phytobiomes network might be used to manage agroecosystems which might turn out to be the best alternative for the

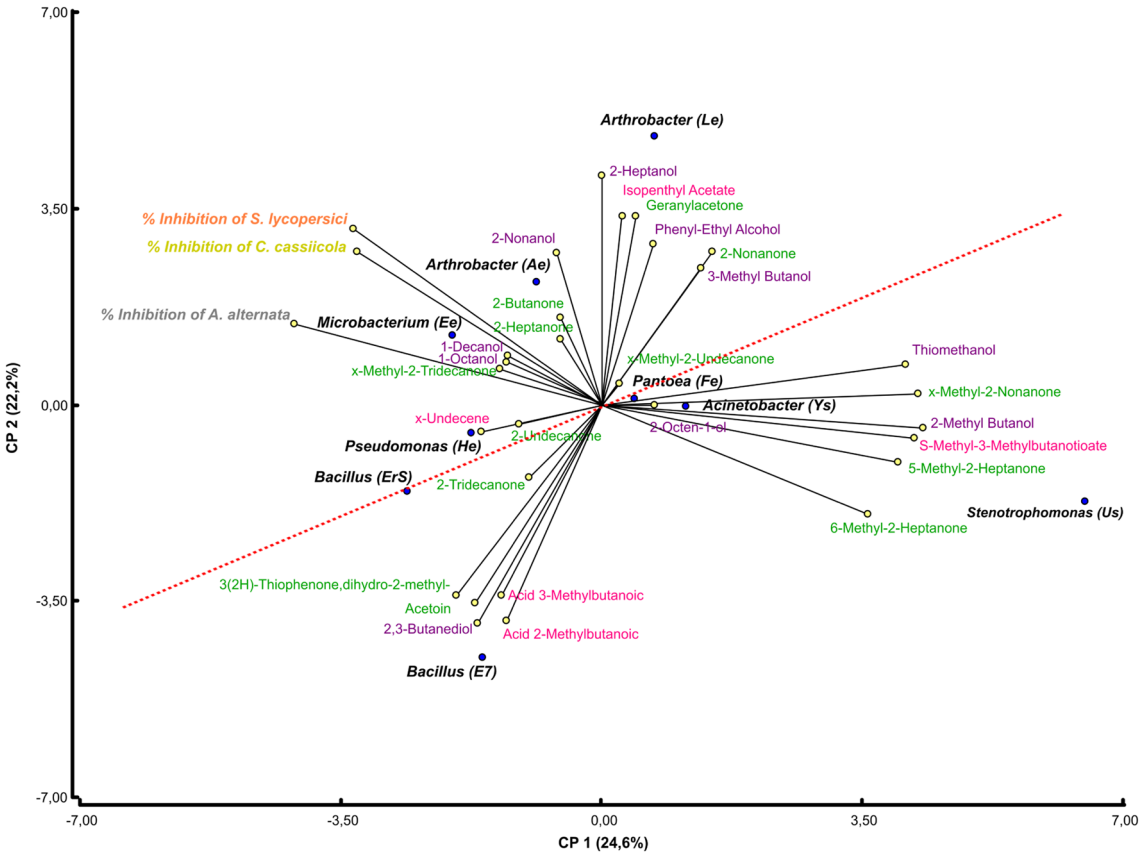

Fig. 4 Biplot showing the putative associations between secondary metabolites, organisms and plant pathogens inhibition. CP1 and CP2 stand for the first and second ordination index that accounted together for 46.8% of variation. Letters within brackets identify the isolate of the bacterial species. Infostat software version 2020p (Balzarini et al. 2008)

development of sustainable management and efficient crop production systems, minimizing in this way the negative effect production has on the environment. Within phytobiomes, microorganisms interact with plants in many different ways since they include rhizospheric associative, symbiotic as well as endophytic interactions. Among them, the latter ones gained importance in terms of their role in promoting plant growth and health. In this regard, this relevant component of the phytobiome has not been profoundly explored as well as its role in plant growth, development and health.

The control of soil-borne plant diseases with "beneficial microorganisms" is probably one of the most important alternatives to replace the management of plant diseases with chemical pesticides in the near future. Many antagonists inhibit fungal growth by various mechanisms (Weiskopk 2013; Brader et al. 2014) indirectly affecting plant growth, which might be increased. In this sense, nine endophytic bacteria of tomato, comprising *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Microbacterium*, *Pantoea*, *Pseudomonas* and *Stenotrophomonas*, were selected based on their ability to inhibit the growth of plant fungal pathogens. All these bacteria synthesize compounds that antagonize the three foliar plant pathogens of tomato *A. alternata*, *C. cassiicola* and *S. lycopersici*. The observed antagonistic interactions between endophytic bacteria and pathogenic fungi are the result of many interactions within plant microbial communities (Brader et al. 2014). This is in contrast with other studies that indicate that specific members of the endophytic bacterial community with a well-established antagonistic mechanism play a prominent role in the inhibition of certain pathogens (de Boer 2017). However, when considering isolated microorganisms and individual mechanisms, it is possible to identify strains or compounds that can be used to develop biotechnological bioproducts. Although there is functional redundancy in bacterial antagonism against pathogens (Table 2) different bacteria synthesize at least a subset of different secondary metabolites that might share inhibitors of the same pathogen, however, they also might have a synergistic effect within the endophytic community of the plant (Lecomte et al. 2016; de Boer 2017). This is not surprising since most probably consortia of microorganisms are much more efficient at biocontrolling plant pathogens that just one microbe.

Regarding plant pathogens, our results highlighted that they differed in their sensibility to biocontrol since when bacteria were co-cultured with plant pathogens *Stemphylium lycopersici* was found to be more sensible than *Alternaria alternata*, being *Corynespora cassiicola* the least sensible. This was confirmed when the inhibitory activity of VOCs released by bacteria was evaluated and again *Stemphylium* was the most sensible. This suggests that the development of biocontrol strategies should include a test with an ample array of plant pathogens if the idea is to develop a product that might be used to control several diseases provoked by different etiological agents.

The volatilome of several organisms (Tilocca et al. 2020) have been studied and among them, the bacterial ones were found to contain alcohols, aldehydes, benzene and organic acids derivatives, terpenes and ketones (Wheatley et al. 1997; Chiron and Michelot 2005; Morath et al. 2012). This is in agreement with the endophytic bacteria volatilome that are pretty similar to other ones already described. Maruzzella et al. (1961) proposed that the antifungal activity of VOCs decrease in the following order: organic acids > aldehydes > alcohols > ethers > ketones > esters > lactones, which was associated with the functional group carried by the compounds (Liu et al. 2008). Also, the activity was dependent on the hydrophobicity of the solute, which affects the penetration in the cell membrane bilayer, which should provoke changes in the physicochemical properties (Urbanek et al. 2012). In summary, the endophytic bacteria included in this study synthesized a volatilome pretty similar to that of rhizospheric bacteria containing compounds that promote plant growth as well as compounds that inhibit fungal growth.

The fact that bacteria were isolated from different plant tissues might explain their ability to synthetize and release different type of VOCs. *Microbacterium* and both isolates of *Arthrobacter* (Ae and Le) were isolated from aerial plant parts tissues and synthesized mainly alcohols. *Arthrobacter* sp. isolates (Ae and Le), produced 3-methyl butanol and phenyl ethyl alcohol that belong to the alcohol type of VOCs. Interestingly, the antagonistic activity of *Microbacterium* sp. (Ee) also appeared to be related to the synthesis of alcohols like 1-decanol and 1-octanol, which were the only ones detected in the VOC profiles of this organism. These three bacterial isolates also synthetized ketones such as 2-nonanone, 2-heptanone, and 2-undecanone that were synthesized also by *B. subtilis* (Er/S) that additionally synthetized 2-tridecanone and acetoin (Table 2). Thus, probably the ability of *B. subtilis* to produce more ketones might be providing antagonistic activity against the three fungal pathogens evaluated. The *Pseudomonas* isolate He, a bacterium that was living endophytically in tomato roots, synthesized in addition to some alcohols and ketones large amounts of x-undecene.

Bacteria living endophytically in tomato plant tissues synthesized alcohols, such as 3-Methyl-1-Butanol, though in varying amounts, this was such that within the VOCs profile of *Pantoea* and both representatives of *Arthrobacter*, and *Acinetobacter*, a greater relative abundance of this alcohol was found within their VOC profiles. This alcohol was the most abundant one exceeding 70% in the case of the *Pantoea* Fe strain, suggesting that it played a key role on the antagonistic activity on the three fungal pathogens assayed. Furthermore, within bacterial isolates, whose VOCs profiles

were rich in alcohols, *Microbacterium*, and both isolates of *Arthrobacter*, were more effective in controlling the growth of the three phytopathogenic fungi. Among these bacteria, *Arthrobacter* isolates also released large amounts of phenyl ethyl alcohol that is known for its remarkable antimicrobial activity (Li et al. 2010; Naznin et al. 2014; Prakash et al. 2015), which might be due to the impaired plasma membrane permeability, amino acid and sugar transport systems, and/or inhibition of macromolecule synthesis (Ingram and Buttke 1984; Lucchini et al. 1993; Etschmann et al. 2002). Furthermore, alcohols are not selectively adsorbed and are mainly accumulated in the cell membrane whose activity is affected the most, altering pathogens viability, becoming in this way an antimicrobial tool (Ingram and Buttke 1984). Moreover, some alcohols, such as isoamyl alcohol (3-Methyl-1-Butanol), might be adsorbed on the spores' surface, adhering to them for a long time, inhibiting in this way spore germination (Ando et al. 2012). So, our findings suggest that endophytic bacteria antagonize fungi with mechanisms already described in free-living bacteria such as the synthesis of alcohols specifically methyl butanol, an alcohol known for its antagonistic activity against fungi (Morita et al. 2019).

Other alcohols such as the long-chain 6–20 carbon atom aliphatic ones also inhibited the growth of several bacteria and fungi (Tanaka et al. 2000; Elgaali et al. 2002). These compounds may function as nonionic surfactants that as described above disrupt membranes (Kubo et al. 2003), in this case by provoking changes in their fatty acid composition (Kabelitz et al. 2003), or by functioning as organic solvents (Hamilton-Kemp et al. 2005). In vitro studies using synthetic alcohols showed that 1-decanol and 1-dodecanol were among the most active ones inhibiting microbial growth. Kubo et al. (1995) demonstrated that aliphatic alcohols were specific in their inhibitory activity against Gram-positive bacteria and fungi, but not against Gram-negative bacteria. Our results confirmed previous findings since bacteria that antagonized the growth of fungal pathogen the most were those that presented the highest levels of alcohols within their VOCs profiles. In agreement with this, it has been found that Gram-positive bacteria synthesize low levels of long-chain alcohols, and also produce a series of ketones (Elgaali et al. 2002). However, we found that *Microbacterium* sp. Ee a Gram (+) bacteria, synthesized high levels of long-chain aliphatic alcohols such as 1-decanol and 1-octanol and these bacteria proved to be efficient at controlling fungal growth in vitro. Therefore, bacterial endophytes that can synthesize alcohols, like bacteria inhabiting other ecological niches, might antagonize plant pathogens; however, it remains to be demonstrated their synthesis and activity within plant tissues.

Another alcohol with higher relative abundance in some isolates was 2,3-butanediol that was found, together with Acetoin, within the VOCs profiles of *Bacillus* sp. and *Pantoea* sp. isolates. Acetoin is a precursor to 2,3-butanediol and can be bio-transformed by plants and microorganisms into 2,3-butanediol stereoisomer's (Javidnia et al. 2016). Interestingly, both compounds were found to promote plant growth and also trigger the induced systemic resistance of plants (Rudrappa et al. 2010; Wu et al. 2019). Therefore, endophytic bacteria have the ability, like other bacteria, to synthesize plant growth-promoting compounds. Future work should evaluate if these bacteria alter plant growth and work endophytically as Plant Growth Promoting Bacteria (PGPB), which does not rule out their role as antagonists of pathogens by activating plant defense mechanisms.

Andersen et al. (1994) found that aliphatic aldehydes and ketones were more effective than alcohols in the inhibition of germ tube formation of *Alternaria alternata* and the presence of unsaturated bond adjacent to carbonyl moiety might make the molecule more reactive and more efficient antifungal. A somewhat similar mechanism might be present in *Bacillus* isolate Er/S that synthesized more ketones that inhibited fungal growth of *A. alternata*. Yuan et al (2012) showed that ketones activity was negatively correlated with the number of carbon atoms. Thus, 2-nonanone and 2-decanone particularly showed a specific strong inhibition activity against *Fusarium oxysporum* (Yuan et al. 2012; Raza et al. 2015). Conversely, 2-tetradecanone and 2-pentadecanone, even though they were abundant at least based on the large peaks areas that can be observed in the GC chromatographs, are known to be poor inhibitors of fungal growth (Yuan et al. 2012; Li et al. 2014). Therefore, according to these results 2-nonanone, 2-undecanone and 2-tridecanone that were synthetized by *B. subtilis* Er/S were responsible for reducing mycelial growth of pathogens and also inhibit spore germination (Yuan et al. 2012). Our results confirmed that the organisms with the ability to synthesize volatile ketones have a higher antagonistic activity. Future work using purified compounds of each of these volatiles should be of help to identify their role in the antagonistic interactions.

Within the genus *Pseudomonas*, it appears that the relative contribution of undecene to the volatile profile of the strains is highly variable. In vitro assays with VOCs showed that 1-undecene was toxic for fungal pathogens as well as plants, but it also stimulated growth (Wang et al. 2013; Popova et al. 2014; Hunziker et al. 2015). A volatile profile analysis showed that those *Pseudomonas* with the ability to synthesize 1-undecene, a fungal growth inhibitor, were responsible for the growth inhibition of *Phytophthora infestans* in vitro. Furthermore, 1-undecene provoked a significant reduction of mycelial growth, spore germination, sporangia formation, and zoospore release of *P. infestans* in a dose-dependent manner. Our studies showed that only *Pseudomonas* sp. inhibited the growth of *Alternaria*, *Corynespora*, and *Stemphylium* by the emission of VOCs,

which incidentally were highly abundant in x-undecene. However, there is not yet much information regarding the effect of alkenes on these pathogens.

In summary it can be concluded that the endospheric environment of tomato host a complex bacterial community, among them representatives of the genera *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Microbacterium*, *Pantoea*, *Pseudomonas*, and *Stenotrophomonas* that are adapted to live within different plant tissues. Each of these bacteria inhibited the growth of *A. alternata*, *C. cassiicola* and *S. lycopersici* that incidentally differed in their sensitivity to VOCs. We found that endophytic bacteria synthesize volatile compounds of different chemical nature, whose activity on growth of fungal pathogens have already been described. However, it remains to be described the regulatory pathways and genes involved in the biosynthesis of volatiles in endophytic bacteria, determine their biologically relevant concentrations and resolve the importance of volatiles in the plant endobiome processes, where interactions between pathogens, antagonists and the plant occur. Volatiles play an important role in communication and competitiveness between physically separated microorganisms (Kai et al. 2009; Effmert et al. 2012; Garbeva et al. 2014a). It is plausible that in the endosphere of plants, latent microorganisms can detect changes in their environments through the volatiles emitted and change their behavior accordingly and, in turn, influence the behavior of endophytic microorganisms (Kai et al. 2009). Although several studies have shown that volatile compounds can be used as signaling molecules in microbial communication, until now it is unclear how microorganisms perceive volatiles as signals.

Acknowledgments We wish to thank Dr. Sergio Mijailovsky from Institute of Investigaciones Bioquímicas de La Plata (CONICET-UNLP, La Plata, Argentina) for his help in performing the extraction, identification and quantification of VOCs and their analysis.

Author contributions The contributions of the authors in carrying out this work are detailed below: conceptualization, López SMY and Balatti PA; methodology, López SMY and Pastorino GN; software and validation, López SMY and Balatti PA; formal analysis, López SMY and Pastorino GN; research, López SMY; writing—preparation of the original draft, López SMY and Balatti PA; writing—review and editing, López SMY and Balatti PA; Project Management and Fund Acquisition, López SMY and Balatti PA.

Funding This research was partially supported by the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICBA), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) of the Ministerio de Ciencia, Tecnología e Innovación Productiva through the projects PICT-2016–0794 Jóvenes Investigadores (Silvina López) and the CICBA through the Subsidio 2017 (Pedro Balatti). http://www.agencia.mincyt.gob.ar; https://www.gba.gob.ar/cic.

Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interest.

References

Ab Rahman SFS, Singh E, Pieterse CM, Schenk PM (2018) Emerging microbial biocontrol strategies for plant pathogens. Plant Sci 267:102–111

Abisado RG, Benomar S, Klaus JR, Dandekar AA, Chandler JR (2018) Bacterial quorum sensing and microbial community interactions. MBio 9(3):e02331-e2417

Andersen RA, Hamilton-Kemp TR, Hildebrand DF, McCracken CT Jr, Collins RW, Fleming PD (1994) Structure-antifungal activity relationships among volatile C6 and C9 aliphatic aldehydes, ketones, and alcohols. J Agric Food Chem 42(7):1563–1568

Ando H, Hatanaka K, Ohata I, Yamashita-Kitaguchi Y, Kurata A, Kishimoto N (2012) Antifungal activities of volatile substances generated by yeast isolated from Iranian commercial cheese. Food Control 26(2):472–478

Bader AN, Salerno GL, Covacevich F, Consolo VF (2020) Native *Trichoderma harzianum* strains from Argentina produce indole-3 acetic acid and phosphorus solubilization, promote growth and control wilt disease on tomato (*Solanum lycopersicum* L.). J King Saud Univ Sci 32(1):867–873

Balzarini MG, Gonzalez L, Tablada M, Casanoves F, Di Rienzo JA, Robledo CW (2008) Infostat. Manual del usuario, Editorial Brujas, Córdoba, Argentina, p 336

Baysal Ö, Lai D, Xu HH, Siragusa M, Çalışkan M, Carimi F, Teixeira da Silva JA, Tör M (2013) A proteomic approach provides new insights into the control of soil-borne plant pathogens by *Bacillus* species. PLoS One 8(1):e53182

Brader G, Compant S, Mitter B, Trognitz F, Sessitsch A (2014) Metabolic potential of endophytic bacteria. Curr Opin Biotechnol 27:30–37

Chiron N, Michelot D (2005) Odeurs des champignons: chimie et rôle dans les interactions biotiques-une revue. Cryptogam Mycol 26(4):299–364

Claeson AS, Sandström M, Sunesson AL (2007) Volatile organic compounds (VOCs) emitted from materials collected from buildings affected by microorganisms. J Environ Monit 9(3):240–245

Collinge DB, Jørgensen HJ, Latz M, Manzotti A, Ntana F, Rojas Tayo EC, Jensen B (2019) Searching for novel fungal biological control agents for plant disease control among endophytes. Endophytes Grow World 21:25–51

De Boer W (2017) Upscaling of fungal–bacterial interactions: from the lab to the field. Curr Opin Microbiol 37:35–41

Dukare AS, Paul S, Nambi VE, Gupta RK, Singh R, Sharma K, Vishwakarma RK (2019) Exploitation of microbial antagonists for the control of postharvest diseases of fruits: a review. Crit Rev Food Sci Nutr 59(9):1498–1513

Effmert U, Kalderás J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38(6):665–703

Elgaali H, Hamilton-Kemp TR, Newman MC, Collins RW, Yu K, Archbold DD (2002) Comparison of long-chain alcohols and other volatile compounds emitted from food-borne and related Gram positive and Gram negative bacteria. J Basic Microbiol 42(6):373–380

Etschmann M, Bluemke W, Sell D, Schrader J (2002) Biotechnological production of 2-phenylethanol. Appl Microbiol Biotechnol 59(1):1–8

Franco MEE, Troncozo MI, López SMY, Lucentini G, Medina R, Saparrat MCN, Ronco LB, Balatti PA (2017) A survey on tomato leaf grey spot in the two main production areas of Argentina led to the isolation of *Stemphylium lycopersici* representatives which were genetically diverse and differed in their virulence. Eur J Plant Pathol 149(4):983–1000

Garbeva P, Hordijk C, Gerards S, de Boer W (2014) Volatiles produced by the mycophagous soil bacterium Collimonas. FEMS Microbiol Ecol 87:639–649. https://doi.org/10.1111/1574-6941.12252

Hamilton-Kemp T, Newman M, Collins R, Elgaali H, Yu K, Archbold D (2005) Production of the long-chain alcohols octanol, decanol, and dodecanol by *Escherichia coli*. Curr Microbiol 51(2):82–86

Hunziker L, Bönisch D, Groenhagen U, Bailly A, Schulz S, Weisskopf L (2015) *Pseudomonas* strains naturally associated with potato plants produce volatiles with high potential for inhibition of *Phytophthora infestans*. Appl Environ Microbiol 81:821–830. https://doi.org/10.1128/AEM.02999-14

Ingram LO, Buttke TM (1984) Effects of alcohols on microrganismos. Adv Microbiol Physiol Bethesda 25:256–300

Javidnia K, Faghih-Mirzaei E, Miri R, Attarroshan M, Zomorodian K (2016) Biotransformation of acetoin to 2, 3-butanediol: Assessment of plant and microbial biocatalysts. Res Pharm Sci 11(4):349

Kabelitz N, Santos PM, Heipieper HH (2003) Effect of aliphatic alcohols on growth and degree of saturation of membrane lipids in *Acinetobacter calcoaceticus*. FEMS Microbiol Lett 220:223–227

Kai M, Effmert U, Berg G, Piechulla B (2007) Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. Arch Microbiol 187(5):351–360

Kai M, Haustein M, Molina F, Petri A, Scholz B, Piechulla B (2009) Bacterial volatiles and their action potential. Appl Environ Microbiol 81:1001–1012. https://doi.org/10.1007/s00253-008-1760-3

Kakoti P, Gogoi P, Yadav A, Singh BP, Saikia R (2020) Foliar Fungal Diseases in Pulses: Review and Management. Management of Fungal Pathogens in Pulses. Springer, Cham, pp 131–142

Kerdraon L, Laval V, Suffert F (2019) Microbiomes and pathogen survival in crop residues, an ecotone between plant and soil. Phytobiomes J 3(4):246–255

Kim KS, Lee S, Ryu CM (2013) Interspecific bacterial sensing through airborne signals modulates locomotion and drug resistance. Nat Commun 4(1):1–12

Köhl J, Kolnaar R, Ravensberg WJ (2019) Mode of action of microbial biological control agents against plant diseases: relevance beyond efficacy. Front Plant Sci 10:845

Kubo I, Fujita T, Kubo A, Fujita K (2003) Modes of antifungal action of alcohols against *Saccharomyces cerevisiae*. Biorg Med Chem 11:1117–1122

Kubo I, Muroi H, Kubo A (1995) Structural functions of antimicrobial long-chain alcohols and phenols. Bioorg Med Chem 3(7):873–880

Le Cocq K, Gurr SJ, Hirsch PR, Mauchline TH (2017) Exploitation of endophytes for sustainable agricultural intensification. Mol Plant Pathol 18(3):469–473

Lecomte C, Alabouvette C, Edel-Hermann V, Robert F, Steinberg C (2016) Biological control of ornamental plant diseases caused by *Fusarium oxysporum*: a review. Biol Control 101:17–30

Lemfack MC, Nickel J, Dunkel M, Preissner R, Piechulla B (2014) mVOC: a database of microbial volatiles. Nucleic Acids Res 42(D1):D744–D748

Li L, Wang Y, Li K, Su F, Ma C, Xu P (2014) Genome sequence of meso-2, 3-butanediol-producing strain *Serratia marcescens* ATCC 14041. Genome Announce. 2(3)

Li Q, Ning P, Zheng L, Huang J, Li G, Hsiang T (2010) Fumigant activity of volatiles of *Streptomyces globisporus* JK-1 against *Penicillium italicum* on Citrus microcarpa. Postharvest Biol Technol 58(2):157–165

Liu H, Carvalhais LC, Crawford M, Singh E, Dennis PG, Pieterse CM, Schenk PM (2017) Inner plant values: diversity, colonization and benefits from endophytic bacteria. Front Microbial 8:2552

Liu WW, Wei MU, Zhu BY, Du YC, Feng LIU (2008) Antagonistic activities of volatiles from four strains of Bacillus spp. and Paenibacillus spp against soil-borne plant pathogens. Agric Sci China 7(9):1104–1114

López SMY, Pastorino GN, Fernández-González AJ, Franco MEE, Fernández-López M, Balatti PA (2020) The Endosphere bacteriome of diseased and healthy tomato plants. Microbiol, Arch. https://doi.org/10.1007/s00203-020-01987-9

López SMY, Pastorino GN, Franco MEE, Medina R, Lucentini CG, Saparrat MCN, Balatti PA (2018) Microbial endophytes that live within the seeds of two tomato hybrids cultivated in Argentina. Agronomy 8(8):136

Lucchini JJ, Bonnaveiro N, Cremieux A, Le Goffic F (1993) Mechanism of bactericidal action of phenethyl alcohol in *Escherichia coli*. Curr Microbiol 27(5):295–300

Ludwig-Müller J (2015) Plants and endophytes: equal partners in secondary metabolite production? Biotechnol Lett 37(7):1325–1334

Maruzzella JC, Chiaramonte JS, Garofalo MM (1961) Effects of vapors of aromatic chemicals on fungi. J Pharm Sci 50(8):665–668

Medina R, Franco ME, Lucentini CG, Rosso JA, Saparrat MC, Bartel LC, Balatti PA (2019) Secondary metabolites synthesized by *Stemphylium lycopersici* and *Fulvia fulva*, necrotrophic and biotrophic fungi pathogen of tomato plants. Curr Plant Biol 20:100122

Molina-Santiago C, Daddaoua A, Fillet S, Duque E, Ramos JL (2014) Interspecies signalling: *P seudomonas putida* efflux pump TtgGHI is activated by indole to increase antibiotic resistance. Environ Microbiol 16(5):1267–1281

Morath SU, Hung R, Bennett JW (2012) Fungal volatile organic compounds: a review with emphasis on their biotechnological potential. Fungal Biol Rev 26(2–3):73–83

Morita T, Tanaka I, Ryuda N, Ikari M, Ueno D, Someya T (2019) Antifungal spectrum characterization and identification of strong volatile organic compounds produced by *Bacillus pumilus* TM-R. Heliyon. https://doi.org/10.1016/j.heliyon.2019.e01817

Nair DN, Padmavathy S (2014) Impact of endophytic microorganisms on plants, environment and humans. Sci World J. https://doi.org/10.1155/2014/250693

Naznin HA, Kiyohara D, Kimura M, Miyazawa M, Shimizu M, Hyakumachi M (2014) Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in Arabidopsis thaliana. PLoS ONE 9:e86882. https://doi.org/10.1371/journal.pone.0086882

Piechulla B, Degenhardt J (2014) The emerging importance of microbial volatile organic compounds. Plant Cell Environ 37(4):811–812

Popova AA, Koksharova OA, Lipasova VA, Zaitseva JV, Katkova-Zhukotskaya OA, Eremina SI et al (2014) Inhibitory and toxic effects of volatiles emitted by strains of *Pseudomonas* and *Serratia* on growth and survival of selected microorganisms, *Caenorhabditis elegans*, and Drosophila *melanogaster*. Biomed Res Int. https://doi.org/10.1155/2014/125704

Prakash B, Singh P, Goni R, Raina AKP, Dubey NK (2015) Efficacy of Angelica archangelica essential oil, phenyl ethyl alcohol and α-terpineol against isolated molds from walnut and their antiaflatoxigenic and antioxidant activity. J Food Sci Technol 52(4):2220–2228

Raza W, Yuan J, Ling N, Huang Q, Shen Q (2015) Production of volatile organic compounds by an antagonistic strain *Paenibacillus polymyxa* WR-2 in the presence of root exudates and organic fertilizer and their antifungal activity against *Fusarium oxysporum* f. sp. *niveum*. Biol Control 80:89–95

Rodríguez-Ortega WM, Martínez V, Nieves M, Simón I, Lidón V, Fernandez-Zapata JC, Martinez-Nicolas JJ, Cámara-Zapata JM, García-Sánchez F (2019) Agricultural and physiological responses of tomato plants grown in different soilless culture systems with saline water under greenhouse conditions. Sci Rep 9(1):1–13

Romoli R, Papaleo MC, De Pascale D, Tutino ML, Michaud L, LoGiudice A, Fani R, Bartolucci G (2014) GC–MS volatolomic approach to study the antimicrobial activity of the

antarctic bacterium Pseudoalteromonas sp. TB41. Metabolomics 10(1):42–51
Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19(8):827–837
Rudrappa T, Biedrzycki ML, Kunjeti SG, Donofrio NM, Czymmek KJ, Paré PW et al (2010) The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. Commun Integr Biol 3:130–138. https://doi.org/10.4161/cib.3.2.10584
Schmidt R, Cordovez V, De Boer W, Raaijmakers J, Garbeva P (2015) Volatile affairs in microbial interactions. ISME J 9(11):2329–2335
Snelders NC, Kettles GJ, Rudd JJ, Thomma BP (2018) Plant pathogen effector proteins as manipulators of host microbiomes? Mol Plant Pathol 19(2):257
Tanaka Y, Fukuda S, Kikuzaki H, Nakatani N (2000) Antibacterial activity of aliphatic long chain compounds against upper airway respiratory tract bacteria. ITE Lett. Batteries. New Technol Med 1:C35–C38
Thomashow LS, Kwak YS, Weller DM (2019) Root-associated microbes in sustainable agriculture: models, metabolites and mechanisms. Pest Manage Sci 75(9):2360–2367
Tilocca B, Cao A, Migheli Q (2020) Scent of a Killer: microbial volatilome and its role in the biological control of plant pathogens. Front Microbiol. https://doi.org/10.3389/fmicb.2020.00041
Tyc O, Song C, Dickschat JS, Vos M, Garbeva P (2017) The ecological role of volatile and soluble secondary metabolites produced by soil bacteria. Trends Microbiol 25(4):280–292
Urbanek A, Szadziewski R, Stepnowski P, Boros-Majewska J, Gabriel I, Dawgul M et al (2012) Composition and antimicrobial activity of fatty acids detected in the hygroscopic secretion collected from the secretory setae of larvae of the biting midge *Forcipomyia nigra* (Diptera: Ceratopogonidae). J Insect Physiol 58(9):1265–1276
Wang C, Wang Z, Qiao X, Li Z, Li F, Chen M et al (2013) Antifungal activity of volatile organic compounds from *Streptomyces alboflavus* TD-1. FEMS Microbiol Lett 341:45–51. https://doi.org/10.1111/1574-6968.12088
Weisskopf L (2013) The potential of bacterial volatiles for crop protection against phytophathogenic fungi Microb Pathog Strategies for combating them. Sci Technol Educ 2:1352–1363
Wheatley R, Hackett C, Bruce A, Kundzewicz A (1997) Effect of substrate composition on production of volatile organic compounds from *Trichoderma* spp inhibitory to wood decay fungi. Int Biodeterior Biodegrad 39(2–3):199–205
Wu Y, Zhou J, Li C, Ma Y (2019) Antifungal and plant growth promotion activity of volatile organic compounds produced by *Bacillus amyloliquefaciens*. MicrobiologyOpen 8(8):e00813
Yuan J, Raza W, Shen Q, Huang Q (2012) Antifungal activity of *Bacillus amyloliquefaciens* NJN-6 volatile compounds against *Fusarium oxysporum* f. sp. *cubense*. Appl Environ Microbiol 78(16):5942–5944