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Research Paper

Inducing phenolic production and volatile organic compounds emission by inoculating *Mentha piperita* with plant growth-promoting rhizobacteria

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) work primarily by improving the nutrient status of plants and increasing plants' phytohormone production. Although this response has been demonstrated in many plant species, it is not well understood in aromatic plant species. To examine the effect of PGPR strains on the emission of volatile organic compounds (VOCs), total phenolic production and phenylalanine ammonia-lyase (PAL) activity, shoots of peppermint (*Mentha piperita*) plants were inoculated or co-inoculated with strains of beneficial rhizobacteria.VOC emissions for the inoculated groups were ~3-fold higher than for controls. Co-inoculated plants produced the greatest increase in VOC emission in comparison to singly inoculated and control plants. The synthesis of phenolic compounds in leaves of all the treated plants was enhanced in comparison with controls; and higher activity of the PAL enzyme was observed in inoculated plants. In view of the economic importance of VOCs and phenolic compounds for a variety of applications in the food and cosmetic industries, *P. fluorescens, B. subtilis* and *P. putida* SJ04 have the potential to improve the productivity of cultivated aromatic plants. Better understanding of the processes that affect secondary metabolites accumulation will lead to increased yields of these commercially valuable natural products.

1. Introduction

Plants are sessile organisms that must discriminate among a variety of challenges posed by the surrounding biotic and abiotic environment, and respond to them. Appropriate responses allow them to allocate their resources in an optimal manner for growth, reproduction, and defense. Over time, plants have evolved many physical and chemical defense systems to combat stress (Kessler and Heil, 2011). The huge diversity of existing phytochemicals and the evolution of secondary metabolism have resulted in increasingly complex interactions with biotic stresses (e.g., herbivores, pathogens, competitors). These interactions have numerous ecological and physiological implications (Arimura et al., 2005; Kessler and Heil, 2011).

Volatile organic compounds (VOCs) are products emitted into the atmosphere from natural sources (Holopainen and Gershenzon, 2010). VOCs are involved in defense systems, communication, pollinator attraction, and resistance to abiotic stress factors (Dicke and Baldwin, 2010; Wenke et al., 2010). Such compounds are released in small amounts into the environment by undamaged healthy plants, whereas during infestation by herbivores or pathogens, they are released in large amounts, from leaves to roots.VOC emission may be either constitutive or induced as a result of stress factors (Niinemets et al., 2013). Terpenoids, the predominant group of plant secondary metabolites, are particularly abundant in peppermint (*Mentha x piperita*), an aromatic species cultivated worldwide for the production of essential oils (EOs), and used fresh or dried (Lawrence, 2007). Terpenes, the primary constituents of EOs from aromatic plants, are volatile, typically lipophilic compounds with low molecular weight and high vapor pressure (Maffei et al., 2011; Wink, 2003). They are produced exclusively by glandular hairs (Gershenzon et al., 2000). EOs and VOCs indirectly protect the plant via tritrophic interactions. By emitting VOCs from their vegetative tissue, plants create a defense system that may ward off natural predators of the attacking herbivores or ward off microbes and animals (Das et al., 2013; Gershenzon, 2007).

Phenolic compounds are plant secondary metabolites that can be released under the influence of multiple biotic and abiotic stresses (Cheynier, 2012). Generally, the role of phenolic compounds in defense is related to their antibiotic or antinutritional effects. In addition to

Abbreviations: EO, essential oil; PAL, phenylalanine ammonia-lyase; PGPR, plant growth-promoting rhizobacteria; TOC, total phenolic compounds; VOC, volatile organic compound

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their involvement in relationships between plants and −animals and/or plants and microorganisms, plant phenolics also have key roles as pigments; as antioxidants and metal chelators; and as signalling agents both above- and below-ground between plant and other organisms (Lattanzio, 2013). Recently, there has been growing interest in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Zheng and Wang, 2001). It was reported that the antioxidant activity of plant materials correlated well with the content of their phenolic compounds (Moein and Moein, 2010).

Soil bacteria (rhizobacteria) of many genera have demonstrated beneficial effects on plant growth, crop yield, and crop quality. Such bacteria, collectively termed "plant growth-promoting rhizobacteria" (PGPR), promote growth through the production of phytohormones, reduction of plant ethylene level, enhancement of nutrient status, and enhancement of disease-resistance mechanisms, and biocontrol function (Babalola, 2010).

Many of our previous investigations have demonstrated that PGPR inoculation increases biomass and EO production in aromatic plants (Banchio et al., 2010; Cappellari et al., 2015; Santoro et al., 2015). On the other hand, biotic and abiotic stresses, such as high temperature, high light and herbivore attack, are well known to increase the emission of VOCs from plants (Holopainen and Gershenzon, 2010). However, VOC emission and total phenol content by aromatic plants inoculated with PGPR has not yet been thoroughly studied.

Here we describe a comparative study of the influences of *M. piperita* inoculation with three beneficial rhizobacterial strains (singly or in combination) on plant VOC emission and total phenol contents.

2. Material and methods

2.1. Bacterial strains, culture conditions, media, and treatments

Three bacterial strains previously reported as PGPR were studied: *Pseudomonas fluorescens* WCS417r; *P. putida* SJ04, a native fluorescent strain isolated from rhizospheric soil collected from a commercial crop of *Mentha* × *piperita* (San José) in Córdoba, Argentina, and tested for plant growth-promoting activity (GenBank KF312464.1); and *Bacillus subtilis* GB03 (Banchio et al., 2010). Bacteria were grown on LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl) for routine use and maintained in nutrient broth with 15% glycerol at −80 °C for long-term storage.

Each bacterial culture was grown overnight at 30 °C with rotation at 120 rpm until reaching the exponential phase, washed twice in 0.9% NaCl by Eppendorf centrifugation (4300 x g, 10 min, 4 °C), resuspended in sterile water, and adjusted to a final concentration of ~10^9 CFU/mL for use as inoculum.

Plants were grown in plastic pots (diameter 12 cm, depth 22 cm) containing sterilized vermiculite. *M. piperita* seedlings were planted (one per pot) in vermiculite and inoculated with 100 µL bacterial suspension. Six experimental treatments were performed: sterile water (control); SJ04; WCS417r; GB03; WCS417r + GB03; WCS417r + SJ04. Ten plants were used for each treatment.

2.2. Greenhouse experiments

Young shoots from *M. piperita* plants grown in Traslasierra Valley (Córdoba Province, Argentina) were surface-disinfected by being soaked for 1 min in 17% sodium hypochlorite solution and rinsed 3 x in sterile distilled water. Disinfected shoots were cultured in 100 mL MS culture medium containing 0.7% (w/v) agar and 1.5% (w/v) sucrose (Murashige and Skoog, 1962). All culture media contained 30 g/L sucrose and 7.5 g/L agar.

Following 30 days of culture as above, the apical meristems with foliar primordia of shoots that did not show contamination were aseptically removed from terminal buds. Explants were cultured in test tubes in 10 mL MS medium with 0.53 µM naphthalene acetic acid (NAA) and 0.26 µM benzyladenine (BA) (Santoro et al., 2011). Plantlets obtained from tips were multiplied by single-node culture in MS medium with 0.53 µM NAA and 0.28 µM BA. pH was adjusted to 5.8, growth regulators were added, and cultures were autoclaved (20 min, 121 °C). Temperature was maintained thereafter at 22 °C, with photoperiod 16 h/d and ~2000 Lux light radiation from cool-white fluorescent tubes. On day 7 of culture, rooting plantlets were obtained at the *in vitro* multiplication stage, transplanted directly into vermiculite in a greenhouse, and watered by a micro-irrigation system (Cappellari et al., 2013).

Plants were grown in a growth chamber under controlled conditions of light (16/8-h light/dark cycle), temperature (22 ± 2 °C), and relative humidity (~70%). Bacterial suspensions as described above were applied to experimental seedlings, and sterile water was applied to control seedlings. All plants received Hoagland's nutrient medium (20 mL/pot) once per week (Cappellari et al., 2013). Experiments were performed under non-sterile conditions.

Experiments were replicated 3 times (10 pots per treatment; 1 plant per pot). Pots were arranged randomly in the growth chamber. Thirty days after inoculation, plants were removed from pots, roots were washed to remove vermiculite, and shoot fresh weight (FW) was measured. The biological material for the collection of VOCs was the same as that used for the extraction of EOs published in (Cappellari et al., 2015), and a new replicate was performed to verify the data.

2.3. Collection of plant VOCs

Plant VOCs were collected 30 days after inoculation, prior to the removal of plants. The collection system consisted of a vacuum pump that created a constant airflow (300 mL/min) through a polyethylene terephthalate (PET) chamber (volume 1500 mL) containing a plant (inoculated or uninoculated). The chamber was closed at one end with a cap pre-drilled to fit the collection trap. At the other end, a cap with a hole through which the plant stem passed separated the bottom of the chamber from the base of the pot. Air exited the chamber through a reusable glass collection trap packet with 30 mg Super-Q absorbent (80–100 mesh; Alltech), which was rinsed 5 x with 10 mL dichloromethane prior to each collection to remove impurities. Headspace VOCs were collected for 2 h and eluted immediately from the absorbent traps with 200 mL dichloromethane, after which internal standard was added (1 µL α-pinene in 50 µL ethanol) (Banchio et al., 2007). Collected VOCs were analyzed by gas chromatography as described below. VOCs emitted by *M. piperita* plants consisted of ~30 different compounds. Thirty percent of the VOC terpene components included (−) menthone, (−) menthol, and (+) pulegone. Following VOC collection, each plant was cut and weighed. VOCs were also collected from control (uninoculated) plants. Collections from an empty chamber showed that the background level of monoterpenes was negligible.

2.4. Determination of total phenols

Total phenols were determined using Folin-Ciocalteu reagent (Singleton and Rossi, 1965). Each plant extract (0.5 mL) or gallic acid (standard phenolic compound) was mixed with Folin-Ciocalteu reagent (0.5 mL, diluted with 8 mL distilled water) and aqueous Na_2CO_3 (1 mL, 1 M). After 1 h, the level of total phenols was determined by colorimetry at a wavelength of 760 nm. Total phenol values were expressed in terms of mg gallic acid (a common reference compound) equivalent per g plant dry weight (Cappellari et al., 2013).

2.5. Determination of PAL enzyme activity

PAL was extracted from100 mg mint leaves; plant material was homogenized with liquid nitrogen using a mortar and pestle containing appropriate buffer solution (50 mM potassium phosphate and 1 mM

EDTA, pH 7.8) and 1% PVP (polyvinylpyrrolidone) and then filtered through a 0.20 mm nylon filter into a centrifuge tube. The tissue extract was centrifuged at 12,000*g* for 40 min at 4C. The supernatant to be used for enzymatic activity determination was stored at 20C. Protein concentration was determined by the method described by Bradford (1976).

PAL activity was assayed following the method described by Beaudoin-Eagan and Thorpe (1985) by measuring the amount of trans-cinnamic acid formed at 290 nm. The reaction mixture consisted of 100 mL of enzyme extract, 900 mL 6 mM of L-phenylalanine and 500 mM Trise HCl buffer solution (pH 8). The mixture was placed in a water bath at 37 °C for 70 min, and the reaction was stopped by the addition of 50 mL of 5 N HCl. Trans-cinnamic acid (1 mg ml^{-1}) was used as a standard, and PAL activity was expressed as mmol trans-cinnamic acid min^{-1} mg^{-1} protein.

2.6. Chemical analyses

Chemical analyses were performed using a Perkin-Elmer Q-700 gas chromatograph (GC) equipped with a CBP-1 capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and a mass selective detector. Analytical conditions: injector temperature 250 °C; detector temperature 270 °C; oven temperature programmed from 60 °C (3 min) to 240 °C at 4°/min; carrier gas = helium at a constant flow rate of 0.9 mL/min; source 70 eV. Oil components were identified based on mass spectral and retention time data in comparison to standard compounds (Banchio et al., 2007). GC analysis was performed using a Shimadzu GC-RIA gas chromatograph fitted with a 30 m x 0.25 mm fused silica capillary column coated with Supelcowax 10 (film thickness 0.25 µm). GC operating conditions: injector and detector temperatures 250 °C; oven temperature programmed from 60 °C (3 min) to 240 °C at 4°/min; detector = FID; carrier gas = nitrogen at constant flow rate 0.9 mL/min.

2.7. Statistical analysis

Data were pooled and subjected to analysis of variance (ANOVA) followed by a comparison of multiple treatment levels with controls using Fisher's post-hoc LSD (least significant difference) test. Differences between means were considered significant for p values < 0.05. The Infostat software program, v. 2008 (Group Infostat, Universidad Nacional de Córdoba, Argentina) was used for all statistical analyses.

3. Results

3.1. Emission of plant VOCs

In headspace experiments, concentrations of total VOCs emitted by inoculated vs. uninoculated *M. piperita* plants were significantly altered (Fig. 1). Singly inoculated plants released ~2.6-fold more monoterpenes than did control plants. VOC emission of theWCS417r + SJ04 co-inoculated plants (920 ng h^{-1} g^{-1}FW) was much higher than that of the control group (290 ng h^{-1} g^{-1}FW).

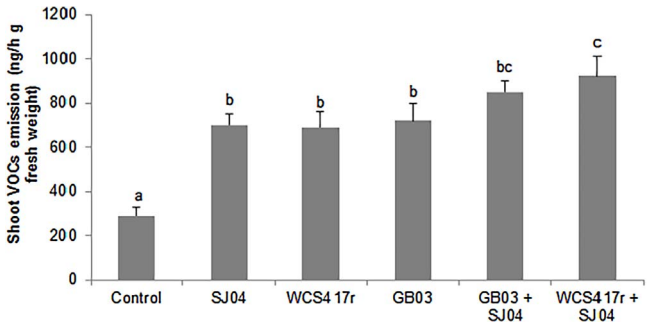

Fig. 1. Emission of shoot VOCs by *M. piperita* plants singly inoculated and co-inoculated with various PGPR strains. Letters above bars indicate significant differences according to Fisher's LSD test ($p < 0.05$).

Emission of major VOCs was altered by PGPR inoculation (Table 1). The amount of headspace menthone was ~2-fold higher for inoculated than for control plants, and singly inoculated and co-inoculated groups did not differ in this regard. Results were similar for menthol and pulegone emission; however, menthol emission was less for the singly inoculated SJ04 and GB03 groups than for other groups (Table 1). Pulegone emission was also ~2-fold higher in treated plants, except in theGB03 + SJ04 group.

PGPR inoculation also resulted in significantly higher relative percentage (R%) values for major VOCs (Table 1). R% of menthone was 6- 9%for the inoculated groups, but only 3% for controls. Similarly, R% of menthol was 5% for controls and 6–9% for inoculated plants; R% of pulegone was 3% for controls and 4–7% for inoculated plants.

3.2. Total phenol content (TPC)

An increased accumulation of total phenols was observed in inoculated mint plants compared to untreated controls (Fig. 2). TPC in leaves of SJ04 inoculated plants presented the higher values (606,45 mg/mg fw). Phenol accumulation was similar for singly or co-inoculated plants. TPC from roots was not affected by inoculation or co-inoculation.

3.3. Phenyl alanine ammonia lyase activity (PAL)

Significantly higher PAL activity was observed in treated plants compared to untreated controls (Fig. 3). The trend observed for TPC was similar to that observed for PAL activity. Inoculated or co-inoculated plants showed the same levels of PAL activity: 15–19 µg trans- cinnamic acid min^{-1} g^{-1} compared to 4 µg for non-inoculated plants.

4. Discussion

Increased emission of VOCs following herbivory has been reported for a variety of plants (Banchio et al., 2007; Dicke and Baldwin, 2010; Dong et al., 2016). Such VOCs may directly defend plants through their repellant or toxic effects on herbivores or pathogens (Kessler and Heil, 2011; Dong et al., 2016), or they may function in plant–plant communication (Dicke and Baldwin, 2010; Dong et al., 2016) to enhance the fitness of the attacked plants (Niinemets et al., 2013). Surprisingly, there is little research on the effects of beneficial microbes on VOC emission in aromatic plants – surprisingly, because such studies could help explain the role that these compounds have in plant reproduction as well as their contribution to plant biodiversity and their applications in agriculture (Dudareva et al., 2013).

PGPR enhance nutrition and tolerance of abiotic stress factors (Kloepper, 1993; Babalola, 2010), and also promote resistance in systemic tissues to microbial pathogens and above-ground herbivorous insects (Pineda et al., 2010). In certain commercial aromatic species (*Origanum majoricum*, *O. majorana*, *Tagetes minuta*, *M. piperita*), PGPR inoculation notably increases growth parameters and EO production. These inoculation effects differ according to plant species (Banchio et al., 2010; Santoro et al., 2011; Cappellari et al., 2013).

In the present study, VOC emission was enhanced (~3-fold higher than control levels) in all five PGPR inoculation groups (SJ04; WCS417r; GB03; WCS417r + GB03; WCS417r + SJ04). From a functional point of view, the single or co-inoculated rhizobacteria produce similar effects. The increase in VOC emission is not proportional to the increase in EOs. EO accumulation in *M. piperita* inoculated with beneficial rihizobacteria is increased 6-fold (Cappellari et al., 2015). This increase in EOs is double that which is observed in the emission of

Table 1
Concentrations of (−) menthone, (−) menthol, and (+) pulegone (mean ± SE) and their relative percentage present in VOC emissions of *M. piperita* plants singly inoculated and co-inoculated with various PGPR strains. Means followed by the same letter within a column are not significantly different according to Fisher's LSD test ($p > 0.05$).

	(−) menthone		(−) menthol		(+) pulegone	
	VOCs (μg/gFW)	%	VOCs (μg/gFW)	%	VOCs (μg/gFW)	%
Control	0.037 ± 0.003*a*	3.29 ± 0.61*a*	0.020 ± 0.002*a*	4.93 ± 0.60*a*	0.018 ± 0.001*a*	3.16 ± 0.48*a*
SJ04	0.048 ± 0.006*ab*	8.41 ± 1.58*b*	0.031 ± 0.002*b*	6.17 ± 0.72*ab*	0.046 ± 0.007*c*	6.07 ± 0.50*c*
WCS417r	0.069 ± 0.007*b*	9.34 ± 0.71*b*	0.048 ± 0.006*c*	8.13 ± 0.75*b*	0.045 ± 0.010*c*	6.57 ± 0.44*c*
GB03	0.067 ± 0.011*b*	9.41 ± 0.80*b*	0.035 ± 0.004*b*	8.01 ± 1.56*b*	0.052 ± 0.008*c*	6.66 ± 0.55*c*
GB03 + SJ04	0.063 ± 0.008*b*	8.87 ± 0.66*b*	0.040 ± 0.005*c*	8.61 ± 0.81*b*	0.028 ± 0.003*b*	4.33 ± 0.79*b*
WCS417r + SJ04	0.062 ± 0.008*b*	6.60 ± 0.74*b*	0.047 ± 0.006*c*	8.80 ± 0.05*b*	0.037 ± 0.006*c*	5.24 ± 0.49*bc*

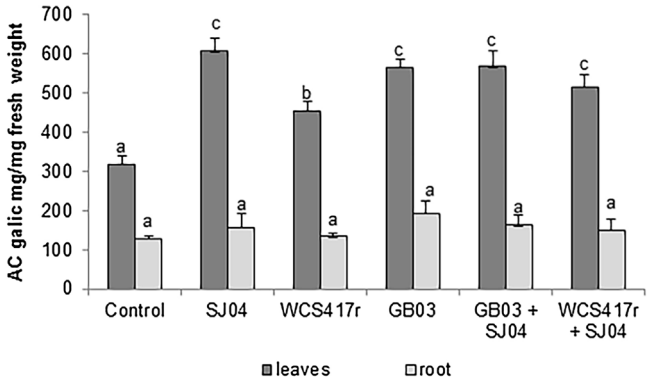

Fig. 2. Total phenol content in *M. piperita* singly inoculated and co-inoculated groups. Letters above bars indicate significant differences according to Fisher's LSD test ($p < 0.05$).

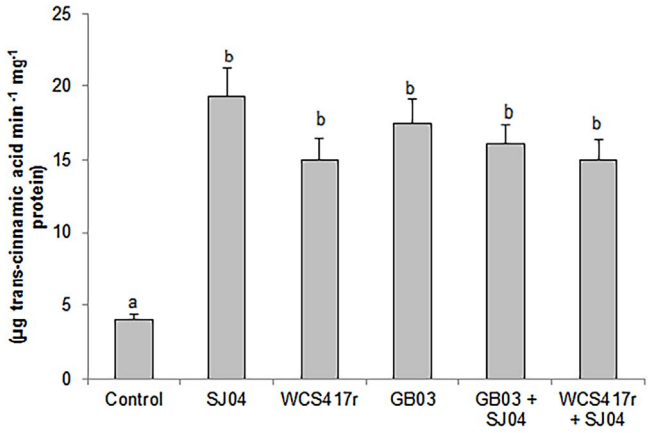

Fig. 3. Phenylalanine ammonia lyase activity determined in peppermint leaves inoculated and co-inoculated with PGPR strains. Means followed by the same letter within a column are not significantly different according to Fisher's LSD test ($p > 0.05$).

VOCs.

PGPR inoculation also alters the proportions of major VOC components. It is interesting to note that proportions of major monoterpenes in volatile emissions typically differ from those in EOs. In both VOCs and EOs, the main compounds are menthone, mentol and pulegone (Cappellari et al., 2015), but the relative percentage of each compound differs. It is important to mention that the VOC were collected with the same biological material Cappellari et al. (2015) used to extract the EOs: the R% (−) of menthone was ~ 5%, R% (−), menthol ~4%, and R% (+) of pulegone ~50%.

The presence of pulegone in the emitted VOCs is only ~3% (Table 1), in contrast with ~50% in EOs. Studies of other plant species have shown similar differences in the monoterpene composition of volatile emissions versus internal oils (Banchio et al., 2007; Gershenzon et al., 2000). Membranes of the trichome, where the EOs are synthesized, may be more permeable to specific monoterpenes, or may released monoterpene that are related to secretory compartments that differ from those of stored compounds (Gershenzon et al., 2000).

How volatiles are released from plant cells is currently largely unknown. Before being emitted into the environment, plant volatiles must cross the membrane, the aqueous cell wall, and, sometimes, the cuticle. Comparative analysis of volatile compounds emitted and present within the plant tissue revealed that the emission of volatiles is not merely a function of their differential volatility but may also involve a cytologically organized excretory process (Dudareva et al., 2013). The membranes of the storage compartment might be selectively more permeable to some volatile compounds, or the emitted substances may be associated with a secretory compartment that is entirely different from that of the stored volatiles (Dudareva et al., 2013). Presumably volatiles move through each barrier via passive diffusion. However, Widhalm et al. (2015) proposed that some mechanisms involved in the transport of other hydrophobic compounds must contribute to volatile emission, and these may reduce resistance. Little knowledge exists to explain metabolite movement between various subcellular compartments and the mechanism of the release process.

The effects of PGPR inoculation on VOCs may reflect a defensive response that protects damaged leaves from further attack. In studies of various insect species, pulegone has been known to exert repellent and toxic effects (Harrewijn et al., 2001). Additionally, it can produce a biochemical boundary to herbivory by eliminating symbionts of the herbivores (Harrewijn et al., 2001). Menthone displays insecticidal and genotoxic effects (Franzios et al., 1997), though to a lesser degree than pulegone.

Apart from the influence of PGPR inoculation on VOC emission, our study also showed the strong influence of rhizobacteria on phenolic production in leaves. Plants synthesize compounds with biological activity − namely, antioxidants − that are secondary products; these are mainly phenolic compounds serving in plant defense mechanisms to counteract reactive oxygen species (ROS) and thereby avoid oxidative damage. Leaves of *M. piperita* contain high levels of polyphenolic compounds, including caffeic acid, rosmarinic acid, eriocitrin, luteolin-7-*O*-glucoside (Dorman et al., 2009; Farnad et al., 2014). In the present study, TPC was 2-fold higher in singly inoculated or co-inoculated plants than in controls. Similar results were obtained in *Tagetes minuta* inoculated with *P. fluorescens* WCS417r and *Azospirillum brasilense* (Cappellari et al., 2013) and in *Piper betle* L. inoculated with *Serratia marcescens* (Lavania et al., 2006). Salla et al. (2014) showed that the inoculation of Eucalyptus with *Streptomyces* increased total phenol content in leaves but not in roots, which confirms our observations. In addition, Panka et al. (2013) reported that the presence of the endophyte fungus *Neotyphodium lolli* increased the content of phenolic compounds in the aerial part of three different genotypes of the perennial grass *Lolium perenne*.

In accordance with the effects found of rhizobacteria inoculation in TPC, levels of PAL activity also increased after different treatments; there were no differences between single inoculated or co-inoculated

plants. The enzyme PAL plays a central role in the inducible defense response (Compant et al., 2005), since it is the first enzyme in the general pathway of phenylpropanoid metabolism in plants and catalyzes the elimination of ammonia from phenylalanine to produce trans-cinnamic acid. These compounds can serve as substrates for several important biochemical pathways, including the formation of phenolic compounds (Tonelli et al., 2011).

Interest in phenolic compounds has increased considerably in recent years because of their antioxidant properties; in addition, these compounds have been reported to be potential candidates in lowering cardiovascular diseases (Huxley and Neil, 2003) and anticarcinogenic activities, antiallergenic, anti-arthrogenic, anti-inflammatory, antimicrobial and antithrombotic effects (Huang et al., 2010). Extracts of fruits, herbs, vegetables, and cereals rich in phenolics are increasingly of interest in the food industry, because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food (Lattanzio, 2013; Cheynier, 2012).

Changes in secondary metabolism such as VOCs and phenolic compounds are often seen in plant defense responses (Van Loon, 2007). Several PGPR strains have been shown to induce systemic resistance to wide-ranging phytopathogens in plants (Kloepper, 1993; Van Loon, 2007; Babalola, 2010). Induced resistance generally establishes an improved level of protection against attackers (Walters, 2011) and is managed by interconnected signalling pathways in which plant hormones act as major regulators (Pieterse et al., 2014). The signalling pathways elicited by PGPR are generally regulated by jasmonic acid (JA) and ethylene (Menzel et al., 2014; Pieterse et al., 2014). The role of the JA pathway in regulating VOC synthesis has been well studied. In many plant species, JA production induces the expression of secondary metabolite biosynthetic genes and the consequent accumulation of secondary metabolites (Schmelz et al., 2003; Zebelo et al., 2011; Dong et al., 2016); and PAL-increased activity and accumulation are believed to depend mainly on the plant hormone ethylene (Chen et al., 2003)

5. Conclusion

Our results suggest that the inoculation with beneficial rhizobacteria strains SJ04, WCS417r and GB03 individually or co-inoculated (with WCS417r + SJ04, GB03 + SJ04) caused a systemic induction of VOCs emission and phenolic compound pathways in *M. piperita* plants.

At this point, little is known regarding the effects of rhizobacteria on the release of VOC emission in aromatic and medicinal plants.

PGPR can significantly increase productivity and reduce the amount of fertilizer required for the economically important *M. piperita*. Bacterial inoculants such as PGPR are efficient biotechnological tools for stimulating secondary metabolism in plants; the magnitude of the changes depends on the bacterial strains or their combination.

Biotic and abiotic events below-ground can alter above-ground defense responses, through mechanisms that are not yet well understood. Improved knowledge of the effects of beneficial rhizobacteria may enhance the tools used to manipulate plant secondary metabolites and therefore benefit human applications.

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