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Microbial volatiles as plant growth inducers

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ABSTRACT

Agricultural practices require novel products that allow sustainable development and commercial production according to the needs of farmers and consumers. Therefore, in the last decade, eco-friendly alternatives have been studied, so volatile organic compounds (VOCs) emitted by microorganisms have emerged as a cheaper, effective, efficient, and an eco-friendly alternative. VOCs are lipophilic compounds derived from microbial metabolic pathways with low molecular weight ($< 300\,\mathrm{g\,mol^{-1}}$), low boiling point, and high vapor pressure that allow them to act as signal molecules over short and long distances. Main case studies provide evidence that VOCs released from diverse microorganisms (i.e. *Bacillus*, *Pseudomonas*, *Arthrobacter*, *Fusarium,* and *Alternaria*) can stimulate growth on a specific "target" seedling, such as *Arabidopsis* and tobacco. Some identified compounds, such as 3-hydroxy-2-butanone (acetoin), 2,3-butanediol, 2-pentylfuran, or dimethylhexadecylmine have shown their ability to elicit growth at root or leaf level. Few studies indicate that VOCs act in the regulation at phytohormone, metabolic pathways and nutrition levels according to genetic, proteomic, and metabolic analyses; but action mechanisms associated with growth-inducing activity are poorly understood. In this work, we reviewed case studies regarding identified compounds and action mechanisms for a better understanding of the information collected so far. Additionally, a brief description about the effects of VOCs for induction of resistance and tolerance in plants are presented, where compounds such as acetoin, dimethyl disulfide, 3-pentanol and 6-pentyl-α-pyrone have been reported. Furthermore, we summarized the knowledge to direct future studies that propose microbial VOCs as a technological innovation in agriculture and horticulture.

1. Introduction

Currently, the high demand for food and the need for increasing both performance and quality of agricultural crops have led to the applications of large amounts of chemical products (i.e. mineral fertilizer and commercial phytohormones), which have been used primarily to increase nutrient availability and stimulate the growth of species grown under field and greenhouse conditions, respectively (Zaman et al., 2015). Nevertheless, their applications have caused serious environmental problems, resulting in loss of soil biological activity, erosion derived from runoff, and leaching from spray components of these products (Savci, 2012). In addition, the synthetic compounds applied in greenhouse conditions have caused food contamination associated with toxic substance accumulation (e.g. nitrosamine compounds in lettuce) (Ward, 2009). Therefore, the search for sustainable alternatives has been carried out in order to reduce the input of chemical products in crops and to produce chemical-free food, so rhizosphere microorganisms have emerged as potential growth inducers.

Microorganisms, both bacteria and fungi, are found in high quantity and wide diversity in the rhizosphere zone, defined as "the narrow zone influenced by plant roots and characterized by their intense association with microbial activity" (Mendes et al., 2013; Dessaux et al., 2016; Van Dam and Bouwmeester, 2016). These microorganisms utilize root exudates, which contain ions, oxygen, water, enzymes, mucilage, and primary and secondary metabolites, representing between 20–40% of fixed carbon located in the underground root system (Philippot et al., 2013; Venturi and Keel, 2016). The plant exudates can determine or modify the microbial community along the root system (Badri et al., 2009). Meanwhile, microorganisms secrete diverse non volatile metabolites with beneficial effects to induce plant growth through direct and indirect pathways, which constitutes a traditional mechanisms studied to date (Dotaniya and Meena, 2015). Several studies conducted in the last decades indicate that direct pathways involve the release of phytohormones (i.e auxin, ethylene, and cytokinins) and organic substances (i.e organic acids) that contribute to growth stimulation and nutrient availability, respectively. Indirect pathways comprise

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substances that prevent pathogens attack through the production of hydrolytic enzymes, antibiotics, siderophores, and hydrogen cyanide (Goswami et al., 2016; Vejan et al., 2016). However, a new mechanism mediated by volatile organic compounds (VOCs) was reported for the first time by Ryu et al. (2003), who showed that volatiles released by *Bacillus subtilis* GB03 induced growth on *Arabidopsis thaliana*, being the first evidence that volatile organic compounds can modulate growth, stress, nutrition, and health processes in plants. To date, studies have achieved considerable progress in elucidating the mode of action of this type of compounds; however, it is still poorly understood.

Up to the present, the most studies have been conducted under controlled laboratory conditions using two compartment Petri dishes hermetically sealed with parafilm, which only allows air contact between the microorganism and the tested plant. These experiments have allowed to determine: the exposure time, microorganisms species, plant target, culture medium, amount or concentration of inoculums for the emission of volatiles with growth activity and to identify some bioactive compounds. The experiments have revealed the important role of VOCs as signal molecules in the modulation of physiological processes in the plant, constituting to an important area of unexplored research products (Piechulla and Degenhardt, 2014; Kanchiswamy et al., 2015a).

In summary, the effect of microbial volatiles on the induction of plant growth comprises an interesting field of investigation, so far studied mainly in *A. thaliana*. Studies at cellular, molecular, and metabolic levels have been able to clarify the effect of VOCs in this plant, but further studies are needed to elucidate the mode of action from perception to its concrete action to induce growth. In addition, it is necessary to investigate the effect of VOCs on vegetable, fruit, and forage crops to prospect their application as a sustainable bioproduct and a strategy to reduce the use of chemical products. Therefore, this review focuses on collecting information published since 2003 to date with the purpose of describing (1) the plant – microorganism interactions, (2) the effect of the culture conditions of the microorganism for the emission of volatiles inducing the growth, (3) the chemical nature of the identified VOCs, (4) the mechanisms of action, and (5) the VOCs effects on resistance and tolerance described to date.

2. Microbial VOCs: concept and chemical properties

Microbial VOCs are signal molecules with low molecular weight ($< 300\ \mathrm{g\ mol^{-1}}$), low boiling point, high vapor pressure (0.01 kPa at 20 °C) and lipophilic nature that acts as ideal infochemicals for modulating physiological processes and traveling through the air, soil, and water (Kanchiswamy et al., 2015a,b). VOCs released from a determinate microorganism have a specific profile that includes compounds derived from different metabolic pathways depending on the living environment. Some compounds belong to alkanes, alkenes, alcohols, esters, ketones, terpenoids, and sulfur families (Schulz and Dickschat, 2007; Korpi et al., 2009; Audrain et al., 2015). VOCs are produced by microorganisms in a given range of scales, and they play a key role as signaling molecules that can act as a wide range of stimuli giving rise to the activation of a series of signals, which regulate physiological processes involved in plant health (Bailly and Weisskopf, 2012; Bitas et al., 2013; Kai et al., 2016). In the next section, case studies that provide relevant information regarding the role of VOCs as growth inducers are described.

3. Plant growth elicited by microbial VOCs: case studies

Several studies on the inducer effects of bioactive VOCs on plant seedlings have been carried out since 2003 to date (Table 1). The first study was performed by Ryu et al. (2003), who showed that volatiles released by *B. subtilis* GB03 elicited a ~5-fold increase of total leaf area of *A. thaliana* after 10 days of exposition. Subsequently, Banchio et al. (2009) demonstrated that the same bacterial species increased growth on shoot-root biomass of *Ocimum basilicum*, which increased 2-fold respect to control while leaf surface was increased ~2.5-fold. Furthermore, Xie et al. (2009) showed that *A. thaliana* seedlings exposed to volatiles released by GB03 exhibited 58 and 71% increases in fresh and dry weight after 2 weeks of exposition. The same interaction was tested by Zhang et al. (2009), who concluded that chlorophyll concentration in *A. thaliana* had an 84% increase. Afterward, Kwon et al. (2010) demonstrated that GB03 elicited significantly the increase of root and shoot fresh weight on *A. thaliana*, after 6 days of inoculation.

Additionally, others *Bacillus* strains have been tested as growth inducers through the emission of volatiles. Zou et al. (2010) showed that volatiles emitted by *B. megaterium* XTBG-34 exhibited a 1.7-fold increase in fresh weight of *A. thaliana* on day 7. Moreover, the effect of VOCs on root system was demonstrated by Gutiérrez-Luna et al. (2010), who concluded that volatile emitted by *Bacillus* species modified root architecture, eliciting the increase of total fresh weight, primary root length, lateral root number, and lateral root length on *A. thaliana*; and they also evidenced a strong association between fresh weight and lateral root length on day 10 ($r^2 = 0.82$). Subsequently, Santoro et al. (2011) proved that volatiles emitted by *B. subtilis* caused the increase of root dry weight (3.5-fold) and shoot fresh weight (2-fold) on *Mentha piperita*. Afterward, Meldau et al. (2013) reported that *Nicottiana atenuata* exposed to volatiles released from *Bacillus* sp. B55 exhibited 5-fold increase in leaf surface and, true leaves were enhanced in ~200%. In addition, the exposition to B55 increased lateral root for $\mathrm{cm^{-1}}$ over 400% compared with control. Furthermore, Ann et al. (2013) indicated that volatiles emitted by *B. vallismortis* EXTN-1 induced the increase ~9-fold in fresh weight of tobacco. Recently, Hao et al. (2016) reported that volatiles released from *B. amyloliquefaciens* FZB42 induced the increase of dry and fresh weight on *A. thaliana*, and a study conducted by Asari et al. (2016) revealed that seedlings of *A. thaliana* exhibited 2-fold increase in fresh and dry weight after 18 days of exposition to volatiles emitted from *B. amyloliquefaciens*.

Other bacterial species that belong to Gram-positive species have been reported for its ability to release volatile organic compounds with growth-inducing activity. A study carried out by Velázquez-Becerra et al. (2011) concluded that *Arthrobacter agilis* UMCV2 had the ability to emit VOCs inducing growth in *Medicago sativa*, enhancing plant fresh weight (~40 mg versus ~60 mg), stem length (~3.0 cm respect to ~1.7 cm), and lateral root density (~2.5 versus ~1.7). Subsequently, Orozco-Mosqueda et al. (2013) demonstrated that seedlings of *Medicago truncatula* exposed to volatiles released from *A. agilis* UMCV2 for 5 days increased shoot fresh weight, root fresh weight, and chlorophyll concentrations in 40%, 35%, and 35%, respectively. Afterward, a study conducted by Castulo-Rubio et al. (2015) showed that the exposition to VOCs of *A. agilis* UMCV2 had a growth-inducing effect on *Sorghum bicolor*, increasing shoot fresh weight in 66% aprox. Besides, Lee et al. (2012) reported that *Paenibacillus polymyxa* E681 emitted a volatile mixture that elicited the increase of surface leaf area foliar (1.6-fold) and fresh weight enhances 2-fold.

Moreover, Gram-negative species have been reported to emit volatile compounds with growth-promoting activity. A study performed by Blom et al. (2011) reported that bacterial species belonging to *Burkholderia*, *Pandoraea*, *Serratia*, and *Chromobacterium* genera increased biomass on *A. thaliana* between ~125–620%. Subsequently, Groenhagen et al. (2013) concluded that exposition of *A. thaliana* to volatiles released from *Burkholderia ambifaria* LMG19182 increased the number of lateral root number around 100% as well as the shoot biomass in 160%. Furthermore, Bailly et al. (2014) indicated that *A. thaliana* exhibited 3-fold increase in plant biomass and number of lateral root after exposition to volatiles released from *Escherichia coli*. Moreover, Bhattacharyya et al. (2015) demonstrated that *A. thaliana* exposed during 14 days to volatiles from *Proteus vulgaris* JBLS202 exhibited a 75–80% increase in fresh weight and induced an increase in primary root length and shoot length by 33.3–37.1% and 24.4–26.7%, respectively. In addition, Park et al. (2015) reported that tobacco seedlings had 8.8 and 9.5-fold increase approximately in fresh weight