Science Letters
2016 | Volume 4 | Issue 1 | Pages 40-43

RESEARCH ARTICLE OPEN ACCESS

Plant Growth Promoting Activity of Volatile Organic Compounds Produced by Biocontrol Strains

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Abstract
The volatile organic compounds (VOCs) produced by plant growth promoting rhizobacteria have been reported to improve growth and induced systemic resistance in plants against different phytopathogens, which revealed the importance of VOCs in plant-microbe interactions. In this study, we evaluated the effect of VOCs produced by nine different biocontrol strains on the plant growth promotion, chlorophyll content and leaf surface area. The biocontrol strains used in this study were isolated from different sources and showed excellent biocontrol activity against different plant pathogens. The results showed that except one biocontrol strain SQR-9, all other biocontrol strains produced VOCs that improved the growth of plants *in vitro* and enhanced the chlorophyll content and leaf surface area. Among different strain, *Pseudomonas fluorescence* strain PF-5 showed maximum growth promotion of plants followed by *Bacillus amyloliquefaciens* strain T-5 and *Pseudomonas fluorescence* strain Q2-87. The results of this study revealed that importance of VOCs in plant growth promotion and also showed that all biocontrol strains do not produce plant growth promoting VOCs.
Keywords Biocontrol, leaf surface area, plant growth promotion, volatile organic compounds.

Received August 26, 2015 **Accepted** October 25, 2015 **Published** April 15, 2016
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To cite this manuscript: Raza W, Yousaf S, Rajer FU. Plant growth promoting activity of volatile organic compounds produced by biocontrol strains. Sci Lett 2016; 4(1):40-43.

Introduction

Rhizobacteria are root colonizing bacteria that form a symbiotic association with plants, although soil also contains deleterious bacteria, but this term is usually used for bacteria that form a relationship beneficial for both parties. Rhizobacteria are also referred as plant growth promoting rhizobacteria (PGPR), which can be rhizospheric or endophytic [1]. The PGPRs promote plant growth by producing siderophores, 1-aminocyclopropane-1-carboxylate and plant hormones and by solubilizing nutrients and fixing nitrogen etc. [2]. In addition, PGPR strains also protect plants from phytopathogens by producing antibiotics, hydrolytic enzymes and volatile organic compounds (VOC), competing for space and nutrients with pathogens and inducing systematic resistance in plants [3, 4]. Among different mechanisms of action, the production of VOCs has its own importance. The VOCs produced by biocontrol strains have been reported to promote plant growth, inhibit bacterial and fungal pathogens and nematodes and induce systemic resistance in plants against phytopathogens [3, 5], like *Bacillus amyloliquefaciens* NJN-6 produced VOCs that inhibited the growth and spore germination of *Fusarium oxysporum* f. sp. *cubense* [6]. *Veillonella* species and *Bacteroides fragilis* produced VOCs that showed antibacterial activity [7]. A VOC tridecane produced by *Paenibacillus polymyxa* E681induced salicylic acid and ethylene signaling marker genes PR1 and VSP2, respectively [8]. Similarly, Ryu et al. [9] reported the plant growth promotion by the VOCs produced by *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a. These reported results showed the importance of VOCs in plant growth promotion and inhibition of plant pathogens.

It is important to evaluate more and more biocontrol strains to find novel strains with maximum plant growth promoting activity. In this study, we evaluated nine biocontrol strains, which have been reported earlier as excellent biocontrol agents, for their ability to produce plant growth promoting VOCs. The plant growth promoting activity was determined by measuring plant fresh weight, leaf surface area and chlorophyll content.

Materials and methods

Microbial strains

The microbial stains selected for this study were provided by our laboratory. The microbial strains were *B. amyloliquefaciens* T-5 (GeneBank accession no. JQ217371), NJN-6 (China General Microbiology Culture Collection Center, CGMCC accession number 3183), FZB42 (Bacillus Genetic Stock Center, Columbus, OH, USA) and SQR-9 (CGMCC accession number 5808); *Pseudomonas fluorescence* WR-1 (GeneBank accession number JQ317786), PF-5 [10], Q2-87 [11]; *Paenibacillus polymyxa* WR-2 (GeneBank accession number KF224925) and C5 (CGMCC number 3303). These

strains were maintained on nutrient agar medium at 4°C for use in experiments and in nutrient broth supplemented with 50% glycerol at -80°C for storage.

Plant growth promotion assay

The seeds of *Arabidopsis thaliana* were surface-sterilized in 70% ethanol for 2 min and then in 1% sodium hypochlorite for 20 min. Later, the seed were washed with sterilized distilled water for four times and placed on petri dishes containing half-strength Murashige and Skoog salt (MS) medium (0.8% agar, 1.5% sucrose and pH 5.7), and vernalized for 2 days at 4°C in the absence of light. Later, the seedlings were placed in a growth chamber (22°C temperature, 12h light, 12h dark, 40W fluorescent light). After two days, the seedlings were transferred to divided petri plates (85mm diameter) for plant growth promotion experiment.

The overnight cultures of biocontrol strains in nutrient broth were centrifuged (10,000×g for 10 min), washed twice with sterile distilled water and then suspended in distilled water (10^7 CFU/ml). One compartment of the divided plates was inoculated with 20 µl of water suspended culture of each strain except control and spread on the modified minimal salt agar medium containing 1.5% sucrose, 0.4% tryptic soya medium and 1.5% agar (w/v). While two days old *A. thaliana* seedlings (six per plate) were placed onto the other compartment containing MS medium and sealed with parafilm. In the control treatment, water was used in place of strains. Each treatment has 10 replicates and all plates were arranged in a growth chamber (22°C temperature, 12h light, 12h dark) in a completely randomized design for fourteen days.

Plant growth measurements

Leaf surface area and fresh biomass

After fourteen days, the plants were randomly removed from the plates and their fresh weight was measured. The total leaf surface area was measured by an integrated digital video image analysis system, Agvision system (AGIMAGE PLUS 1.08; Decangon Devices, Pullman, WA; Panasonic CCTV camera WV-BL200; Secaucus, NJ).

Chlorophyll content measurement

After fourteen days, the Arabidopsis leaves were detached, homogenized in 80% aqueous acetone (1 ml) and centrifuged at 13,000×g for 5 min. The supernatant absorbance readings were taken at 470, 646.8 and 663.2 nm. The total chlorophyll content was calculated as [(7.15× $A_{663.2}$) + (18.71 × $A_{646.8}$)] / [1000 × (fresh weight of leaves)], and was reported as mg chlorophyll per g fresh weight [12].

Results and discussion

The plant growth promotion assay results showed that, except strains SQR-9, all other biocontrol strains produced VOCs that increased the growth of plants. The strains SQR-9 showed 16% decrease in plant growth. The strain SQR-9 has been reported as an efficient biocontrol agent [13], but it might produce some VOCs that inhibited the growth of plants. Some plant growth promoting rhizobacteria *Pseudomonas fluorescence* S97 and S241 have been reported to produce VOCs like hydrogen cyanide that inhibited the growth of plants [14]. It might be a reason of plant growth inhibition by strains SQR-9.

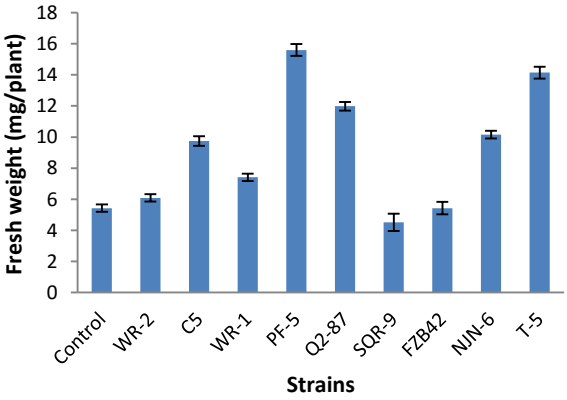

Fig. 1 Fresh weights of Arabidopsis plants after exposure to the VOCs produced by different biocontrol strains after fourteen days.

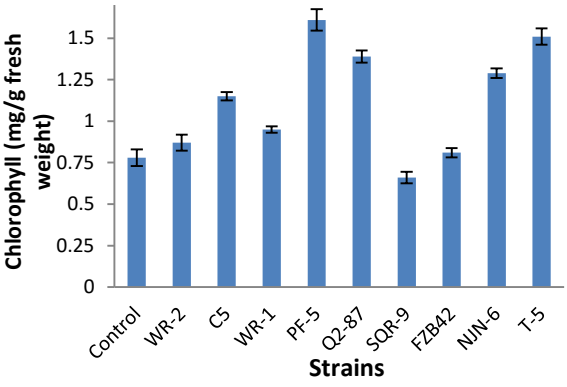

Fig. 2 The chlorophyll content of Arabidopsis plants after exposure to the VOCs produced by different biocontrol strains.

The VOCs produced by strain PF-5 showed maximum increase in plant growth followed by strains T-5, Q2-87, NJN-6 and C5, and this increase was 189%, 162%, 122%, 88% and 80% over control, respectively (Fig. 1). The VOCs produced by strain FZB42 showed minimum plant growth promotion activity. Different VOCs have been reported to promote plant growth. Like 2,3-butanediol and acetoin were released by two

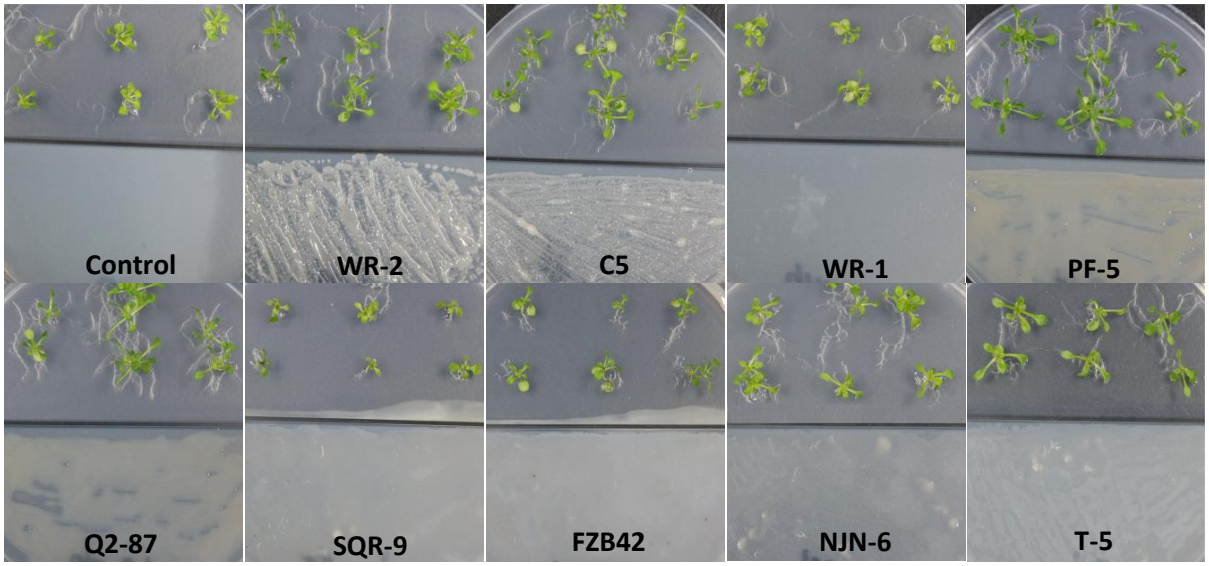

Fig. 3 Growth of Arabidopsis after exposure to the VOCs produced by different biocontrol strains. The photographs were taken after 14 days incubation in growth chamber.

bacterial strains *B. subtilis* GB03 and B. *amyloliquefaciens* IN937a that stimulated the plant growth, whereas bacterial mutants blocked in 2,3-butanediol and acetoin synthesis were devoid in this growth-promotion capacity [9]. Similarly, *Pseudomonas fluorescens* SS101 produced three VOCs, 3-tetradecadien-1-ol, 2-butanone and methyl-n-1-2-tridecene that improved the growth of tobacco [15].

The results of the effect of VOCs on the chlorophyll content of Arabidopsis plants showed that the VOCs produced by all biocontrol strains except SQR-9 increased the chlorophyll content of plant leaves. The VOCs of SQR-9 showed a 15 % decrease in the chlorophyll content of plant leaves over control which is in accordance with the results of plant growth promotion assay results. The strain PF-5 showed a maximum increase of 106% in the chlorophyll content of plants over control followed by strains T-5, Q2-87, NJN-6 and C-5 that showed 94%, 78%, 65% and 47% increase in the chlorophyll content of plant leaves over control, respectively. The VOCs produced by strain FZB42 showed minimum increase in the chlorophyll content of plant leaves (Fig. 2).

The results of the leaf surface area showed that the exposure of VOCs produced by all biocontrol strains except SQR-9 significantly enhanced the leaf surface area of plant leaves. The VOCs of SQR-9 showed a 10 % decrease in the leaf surface area of plant leaves over control which is in accordance with the results of plant growth promotion assay and chlorophyll content results (Fig. 3). The strains PF-5 showed a maximum increase of 49% in the leaf surface area of plants over control followed by strains T-5, Q2-87, NJN-6 and C-5 that showed 38%, 36%, 33% and 23% increase in the leaf surface area of plants over control, respectively (Fig. 4). The VOCs produced by strain FZB42 showed a minimum increase in the leaf surface area of plant leaves. There is not much information available about the actual mechanism of VOCs to improve plant growth and other characteristics. However, some studies reported the partial mechanism information like Ryu et al. [9] reported that cytokinin signaling pathway appeared to play some role in growth promotion with exposure to *B. subtilis* GB03 VOCs. Similarly, *Bacillus* sp. B55 produced dimethyl disulfide that improved plant growth of tobacco by enhancing sulfur nutrition [16]. In another report, VOCs like *m*-cresol and methyl benzoate produced by plant growth-promoting fungi, *Cladosporium* sp. and *Ampelomyces* sp. elicit induced systemic resistance (ISR) in plants against the pathogen *Pseudomonas syringae* pv. tomato DC3000 and salicylic acid and jasmonic acid/ethylene might be involved in the ISR mediated by the VOCs [17]. All tested biocontrol strains produced VOCs that improved

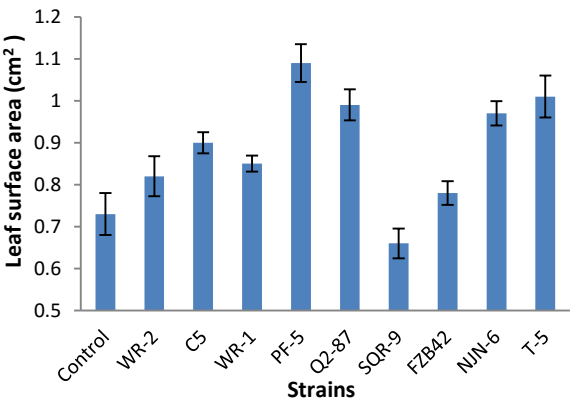

Fig. 4 The leaf surface area of Arabidopsis plants after exposure to the VOCs produced by different biocontrol strains.

the plant growth; however, biocontrol strains SQR-9 produced VOCs that inhibited the growth of plants. The strain SQR-9 has been reported as an excellent biocontrol agent to colonize roots and control soil-borne diseases effectively [18, 19].

The results of this study revealed that all biocontrol strains are not able to produce plant growth promoting VOCs. More research is required to elucidate the mechanisms of action involved in the plant-VOCs interactions and to develop safer strategies with VOCs to improve plant growth and control plant disease.

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