Biological Control 98 (2016) 18–26

Contents lists available at ScienceDirect

Biological Control

journal homepage: www.elsevier.com/locate/ybcon

Effects of *Bacillus velezensis* strain BAC03 in promoting plant growth

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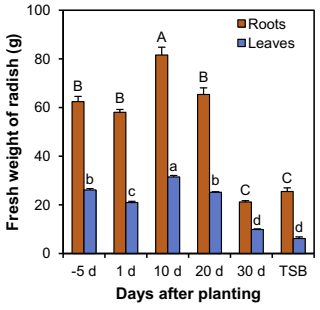
HIGHLIGHTS

- *Bacillus velezensis* BAC03 enhanced growth on nine selected types of plants.
- BAC03 applied 10 days after planting had the best effect in radish growth promotion.
- Multiple applications of BAC03 benefited radish biomass increasing.
- BAC03 produces IAA and NH_3, has ACC deaminase activity as well.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 January 2015
Revised 22 March 2016
Accepted 24 March 2016
Available online 24 March 2016

Keywords:
Plant growth promotion
Bacillus velezensis
PGPR
Streptomyces scabies

ABSTRACT

Bacillus velezensis (formerly referred to as *Bacillus amyloliquefaciens*) strain BAC03 is a plant growth promoting rhizobacterium. It has shown to be antagonistic against *Streptomyces scabies* in our previous studies, but its efficacy on plant growth promotion needs to be determined. In this study, BAC03 was tested for potential growth promotion on nine selected types of plants, including beet, carrot, cucumber, pepper, potato, radish, squash, tomato, and turnip, at the concentration of 10^5 colony forming unit cm^{-3} potting mix under greenhouse conditions. Results showed that BAC03 increased the growth of some tested plants at various levels in different plant parts. Application of BAC03 at 10 days after planting was associated in radish with the highest biomass gain compared to applications at other stages. Multiple applications of BAC03 giving the same total amount of inoculum resulted in higher weights of radish roots and leaves. That BAC03 produced indole-3-acetic acid and ammonia, as well as showed a 1-aminocyclopropane-1-car boxylate deaminase activity may be related to plant growth promotion. Acetoin and 2,3-butanediol were detected as major components of the volatile released from BAC03 by using gas chromatography–mass spectrometry analysis.

1. Introduction

Plant growth promoting rhizobacteria (PGPR) are commonly present within or in the vicinity of the plant rhizosphere (Kloepper et al., 1980). They have frequently been documented to improve plant growth by providing promotive substances or facilitating plants uptake of nutrients from the environment (Lugtenberg and Kamilova, 2009). A large array of bacteria, such as *Enterobacter*, *Arthrobacter*, *Burkholderia*, *Pseudomonas*, *Bacillus*, *Azospirillum*, *Klebsiella*, *Serratia*, and *Paenibacillus*, have been reported to enhance plant growth (Spaepen et al., 2009). In addition to the trait of direct plant growth enhancement, some PGPRs may increase plant growth indirectly by preventing deleterious effect of plant pathogenic microorganisms (Kim et al., 2011) and increasing plant tolerance to environmental stresses, such as flooding (Grichko and Glick, 2001), salt stress (Mayak et al., 2004a), water deprivation (Mayak et al., 2004b), and excess of heavy metals (Zhuang et al., 2007).

PGPRs can directly enhance plant growth through a broad range of mechanisms, including: (1) producing or changing the

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http://dx.doi.org/10.1016/j.biocontrol.2016.03.010
1049-9644/

concentration of various phytohormones, such as auxins, cytokinins, and gibberellins (Santner et al., 2009); (2) secreting enzymes that can modulate plant growth and development, such as reducing ethylene level by synthesis of 1-aminocyclopropane-1-carboxy late deaminase (Yang and Hoffman, 1984; Penrose et al., 2001); (3) supplying plants with nutrients, such as enhancing asymbiotic nitrogen fixation (Doberein and Campelo, 1971); (4) increasing the solubilization of phosphorus and other trace element for plant uptake (Gyaneshwar et al., 2002); and (5) synthesizing siderophores which can provide soluble iron to plants (Scher and Baker, 1982).

Enhancement of plant growth by root-colonizing *Bacillus* spp. is well documented (Chanway et al., 1988; Turner and Backman, 1991; Ryu et al., 2003). For example, *Bacillus velezensis* strain FZB42 (formerly referred to as *Bacillus amyloliquefaciens*) produces indole-3-acetic acid (IAA) (Idris et al., 2007; Dunlap et al., 2015). *Bacillus velezensis* (formerly referred to as *Bacillus subtilis*) and *Bacillus megaterium* produce cytokinin (Arkhipova et al., 2007). Some beneficial microorganisms produce gibberellin or jasmonic acid (Forchetti et al., 2007). These chemical compounds are all related to plant growth promotion. Moreover, volatile organic compounds from some *B. velezensis* have shown to trigger growth promotion in *Arabidopsis* by regulating auxin homeostasis (Zhang et al., 2007).

Bacillus velezensis strain BAC03 (formerly referred to as *B. amyloliquefaciens*) has antimicrobial (Meng et al., 2012) and biological control activities in greenhouse and field conditions (Meng et al., 2013). It also displayed potential growth promotion ability under the stress of plant pathogen exposure (Meng et al., 2012). To utilize this bacterium for enhancing plant growth to get a better result, it is necessary to evaluate BAC03 for plant growth activity and determine the optimal strategies of BAC03 application for plant growth promotion. The aims of this work were to (1) assess plant growth responses to BAC03 treatment; (2) test different strategies for BAC03 application in radish growth promotion; (3) test the effect of BAC03 on seed germination and seedling growth of a variety of plants; (4) detect substance(s) synthesized by BAC03 that may be associated with plant growth promotion; and (5) characterize volatiles released by BAC03 that may impact plant growth.

2. Materials and methods

2.1. Bacterial cultures

Bacillus velezensis BAC03 was obtained and maintained by the Hao Laboratory (Meng et al., 2012). *Bacillus velezensis* FZB42, the active ingredient in commercial biocontrol product RhizoVital42 (ABiTEP GmbH Inc., Berlin, Germany), was obtained from the *Bacillus* Genetic Stock Center (Columbus, OH, USA). *Bacillus velezensis* QST713 (formerly referred to as *B. subtilis*) was obtained from the commercial product Serenade (Bayer CropScience Inc., Monheim, Germany) by culturing the product (powder) in tryptic soy broth (TSB; EMB Chemical Inc., Gibbstown, NJ, USA). All Bacillus strains were cultured on tryptic soy agar (TSA; EMB Chemical Inc.).

2.2. Plant growth promotion assay in different plants

To examine the effect of BAC03 on plant growth, nine different types of plant were used. Seeds of radish (cv.'Cherry Belle', Burpee Inc. Warminster, PA, USA), beet (cv.'Burpee's Red Ball'), carrot (cv.'Touchon'), cucumber (cv.'Bush Champion'), pepper (cv.'Bush Belle Hybird'), squash (cv.'Black Beauty'), tomato (cv.'Summer Girl Hybrid'), and turnip (cv.'Purple Top') were pre-germinated on sterile moist filter paper (No.1, Whatman, Pittsburgh, PA) in a Petri dish (VWR International, LLC, Radnor, PA, USA), incubated at 25 °C. The germinated seeds were placed in a 1-L pot containing potting mix (ASB Greenworld Inc., New Brunswick, VA, USA), and placed in a growth chamber (24 °C, 14 h light, and 90% relative humidity), with 2 seedlings per pot.

To prepare the inoculum of BAC03, a bacterium was grown in Tryptic soy broth (TSB; EMB Chemical Inc.) at 28 °C on an incubator shaker (Thermo Fisher Scientific Inc, Rockford, IL, USA) at 180 rpm for 48 h. Liquid culture of BAC03 was applied as a drench 15 days after planting (DAP), to a final concentration of 10^5 CFU cm^{-3} potting mix (ASB Greenworld Inc.). The bacterial concentration was confirmed by dilution plating. This final concentration of BAC03 was used in the same situations throughout the study. Sterile TSB was used to treat the potting mix as a negative control. Four replicates were used for each treatment. Radish, squash, cucumber, and turnip were harvested six weeks after planting, while beet, tomato, pepper, and carrot were harvested two months after planting. Height, number of flowers, and/or fresh and dry weight of both leaves and roots, were measured after harvesting, depending on the plant type (Table 1). This experiment was conducted twice.

For potato, tuber pieces (cv. 'Snowden') with at least one eye were incubated in the potting mix in a growth chamber (25 °C, 14 h light, and 90% relative humidity) until sprouting, then transferred in 3.78 L plastic pots containing potting mix in the greenhouse (18–22 °C with a 14-h photoperiod supplemented by light at 200 μmol m^{-2} s^{-1}). A liquid culture of BAC03 (10^7 CFU ml^{-1}) was added to the potting mix as a drench 10 days after planting (DAP), to give a final concentration of 10^5 CFU cm^{-3} potting mix. A negative control of non-infested potting mix mixed with TSB was used. Six weeks after transplanting, the height of the plant from the soil line to the apex of potato was measured with a ruler. Plants were harvested three months after transplanting. Tuber yield was determined by measuring the weight of potato tubers from each pot.

2.3. Determination of strategies of BAC03 application for radish growth promotion

2.3.1. BAC03 application at different stages

BAC03 was applied at five different time during radish growth, including five days before planting (DBP), 1, 10, 20, and 30 DAP

Table 1
Growth responses of select plants to *Bacillus velezensis BAC03* at concentration of 10^5 CFU cm^{-3} potting mix.

Plant type	Height	No. of flowers	Fresh leaf weight	Dry leaf weight	Fresh tuber/root weight	Dry root weight
Beet	[a]+	[b]–	+++	++	+++	+++
Carrot	+	–	+++	+++	+++	+++
Cucumber	++	+	++	++	–	–
Pepper	+	+	+++	++	–	–
Potato	+	+	+	+	+	–
Radish	+	–	+++	++	+++	+++
Squash	+	+	++	++	–	–
Tomato	+	+	++	++	–	–
Turnip	+	–	++	+	+++	+++

[a] Plant growth promotion efficacy was calculated as [(BAC03 – control)/control] * 100%. The values were expressed as + (0–100%), ++ (100–200%), and +++ (>200%).
[b] –: not detected.