

Bacillus amyloliquefaciens Strain W19 can Promote Growth and Yield and Suppress *Fusarium* Wilt in Banana Under Greenhouse and Field Conditions



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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are considered to be the most promising agents for cash crop production *via* increasing crop yields and decreasing disease occurrence. The *Bacillus amyloliquefaciens* strain W19 can produce secondary metabolites (iturin and bacillomycin D) effectively against *Fusarium oxysporum* f. sp. *cubense* (FOC). In this study, the ability of a bio-organic fertilizer (BIO) containing strain W19 to promote plant growth and suppress the *Fusarium* wilt of banana was evaluated in both pot and field experiments. The results showed that application of BIO significantly promoted the growth and fruit yield of banana while suppressing the banana *Fusarium* wilt disease. To further determine the beneficial mechanisms of the strain, the colonization of green fluorescent protein-tagged strain W19 on banana roots was observed using confocal laser scanning microscopy and scanning electron microscopy. The effect of banana root exudates on the formation of biofilm of strain W19 indicated that the banana root exudates may enhance colonization. In addition, the strain W19 was able to produce indole-3-acetic acid (IAA), a plant growth-promoting hormone. The results of these experiments revealed that the application of strain W19-enriched BIO improved the banana root colonization of strain W19 and growth of banana and suppressed the *Fusarium* wilt. The PGPR strain W19 can be a useful biocontrol agent for the production of banana under field conditions.

Key Words: biocontrol, bio-organic fertilizer, colonization, crop yield, fungal disease, indole-3-acetic acid, plant growth-promoting rhizobacteria (PGPR), root exudates

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INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are rhizosphere bacteria that can improve plant growth and increase crop yield (Idris *et al.*, 2007). They have been considered to be the most promising agents for cash crop production due to increasing crop yields and decreasing disease occurrence (Raguchander *et al.*, 1997). Many *Bacillus amyloliquefaciens* strains have been shown to be excellent PGPR with many advantages, including wide distribution, easy isolation and culturing, strong colonization and suppression of soil-borne diseases to sustain plant health (El-Hassan and

Gowen, 2006; Kumar *et al.*, 2011; Fan *et al.*, 2012). However, the biological and growth-promoting effects of PGPR largely depend on their ability to survive or reproduce during the period of application and root system colonization because they require suitable nutrition from either organic fertilizers or root exudates (El-Hassan and Gowen, 2006). Lots of reports have demonstrated that a special bio-organic fertilizer (BIO) that combines PGPR with mature compost could enhance the activity of PGPR by promoting colonization in a variety of plant rhizospheres (Wu *et al.*, 2009; Kavino *et al.*, 2010; Zhang *et al.*, 2014). The mature compost, not only acts as a food source and shelter

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for the PGPR, but also supports them to compete with plant pathogens and other indigenous organisms (Raviv *et al.*, 2005).

It is generally accepted that the efficient colonization of plant roots by PGPR is a critical step for further interactions (Fan *et al.*, 2011), and several reports have demonstrated the existence of a correlation between PGPR root colonization, especially biofilm formation, and protection against pathogens in plants (Beauregard *et al.*, 2013). In the interactions between plants and PGPR, root exudation plays an important role due to the diverse array of carbon-containing primary metabolites, such as sugars and amino acids, and more complex secondary compounds, which provide nutrition for colonizing microbes in the rhizosphere (Bais *et al.*, 2004; Chen *et al.*, 2012). Thus, determining the correlation between the PGPR colonization ability and root exudates from the target plant is important. After colonization, several plant growth-promoting traits, such as phosphate solubilization and the production of ammonia, indole-3-acetic acid (IAA), siderophore, chitinase, and hydrocyanic acid (HCN), can be improved (Park *et al.*, 2005; Ahmad *et al.*, 2008). Among these compounds, the phytohormones produced by bacteria can directly and significantly influence plant growth (Idris *et al.*, 2007). The IAA from bacteria has been shown to be the most pivotal phytohormone, and it plays an important role in the symbiotic relationship between PGPR and plants (Goswami *et al.*, 2014).

Banana is one of the most popular fruit crops and cultivated in many tropical and subtropical regions. However, the banana yield per acre has rapidly decreased due to the invasion of *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* race 4 (FOC) (Snyder and Hansen, 1940). *Fusarium* wilt is one of the most serious soil-borne fungal diseases and the most prominent limiting factor both for the quality and quantity of banana production worldwide (Lin *et al.*, 2009). In addition to the contribution of more scientific basis for the field management, the exploration of efficient PGPR is becoming more popular in key areas of research for banana production because the PGPR not only promote plant growth but also protect plants from soil-borne pathogens (Martins *et al.*, 2013; Prashar *et al.*, 2014). The application of many potential PGPR alone or in combination with compost has shown excellent plant promotion and pathogen inhibition *in vitro* and in the greenhouse but not under field conditions (Ramírez and Kloepper, 2010). Field experiments are the last stage in which the antagonistic microorganism must be tested for any effective biological control system. Neverthe-

less, to our knowledge, few studies have described the promotion of growth in banana and the biological control of banana *Fusarium* wilt disease under field conditions using PGPR, especially in combination with composts. A novel bio-organic fertilizer BIO6 (combining *B. amyloliquefaciens* strain W19 with manure compost), developed in our laboratory, could efficiently control banana *Fusarium* wilt disease and promote banana growth in pot experiments with diseased soil (Wang *et al.*, 2013). Moreover, the potential mechanism of biocontrol by *B. amyloliquefaciens* strain W19 was also studied. However, the direct mechanisms of biocontrol and growth promotion by this strain and the biocontrol efficiency of BIO6 under field conditions should be studied further.

A previous study was performed with strain W19 to elucidate its biocontrol activities (Wang *et al.*, 2013); however, little is known about its colonization behavior in host rhizosphere, its plant growth-promoting ability in pot and field experiments and its biocontrol activity in field experiments. The hypothesis was that strain W19 could promote the growth of banana plants by producing IAA after root colonization, thereby increasing banana yield. Thus, the overall objectives of this research were to investigate the root colonization ability of strain W19 tagged with green fluorescent protein (GFP) using both hydroponic and vermiculite culture systems, to evaluate the ability of strain W19-enriched BIO in banana growth promotion under greenhouse and field conditions and to verify the biocontrol efficiency of BIO under field conditions. Results of this study would increase the understanding of some of the positive effects of PGPR strain W19 on plant growth and suggest that the strain could be used effectively under field conditions.

MATERIALS AND METHODS

Strain, plant material and BIO preparation

The *B. amyloliquefaciens* strain W19 (CCTCC accession No. AB 2012028, China Center for Type Culture Collection) was isolated and identified in Jiangsu Key Lab and Engineering Center for Solid Organic Waste Utilization, Nanjing Agricultural University, Nanjing of China and grown on Luria-Bertani (LB)-agar plates prior to use. The BIO and banana seedlings (*Musa* AAA Cavendish cv. Brazil) used for pot and field experiments were prepared as previously reported (Wang *et al.*, 2013). In short, the organic material in the BIO was a mixture of amino acid fertilizer and pig manure compost (2:3, weight/weight). The amino acid fertilizer was prepared from rapeseed oil cakes after treatment with microbial hydrolase for 7 d. The pig

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