Journal of Agriculture and Crops
ISSN(e): 2412-6381, ISSN(p): 2413-886X
Vol. 9, Issue. 3, pp: 372-375, 2023
URL: https://arpgweb.com/journal/journal/14
DOI: https://doi.org/10.32861/jac.93.372.375

Academic Research Publishing Group

Original Research | Open Access

In Vitro Antagonistic Action by *Bacillus Velezensis* Strain LP16S Against Cotton Wilt Pathogens

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Article History
Received: 25 February, 2023
Revised: 19 April, 2023
Accepted: 13 May, 2023
Published: 19 May, 2023

Abstract

Cotton productivity and profitability are hampered by several biotic stresses, including the two most destructive wilt pathogens *Verticillium dahliae* and *Fusarium oxysporum* f. sp. *vasinfectum* (FOV). In this study, an *in vitro* assay was conducted to determine the activity of *Bacillus velezensis* LP16S against three *V. dahliae* isolates and six *F. oxysporum* f. sp. *vasinfectum* isolates. Among the fungal isolates, the response when exposed to the *B. velezensis* LP16S strain in a half-strength potato dextrose agar plate varied markedly. *Fusarium oxysporum* f. sp. *vasinfectum* isolates FOV11, FOV 944, and FOV Tx8 were tolerant, while FOV Tx39, FOV 1073, and the three *V. dahliae* isolates were highly sensitive to *B. velezensis* LP16S. In conclusion, this *Bacillus* sp. strain has potential for use in managing these damaging cotton diseases.

Keywords: *In vitro* assay, cotton, *Bacillus velezensis*, , Verticillium wilt, Fusarium wilt.

How to Cite: Louis K. Prom, Enrique G. Medrano, Jinggao Liu, 2023. "*In Vitro* Antagonistic Action by *Bacillus Velezensis* Strain LP16S Against Cotton Wilt Pathogens." *Journal of Agriculture and Crops*, vol. 9, pp. 372-375.

1. Introduction

Cotton (*Gossypium* spp.) is a valuable commodity in global commerce and is used primarily in the textile industry [1]. The US is the leading exporter and during the 2019-2020 season, 20 million bales of cotton were produced amounting to a monetary value of $7 billion [1]. In the US, Upland cotton (*Gossypium hirsutum*), accounting for 97% and Pima cotton (*Gossypium barbadense*) 3% are grown in 17 States [1]. Globally, cotton production is hampered by several biotic stresses, including Verticillium wilt incited by *Verticillium dahliae*, and Fusarium wilt incited by *Fusarium oxysporum* f. sp. *vasinfectum*, resulting in significant yield losses and decreases in fiber quality [2-5]. These wilt pathogens are considered the most destructive pathogens of cotton [3, 5, 6]. The occurrence of both pathogens in the same wilted cotton plant had been reported [7]. Although resistant sources to *V. dahliae* and *F. oxysporum* f. sp. *vasinfectum* in cotton have been identified, long-term sustainability and environmentally friendly options for cotton production are being explored using bioagents [3, 6, 8-11].

In this study, the antagonistic effect of *Bacillus velezensis* strain LP16S against *V. dahliae* and *F. oxysporum* f. sp. *vasinfectum* was examined *in vitro*. Our hypothesis is that utilization of an environmentally friendly control practice will minimize the use of hazardous chemical application protocols currently implemented. Thus, producers could reduce management costs associated with disease inflicted by the fungal agents and minimize related environmental dangers.

2. Materials and Method

Bacillus Strain – The *Bacillus velezensis* LP16S strain was originally obtained from a half-strength potato dextrose agar (½PDA; Becton, Dickinson and Company, Sparks, MD) culture plate containing seeds harvested from sorghum (*Sorghum bicolor* (L.) Moench) plants grown at the Texas A&M AgriLife Research Farm, Burleson County, Texas. The *B. velezensis* strain LP16S was sequenced, and data deposited in the NCBI (Accession # SRX5801078; Whole-Genome deposited at DDBJ/EMBL/GenBank, Accession # SSKM00000000.1 [12, 13].

Cotton pathogens: Table 1 shows the isolates, identification number, sources, and locations of the three isolates of *V. dahliae* and six *F. oxysporum* f. sp. *vasinfectum* (FOV) used in this study.

Screening for antifungal activity on mycelial growth: The fungal species *V. dahliae* and FOV used in the study are stored at the ARS-USDA-Southern Plains Area Research Center, College Station, Texas. Three 2.5 cm Whatman paper discs were soaked in *Bacillus velezensis* LP16S spore suspension and placed in equidistant spots on Petri dish containing ½PDA and the fungal species agar plugs (three isolates of *V. dahliae* and six FOV isolates) were placed between the treated paper discs on November 28, 2022 (Fig. 1). The culture plates were placed in an incubator set at 27± 1^0C for 11 days. During the incubation period, pictures of the plates were taken at 7 days and again at 11 days (Fig. 2).

3. Results and Discussion

Utilization of biocontrol agents or their metabolites could be an effective option where resistant sources are lacking, and availability of fungicides are cost prohibitive or ineffective. For sustainability of crop production and to avoid continued environmental degradation of toxic synthetically produced chemical agent applications, the use of biocontrol agents could be a valuable option. In this study, an *in vitro* assay was conducted to determine the effectiveness of *B. velezensis* LP16S in suppressing the mycelial growth of two of the most important cotton wilt fungi. Inhibition of mycelial growth was distinctly indicated by a clear zone between the paper discs soaked in *B. velezensis* LP16S spore suspension and the fungal spp. as shown in Figure 2. Notably, the responses among the fungal spp. varied with FOV11, FOV 944, and FOV Tx8 exhibiting tolerance, while all the *V. dahliae* isolates tested in this study, FOV Tx39, and FOV 1073 were highly sensitive when exposed to *B. velezensis* LP16S. In a previous study using the same *B. velezensis* LP16S strain, Prom, *et al.* [12] reported the inhibition of both mycelial growth and spore germination of three sorghum pathogens *Fusarium thapsinum*, *Colletotrichum sublineola*, and *Curvularia lunata*. In a dual culture study, the metabolites obtained from *B. velezensis* strain HNH9 inhibited the mycelial growth of *V. dahliae* and under greenhouse experiment, exposure of the strain to cotton plants significantly reduced the severity of Verticillium wilt when compared to the controls [3]. Zhang, *et al.* [6], reported the inhibition of both mycelial growth and spore germination of *V. dahliae* when challenged with *Bacillus* sp. T6 even in the absence of direct contact with the strain, indicating that volatile compounds may be responsible for the inhibitory action. Volatile and nonvolatile metabolites from *B. subtilis* EBSO3 also were shown to markedly reduce the mycelial growth, spore and microsclerotia germination of *V. dahliae*, and culture filtrate reduced the severity of Verticillium wilt under field trial [14]. Raut and Hamde [10], isolated 114 rhizobacteria from soil samples collected in field planted with Bt-cotton, 13 of the isolates were able to suppress the mycelial growth of FOV by almost 69%.

In conclusion, this study and previous works noted above had shown the capacity of *B. velezensis* as a potential option for use in the control of cotton wilt diseases. Nevertheless, in this current study *B. velezensis* LP16S strain was most effective in suppressing the mycelial growth of *Verticillium dahliae* than some of the FOV isolates. Based on our results, further study will be needed to determine the metabolites or components responsible for the differential responses of the FOV isolates when challenged with *Bacillus velezensis* LP16S strain.

Table-1. Isolates, source, and location of the fungi isolation used in the study

Isolate	Source	Location
Verticillium dahliae	Cotton	Xinjiang, China
Verticillium dahliae	Tomato	California, USA
Verticillium dahliae	Cotton	Xingjian, China
Fusarium oxysporum f. sp. vasinfectum Tx39	Cotton	Texas, USA
Fusarium oxysporum f. sp. vasinfectum 944	Cotton	Texas, USA
Fusarium oxysporum f. sp. vasinfectum Tx8	Cotton	Alabama, USA
Fusarium oxysporum f. sp. vasinfectum 11	Cotton	California, USA
Fusarium oxysporum f. sp. vasinfectum 1073	Soil	Alabama, USA
Fusarium oxysporum f. sp. vasinfectum 983	Cotton	Alabama, USA

Figure-1. Model of fungal species placed on the plates on November 28, 2022 (initial inoculation date)

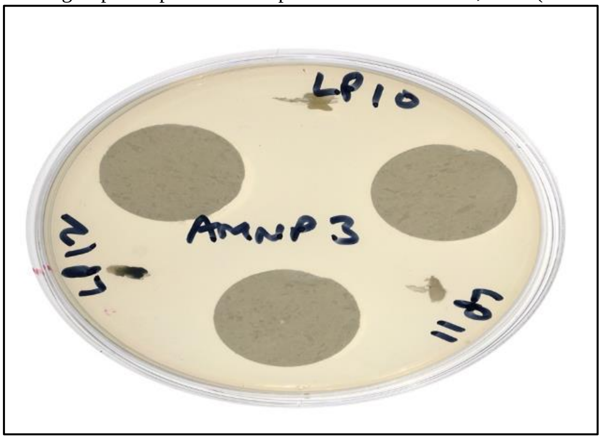
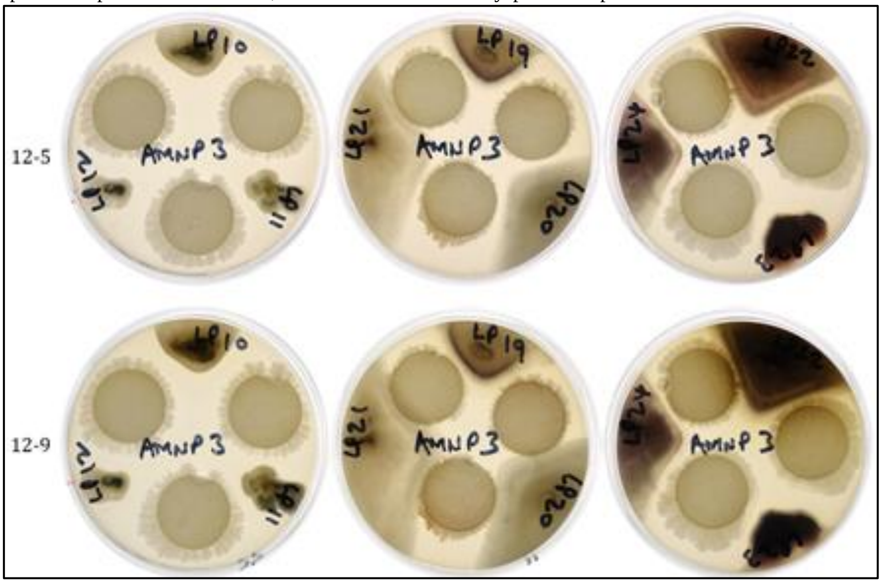


Figure-2. Pictures taken at 7 days (12-5) and again at 11 days (12-9) during incubation. LP10 = Verticillium dahliae 1899 NP; LP11 = Verticillium dahliae TS 2; LP12 = Verticillium dahliae 1966 ND; LP19 = Fusarium oxysporum f. sp. vasinfectum Tx39; LP20 = Fusarium oxysporum f. sp. vasinfectum TX8; LP21 = Fusarium oxysporum f. sp. vasinfectum 944; LP22 = Fusarium oxysporum f. sp. vasinfectum 11; LP23 = Fusarium oxysporum f. sp. vasinfectum 1073; and LP24 = Fusarium oxysporum f. sp. vasinfectum 983



Acknowledgement

CRIS project from the United States Department of Agriculture. Project number 3091-22000-040000D. USDA is an equal opportunity provider and employer.

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