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EVALUATION OF *BACILLUS STERCORIS* STRAIN D7XPN1 AS A PLANT GROWTH-PROMOTING AND BIO-CONTROL AGENT AGAINST FUSARIUM OXYSPORUM-INDUCED WILT IN BANANA AND TOMATO

Preeti Mehta[1] and Ashish Saraf [2]

[1,2] School of Sciences, MATS University, Raipur, C.G

Abstract

The fungal pathogen *Fusarium oxysporum* causes fusarium wilt. A disease that affects tomato and banana plants globally. Present research reveals the plant growth-promoting rhizobacterium (PGPR) *Bacillus stercoris* strain D7XPN1 for its efficacy in inhibiting the development of *Fusarium oxysporum*. *Bacillus stercoris* D7XPN1 has antifungal action, promotes plant growth, and activates defense systems in host plants. *Bacillus stercoris* D7XPN1 inhibited the development of *Fusarium oxysporum* by producing an antifungal chemical. The plant treated with D7XPN1 exhibits augmented growth traits, including increased biomass in both roots and shoots. The findings indicate that Bacillus stercoris D7XPN1 is a potential biocontrol agent for managing fusarium wilt infestation.

Key Words: PGPR, *Bacillus stercoris,* Biocontrol, Fusarium wilt, Sustainable agriculture

Introduction

One of the most important soil borne diseases that affect tomato and banana production is fusarium wilt .The disease is caused by fusarium *oxysporum* .The disease is responsible for significant economic losses due to fungal infection [1,2]. The transmission of fungus is occurred through contaminated soil, water, and plant material. The fungus invade the vascular system of plant which causes severe wilting, leaf yellowing, and ultimately plant death [2, 3]. The traditional method to control the diseases is utilization of chemical fungicides but because of their negative effects on soil microbiota, their application is often limited. [4]

The application of microbial based biocontrol is alternative and safe tool to control the growth of plant pathogen [5, 6]. The group of microorganisms called plant growth-promoting rhizobacteria (PGPR) occupy around root region of plant and inhance the plant growth by secreating growth hormone and many other metabolites [7, 8, 9]. From recent few year the PGPR based biological control agent has become ecofriendly substitute of chemical pesticides [10]. Among all the PGPR groups the *Bacillus* species have drawn a lot of interest because of their capacity to inhibit plant

10.48047/jocaaa.2022.30.02.40

diseases via a variety of strategies, such as the release of antifungal metabolites,resource competition and the acceleration of plant defensive mechanism[11,12].

The research work mainly focus the biocontrol role of *Bacillus stercoris* D7XPN1 isolated from the rhizosporic region of chickpea plant against *Fusarium oxysporum*. The research support the hypothesis that the *Bacillus stercoris D7XPN1* has activate the plant defence mechanism and suppress the growth of fungal pathogen fungal growth. Apart from their role as biocontrol agent it also enhance the growth parameter of tomato and banana plant.

Material and methods

Isolation of PGPR –Rhizospheric samples of chickpea were collected from several locations from Raipur to isolate PGPR. LB agar medium was used to isolate the bacteria using the serial dilution technique. Bacterial colonies were isolated and inoculated onto new LB agar plates based on their morphological characterstic . The culture is named as CPR 1, 2, 3. The isolates were identified morphologically.For molecular identification the selected strain was sent to Biokart India Pvt Lt, Bangalore, Karnataka, for 16srRNA sequencing [13].

Isolation of *Fusarium oxysporium*- Fusarium-infected plant samples were collected from several locations throughout the Raipur district. The roots of infected banana and tomato plants were cleaned and surface sterilized, then placed on a PDA medium and incubated at 27°C in the dark. After five days, fungal colonies formed were moved to fresh PDA plates and identified using the available literature [13].

HCN production- The isolated PGPR culture was streaked on a modified agar plate after 4.4 g glycine/L was added to the nutrient agar. The lid of petriplate was covered with a Whatman filter paper (No. 1) soaked in 2% sodium carbonate (0.5% picric acid). After being parafilm-sealed, the plates were incubated for four days at 36±2°C. The paper's transition from orange to red suggested the formation of HCN [14].

Siderophore production – To assess siderophore production Chrome Azurol S (CAS) assay was used. Isolates were inoculated into plates containing the CAS dye to perform the test. After that, the plates were incubated for seven days at 37°C. A positive outcome for siderophore production was shown by the development of an orange halo or zone surrounding the bacterial colonies following incubation [15].

Antifungal activity of isolates against *Fusarium oxysporum* -The antagonistic ability of bacterial isolates against the fungal pathogen *Fusarium oxysporum* on Potato Dextrose Agar

10.48047/jocaaa.2022.30.02.40

(PDA) was assessed using a dual culture experiment. The bacterial isolate was streaked on one side of the plate, while a fungal mycelial disc was inoculated on the other. A control with solely fungal growth was added, and the plates were incubated for seven days at 28°C. Antifungal activity was demonstrated by the development of a distinct inhibitory zone surrounding the bacterial colonies. The antagonistic effect of the bacteria was measured by measuring the size of the inhibition zone [16].

Pot experiment – In vivo affect of PGPR on growth of fungal pathogen and disease incidence was done by pot experiment. F our-week-old banana plantlets of comparable size from a nursery in method and cultivated in sterilized soil (4:1 sand to clay). Before immersing the roots in 500 mL of the solution for 90 minutes, the bacterial strain was cultivated, centrifuged, and adjusted to 10^8 cells mL^{-1}. Sterile and pathogen-contaminated soil was used to sow treated seedlings and biometric characteristics (height, width, leaf number, and area) and infection levels were evaluated after 60 days [17]. Surface sterilized tomato seeds were first treated with isolated and PGPR .the seed were saw in soil infested with fungal pathogen. Every two weeks, seedling growth metrics and wilting scores were noted; wilting was evaluated using leaf symptoms [18].

Result and Discussion

Isolation, identification and antagonistic ability of *Bacillus stercoris* strain D7XPN1:

The strain of *Bacillus stercoris* strain D7XPN1 was isolated from the root region of chickpea plant and identified by 16s RNA sequencing. The isolated strain have ability to secrete siderophore and HCN. When tested against fungal pathogen it successfully inhibit the growth of fungi.

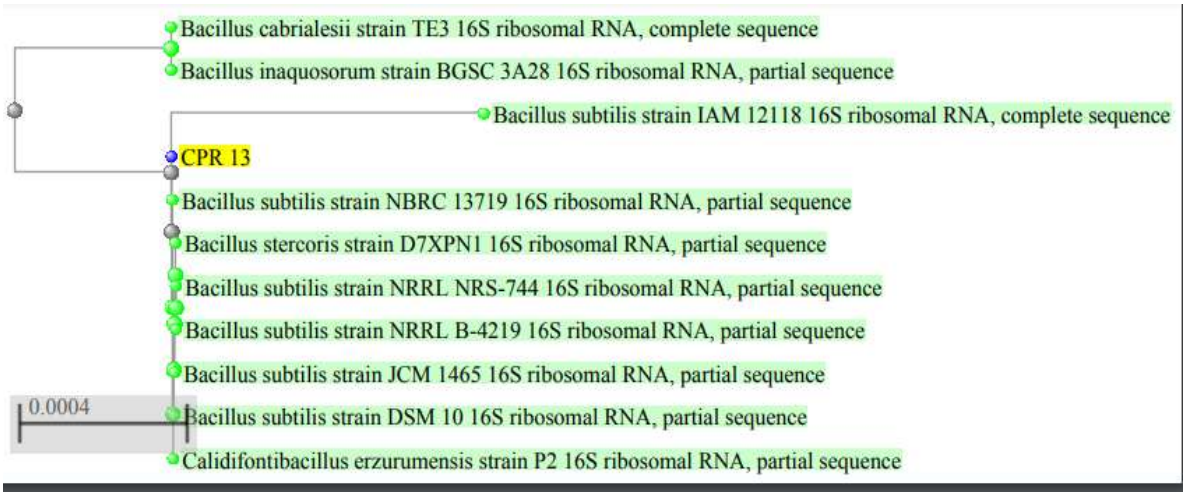

Fig.1. 16sRNA sequencing analysis result

Table 1. Plant growth promoting trait of *Bacillus stercoris* D7XPN1

10.48047/jocaaa.2022.30.02.40

PGP traits	Result
HCN production	+
Siderophore production	+

Fig. 2. Siderophore production

Fig. 3. HCN production

Mycellial growth inhibition –The mycellial growth inhibition was studied by dual culture method

Table 2. Percentage of mycellial growth inhibition

Fungal pathogen	Mycellial growth inhibition % (C-T)/C×100
F. oxysporum f. sp. lycopersici	32.7%
F. oxysporum f. sp. cubense	44.4%

The table explain the mycelial growth inhibition percentage of different fungal pathogens. The formula to calculate the inhibition is

Inhibition % = {(C - T)}/{C} × 100

Where:

C = control value (represent the growth of the pathogen in untreated environment).

T = The treatment value (the growth of the pathogen in the presence of PGPR meant to inhibit growth). *F. oxysporum f. sp. lycopersici* has a mycelial growth inhibition percentage of 32.7%. This means that the treatment reduced its growth by 32.7% compared to the control. *F. oxysporum f. sp. cubense* has a mycelial growth inhibition percentage of 44.4%. This means that the treatment reduced its growth by 44.4% compared to the control .The comparison clearly show that bacterial PGPR is more effective against *F. oxysporum f. sp. cubense.*

10.48047/jocaaa.2022.30.02.40

Fig. 2. Dual culture Plate Technique

In vivo effect of PGPR on control of fusarium wilt of tomato and banana plant

Table 3. In vivo effect of PGPR on disease reduction

Groups	Tomato		Banana	
	Control	Treated	Control	Treated
Total plant	18	18	9	9
Infected plant	16	8	7	3
Disease incidence (%)	88.9	44.4	77.7	33.3
Disease severity	81.1	8.9	86.7	60.0
Disease Reduction (%)		50.0		57.8

- Data are multiple of three observations

The data on the table shows the disease incidence and severity in tomato and banana plants. By comparing a control plants (untreated) with a treated plants (received treatment) it clearly indicate that treatment was effective in reducing disease incidence and severity. In Tomato plants the reduction in disease incidence is 50.0%, that means treatment with PGPR suppress the number of infected plants. Where as in banana plant the reduction in disease incidence was recorded 57.8%, it shows that PGPR is provide a good level of protection against wilt pathogen. The disease severity is reduced in both group of treated plant but in comparison to banana, the PGPR is more effective against *F. oxysporum f.* sp. *lycopersici*.

Discussion

10.48047/jocaaa.2022.30.02.40

Previous research work done on PGPR also show that colonization of *Bacillus spp*. on roots is essential for biocontrol processes .They also amplify the plant growth by solubilizing insoluble phosphorus, secreating siderophore and releasing HCN[19,20,21]. Pengproh *et al* 2023 also reported similar result and isolated 11 Bacillus species from soil extinct volcanoes, Phanom Rung, Plai Bat, and Khao Khok, in Buriram Province, Thailand and check their antagonistic and plant growth-promoting activity Through whole genome sequencing research and the 16S rRNA gene sequence, isolate B.PNR1, , was determined to be *Bacillus stercoris* strain D7XPN1 has the best biocontrol and PGP [22]. Isolated from a healthy banana plant, *Bacillus amyloliquefaciens strain* NJN-6 generates antibiotic substances such as bacillomycin D and iturin A[23].The gene clusters linked to antifungal and plant growth-promoting properties were present in various other spii of Bacillus like *B. subtilis, B. mojavensis, B. amyloliquefaciens, B. velezensis,* and *B.* cereus [24]. The secon dary antifungal metabolites like iturin, fengycin, surfactin, and bacillomycin secreted by *Bacillus licheniformis* CSR-D4 reduced the incidence of wilt disease in banana plant [25]. Ting *et al* 2011 isolated Bacillus *subtilis strain B25* from banana rhizosphere soil in Hainan which not only act against *Fusarium oxysporum f. sp. cubense* but also inhibit other plant pathogenic fungi, including *Alternaria solani, Botrytis cinerea,* and *Colletotrichum gloeosporioides* [26]. Climate-flexible bacterial isolates *Bacillus albus* and *Bacillus megaterium* are potent biocontrol agents against Fusarium oxysporum in tomatoes [27].

Conclusion

The incidence and severity of fusarium wilt is decreased by the treatment in tomato and banana plant. In tomatoes, it significantly decreased the severity of the disease, whereas in bananas, it had a lesser effect as compare to tomato. This implies that the treatment is a potential approach to managing disease, but the success of the treatment is depending on types of plant.

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Conflict of Interests: The authors declare that they have no competing interest.

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