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THE UTILIZATION OF *Bacillus* spp. AS ENDOPHYTES IN ENHANCING PLANT RESISTANCE AND HEALTH AGAINST *Fusarium oxysporum* : A MINI REVIEW)

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Abstract

Fusarium oxysporum is a destructive pathogen responsible for significant losses in chili (*Capsicum spp*.) and other crops. Traditional chemical control methods pose environmental and health risks, highlighting the need for sustainable alternatives. This review explores the role of Bacillus spp. as endophytic biocontrol agents in combating F. oxysporum. Species such as B. subtilis, B. mojavensis, and B. velezensis exhibit antifungal activity through the production of hydrolytic enzymes (e.g., glucanase, cellulase, and chitinase) and secondary metabolites like lipopeptides and antibiotics, which inhibit pathogen growth. Furthermore, these bacteria enhance plant health by inducing systemic resistance, promoting root development, and creating a rhizosphere environment unfavorable to pathogens. Their synergistic interactions with other beneficial microorganisms further bolster plant defense and productivity. Molecular and physiological analyses confirm the efficacy of Bacillus spp. in reducing disease severity and improving plant resilience. Continued research into their mechanisms and field application can support more sustainable agricultural practices.

Key words: Fusarium oxysporum, Bacillus spp., Biocontrol agents, Endophytic bacteria, Antifungal

Introduction

Wilt disease caused by *Fusarium oxysporum* is a significant challenge for crops like chili (*Capsicum* spp.), causing severe yield and quality losses. The pathogen disrupts the plant's vascular system, leading to symptoms such as yellowing leaves, wilting, and eventual plant death (Ferniah *et al*., 2014). Conventional management relies heavily on chemical pesticides, which can negatively impact the environment, human health, and the sustainability of agricultural ecosystems.

As an eco-friendly solution, *Bacillus* spp., including *B. subtilis*, *B. mojavensis*, and *B. velezensis*, have gained attention as biocontrol agents. These bacteria produce hydrolytic enzymes such as chitinase and glucanase, which degrade the pathogen's cell wall, and secondary metabolites with antifungal properties that suppress *F. oxysporum* (Diabankana *et al*., 2021). Additionally, *Bacillus* spp. can act as endophytes, colonizing plant tissues without causing harm. This interaction enhances plant growth, increases resistance to pathogens, and improves tolerance to abiotic stress. *Bacillus* spp. also induce systemic resistance mechanisms, such as Induced Systemic Resistance (ISR), which primes plants against pathogen attacks (Putri *et al*., 2023).

Research suggests that endophytic *Bacillus* spp. are highly effective in producing antifungal compounds within plant tissues and promoting beneficial microbial interactions in the rhizosphere. However, the detailed mechanisms underlying these processes remain poorly understood. This review aims to to find out how to utilize

Bacillus spp. as endophytes in enhancing plant resistance and health against *Fusarium oxysporum*

Research Methods

The following are the methods from several research articles conducted on the utilization of *Bacillus* spp. to enhance plant resistance and health against *Fusarium oxysporum*.

Isolation, Identification, and Characterization of Bacillus spp.

Bacillus spp. were isolated from chili rhizosphere soil (0–15 cm depth) and plant tissues using serial dilution and plating on Luria-Bertani (LB) Agar. Isolates were purified based on colony morphology. Identification included Gram staining and 16S rRNA gene sequencing using primers 1387R and 63F, with phylogenetic analysis performed using MEGA 6 and BLAST (Putri *et al*., 2023).

Characterization involved macroscopic (colony size, shape, color) and microscopic (Gram staining, spore formation) observations, as well as enzyme assays. Enzymatic activities (β-glucanase, cellulase, protease, chitinase, lipase) were tested on selective media, and biosurfactant production was evaluated using the drop-collapse method. Enzymatic activity indices were calculated by comparing clearance zone diameters to colony diameters (Asha et al., 2016; Diabankana et al., 2021; Hendricks et al., 1995).

Isolation, Identification, and Pathogenicity Test of *Fusarium oxysporum*

Ferniah *et al*. (2014) isolated *Fusarium oxysporum* from wilting chili plants by sterilizing infected root or stem pieces with 0.1% $HgCl_2$, then culturing them on Potato Dextrose Agar (PDA) with streptomycin for 5–7 days. Fungal colonies were identified based on macroscopic and microscopic characteristics, using lactophenol blue staining. Molecular identification involved amplifying the ITS region with PCR and confirming sequences through gel electrophoresis and analysis with MEGA 5.1. Inoculation of *Fusarium oxysporum* was carried out by soaking healthy chili roots in a fungal suspension (10^6 conidia/mL) for 30 minutes after surface sterilization. A control was treated with sterile water (Ferniah *et al*., 2014).

Antagonism Test of *Bacillus* spp. Against *Fusarium oxysporum*

Etebarian *et al*. (2005) used the dual culture method on PDA media to test the antagonism of *Bacillus* spp. against *Fusarium oxysporum*. *Fusarium oxysporum* was inoculated at the center of the plate, while *Bacillus* spp. isolates were placed 2 cm apart from the fungal colony. After 5 days of incubation at 25°C, the inhibition zone was measured, and the inhibition percentage was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{R1-R2}{R1} \times 100$$

Where R1 is the radius of fungal growth in the control, and R2 is the radius of growth in the presence of *Bacillus* spp. This method helps evaluate the potential of *Bacillus* spp. as a biocontrol agent against *F. oxysporum*.

Analysis of Antifungal Secondary Metabolites

Listyorini *et al.* (2021) employed the LC-MS/MS system (Waters, AS) to analyze antifungal metabolites in crude ethyl acetate (EA) extracts of *Bacillus subtilis* W3.15. A

5 µl crude EA extract (dissolved in methanol) was injected into the LC-MS system with a UPLC C18 column. The flow rate was 0.2 mL/min, and the analysis was performed at 25°C. The mobile phases consisted of 5 mM formic acid in water (Phase A) and 0.05% formic acid in acetonitrile (Phase B). For mass spectrometry, electrospray ionization (ESI) in positive ion mode was used (Xevo G2-S Qtof, Waters, AS), and MS/MS spectra were recorded in the 50–1,200 m/z range. Dominant peaks were identified using ChemSpider and MassBank databases (Putri et al., 2023).

Endophytic Bacteria Assay for Fusarium Wilt Control

The procedure for assessing the potential of indigenous endophytic bacteria (IEB) to control Fusarium wilt involved culturing a pure colony of IEB in Tryptic Soy Broth (TSB) for 24 hours, followed by transfer to sterile coconut water for a 48-hour main culture. The IEB suspension was then diluted to a concentration of 10^8 CFU/mL (McFarland scale 8). Chili pepper seeds were immersed in the IEB suspension before being sown in seed trays and later transplanted into polybags filled with a soil and organic matter mixture. To test the pathogenicity of *Fusarium oxysporum*, isolates were obtained from infected plants, cultured on Potato Dextrose Agar, and tested on 21-day-old chili seedlings to identify the most virulent strains. The selected virulent isolates were subsequently cultured in rice and inoculated into the plants before planting, Parameter observed in this research were incubation time, incidence, severity, as described by Yanti et al. (2018).

Colonization of *Bacillus* spp. on Plants

Colonization was tested by modifying the method of Liang *et al.* (2024). Chili seeds were disinfected and planted in a perlite-peat soil mix. After 45 days, roots were transferred to a half-strength Hoagland solution and incubated for 2 days. *Bacillus subtilis* Ydj3 culture (1×10^7 CFU/mL) was added, and the treatment was incubated for 24 hours. Colonization was observed using optical microscopy for root hair length measurement and scanning electron microscopy (SEM) for detailed root surface analysis.

Results and Discussion

Isolation, Identification, and Characterization of Bacillus spp.

Putri et al. (2021) conducted the isolation and characterization of *Bacillus* spp., with isolate W3.15 showing promising features. This isolate forms milky white colonies with raised elevation, smooth edges, and a regular circular shape (Figure 1.A). It is Gram-positive, rod-shaped, and forms endospores that are green when stained with Gram (Figure 1.C).

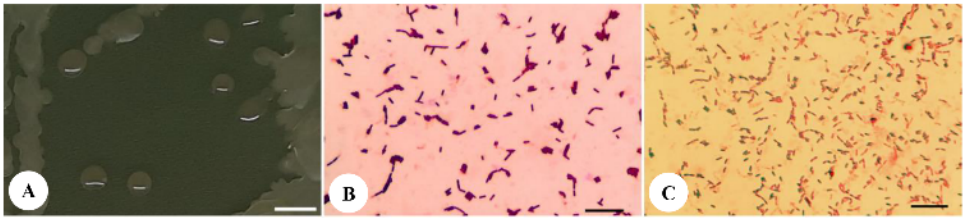

Figure 1. Colony morphology and staining analysis of cell Gram and endospores of potential isolate W3.15. (A) W3.15 isolate on TSA medium after incubation for 24 hours. (B-C) Rod-shaped cells and endospores of the W3.15 isolate (Putri *et al.*, 2021).

Biochemical tests revealed that W3.15 is positive for starch hydrolysis, catalase activity, and citrate utilization, and also yielded positive results in VP and anaerobic agar tests that showed in table 1. Phylogenetic analysis indicated that W3.15 shares 98.8% similarity with *Bacillus subtilis* strain MPF73, although with a bootstrap value of 68/100 (Figure 2). The 16S rRNA gene sequence has been deposited in GenBank under the access number MW345907.

Table 1. Morphological and biochemical characteristics of W3.15 bacterial isolates that produce glucanase and antifungal activity (Putri *et al*., 2021)

Characteristic	W3.15 Isolate
Morfologi Colon	
Konfigurasi	round
Margin	soft
Elevation	Raised
Shape	circle
Pigmentation	Opaque
Cell Morphology	
Gram Reaction	+ (positive)
Shape	trunk
Preparation	single
Biological Characteristics	
Catalase	+ (positive)
Uji Voges-Proskauer	+ (positive)
H_2S Production	- (negative)
Urease detected	- (negative)
Anaerobic agar	- (negative)
Citrate utilization test	+ (positive)

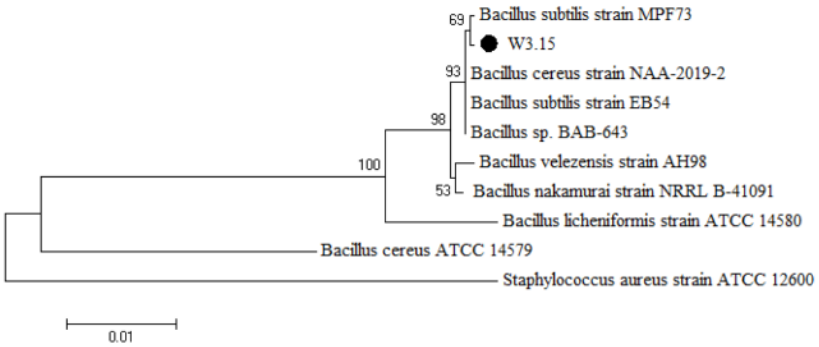

Figure 2. Analysis of phylogeny trees of 16S rRNA gene sequence from W3.15 potential isolate (Putri *et al*., 2021).

Additionally, W3.15 was tested for its ability to produce various hydrolytic enzymes. It demonstrated significant glucanolytic activity, forming a clear zone around its colonies on glucan agar (Figure 3.A), indicating its potential in β-glucanase production. This isolate produced larger and clearer zones compared to other strains, confirming its superior glucanolytic activity (Putri *et al*., 2021).

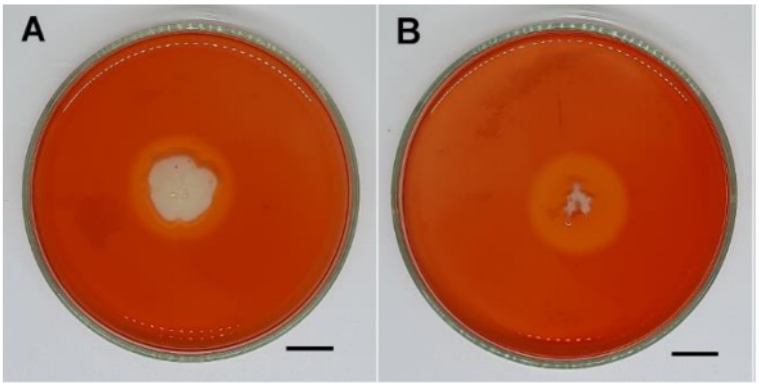

Figure 3. Glucolytic activity and positive control glucanolytic index values. Clear zone around bacterial colonies on glucan agar after incubation for 48 hours. (A) *Bacillus velezensis* BT2.04 and (B) potential isolate of *B. subtilis* W3.15 (Putri *et al*., 2021).

Further tests showed that W3.15 could produce cellulase and protease, but not chitinase, as no clear zones appeared for chitinase after incubation (Table 2, Table 3).

Table 2. Glucolytic Index Obtained from Bacterial Clear Zones in Several Tested Bacteria and Positive Control of *B. velezensis* Isolate BT2.04 (Putri *et al*., 2021).

Isolation Code	Glucolytic Index
CR.9	5.93 hrs
W3.15	4,94 f
W3.1	5,29 g
C1.2	1,46 b
C2.1	1,61 c
C3.3	2.96 d
PM.2	1,09 a
BT2.04	3.05 and

Note: Numbers followed by the same letter in the same column did not differ significantly in the DMRT test ($p \leq 0.05$).

Table 3. Hydrolytic Enzyme Activity of W3.15 Potential Isolate (Putri *et al.*, 2021).

Bacterial Species	Cellulase Activity	Protease Activity	Chitinase Activity
Isolat *B. subtilis* W3.15	+a	+	-[b]
Isolat *B. velezensis* BT2.04	n.t.c	+	-
Escherichia coli	-	-	-

Note: B. velezensis *BT2.04 and* E. coli *isolates* were used as positive and negative controls. N.T. = not tested.

These findings align with the research by Diabankana *et al*. (2021) on *Bacillus mojavensis* PS17, which also produced hydrolytic enzymes such as cellulase, lipase, protease, and chitinase. In addition, *B. mojavensis* PS17 demonstrated hydrophobic properties in its cell-free culture (Figure 4). These results suggest that both W3.15 and PS17 have the potential to act as biological control agents by producing enzymes and biosurfactants, crucial for adapting to abiotic stress and combating pathogens like *Fusarium*.

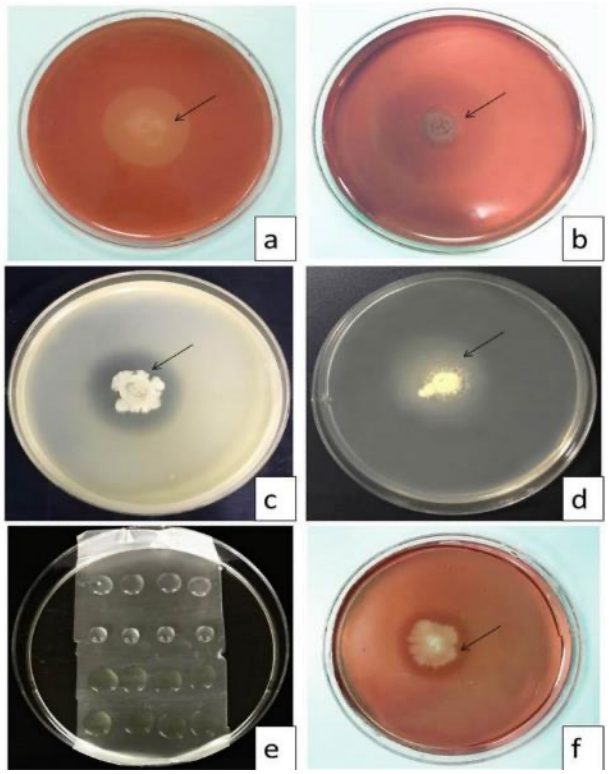

Figure 4. Production of hydrolytic enzymes by *B. mojavensis* PS17 with arrows indicating zones of enzyme activity. a) Cellular activity; b) β-glucanase activity; c) Protease production; d) Lipase activity; e) Biosurfactant activity (first row – SDS 20%, second row – water, third and fourth rows – *B. mojavensis* PS17); f) Chitinase activity (Diabankana *et al.,* 2021).

Isolation, Identification, and Pathogenicity Test of *Fusarium oxysporum*

Ferniah *et al*. (2014) conducted a study in Tawangmangu, a region endemic to Fusarium wilt, during the rainy season of 2013. Infected plants exhibited wilting symptoms on the leaves and yellowing on the stems, which were caused by *Fusarium oxysporum*. The infection begins at the roots, where the pathogen causes severe water stress by obstructing the xylem vessels. The primary symptom is the browning of vascular tissues, followed by upward leaf curling, which leads to secondary wilting symptoms (Figure 5).

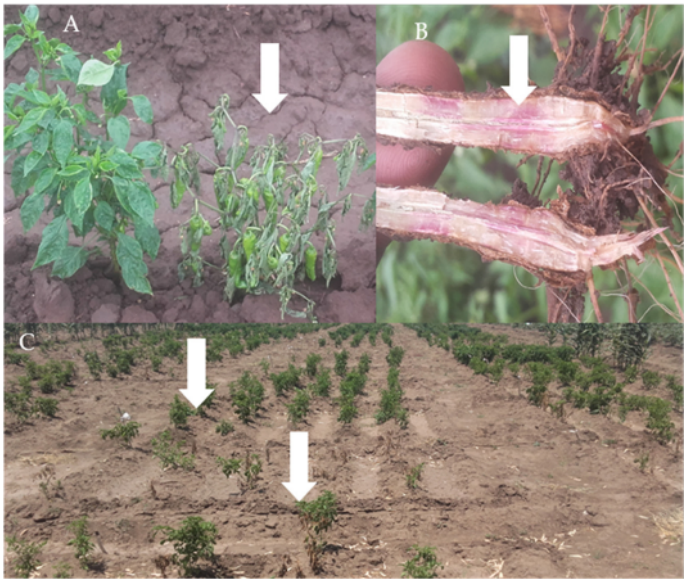

Figure 5. Symptoms of fusarium wilt disease in cayenne pepper. (a) Plants wither due to fusarium wilt, (b) Stems change color, (c) Fields severely infected by *F. oxysporum* (Gabrekiristos & Demiyo, 2020)**.**

One strain of *F. oxysporum*, named P1a, was isolated from infected chili plants in Tawangmangu. The morphological characteristics of this pathogen, as observed by Ferniah *et al*. (2014), include white mycelium with a cotton-like texture on the upper side and purple on the underside of the colony, with a 4-5 cm diameter after five days of incubation on PDA medium. The conidia grow from short phialides with a false head. Macroconidia are fusiform and straight, measuring 27-46 x 3-4.5 µm with 3-5 septa. Microconidia are abundant, ellipsoid or fusiform, with or without 1-2 septa, measuring 5-15 x 2.2-3.5 µm. Chlamydospores are formed terminally or intercalary, singly or in pairs (Figure 6).

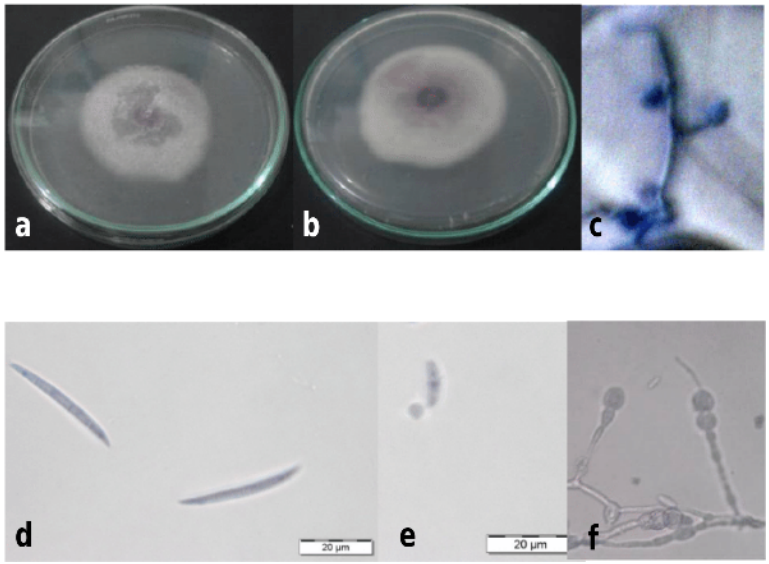

Figure 6. Morphology *of Fusarium oxysporum* a. Colonies, b. Lower colonies, c. Phialid, d. Macro-conidia, e. Micro-conidia, f. Chlamydospora (Ferniah *et al.,* 2014).

In pathogenicity tests, *F. oxysporum* isolate P1a was shown to cause wilting in chili plants. Symptoms of the disease worsened significantly 15 days after inoculation (DAI) (Figure 7).

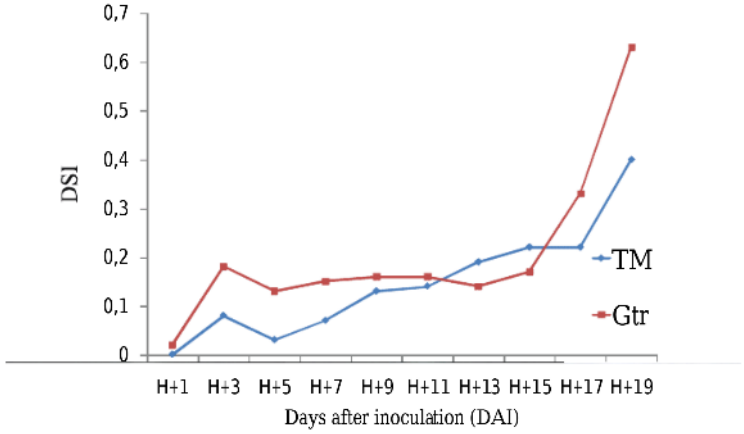

Figure 7. Disease severity index in chili plants by TM: Cultivar TM999, Gtr: Cultivar Gantari. *F. oxysporum* (Ferniah *et al.*, 2014).

By day 19, the fungi caused wilting in TM999 and Gantari cultivars. The pathogen caused stunting and wilting of chili plants, with or without yellowing of the leaves. Wilting started from older leaves and spread to younger ones. The stem exhibited discoloration or yellowing internally. The wilting symptoms became more severe after 15 days post-infection when the pathogen had successfully infected the plants. The pathogen infiltrates the roots through wounds and then colonizes the vascular system, obstructing the transport of water and nutrients, leading to wilting. The pathogen requires 13-17 days post-infection to manifest symptoms. As it can grow on nutrient media, it is classified as a non-obligate pathogen of *F. oxysporum*, which can survive in soil in the form of dormant chlamydospores until encountering a suitable host. Crop rotation is recommended to control this pathogen, as planting crops like soybeans does not trigger the pathogen, but planting chili does (Ferniah *et al*., 2014).

Antagonism Test of *Bacillus* spp. and *Fusarium oxysporum*

Putri *et al*. (2021) conducted an antagonism test between *Bacillus subtilis* W3.15 and *Fusarium oxysporum* and found that the W3.15 isolate inhibited 58.8% of fungal hyphal growth after 6 days (Figure 8). The antifungal activity of bacterial metabolites was assessed by adding 300 µl of the W3.15 culture filtrate to PDA medium. This resulted in a 66.5% inhibition of *F. oxysporum* growth after 6 days of incubation (Figure 7). The mycelium of the treated fungi showed dense, pink-white colonies, while the control fungi exhibited transparent pink-violet colonies (Figure 8).

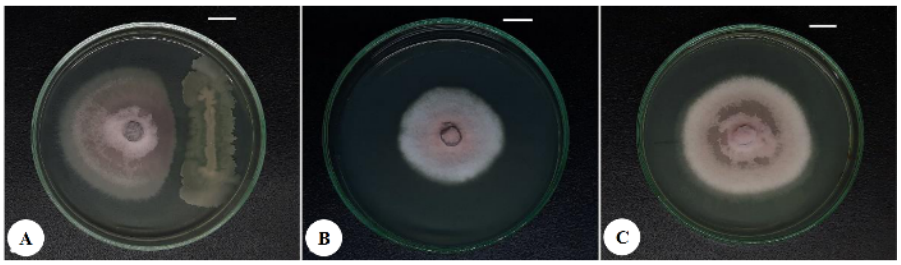

Figure 8. In vitro antibacterial activity of bacterial cells and culture filtrates against Fusarium oxysporum. Double culture test of W3.15 isolate (A) and culture filtrate test (300 µl) of W3.15 (B) isolate compared to the growth of F. oxysporum in a culture aged 6 days (C) (Putri *et al*., 2021).

Putri *et al*. (2021) also observed changes in fungal hyphal morphology caused by the antifungal activity of the W3.15 isolate. Microscopic examination revealed that dual-culture tests resulted in reduced hyphal diameter, distorted and reduced branching, and intracellular granulation. Filtrate application also induced vacuole formation along the hyphae (Figure 9).

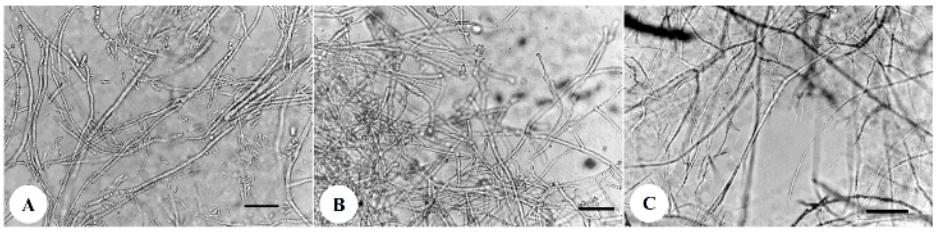

Figure 9. Changes in hyphae morphology in the presence of bacterial cells and bacterial culture filtrates after 6 days of incubation. Normal hyphae of Fusarium oxysporum (A), hyphae with short and irregular branching in antagonist treatment with W3.15 isolate (B), hyphae showed granule or vacuole formation along hyphae, hyphae deformatio, and hyphae death in the addition of culture filtrate (C) (Putri *et al*., 2021).

The reduction in *F. oxysporum* growth was linked to disruption in the fungi's cell metabolism during hyphal extension, leading to structural deformation, similar to previous findings where *Bacillus subtilis* compounds caused hyphal swelling, excessive branching, and abnormal growth in *Magnaporthe oryzae*. Lin et al. (2022) also observed severe distortion in *Fusarium verticillioides* mycelium due to Bacillomycin D from *B. subtilis*. The rough extract also caused irregular hyphal twisting, likely due to the accumulation of antifungal compounds, which may induce oxidative stress, increasing ROS production, damaging cells, and causing cell degradation (Hwang et al., 2022; Kulbacka et al., 2009)

Analysis of Antifungal Secondary Metabolites

The Base Peak Ion (BPI) chromatogram of the crude ethyl acetate (EA) extract from *Bacillus subtilis* W3.15, analyzed via LC-MS/MS, showed several dominant peaks in the retention time range of 0–22 minutes (Figure 10). Four dominant peaks were detected at retention times of 8.16 minutes, 9.12 minutes, 10.07 minutes, and 10.71 minutes. Mass spectrum and monoisotopic mass analysis suggested that two of these peaks contain active antifungal compounds. The identified compounds included epicatechin (290.15 m/z) at 10.71 minutes, diaveridin (260.17 m/z) at 10.07 minutes, and

benzophenone-8 (244.18 m/z) at 9.11 minutes. The peak at 10.71 minutes had the largest area (22.19%), followed by the 10.07-minute peak (15.12%).

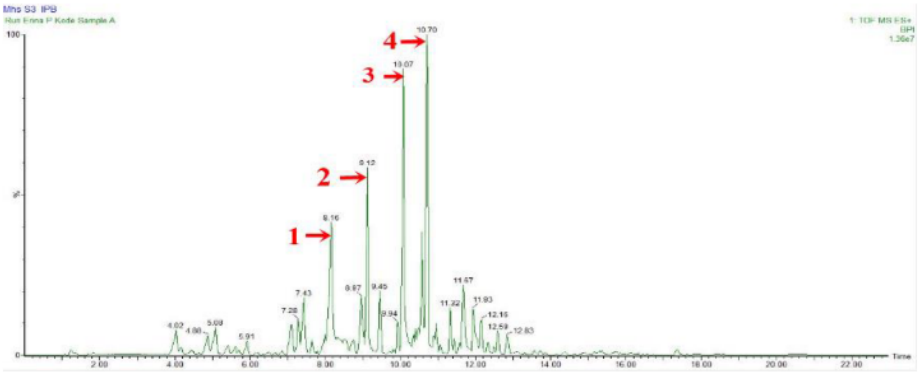

Figure 10. Basic peak ion chromatograms (BPI) corresponding to LC-MS/MS analysis of crude ethyl acetate extracts produced by Bacillus subtilis strain W3.15 showed four dominant peaks at retention times of 0–22 minutes (Putri *et al*., 2023).

Epicatechin has been shown to enhance resistance against *Botrytis cinerea* in apples by activating the phenylpropanoid pathway and increasing flavonoid accumulation (Zhang et al., 2020). Diaveridin is known for its antiprotozoal activity, while benzophenone is reported to be both antibacterial and antifungal. Putri *et al*. (2023) revealed that the supernatant extract of *B. subtilis* W3.15 exhibited significant antifungal activity against *F. oxysporum*, reducing radial growth and causing hyphal deformation, likely triggered by the active antifungal compounds present in the extract.

Table 4. Fusarium wilt disease progression in chili pepper plants treated with indigenous endophytic bacteria (Yanti *et al.*, 2018).

Strains	Disease Development Time	Effectivity	Disease Incidence (%)	Effectivity	Severity	Effectivity
B. pseudomycoides strain NBRC 102132	42.00[a]	106.59	0.00	100.00	0.00[d]	100.00
B. thuringiensis strain ATCC 10792	42.00[a]	106.59	0.00	100.00	0.00[d]	100.00
B. mycoides strain 273	42.00[a]	106.59	0.00	100.00	0.00[d]	100.00
B. cereus strain NBRC 15305	42.00[a]	106.59	0.00	100.00	0.00[d]	100.00
B. bingmayongensis strain FJAT-13831	42.00[a]	106.59	0.00	100.00	0.00[d]	100.00
B. manliponensis strain BL4-6	39.33[ab]	93.46	66.67	33.33	0.67[cd]	83.25
B. thuringiensis strain ATCC 10792	31.00[c]	52.48	66.67	33.33	2.67[ab]	33.25
B. weihenstephanensis strain DSM 11821	32.67[bc]	60.70	100.00	0.00	2.00[bc]	50.00
B. mycoides strain 273	30.67[c]	50.86	66.67	33.33	2.67[ab]	33.25
Control	20.33[d]	100	4.00	-	4.00[a]	-

Note: Means with the same letter are not significantly different by Duncan multiple range test at $p < 0.05$.

The findings of Yanti *et al.* (2018) demonstrated that the introduction of *Bacillus* spp. strains significantly reduced the incidence, severity, and development time of Fusarium wilt (Table 4). Five Bacillus strains, namely *B. pseudomycoides* strain NBRC 101232, *B. thuringiensis* strain ATCC 10792, *B. mycoides* strain 273, *B. cereus* strain NBRC 15305, and *B. bingmayongensis* strain FJAT-13831, effectively prevented Fusarium wilt, with no disease symptoms observed throughout the experiment. These strains were also shown to suppress Fusarium wilt efficiently. The mechanisms employed by antagonistic microorganisms include antibiosis, competition, predation, and parasitism. Antagonism assays have demonstrated that bacterial isolates can inhibit fungal growth by producing secondary metabolites with toxic properties and mycolytic enzymes that degrade the cell walls of pathogenic fungi. These phenomena of antibiosis and hydrolytic enzyme activity have been identified as the primary mechanisms of biocontrol agents in suppressing plant pathogens (Alabouvette et al., 2006).

Colonization of *Bacillus* spp. on Plants

Liang *et al*. (2024) demonstrated that the application of *Bacillus subtilis* Ydj3 enhanced root hair growth and altered the root architecture of chili pepper plants compared to the control group. Microscopic observations revealed that the roots of *B. subtilis* Ydj3-treated plants had denser and longer root hairs in the apical meristem, elongation, and maturation zones. Scanning electron microscopy (SEM) images (Figure 11) showed no bacterial cells on the root surface in the control group, whereas bacterial cells were clearly observed colonizing the root surface and root hairs in the *B. subtilis* Ydj3 treatment. These findings highlight the ability of *B. subtilis* Ydj3 to colonize the root system of chili pepper plants.

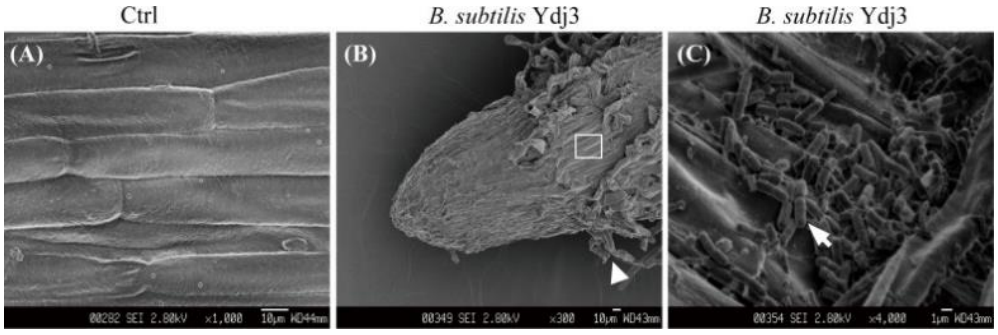

Figure 11. SEM on the roots of chili plants. (A) Treatment with distilled water as control (Ctrl); (B, C) treatment with B. subtilis Ydj3 with a scale of 10 µm. (A) plant cell parts; (B) the tip of the root, the arrow shows the root hair; the square area is enlarged on C; (C) Enlarged image of the square area in B, the arrow shows bacteria (Liang *et al*., 2022).

Endophytic bacteria utilize hydrolytic enzymes to break down plant cell walls and enter host tissues (Kaplan et al., 2013). *B. subtilis* boosts its competitiveness in the rhizosphere by producing antibiotics (Maan et al., 2021) and adapting to root colonization, enhancing biofilm formation in response to plant polysaccharides (Nordgaard et al., 2022). It also suppresses plant defense responses and modulates gene expression to facilitate colonization (Rekha et al., 2018).

B. subtilis Ydj3 demonstrated its ability to colonize the root system and suppress *Fusarium oxysporum* pathogens through mechanisms such as space competition,

antimicrobial compound production, and enhancing plant resistance (Shahzad et al., 2017). Lipopeptide compounds from *B. subtilis* can damage the pathogen's cell membranes, causing hyphal lysis in *F. oxysporum*. Yuan et al. (2012) also observed that *B. subtilis* colonization in plant roots enhances resistance by increasing the accumulation of phenolic compounds that inhibit pathogens. Therefore, *B. subtilis* Ydj3 not only supports root growth but also creates a microenvironment that discourages the development of *Fusarium oxysporum*, making it an effective candidate for biocontrol against fusarium wilt disease.

Figure 12 illustrates the interaction between plants, *Bacillus* spp., and *Fusarium* pathogens in the rhizosphere (Khan et al., 2017). On the negative side, *Fusarium* pathogens attack the plant host through root infection, leading to symptoms such as wilting, stunted growth, and plant death. This negative interaction poses significant challenges to plant health and productivity. However, *Bacillus* spp. provides positive interactions by antagonizing *Fusarium* pathogens. *Bacillus* spp. competes for nutrients and space, produces antimicrobial compounds like antibiotics, and neutralizes pathogen virulence factors.

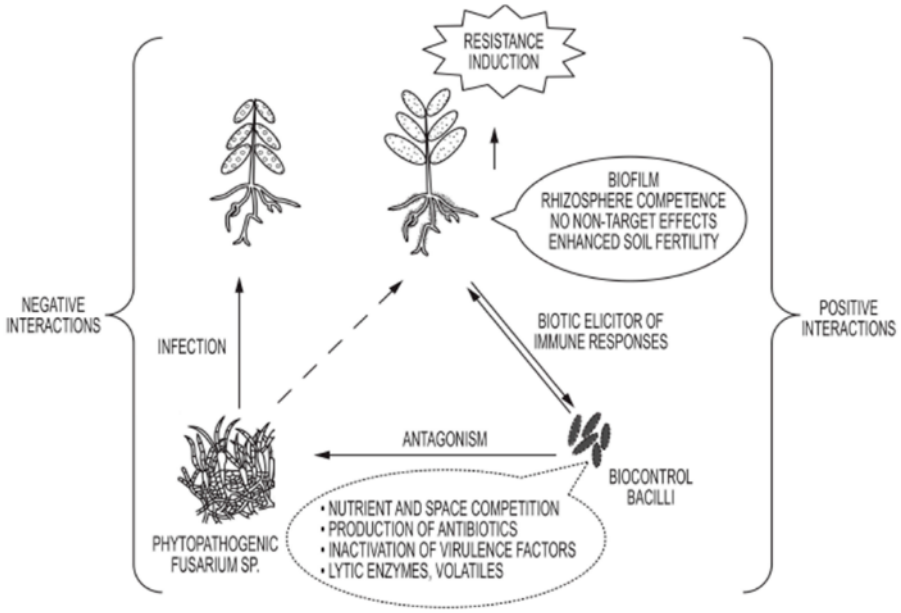

Figure 12. A scheme describing the interaction between Bacillus and Fusarium spp., and their relative impact on plant health (Khan *et al*., 2017).

Additionally, *Bacillus* spp. produces lytic enzymes and volatile compounds that can degrade pathogen cell structures. Beyond its role as an antagonistic agent, *Bacillus* spp. stimulates plant immune responses through biotic elicitors, enhancing plant resistance to infection. *Bacillus* spp. also forms biofilms, improving its competence in the rhizosphere, supporting soil fertility, and preventing negative effects on non-target organisms. This combination of effects provides effective protection for plants while promoting their growth and overall health.

Conclusion

Based on the results of study, the conclusion are; (1) *Bacillus* spp. produce enzymes and secondary metabolites, such as lipopeptides and antibiotics, that disrupt the cell wall of *Fusarium oxysporum*, inhibiting its growth and causing hyphal lysis. (2) As endophytes, *Bacillus* spp. colonize plant tissues, enhancing plant resistance by improving

phenolic compounds and defense enzymes that combat pathogens. And (3) *Bacillus* spp. significantly boost plant health by promoting root growth, nutrient uptake, and stress tolerance, while also creating a microenvironment in the rhizosphere that hinders pathogen development.

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