

Isolation and Evaluation of Antifungal Metabolites from *Bacillus subtilis* Against *Solanum lycopersicum* (Tomato) Fungal Pathogens

Himanshu Sisodia*

*PhD Research Scholar, Department of Botany, University of Rajasthan, Jaipur-302004

Email: himanshusisodiathoi@gmail.com

Abstract:

The increasing resistance of fungal pathogens and environmental concerns over chemical fungicides necessitate alternative control strategies in sustainable agriculture. The present study evaluates the antifungal efficacy of secondary metabolites produced by *Bacillus subtilis* against three significant fungal pathogens—*Fusarium oxysporum*, *Alternaria solani*, and *Rhizoctonia solani*—that commonly infect tomato (*Solanum lycopersicum*). Secondary metabolites from *B. subtilis* were extracted and applied to tomato seeds and fungal cultures. Results indicated a concentration-dependent antifungal effect and enhancement of seed germination at optimal metabolite levels. This study supports the utilization of *B. subtilis* as a biocontrol agent and biofertilizer component for disease suppression and growth promotion in tomato crops.

Keywords: *Bacillus subtilis*, biocontrol, tomato, secondary metabolites, seed germination, Fusarium wilt, damping-off, sustainable agriculture

1. INTRODUCTION

Fungal diseases such as Fusarium wilt, early blight, and damping-off are major constraints in tomato (*Solanum lycopersicum*) cultivation, causing substantial yield losses globally. Conventional disease control methods rely heavily on synthetic fungicides, which pose environmental hazards and lead to fungicide-resistant strains [1]. Consequently, biological control through antagonistic rhizobacteria offers an eco-friendly and sustainable alternative.

Bacillus subtilis, a Gram-positive endospore-forming bacterium, is renowned for its plant-growth-promoting and disease-suppressive traits. It produces a broad spectrum of antimicrobial compounds such as lipopeptides (iturin, fengycin, surfactin), which inhibit phytopathogens by disrupting cell membranes and vital processes [2]. Moreover, *B. subtilis* can colonize root surfaces effectively, forming protective biofilms and inducing systemic resistance in plants [3].

This study aims to isolate *B. subtilis* from rhizospheric soil and assess the antifungal activity of its extracellular metabolites against fungal pathogens of tomato and their impact on seed germination and vigor.

2. MATERIALS AND METHODS

2.1. Isolation of *Bacillus subtilis*

Soil samples were collected from the rhizosphere of healthy tomato plants in an organic farm. Serial dilution and pour plate techniques were used to isolate bacterial colonies on nutrient agar. Colonies were purified and identified as *B. subtilis* based on Gram staining, endospore staining, and standard biochemical tests [2].

2.2. Fungal Pathogen Maintenance

Pathogenic strains of *Fusarium oxysporum*, *Alternaria solani*, and *Rhizoctonia solani* were procured from a plant pathology laboratory and maintained on potato dextrose agar (PDA) at 28°C.

2.3. Extraction of Secondary Metabolites

B. subtilis was cultured in nutrient broth for 72 hours at 30°C with agitation (120 rpm). The broth culture was centrifuged at 10,000 rpm for 15 minutes. The supernatant obtained was then

filtered through Whatman filter paper to collect the crude extract of extracellular metabolites.

2.4. Tomato Seed Germination Assay

Tomato seeds were disinfected by treating them with 0.1% mercuric chloride for 2 minutes, followed by multiple rinses with sterile distilled water to ensure complete removal of the sterilizing agent. Seeds were soaked in *B. subtilis* metabolite filtrate at 25%, 50%, 75%, and 100% concentrations for 4, 16, 24, and 30 hours. Ten seeds were placed in each replicate on moist filter paper in sterile petri dishes and incubated for 5 days.

2.5. In Vitro Antifungal Assay

Antagonistic activity was evaluated using the agar well diffusion method. PDA plates were inoculated centrally with 5 mm plugs of fungal cultures. Wells (6 mm) were filled with *B. subtilis* filtrates of different concentrations. The zones of inhibition were recorded following a 5-day incubation period.

3. RESULTS

3.1. Table-I Seed Germination Performance:

Treatment (Concentration)	Germination Rate (%)	Mean Radicle Length (cm)	Mean Plumule Length (cm)
Control (Distilled Water)	64.00	3.2	2.8
25% Metabolite	78.00	4.0	3.5
50% Metabolite	85.00	4.6	3.9
75% Metabolite	70.00	3.8	3.2
100% Metabolite	60.00	3.0	2.6

Observation: The 50% concentration of *B. subtilis* metabolites demonstrated the highest seed germination rate and seedling vigor.

3.2. Table-II Antifungal Activity of Metabolites:

Pathogen	Inhibition Zone (mm) @ 100% Metabolite
<i>Fusarium oxysporum</i>	20 mm
<i>Alternaria solani</i>	25 mm

- [1] Choudhary, D. K., & Johri, B. N. (2009). Interactions of *Bacillus* spp. and plants – With special reference to induced systemic resistance (ISR). *Microbiological Research*, 164(5), 493–513. <https://doi.org/10.1016/j.micres.2008.08.007>
- [2] Stein, T. (2005). *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions.

<i>Rhizoctonia solani</i>	17 mm
---------------------------	-------

Observation: All three pathogens were significantly inhibited, with *A. solani* being the most susceptible.

4. DISCUSSION

The study demonstrated that metabolites produced by *Bacillus subtilis* have dual roles—enhancing seed germination at moderate concentrations and inhibiting fungal pathogens effectively. The antifungal action is likely attributed to lipopeptides such as surfactin and iturin, which are known to cause membrane disruption and cell leakage in fungi [5].

The improved seedling performance at 50% concentration could be due to beneficial auxin-like compounds (e.g., indole-3-acetic acid) and phosphate-solubilizing enzymes produced by *B. subtilis* [4]. However, at higher concentrations, a reduction in growth parameters indicates possible phytotoxic effects, necessitating concentration optimization in field formulations.

Similar studies reported effective suppression of *F. oxysporum* and *A. solani* by *B. subtilis*-based biocontrol agents [6], supporting our findings. However, in vitro studies must be validated under greenhouse and field conditions for practical application.

5. CONCLUSION

Bacillus subtilis-derived extracellular metabolites exhibit promising antifungal activity against major tomato pathogens while enhancing seed germination at optimal concentrations. These findings underscore the potential of *B. subtilis* as a natural bio-inoculant in integrated pest and nutrient management strategies for sustainable tomato cultivation. Future research should include metabolite purification, mode-of-action studies, and field-scale validation.

6. REFERENCES

- Molecular Microbiology*, 56(4), 845–857. <https://doi.org/10.1111/j.1365-2958.2005.04587.x>
- [3] Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, 94(11), 1259–1266. <https://doi.org/10.1094/PHYTO.2004.94.11.1259>

- [4] Idris, E. E. S., Iglesias, D. J., Talon, M., & Borriss, R. (2007). Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. *Molecular Plant-Microbe Interactions*, 20(6), 619–626. <https://doi.org/10.1094/MPMI-20-6-0619>
- [5] Meena, K. R., & Kanwar, S. S. (2015). Lipopeptides as the antifungal and antibacterial agents: Applications in food safety and therapeutics. *Biocatalysis and Agricultural Biotechnology*, 4(1), 71–79. <https://doi.org/10.1016/j.bcab.2014.10.001>
- [6] Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2017). Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, 8, 667. <https://doi.org/10.3389/fphys.2017.00667>