SCIENCE SET
OPEN ACCESS PUBLISHERS

ISSN: 3067-8366
Research Article

Journal of Environmental Science, Sustainability, and Green Innovation

Preliminary Results of Biological Control of Pectobacterium spp. in Potato Using an Antagonistic Bacillus Strain

Ahlem Sassi[1]*, H. Chouchane[1], N. Khamassy[2], A. Mosbah[1], F. Khouaja Djilani[1,3]

[1]*Laboratory of Biotechnology and Valorization of Bio-Georesources, ISBST, Tunisia*
[2]*Laboratory of Horticultural Sciences, INRA Tunisia*
[3]*Laboratory of Molecular Genetics, Immunology and Biotechnology, Faculty of Sciences, Tunis*

***Corresponding author:** Ahlem Sassi, Laboratory of Biotechnology and Valorization of Bio-Georesources, ISBST, Tunisia.

Submitted: 20 January 2026 **Accepted:** 05 February 2026 **Published:** 11 February 2026

***Citation:** Sassi, A., Chouchane, H., Khamassy, N., Mosbah, A., Khouaja Djilani, F., & Khouaja Djilani, F.(2026). Preliminary Results of Biological Control of Pectobacterium spp. in Potato Using an Antagonistic Bacillus Strain. J of Environ Sci & Sustain & Green Innov, 2(1), 01-06.*

Abstract
Pectobacterium spp. are responsible for soft rot and black leg in potatoes, causing substantial economic losses worldwide due the major losses post-harvest and during storage. This study aimed to isolate and characterize the pathogen from local infected potato tubers and evaluate the antagonistic potential of local bacterial isolates. Eighty actinobacteria and twenty Bacillus strains were tested in vitro against Pectobacterium where one Bacillus strain demonstrated strong antagonistic activity as whole culture. Secondary metabolite extractions using organic solvents indicated that ethyl acetate extracts were the most effective, followed by cyclohexane and acetone fractions, suggesting the production of both semi-polar and hydrophobic antimicrobial compounds. Hydroponic detached leaf assays and in planta assays achieved complete disease suppression (0% incidence vs 100% in pathogen controls). Fisher's exact test confirmed highly significant differences ($p \leq 0.0143$ for all comparisons). No symptoms or phytotoxicity occurred in Bacillus treatments, demonstrating biocontrol potential of this native Bacillus isolate for sustainable Pectobacterium soft rot management in potato, with possible PGPR activity.

Keywords: Soft Rot, Pectobacterium, Bacillus, Biocontrol, Antibacterial, PGPR.

Introduction

Soft rot, caused by Pectobacterium species, poses a significant risk to potato farming, resulting in considerable losses both in the field and during storage worldwide, due to the secretion of pectolytic and cellulolytic enzymes that macerate plant tissues [1].

This pathogen is not only highly transmissible through contaminated water, tools and soil it also presents large genetic variability further complicating the management which relies mainly on using certified seeds. Tubers that appear healthy may develop symptoms during or post-harvest, where this infection promoting secondary infections [2]. Thus, the development of an efficient biocontrol agent against this major threat is essential to control epidemics and reduce crop losses, thereby promoting sustainable agriculture.

This research focused on isolating the pathogen and identifying native bacteria with antagonistic properties that can suppress Pectobacterium spp., as well as assessing their biocontrol potential through both in vitro and in vivo methods. Potato leaves showing typical symptoms were collected, and the pathogen was isolated from these tissues using selective media to evaluate pectinolytic and cellulolytic activities. The isolates obtained were characterized through macroscopic, microscopic observations and molecular identification. The antagonistic activity was tested in vitro using a disk-diffusion assay, this activity was further confirmed in vivo through hydroponic and in planta experiments. Leaves treated with the Bacillus strain exhibited a notable reduction in symptom severity compared to those only inoculated with the pathogen.

Materials and Methods

Revivification of Bacterial Strains

80 actinobacterial isolates were revived on Luedemann medium and incubated at 30 °C for 7–14 days and 20 Bacillus isolates

were revived on TSA medium at 30 °C for 24–48 h.

Isolation and Characterization of Pectobacterium spp.

Infected potato leaves were washed with sterile distilled water. Lesions were sampled, placed in 200 µL sterile saline with glass beads, vortexed, and plated on neutral-pH pectin agar (see Appendix 1) in order to facilitate the selective isolation of pectinolytic bacteria and evaluate enzymatic virulence [2, 3].

Appendix 1: Pectin medium composition

Component	Quantity (g/L)
Pectin	5
$NaNO_3$	2
KH_2PO_4	0.26
$MgSO_4$	0.5
$CaCl_2$	0.1
Agar	25
Distilled water	q.s.p. 1 L

Colonies were purified through successive sub-culturing then subjected to macroscopic, microscopic observations and further cellulolytic activity testing on carboxyméthylcellulose (CMC) agar medium.

Primary Screening of Antagonistic Activity

Primary screening for antagonistic activity against the pathogen was performed using the agar disk diffusion method. After uniformly spreading the Pectobacterium culture onto agar plates, sterile paper disks were impregnated with the tested bacteria. Live cultures adjusted to OD600 ≈ 0.12–0.15 for the actinobacterial strains and 0.10–0.12 for Bacillus were tested on TSA plates.

Secondary Screening: Metabolite Extraction

A secondary screening was performed to identify the bioactive metabolites produced by the antagonist strain. After culturing the strain in TSB, the culture was sonicated and centrifuged. The clarified supernatant was divided and subjected to liquid–liquid extraction with different organic solvents: cold acetone for hydrophobic compounds, ethyl acetate for semi-polar metabolites, and cyclohexane for lipidic and highly non-polar molecules. The resulting fractions were evaporated, resuspended in 30% isopropanol 70% distilled water, and each extract was tested for antagonistic activity using the disk diffusion method.

Tertiary Screening on Hydroponic Substrate

At the end of the incubation period, well-developed colonies were carefully scraped under sterile conditions and resuspended in 1 mL of phosphate-buffered saline (PBS) to obtain homogeneous bacterial suspensions for screening assays.

A solidified hydroponic substrate was used to support potato leaves, following the method described by Hothem et al [5]. Briefly, Hoagland nutrient medium (see Appendix 2), solidified with agar, was prepared. The nutrient solution was poured into sterile Petri dishes and allowed to solidify at room temperature.

Appendix 2: Hoagland Complete Nutrient Solution Recipe (1 L Final)

Main Stock Solutions

Prepare stocks at 1 M (unless noted), store at 4°C (stable for months).

Stock Solution	Concentration	Quantity (mL/L final)
Monoammonium phosphate ($NH_4H_2PO_4$)	1.00 M	1
Potassium nitrate (KNO_3)	1.00 M	6
Calcium nitrate [$Ca(NO_3)_2$- H_2O]	1.00 M	4
Magnesium sulfate ($MgSO_4$)	2.00 M	2

Micronutrients (Stock Solution 5: 1 L)

Dissolve in 1 L distilled water, use 1 mL/L final.

Compound	Quantity (g/L)
Boric acid (H_3BO_3)	2.86
Manganese chloride ($MnCl_2$- $4H_2O$)	1.81
Zinc sulfate ($ZnSO_4$- $7H_2O$)	0.22
Copper sulfate ($CuSO_4$- $5H_2O$)	0.08
Sodium molybdate (Na_2MoO_4- $2H_2O$)	0.02

Iron (Stock Solution 6: 1 L)

1. Dissolve 26.1 mg EDTA in 286 mL water + 19 g KOH.
2. Dissolve 24.9 mg $FeSO_4$- $7H_2O$ in 500 mL water.
3. Slowly add $FeSO_4$ to EDTA under aeration/stirring overnight.
4. Adjust to 1 L (pH ~7.1, wine-red color, minimal precipitate).

Usage: 0.25 mL/L final.

Final assembly (1 L): Mix stocks into distilled water with stirring, adjust pH to 5.5-6.0 if needed

Healthy potato leaves were thoroughly washed with sterile distilled water, surface-disinfected with 70% ethanol, and air-dried on sterile paper. The leaves were then placed on the surface of the solidified hydroponic substrate. Two distinct bacterial suspensions were inoculated directly onto the leaf surface: one containing the pathogenic strain and the other the tested antagonistic strain.

The Petri dishes were incubated at room temperature (25–28 °C) for one week under sterile conditions. The interaction between the two microorganisms was monitored by visual observation of symptom development on the leaf tissues.

In Planta Assays

To confirm the antagonistic strain's potential under conditions close to the natural environment and to evaluate its ability to prevent or reduce disease development, in vivo assay was conducted on potato plants maintained under controlled conditions (constant temperature, photoperiod, and humidity). Bacterial inoculation was performed directly into the soil at the base of the potato plants in order to simulate a natural rhizospheric interaction.

The bacterial strains were cultured on solid agar medium for 48 h. Colonies were aseptically scraped and resuspended in phosphate-buffered saline (PBS). The suspensions were vortexed for 20 minutes to ensure complete homogenization. Inoculums concentrations were standardized by adjusting the optical density using a spectrophotometer UV-visible. For Gram-negative bacteria such as Pectobacterium, the optical density was adjusted to OD_{600} = 0.12–0.15, corresponding to approximately 10^8 CFU/mL. For Bacillus spp., the optical density was adjusted to OD_{600} = 0.10–0.12, equivalent to approximately 10^8 CFU/mL, taking into account the slightly lower absorbance resulting from differences in cell size and morphology. These concentrations were used for all treatments in the in vivo assay.

Five treatments were performed to evaluate the pathogenic, preventive, curative, and plant growth–promoting (PGPR) effects of the antagonistic strain:

Positive Control (pathogen alone)

The pathogen was applied alone to induce disease symptoms and serve as a reference control.

Co-inoculation (pathogen + antagonist)

Suspensions of the pathogen and the antagonistic strain were applied simultaneously to the soil to assess their direct interaction.

Preventive Treatment (antagonist → pathogen, 24 h)

The antagonistic Bacillus spp. strain was applied 24 h prior to pathogen inoculation to evaluate its preventive colonization ability and protective effect on the plant.

Curative Treatment (pathogen → antagonist, 24 h)

The pathogen was introduced 24 h before application of the antagonistic strain to assess its curative effect following initial infection establishment.

Antagonist Alone (Biostimulation / Phytotoxicity)

The antagonistic strain was applied alone to the soil to evaluate a potential PGPR effect or, conversely, any phytotoxicity.

Statistical Analysis

Disease incidence was calculated as the proportion of experimental units (individual leaves for hydroponic assays; individual plants for in planta assays) exhibiting soft rot symptoms at the end of the observation period. Each experimental unit was scored binarily as symptomatic (1) or asymptomatic (0).

Treatment effects on disease incidence were analyzed using two-tailed Fisher's exact test on 2×2 contingency tables (treatment × disease status). Hydroponic assays compared: (i) pathogen alone (n = 3 leaves) vs. co-inoculation (n = 9 leaves), and (ii) pathogen alone (n = 3 leaves) vs. Bacillus alone (n = 3 leaves). In planta assays compared pathogen alone (n = 4 plants) vs. each Bacillus treatment co-inoculation, preventive (Bacillus 24 h prior), curative (Bacillus 24 h post-pathogen), Bacillus alone; n = 4 plants each.

All analyses were conducted in R version 4.4.1 (R Core Team, 2024) using the fisher test function. Statistical significance was declared at $\alpha = 0.05$.

Results and Discussion

Pectobacterium isolates displayed characteristic pectinolytic and cellulolytic activities, confirming their pathogenic potential Colonies were circular, cream-colored, mucoid, with clear pectinolytic halos (figure1). Gram staining confirmed Gram-negative bacilli arranged singly or in short chains (Figure 2) [2, 3].

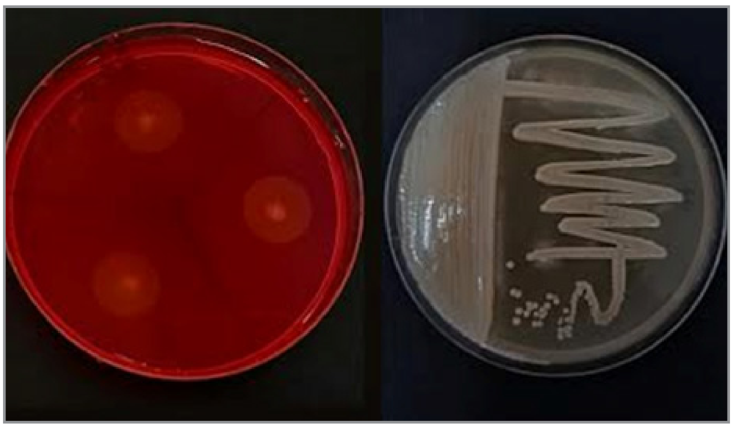

Figure 1: enzymatic activities and macroscopic observation

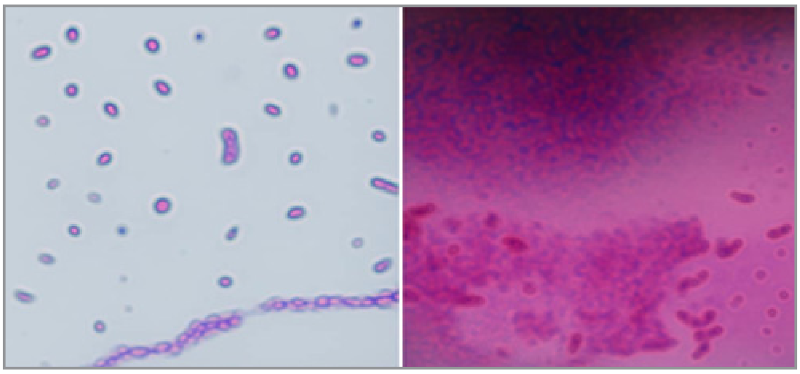

Figure 2: microscopic observation

The use of pectin-enriched medium for isolation allowed selective growth of pectinolytic bacteria, highlighting enzymatic activity linked to virulence [1, 2]. The observed antagonism may involve antibiosis, nutrient competition, and modulation of pathogen quorum sensing [5].

Only one Bacillus strain showed significant antagonism in vitro. Secondary metabolite profiling suggested the production of both semi-polar and hydrophobic antimicrobial compounds, with ethyl acetate extracts being the most active, followed by cyclohexane and acetone fractions (Figure 3) this method demonstrates that metabolites extracted directly from cells can also play a crucial role, opening new perspectives for the formulation of concentrated antimicrobial agents [6, 7].

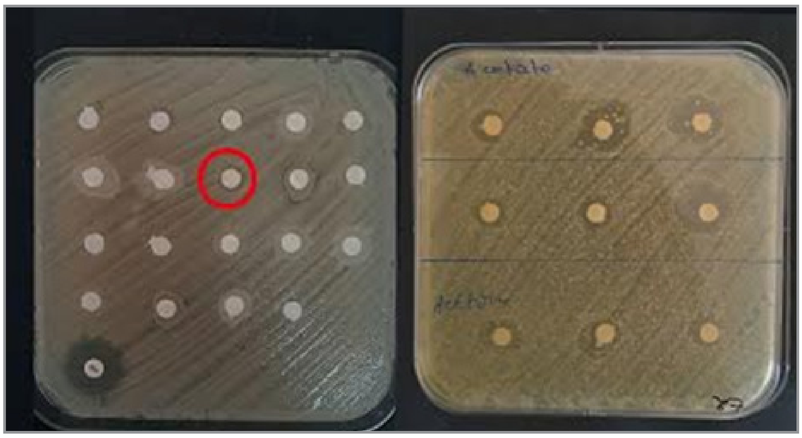

Figure 3: screening results

This indicates that this strain produces bioactive compounds capable of inhibiting Pectobacterium growth. Previous studies demonstrated that Streptomyces, Pseudomonas fluorescens, and Bacillus velezensis produce metabolites capable of suppressing Pectobacterium [8, 9]. These results indicate that direct extraction of metabolites from Bacillus cells provides a targeted approach to identifying active compounds, which may serve as the basis for antimicrobial agent development. The hydroponic assay demonstrated strong antagonistic activity of the Bacillus isolate (Table 1). All leaves inoculated with Pectobacterium alone developed characteristic soft rot symptoms (3/3 leaves; 100% incidence). Co-inoculation with the Bacillus antagonist completely prevented symptom development (0/9 leaves; 0% incidence), with Fisher's exact test confirming highly significant disease suppression ($p = 4.44 \times 10^{-10}$). Leaves treated with Bacillus alone remained asymptomatic (0/3 leaves; 0% incidence), indicating no phytotoxicity ($p = 0.0426$ vs. pathogen alone).

Table 1: Disease incidence in hydroponic detached leaf assay

Treatment	Diseased/Total	Incidence (%)	p-value
Pathogen alone	3/3	100	—
Co-inoculation	0/9	0	4.44×10^{-10}
Bacillus alone	0/3	0	0.0426

In planta assays under rhizospheric conditions confirmed the biocontrol potential across all application strategies (Table 2). All plants receiving Pectobacterium alone exhibited severe soft rot symptoms (4/4 plants; 100% incidence). The Bacillus antagonist achieved complete disease suppression regardless of application timing: co-inoculation (0/4 plants), preventive treatment (0/4 plants), and curative treatment (0/4 plants), each significantly different from the pathogen control ($p = 0.0143$ for all comparisons). Plants treated with Bacillus alone showed no symptoms (0/4 plants), confirming safety for agricultural application.

Table 2: Disease incidence in in planta potato assays

Treatment	Diseased/Total	Incidence (%)	p-value
Pathogen alone	4/4	100	—
Co-inoculation	0/4	0	0.0143
Preventive	0/4	0	0.0143
Curative	0/4	0	0.0143
Bacillus alone	0/4	0	0.0143

positive control: infected plants   Treatment with the antagonistic Bacillus strain   Antagonistic Bacillus strain alone

Figure 4: In Planta Test Results

Representative images of the plants from each treatment are shown in Figure 4. The figure visually highlights the severe symptoms in Pectobacterium-infected plants and the complete protection provided by the Bacillus antagonist across all application strategies.

In planta assays demonstrated both safety (no phytotoxicity: $p \leq 0.0426$ vs. pathogen controls) and efficacy (complete disease suppression: $p \leq 0.0143$ across all treatment comparisons) of this approach. Preventive, curative, and co-inoculation treatments significantly reduced disease severity, without any phytotoxic effects. These findings support its potential as a biocontrol agent and plant growth-promoting rhizobacterium (PGPR).

Overall, this work underscores the importance of microbial diversity in biocontrol strategies and highlights the potential of Bacillus as a potent, safe, and reproducible antagonist of Pectobacterium spp., contributing to sustainable management of potato soft rot suggesting both efficient and accessible approaches in order to maintain plant health as the strain has both preventive and curative effects [10].

Conclusion

This study demonstrates the potential of biological control as an effective and sustainable strategy for managing potato soft rot caused by Pectobacterium spp. An antagonistic bacterium belonging to the genus Bacillus was isolated and shown to significantly inhibit the pathogen under both in vitro and in vivo conditions. The combined use of selective isolation, phenotypic characterization, and bioassays provided reliable evidence of its antagonistic capacity. A major outcome of this work is the identification of bioactive lipophilic and semi-polar fractions from bacterial cell extracts as the primary source of antibacterial activity. This finding indicates that diffusible secondary metabolites, rather than direct microbial competition alone, play a central role in pathogen inhibition.

The fraction-based approach adopted in this study represents a methodological advance, as it narrows the search for active compounds and supports the development of more targeted biocontrol formulations.

Overall, the results confirm that antagonistic Bacillus spp. constitute a promising alternative to conventional chemical control methods for potato disease management. By focusing on active metabolite fractions, this work lays the foundation for future identification, optimization, and formulation of bioactive compounds, contributing to the development of environmentally friendly antimicrobial agents.

The study thus supports the transition toward sustainable agricultural practices and reinforces the potential of microbial metabolites as key tools in plant disease control.

References

1. Pérombelon, M. C. M. (2002). Potato diseases caused by soft rot erwinias: an overview of pathogenesis. Plant pathology, 51(1), 1-12.
2. Toth, I. K., Barny, M. A., Brurberg, M. B., Condemine, G., Czajkowski, R., Elphinstone, J. G., ... & Yedidia, I. (2021). Pectobacterium and Dickeya: environment to disease development. In Plant diseases caused by Dickeya and Pectobacterium species (pp. 39-84). Cham : Springer International Publishing. https://doi.org/10.1007/978-3-030-61459-1_3
3. Hugouvieux-Cotte-Pattat, N., Condemine, G., & Shevchik, V. E. (2014). Bacterial pectate lyases, structural and func-

tional diversity. Environmental microbiology reports, 6(5), 427-440.

4. Hothem, S. D., Marley, K. A., & Larson, R. A. (2003). Photochemistry in Hoagland's nutrient solution. Journal of Plant Nutrition, 26(4), 845-854.
5. Faure, D., & Dessaux, Y. (2007). Quorum sensing as a target for developing control strategies for the plant pathogen Pectobacterium. European Journal of Plant Pathology, 119(3), 353-365.
6. Elhalag, K. M., Al-Anany, M. S., & Megahed, A. A. (2025). Efficacy of plant extracts and bio-fertilizers for the control of bacterial wilt and soft rot agents in potato. Journal of Plant Pathology, 107(2), 897-917.
7. Melkumyan, M., Babayan, B., Yesayan, A., Yesayan, T., Sevoyan, G., Grigoryan, A., & Grigoryan, A. (2025). Pseudomonas fluorescens: Prospective green antimicrobial for crops cultivation. Bioactive Compounds in Health and Disease-Online ISSN: 2574-0334, 8(8), 269-278.
8. Baz, M., Lahbabi, D., Samri, S., Val, F., Hamelin, G., Madore, I., ... & Barakate, M. (2012). Control of potato soft rot caused by Pectobacterium carotovorum and Pectobacterium atrosepticum by Moroccan actinobacteria isolates. World Journal of Microbiology and Biotechnology, 28(1), 303-311.
9. Cheffi, M., Chenari Bouket, A., Alenezi, F. N., Luptakova, L., Belka, M., Vallat, A., ... & Belbahri, L. (2019). Olea europaea L. root endophyte Bacillus velezensis OEE1 counteracts oomycete and fungal harmful pathogens and harbours a large repertoire of secreted and volatile metabolites and beneficial functional genes. Microorganisms, 7(9), 314.
10. R Core Team. (2016). R : A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www. R-project. org/.