*Original Research Article*

Rhizosphere competency of *Bacillus* spp. and their biocontrol efficacy against *Rhizoctonia solani* and *Streptomyces scabies* under field conditions

.

ABSTRACT

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| **Background:** Potato (Solanum tuberosum L.) is susceptible to soil-borne pathogens such as Rhizoctonia solani AG-3 and Streptomyces scabies, which cause black scurf and common scab, respectively. These pathogens persist in the soil and are difficult to control using conventional chemical methods. Therefore, biocontrol agents such as Bacillus subtilis B4 and B. amyloliquefaciens B7 have emerged as promising alternatives due to their strong antagonistic activity and plant growth-promoting properties.**Methods:** Field trials were carried out using talc-based formulations of *Bacillus* spp., applied through tuber dipping (10 g and 15 g/L) and soil application (2.5 kg and 3.5 kg/?), either individually or in combination. Rhizosphere soil samples were collected at 0, 15, 30, 60, and 90 days after sowing to determine colony-forming units (cfu/g) and evaluate rhizosphere persistence.**Results:** The combined treatment of tuber dipping (15 g/L) and soil application (3.5 kg) resulted the highest CFU values. B. subtilis B4 reached 6.4 × 10⁹ and 7.3 × 10⁹ cfu/g in 2019 and 2020, respectively. Though populations declined over time, viable counts of approximately 5.9 log₁₀ CFU /g were still detected at 90 days. ANOVA revealed significant effects (p<0.001) of treatment, bacterial strain, and sampling time, while yearly differences were non-significant. The same treatment enhanced emergence, plant vigor, and yield (213.75 qt/acre), while reducing disease severity compared with the control (143.00 qt/acre).**Conclusion:** Bacillus subtilis B4 and B. amyloliquefaciens B7 confirmed strong rhizosphere competency and disease suppression under field conditions. The integrated application method continued bacterial populations and improved plant performance, indicating that Bacillus-based bioformulations are a viable eco-friendly approach for cotrolling major potato diseases. |

*Keywords: Bacillus subtilis*, Bacillus amyloliquefaciens, Rhizosphere competency, Black scurf and common scab

1. INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important food crop worldwide and is extensively cultivated across temperate and subtropical regions (Mahr, 2021). However, its cultivation is severely constrained by soil-borne diseases, particularly black scurf and common scab. Black scurf is caused by *Rhizoctonia solani* AG-3, a fungus capable of persisting in soil for years through sclerotia (Arora and Khurana, 2004). It affects seed germination, plant vigor, and tuber quality. Common scab, primarily caused by *Streptomyces scabies*, results from the production of thaxtomin A, a phytotoxin involved in pathogenesis (Saber et al., 2015). Both diseases are difficult to manage due to their soil-borne nature and wide host range. Chemical management strategies often lead to environmental contamination and the development of resistance in pathogens (Dukare et al., 2019). Consequently, biological control has emerged as a safer, eco-friendly alternative. Among biological control agents (BCAs), Bacillus spp. is well-recognized for their ability to produce antimicrobial compounds, enzymes like chitinase and glucanase, and for their ability to colonize the rhizosphere (Miljaković et al., 2022). This study aims to evaluate the rhizosphere competency of *Bacillus* spp. and their biocontrol potential against black scurf and common scab of potato under field conditions.2. material and methods

The primary goal of this study was to assess the rhizosphere competency of *Bacillus* spp. alongwith management of black scurf and common scab. *Bacillus* spp. possesses strong rhizosphere competency, which is essential for their success as BCAs.

**2.1 Mass culturing and preparation of bioformulation**

Potent *Bacillus* spp. isolates were conveyed individually in commercial talc powder (magnesium silicate) using a modified method based on Suryadi et al. (2021). The talc powder, serving as a carrier, was sterilized by autoclaving at 121∘C and 15 psi for 30 minutes. A bacterial suspension was prepared in Nutrient Broth, and 600 ml of this broth culture was mixed with 1 kg of the sterilized talcum powder. The resulting mixture was shade-dried. To improve adhesion, 1% Carboxy Methylcellulose (CMC) was incorporated before packaging the bioformulation.

**2.2 Evaluation of *Bacillus* spp. based bioformulations**

Field trials were conducted at the Department of Plant Pathology, Punjab Agricultural University (PAU), to assess the efficacy of bioformulations derived from selected Bacillus spp. strains against *Rhizoctonia solani* and *Streptomyces scabies*, the causal agents of black scurf and common scab in potato, respectively. The potato variety 'Kufri Pukhraj', known for its susceptibility to both diseases (Singh et al., 2021), was used for the trials. Tuber treatment involved immersing the seed potatoes in a solution containing either 10 or 15 g of the Bacillus formulation per litre of water for 10–15 minutes. For soil application, 2.5 kg of the Bacillus formulation was thoroughly mixed with 25 kg of well-decomposed farmyard manure (FYM) and incubated for 72 hours prior to field application. Treatments comprised tuber dip alone, soil treatment alone, a combination of both, a standard chemical control, and an untreated infected control, each replicated three times.

To assess rhizosphere colonization, root-adjacent soil and small root sections (approximately 10 mm in length) were collected. These samples were suspended in 10 ml of 10 mM phosphate buffer (pH 7.2) and agitated at 150 rpm on a rotary shaker at 30°C for one hour. Following serial dilution, aliquots were spread onto nutrient agar (NA) plates, which were then incubated at 28 ± 2°C for 48 hours. Colony counts were used to estimate the population density of Bacillus spp. per gram of soil, following the methodology described by Das et al. (2010) and Killani et al. (2011).

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| CFU per gram of soil | = | No of colonies × Dilution made × Fresh wt. of the soil |
| Oven dry weight of the soil |

**2.3 Statistical Analysis**

For statistical analysis, all data was transformed and analyzed by Analysis of Variance (ANOVA) by using Randomized Block Design (RBD) using relevant R packages. Post-hoc comparisons among treatment means were achieved using Tukey’s Honest Significant Difference (HSD) test and Fisher’s Least Significant Difference (LSD) (Kara and Arici 2019).

3. results and discussion

Two antagonistic strains of *Bacillus* spp., formerly isolated and molecularly identified, were exploited in this study. These strains included: *Bacillus subtilis* B2 (ON479589) and *Bacillus amyloliquefaciens* B7 (ON489306). The isolates were preserved on nutrient agar slants for further experimentation. Different treatments of B4 and B7 showed a positive effect on tuber sprouting compared to untreated control. Under field conditions, maximum sprouting was observed in 15g ± Soil 3.5kg treatment of B4 and B7 i.e. 91.67 % and 90.56.00% respectively as compared to untreated control i.e. 75.56%. Tuber Dip 15g ± Soil 3.5kg of B4 and B7 reduced the common scab severity by 8.89 % and 11.11 %, respectively as compared to untreated control 55.56%.Tuber Dip 15g ± Soil 3.5kg of B4 also showed maximum total length (shoot and root length) 65.39 cm followed by B7 (Tuber Dip 15g ± Soil 3.5kg) 64.14 cm as compared to untreated control 30.05 cm. A similar trend was also recorded in yield as above. Maximum yield in B4 (Tuber Dip 15g ± Soil 3.5kg) under field conditions 213.75 as compared to untreated control i.e. 143.00qt/acre (Singh et al. 2025). The Tuber Dip 15g + Soil 3.5kg (B 4), Tuber Dip 15g + Soil 2.5kg (B 4), Tuber Dip 15g + Soil 3.5kg (B 7) and Tuber Dip 10g + Soil 3.5kg (B 4) had lowest pooled mean black scurf disease severity of 8.00, 9.33, 11.33 and 13.33 per cent (Singh *et al* 2022).

The persistence and colonization skill (rhizosphere competency) of Bacillus spp. bioformulations were monitored throughout the crop growth period to determine the duration of their effectiveness. Among the treatments, the combined use of Tuber Dipping (15 g) and Soil Treatment (3.5 kg/?) consistently exhibited the highest colony-forming unit (cfu) counts per gram of soil across all Bacillus isolates tested. Notably, *Bacillus subtilis* B4 exhibited the highest initial population with 6.4 × 10⁹ cfu/g in 2019 and 7.3 × 10⁹ cfu/g in 2020 at day 0. In comparison, *Bacillus amyloliquefaciens* B7 recorded 9.1 × 10⁸ and 3.2 × 10⁹ cfu/g for the same time points.

Over time, a steady reduction in bacterial population was observed across all treatments. For *B. subtilis* B4, the cfu count declined to 2.2 × 10⁶ and 5.4 × 10⁶ at 60 days post-application during 2019 and 2020, respectively. In the case of *B. amyloliquefaciens* B7, 1.8 × 10⁶ cfu/g was noted at 45 days and 4.2 × 10⁶ at 60 days (Table 1). The overall trend indicated a decline in viable bacterial counts over time; however, higher initial counts were maintained longer in treatments where higher inoculum loads were applied, particularly in soil-only and combination treatments.

The population dynamics of *Bacillus subtilis* (B4) and *Bacillus amyloliquefaciens* (B7) were assessed across different treatments under field conditions over a 90-day period during 2019 and 2020. The bacterial population was expressed as CFU per gram of soil and subsequently transformed into log₁₀ values to normalize variance and facilitate statistical comparison. Across all treatments, the bacterial population declined gradually over time. However, the rate and extent of decline varied significantly between treatments, isolates, and years. Based on log-transformed CFU values the Anova interpretation is that the Year factor does not show a statistically significant effect (F=2.73, p=0.100). This implies that the overall CFU counts did not significantly differ between 2019 and 2020. The Species factor shows a significant effect (F=25.90, p<0.001). This recommends that there is a significant difference in the performance of *Bacillus subtilis* B4 and *Bacillus amyloliquefaciens* B7. The Treatments factor has a highly significant effect on the log-transformed CFU counts (F=220.32, p<0.001). This indicates that different treatments significantly impact the *Bacillus* population in the soil. The Days factor also has a highly significant effect (F=631.69, p<0.001)(Table 1).

This is expected, as microbial populations often change over time, typically decreasing after an initial application. Also showed that treatment effect is significant and Soil + Tuber combination showed much higher rhizosphere persistence (Log ~7+). The Tukey HSD test for treatments reveals several significant differences (p<0.05). Tuber Dip 10g had significantly lower CFU counts compared to Soil 2.5 kg (p=0.0014), Tuber Dipping 10g/? + Soil 2.5kg/? (p=0.0014), Tuber Dipping 15g/? + Soil 2.5kg (p=0.000) and Tuber Diping 15g/? + Soil 3.5kg (p=0.000). The coefficient of variation (CV) was

**Table 1: Rhizosphere competence of *Bacillus* spp. isolates treatments under field conditions**

| Treatments | *Bacillus* spp. (cfu/g of soil) \* |
| --- | --- |
| Days | *Bacillus subtilis* B4 | *Bacillus amyloliquefaciens* B7 |
| 2019 | 2020 | 2019 | 2020 |
| Tuber Dip 10g | 0 | 3.6 × 106 (6.56) | 3.3 × 106 (6.52) | 5.2 × 106 (6.72) | 7.3 × 106 (6.86) |
| 15 | 2.4 × 105 (5.38) | 4.8 × 105 (5.68) | 3.5 × 105 (5.54) | 3.7 × 105 (5.57) |
| 30 | 6.8 × 104 (4.83) | 5.1 × 104 (4.71) | 1.1 × 105 (5.04) | 4.3 × 104 (4.63) |
| 45 | 2.9 × 103 (3.46) | 2.3 × 104 (4.36) | 6.3 × 104 (4.80) | 5.9 × 104 (4.77) |
| 60 | 6.2 × 102 (2.79) | 7.6 × 103 (3.88) | 1.9 × 103 (3.28) | 4.5 × 103 (3.65) |
| 90 | 4.1 × 102 (2.61) | 2.0 × 102 (2.30) | 7.1 × 102 (2.85) | 3.2 × 102 (2.51) |
| Tuber Dip 15g  | 0 | 3.9 × 106 (6.59) | 4.6 × 106 (6.66) | 3.2 × 106 (6.51) | 6.4 × 106 (6.81) |
| 15 | 1.9 × 106 (6.28) | 7.4 × 105 (5.87) | 1.6 × 106 (6.20) | 6.2 × 105 (5.79) |
| 30 | 7.6 × 105 (5.88) | 4.6 × 105 (5.66) | 4.4 × 105 (5.64) | 8.3 × 104 (4.92) |
| 45 | 5.2 × 105 (5.72) | 6.7 × 104 (4.83) | 3.1 × 104 (4.49) | 5.1 × 104 (4.71) |
| 60 | 3.7 × 104 (4.57) | 5.1 × 104 (4.71) | 2.7 × 104 (4.43) | 7.1 × 103 (3.85) |
| 90 | 5.3 × 103 (3.72) | 1.2 × 103 (3.08) | 8.4 × 103 (3.92) | 1.6×103 (3.20) |
| Soil 2.5 kg  | 0 | 4.5 × 108 (8.65) | 5.2 × 108 (8.72) | 3.8 × 108 (8.58) | 3.9 × 108 (8.59) |
| 15 | 7.2 × 107 (7.86) | 7.9 × 107 (7.90) | 4.4 × 107 (7.64) | 6.7 × 107 (7.83) |
| 30 | 8.5 × 106 (6.93) | 7.1 × 106 (6.85) | 4.9 × 106 (6.69) | 1.6 × 106 (6.20) |
| 45 | 3.6 × 105 (5.56) | 7.3 × 105 (5.86) | 6.4 × 105 (5.81) | 6.2 × 105 (5.79) |
| 60 | 4.9 × 104 (4.69) | 5.8 × 104 (4.76) | 4.5 × 104 (4.65) | 3.7 × 104 (4.57) |
| 90 | 6.5 × 103 (3.81) | 2.1 × 104 (4.32) | 1.1 × 104 (4.04) | 7.3 × 103 (3.86) |
| Soil 3.5 kg  | 0 | 4.5 × 108 (8.65) | 5.4 × 108 (8.73) | 3.2 × 108 (8.51) | 3.7 × 108 (8.57) |
| 15 | 3.1 × 107 (7.49) | 4.4 × 107 (7.64) | 2.9 × 107 (7.46) | 3.4 × 107 (7.53) |
| 30 | 6.2 × 106 (6.79) | 7.2 × 106 (6.86) | 5.8 × 106 (6.76) | 6.1 × 106 (6.79) |
| 45 | 7.9 × 105 (5.90) | 8.1 × 105 (5.91) | 4.7 × 105 (5.67) | 7.5 × 105 (5.88) |
| 60 | 6.9 × 104 (4.84) | 7.3 × 104 (4.86) | 5.3 × 104 (4.72) | 5.9 × 104 (4.77) |
| 90 | 1.4 × 104 (4.15) | 2.2 × 104 (4.34) | 1.1 × 104 (4.04) | 1.3 × 104 (4.11) |
| Tuber Dip 10g + Soil 2.5kg | 0 | 6.9 × 108 (8.84) | 7.2 × 108 (8.86) | 2.2 × 108 (8.34) | 3.4 × 108 (8.53) |
| 15 | 1.9 × 107 (7.28) | 3.5 × 107 (7.54) | 1.4 × 107 (7.15) | 5.2 × 107 (7.72) |
| 30 | 5.2 × 106 (6.72) | 4.6 × 106 (6.66) | 4.9 × 106 (6.69) | 2.2 × 106 (6.34) |
| 45 | 3.3 × 105 (5.52) | 5.9 × 105 (5.77) | 1.1 × 105 (5.04) | 4.5 × 105 (5.65) |
| 60 | 8.7 × 104 (4.94) | 9.2 × 104 (4.96) | 6.7 × 104 (4.83) | 7.8 × 104 (4.89) |
| 90 | 5.3 × 104 (4.72) | 3.2 × 104 (4.51) | 1.6 × 104 (4.20) | 2.6 × 104 (4.41) |
| Tuber Dip 10g + Soil 3.5kg | 0 | 2.3 × 109 (9.36) | 3.4 × 109 (9.53) | 2.8 × 108 (8.45) | 4.7 × 108 (8.67) |
| 15 | 5.1 × 108 (8.71) | 7.9 × 108 (8.90) | 4.4 × 107 (7.64) | 6.7 × 107 (7.83) |
| 30 | 7.5 × 107 (7.88) | 3.1 × 107 (7.49) | 3.9 × 106 (6.59) | 1.6 × 107 (7.20) |
| 45 | 4.4 × 106 (6.64) | 7.4 × 106 (6.87) | 9.4 × 105 (5.97) | 7.3 × 106 (6.86) |
| 60 | 6.9 × 105 (5.84) | 5.9 × 105 (5.77) | 3.5 × 105 (5.54) | 2.7 × 105 (5.43) |
| 90 | 2.4 × 105 (5.38) | 3.1× 105 (5.49) | 7.1 × 104 (4.85) | 6.3 × 104 (4.80) |
| Tuber Dip 15g + Soil 2.5kg | 0 | 2.6 × 109 (9.41) | 4.2 × 109 (9.62) | 3.2 × 108 (8.51) | 5.9 × 108 (8.77) |
| 15 | 3.2 × 108 (8.51) | 2.4 × 108 (8.38) | 6.9 × 107 (7.84) | 1.5 × 108 (8.18) |
| 30 | 7.1 × 107 (7.85) | 7.0 × 107 (7.85) | 2.1 × 107 (7.32) | 6.2 × 107 (7.79) |
| 45 | 6.9 × 106 (6.84) | 1.2 × 107 (7.08) | 2.7 × 106 (6.43) | 3.4 × 106 (6.53) |
| 60 | 1.8 × 106 (6.26) | 3.7 × 106 (6.57) | 5.6 × 105 (5.75) | 6.2 × 105 (5.79) |
| 90 | 5.7 × 105 (5.76) | 6.1×105 (5.79) | 2.4 × 105 (5.38) | 3.3 × 105 (5.52) |
| Tuber Dip 15g + Soil 3.5kg | 0 | 6.4 × 109 (9.81) | 7.3 × 109 (9.86) | 9.1 × 108 (8.96) | 3.2 × 109 (9.51) |
| 15 | 4.9 × 108 (8.69) | 5.2 × 108 (8.72) | 2.7 × 108 (8.43) | 1.2 × 108 (8.08) |
| 30 | 7.3 × 107 (7.86) | 8.3 × 107 (7.92) | 6.6 × 107 (7.82) | 7.8 × 107 (7.89) |
| 45 | 8.3 × 106 (6.92) | 2.2 × 107 (7.34) | 1.8 × 106 (6.26) | 1.7 × 107 (7.23) |
| 60 | 2.2 × 106 (6.34) | 5.4 × 106 (6.73) | 8.4 × 105 (5.92) | 4.2 × 106 (6.62) |
| 90 | 8.5 × 105 (5.93) | 9.2 × 105 (5.96) | 4.9 × 105 (5.69) | 5.6 ×105 (5.75) |
| CD(p≤0.05) Treatments: 0.1900, Days: 0.1645, Isolates: 0.0950, Year: 0.0950 |

\* → Mean of three replications, Values in the parenthesis are log-transformed (base 10) values of respective means

calculated as 25.54%, which is within the acceptable biological range, indicating reliable data consistency. Tuber Dip 15g + Soil 3.5 kg was the best. Highest log CFU at day 0 (B4: 6.4e9 → log ≈ 9.81). This treatment-maintained log CFU ~5.9 even after 90 days for both strains and found to have best rhizosphere competency under field conditions. Although CFU counts naturally decline over time, So, combined treatment (tuber + soil) at higher doses is most effective.

The present study clearly validates the efficacy of *Bacillus* spp. in managing major potato diseases- black scurf and common scab, along with their role in encouraging plant growth and colonizing the rhizosphere under field conditions. *Bacillus subtilis* and related species have consistently confirmed antagonistic activity against *Rhizoctonia solani*, the causal agent of black scurf. For instance, Hussain and Khan (2020) described that *B. subtilis* strain HussainT-AMU reduced black scurf incidence by up to 71% in pot trials and 50% in field conditions. Similarly, Brewer and Larkin (2005) found a 30% reduction in disease severity with *B. subtilis* GB03 over three years of field application. High disease suppression efficacy was also observed by Ben Khedher *et al*. (2015), where B. subtilis V26 reduced black scurf by 81% using 10⁹ spores/ml of a mixed culture. Ali *et al*. (2017) further confirmed that both tuber and soil treatments with *B. subtilis* significantly reduced disease index by 72.75% and 63.76%, respectively. In greenhouse conditions, Saber et al. (2015) found that *B. subtilis* ATCC 11774 reduced disease severity by 30%, while Kumar et al. (2013) detected 30-41.45% reductions using *Bacillus* strains D-4 and E-5.

The suppression of common scab, caused by *Streptomyces scabies*, by *Bacillus* spp. was also significant. Lin et al. (2018) showed that *B. amyloliquefaciens* Ba01 application reduced disease severity from 14.4% to 5.6% under natural field conditions. When applied at 2 × 10⁷ cfu/ml, disease incidence dropped from 21% to 5%. Chen et al. (2017) verified that *B. laterosporus* AMCC100017 reduced *Streptomyces* populations and lowered disease severity from 2.60 to 0.77, achieving a biocontrol efficacy of 70.51%. Similar success was described by Li et al. (2019), where *B. altitudinis* AMCC 101304 reduced incidence from 100% to 34.19% (single treatment), with control efficiency up to 82.5%. Cui et al. (2020; 2022) also reported effective scab control by *B. velezensis* strain 8-4 (51.83% efficiency) and *B. amyloliquefaciens* 3-5 (38.9% efficiency), surpassing chemical bactericides. Other reports, including Hassan et al. (2021) and Yu-Cong et al. (2018), confirmed that B. subtilis significantly reduced scab severity (up to 60%) and improved marketable yields.

Apart from disease suppression, *Bacillus* spp. demonstrated considerable plant growth–promoting abilities. Kumar *et al*. (2013) reported increased shoot and root length in Bacillus-treated plants under both pot and field conditions, with plant heights of 53.80 and 58.40 cm compared to 44.20 cm in the control. Similarly, Ali et al. (2017) observed plant heights of 28.67 cm and 25.00 cm with B. subtilis treatments, significantly exceeding the 20.00 cm seen in untreated plants. Ben Khedher et al. (2015) verified a height increase from 35.1 cm to 48.4 cm with B. subtilis V26 application. Bacillus spp. also enhanced nutrient uptake and hormone production. As noted by Sivasakthi et al. (2014), phosphate-solubilizing Bacillus spp. increased the availability of phosphorus, iron, and zinc, improved biological nitrogen fixation, and stimulated growth via metabolites like auxins, cytokinins, and gibberellins. These direct and indirect mechanisms contribute to robust plant growth under biotic stress.

Understanding the rhizosphere competency of *Bacillus* spp. has progressed significantly over time, highlighting their ability to colonize, persist, and function efficiently in the root zone under field conditions. Early indications came from Brewer and Larkin (2005), who observed sustained disease suppression by *B. subtilis* GB03 over three years of field application, suggesting stable rhizosphere presence. Somani and Arora (2010) further supported this by showing that seed dip treatments with *B. subtilis* B4 led to a 24% decrease in black scurf, implying successful root colonization post-planting. By 2013, Larkin and Tavantzis showed that *B. subtilis* GB03 in combination with compost not only reduced disease but enhanced tuber yield, likely due to improved root-zone microbial activity. A major advance came from Chen et al. (2017), who directly quantified *B. laterosporus* AMCC100017 populations in the potato rhizosphere (5.47–6.87 log₁₀ cfu/g soil), confirming strong colonization linked to reduced common scab severity. In similar, Meng and Hao (2017) applied *B. amyloliquefaciens* BAC03 to radish and observed improved biomass and disease suppression, suggesting root-associated activity. Lin et al. (2018) added that Ba01 applied at 2 × 10⁷ cfu/ml significantly reduced common scab, showing a dose-dependent colonization effect and highlighting its ability to compete in the rhizosphere while producing antifungal metabolites. More recently, Hussain and Khan (2020) showed that both bacterial suspensions and culture filtrates of *B. subtilis* HussainT-AMU effectively reduced disease under field conditions, further affirming field-level rhizosphere persistence.

4. Conclusion

This study confirms that *Bacillus subtilis* and *Bacillus amyloliquefaciens* are effective rhizosphere colonizers with strong potential to control black scurf and common scab of potato under field conditions. Their capability to persist in the rhizosphere, suppress pathogens, and promote plant growth highlights their value as eco-friendly alternatives to chemical controls. The collective application of *B. subtilis* B4 or *B. amyloliquefaciens* B7 through tuber dipping (15 g/?) + soil application (3.5 kg/?) was the most effective strategy for ensuring long-term colonization and disease suppression. These strains hold potential for development into commercial bioformulations to support sustainable potato cultivation.

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