

# ENHANCEMENT OF TOMATO GROWTH AND BIOCONTROL OF *FUSARIUM SOLANI* ROOT ROT DISEASE BY *STREPTOMYCES ROCHEI* BT02

LÊ THANH ĐẠM, NGUYỄN LƯU VĨNH HẢO, NGUYỄN THỊ HỒNG HẠNH, THẠCH THỊ THẢO, QUẢNG THỊ ANH ĐÀO, PHAN MINH ĐẠT AND NGUYỄN VŨ PHONG\*

*Faculty of Biological Sciences, Nong Lam University, Ho Chi Minh City 72820, Vietnam.*

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## ABSTRACT

This study investigated the dual role of *Streptomyces rochei* BT02 in promoting tomato growth and suppressing *Fusarium solani*, the causative agent of tomato root rot disease. Various assays were conducted to evaluate the plant growth-promoting attributes of strain BT02 including indole-3-acetic acid (IAA) biosynthesis, phosphate solubilization, biofilm formation, and siderophore production. Under *in vitro* conditions, strain BT02 exhibited plant growth-promoting characteristics such as cellulose degradation, phosphate solubilization, IAA synthesis, biofilm formation, and siderophore production. Application of strain BT02 significantly improved tomato seed germination (26.7%), with enhanced sprout and radicle lengths and seed vigor (38.6%). Greenhouse experiments revealed substantial increases in plant height, leaf and branch numbers, inflorescence, and flower number, emphasizing the positive impact of strain BT02 on overall plant growth. The BT02 strain demonstrated antagonistic efficacy of 45.3 to 58.9% against *Fusarium solani*, as evidenced by the dual culture and agar well diffusion methods. Under greenhouse conditions, strain BT02 significantly reduced disease incidence and demonstrated control efficacy of 41.6% against *Fusarium solani*, highlighting its biocontrol potential. This study provides insights into the mechanisms employed by *Streptomyces rochei* BT02 to enhance plant growth and suppress root rot disease, paving the way for its application as a biocontrol agent in sustainable agriculture.

**Keywords:** *Fusarium solani* biocontrol, plant growth-promoting bacteria (PGPB), root rot disease control, *Streptomyces rochei* BT02; tomato growth promotion

## INTRODUCTION

With ever-increasing global population growth and the consequential negative environmental impacts, ensuring the quality of agricultural products poses a formidable challenge for both farmers and researchers. The adoption of biofertilizers and biotic agents, together with judicious farming management practices, have attracted significant attention within the scientific community, with the utilization of plant growth-promoting bacteria (PGPB) extensively documented.

PGPB are ubiquitously distributed in soil and on root surfaces or endophytically within roots. They exhibit remarkable capabilities of fostering

plant growth and curtailing plant diseases through multifaceted mechanisms encompassing direct processes such as phosphorus solubilization, nitrogen fixation, siderophore production, hydrogen cyanide and ammonia release as well as the synthesis of phytohormones including auxin, cytokinin, and gibberellin. Indirect mechanisms include ACC deaminase activity, antibiotic production, secretion of cell wall degradation enzymes, and induction of systemic resistance (Ryu *et al.* 2003).

Among plant growth-promoting rhizobacteria (PGPR), *Streptomyces* species are widely distributed in soil, water, and agricultural ecosystems. This diversity in nutritional and physiological processes equips them with adaptability to adverse environmental factors including temperature fluctuations, humidity

\*Corresponding author, email: nvphong@hcmuaf.edu.vn

variations, oxygen content, nutrient availability, and the presence of toxic substances. *Streptomyces* species produce an array of secondary metabolites with biocontrol activities against plant parasitic fungi, thereby contributing to their significance in agriculture (Nazari *et al.* 2023).

These secondary metabolites encompass phytohormones, siderophores, enzymes, volatile organic compounds (VOCs), antibiotics, and various other bioactive substances. *Streptomyces* biocontrol strains, leveraging both direct antimicrobial activity and the induction of plant resistance through indirect biosynthetic pathways, show high promise for plant protection and as growth-promoting agents in agricultural contexts. *Streptomyces* species also showcase insecticidal, antibacterial, and antifungal activities, further enhancing their potential applications in sustainable agriculture.

*Fusarium* root rot, induced by *Fusarium solani*, constitutes a significant threat in tomato production regions. *Streptomyces* species have shown inhibitory effects against this phytopathogenic fungus. *Streptomyces kebangsaanensis*, isolated from a Malaysian ethnomedicinal plant, displayed antifungal activity against *F. solani* isolates, with the contained phenazine-1-carboxylic acid (PCA) exhibiting enhanced inhibitory effects in combination with amphotericin B (Elshafie & Camele 2022). Another *Streptomyces* strain, *S. sasae* TG01, inhibited the growth of both *F. solani* and *F. oxysporum*, suggesting its potential as a biocontrol agent (Mazlan *et al.* 2020), while the biological control *Streptomyces* strain, *Streptomyces* sp. Lnu-12 exhibited high inhibition activity against melon *Fusarium*, *Fulvia fulva*, and *Alternaria solani* indicating its potential to control soil-borne diseases in vegetables (Bubici 2018).

*Streptomyces rochei* has been extensively investigated in various contexts, with strain IDWR19 demonstrating significant plant growth-promoting (PGP) activities, enzyme production, and positive effects on plant growth, manifesting as a 12.2% increase in shoot length and a 1.8-fold increase in biomass (Jog *et al.* 2012). In a previous study, *S. rochei* (MT122809) emerged as a potential antagonist to various pathogens, producing microbial volatile organic compounds (mVOCs) that inhibited the growth of sorghum grain mold pathogens while enhancing plant growth (Sudha *et al.* 2022). *Streptomyces rochei* ACTA1551

exhibited robust suppression of *Fusarium oxysporum* *in vitro* and also protected tomato seeds from infection *in vivo*, demonstrating potential as a biocontrol agent against the pathogen (Kanini *et al.* 2013), while *S. rochei* AMET 311 strain exhibited *in vitro* activity against *Rhizoctonia solani* (Venkatramani & Jayaprakashvel 2023).

This study elucidated the characteristics associated with the ability of *Streptomyces rochei* BT02 to promote plant growth and control *Fusarium solani*, the causal agent of tomato root rot disease. Insights garnered from this investigation will provide a foundation for better selection and formulation of bioproducts conducive to sustainable agriculture.

## MATERIALS AND METHODS

### Materials

Strains of *Streptomyces rochei* PN02 and *Fusarium solani* were isolated, identified, and preserved at the Plant Integrated Biology (PIB) Laboratory of Nong Lam University, Ho Chi Minh City (Tân *et al.* 2023). Phu Nong F1 (T-11) tomato seeds were utilized in this study.

### Determination of the ability of *Streptomyces rochei* PN02 to promote plant growth

Bacterial cultures were incubated in Gause 1 or ISP2 media for 5-7 days at 30°C with shaking at 150 rpm. The bacterial suspension was prepared with an optical density of 0.1 at 600 nm. All experiments were conducted in a completely randomized design with four replicates. The cellulolytic capability of bacteria is determined by clear yellow halos around the bacterial colonies on CMC agar medium (Teather and Wood 1982). The ability to dissolve phosphate was assessed by the appearance of a clear zone of  $\text{Ca}_3(\text{PO}_4)_2$  on Pikovskaya's agar medium after 7 days of cultivation at 30°C (Nautiyal 1999). Indole-3-acetic acid (IAA) production by bacterial strains cultured in ISP2 medium was detected and quantified using the Salkowski reagent colorimetric method at a wavelength of 520 nm (Sasirekha *et al.* 2012). Gibberellic acid (GA) production was quantified using the extraction method with ethyl acetate, and absorbance was measured at 254 nm (Berríos *et al.* 2004). The ability to form biofilms was determined using the

method described by O'Toole *et al.* (O'Toole *et al.* 2000) using Gause 1 medium. Siderophore production was assessed on CAS agar medium according to Srinivas *et al.* (Srinivas *et al.* 2020), and nitrogen fixation of the bacterial strains was determined according to Bashan *et al.* (Bashan *et al.* 1998).

### **Investigation of the influence of *Streptomyces rochei* BT02 on tomato plant growth**

As *in vitro* conditions, the tomato seeds were sterilized and soaked in a bacterial suspension ( $10^9$  CFU/mL) for 60 minutes. At least 30 seeds were sown in Petri dishes containing moistened cotton wool and incubated at  $25 \pm 1^\circ\text{C}$ . Seed germination was monitored after 2, 4, and 7 days. Six germinated 2-day-old tomato seeds were placed on water agar medium and divided into two sections. A paper disk soaked in 20  $\mu\text{L}$  of bacterial solution ( $10^9$  CFU/mL) was placed 2.5 cm from the root tip. Distilled water was used as a control. The medium plates were positioned vertically in a growth chamber and co-cultivation was carried out for 5 days under conditions of 16 hours light/8 hours dark at  $25^\circ\text{C}$  and 70% humidity. Parameters including germination rate (%), sprout length (cm), plumule length (cm), radicle length (cm), and seed vigor were recorded (Thilagam & Hemalatha 2019).

Under greenhouse conditions, healthy tomato seedlings with three fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer at a ratio of 4:1:1:1. When the plants had five true leaves, 8 mL of bacterial culture ( $10^7$  CFU/mL) was irrigated at the base of each plant, and repeated once a week for four weeks. Plant height, leaf number, branch number, inflorescence, flower number, and fruit quality were monitored (Dias *et al.* 2017).

### **Evaluation of the antagonistic ability of *Streptomyces rochei* BT02 against *Fusarium solani***

The dual culture method was performed according to Ferreira *et al.* (1991) by measuring the fungal colony radius and calculating the antagonistic efficacy following the formula of Moayed (2010), and the rating scale of Soyong (Soyong 2010). The agar well diffusion method

was performed according to Sharma *et al.* (2017). All antagonistic activity of bacteria was demonstrated through the formation of inhibition zones after a 48-hour incubation period, calculated using the formula of Azman *et al.* (2017). The experiments were arranged in a completely randomized design and repeated three times with four Petri dishes in each replication.

Healthy tomato seedlings with three fully developed leaves were transplanted into pots (25 x 21 cm) containing clean soil, manure, lime, and phosphate fertilizer at a ratio of 4:1:1:1. When the plants had five true leaves, root injury was created, followed by irrigation of 5 mL of *F. solani* spore suspension ( $10^6$  spores/mL) and 8 mL of a bacterial suspension ( $10^7$  CFU/mL) around the plant base, simultaneously or after fungal inoculation depending on the treatment. The experiments were arranged in a randomized complete block design, with each treatment replicated three times and four pots per treatment. The pots were placed in a greenhouse, and watering was provided as per the plant requirements. Disease incidence (%) and disease index (%) after infection at 7, 14, 21, and 28 days were recorded, and the AUDPC value was calculated according to Jeger & Viljanen-Rollinson (2001). The efficacy of disease control was calculated according to Abbott (1925).

### **Data analysis**

The experimental data were analyzed by Analysis of Variance (ANOVA), with means compared using Duncan's test. The data were transformed to ensure compliance with the normal distribution if necessary.

## **RESULTS AND DISCUSSION**

### **Plant growth-promoting ability of *Streptomyces rochei* BT02**

Under *in vitro* conditions, the BT02 strain demonstrated the ability to degrade cellulose, solubilize phosphate, synthesize IAA, form biofilms, and produce siderophores. However, it did not produce gibberellic acid or fix nitrogen (Table 1). Seeds treated with the BT02 strain exhibited a germination rate of 92.5% after 72 hours, compared to the control at 67.8%. Notably, BT02-treated seeds showed longer

sprouts (1.10 cm), radicle length (4.02 cm), and increased vigor (569.8), with statistically significant differences ( $p < 0.01$ ) (Table 2; Figure 1).

Under greenhouse conditions at 28 days after inoculation, plants treated with the BT02 strain reached a height of 79.6 cm, surpassing the control plants at 74.7 cm. The BT02 treatment significantly influenced plant growth,

demonstrating increases in the number of leaves (19.4), branches (1.30), inflorescence (7.30), and flowers (14.3) ( $p < 0.05$ ) (Table 3; Figure 2). After 56 days of inoculation, the BT02 strain treatment resulted in the highest number of 6.50 fruits per plant. Parameters including fruit weight, fruit diameter, and fruit flesh thickness were significantly higher than the control ( $p < 0.05$ ) (Table 4).

Table 1 Characteristics related to plant growth-promoting ability of *Streptomyces rochei* BT02

CMC (mm)	Phosphorus solubilization index	IAA concentration ( $\mu\text{g/mL}$ )	Biofilm formation	Siderophore production	Gibberellic acid ( $\mu\text{g/mL}$ )	Nitrogen fixation
$24.67 \pm 2.67$	$1.56 \pm 0.22$	$11.50 \pm 0.46$	+	+	-	-

Table 2 Effect of *Streptomyces rochei* BT02 on seed germination and vigor

Treatment	Germination rate (%)	Sprout length (cm)	Plumule length (cm)	Radicle length (cm)	Seed vigor
Control	$67.8^a \pm 4.92$	$0.57^a \pm 0.41$	$2.33 \pm 0.29$	$2.83^a \pm 0.35$	349.8
BT02	$92.5^b \pm 2.94$ (26.7%)	$1.10^b \pm 0.19$ (48.1%)	$2.16 \pm 0.43$	$4.02^b \pm 0.33$ (29.6%)	569.8 (38.6%)

Note: Numbers with the same superscript indicate non-significant statistical differences ( $\alpha = 0.05$ ).

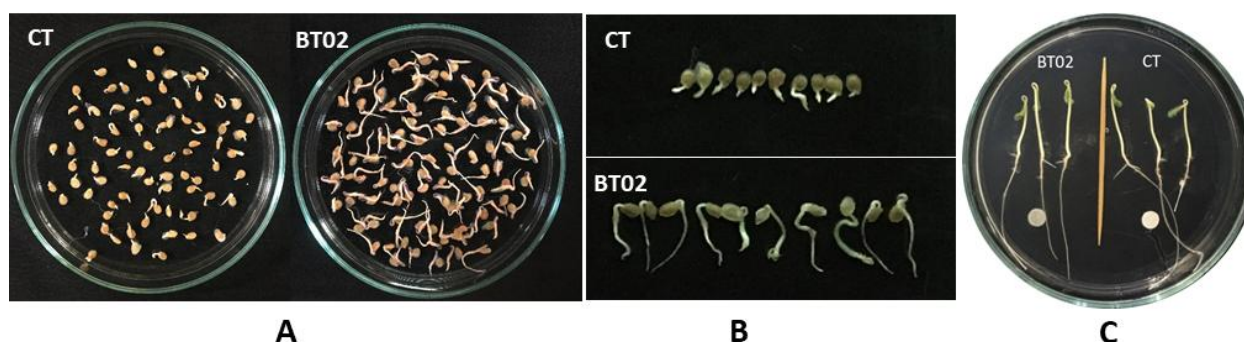


Figure 1 Influence of *Streptomyces rochei* BT02 on tomato seed germination (A-B) 4 days after inoculation; (C) 7 days after inoculation.

Table 3 Influence of *Streptomyces rochei* BT02 on growth of tomato plants under greenhouse conditions

Parameter	Treatment	7 DAI	14 DAI	21 DAI	28 DAI
Plant height (cm)	Control	$16.4^b \pm 1.20$	$29.3^b \pm 2.28$	$52.4^b \pm 2.24$	$74.7^b \pm 2.00$
	BT02	$21.5^a \pm 1.96$ (23.7%)	$35.9^a \pm 2.11$ (18.3%)	$56.9^a \pm 2.16$ (7.9%)	$79.6^a \pm 2.80$ (6.1%)
Number of leaves	Control	$7.10 \pm 0.70$	$10.1 \pm 0.94$	$15.2 \pm 1.07$	$18.9 \pm 0.94$
	BT02	$7.50 \pm 0.67$	$10.7 \pm 0.90$	$15.8 \pm 0.97$	$19.4 \pm 1.20$
Number of branches	Control			$0.10^b \pm 0.30$	$0.60^b \pm 0.49$
	BT02			$0.70^a \pm 0.46$ (85.7%)	$1.30^a \pm 0.46$ (53.8%)
Inflorescence	Control	$0.40^b \pm 0.49$	$2.20^b \pm 0.60$	$3.80^b \pm 0.60$	$5.30^b \pm 0.46$
	BT02	$0.80^a \pm 0.40$ (50%)	$3.10^a \pm 0.53$ (29.0%)	$4.90^a \pm 0.30$ (20.4%)	$7.30^a \pm 0.46$ (27.3%)
Flowers	Control	$0.50 \pm 0.67$	$4.01^a \pm 1.09$	$8.40^a \pm 0.80$	$12.0^a \pm 1.61$
	BT02	$1.10 \pm 0.70$	$5.10^b \pm 0.53$ (21.5%)	$11.0^b \pm 1.41$ (23.6%)	$14.3^b \pm 1.26$ (16.0%)

Numbers with the same superscript indicate non-significant statistical differences ( $\alpha = 0.05$ ). DAI: days after inoculation.

*Streptomyces* species distributed globally possess the capacity to enhance growth in diverse crops, serving as versatile agents in roles such as root bacteria treatments, biopesticides, biofertilizers, and bio stimulants (Ryu *et al.* 2003). The mechanisms underlying the promotion of plant growth by PGPR encompass the synthesis of plant growth regulators and the production of siderophores, hydrocyanic acid, antibiotics, and volatile compounds. *Streptomyces* species contribute to crop productivity through competition, induction of systemic resistance, and mineral solubilization such as phosphorus or potassium (de Andrade *et al.* 2023). Furthermore, these species exhibit biological control activity against phytopathogens by colonizing plant roots and producing antifungal metabolites, providing a sustainable alternative to chemical fungicides.

This study explored the plant growth-promoting impact of *S. rochei* BT02 on tomato plants and its antagonistic activity against *Fusarium solani* through both Petri dish and pot experiments. Our findings revealed that *S. Rochei*

BT02 possessed plant growth-promoting characteristics such as the biosynthesis of IAA, phosphate solubilization, and the formation of biofilms and siderophores. Treatment with the bacterial suspension significantly enhanced the germination and growth performance of seedlings in Petri dishes, attributed in part to IAA biosynthesis and secretion by *S. rochei* BT02. As well as regulating apical dominance, cell elongation, and primary and secondary root growth, IAA induces the relaxation of root cell walls, thereby promoting overall plant growth (Wang *et al.* 2009). The BT02 strain also demonstrated positive effects on plant growth, as evident in the various growth and fruit quality parameters. Our results supported the potential application of *S. rochei* BT02 as a PGPR strain for sustainable agriculture.

#### Antagonistic ability against *Fusarium solani* by *Streptomyces* BT02

In the dual culture method, PN02 exhibited resistance rates of 58.9%, while in the agar well diffusion method, PN02 showed antagonistic efficiency of 45.3% (Figure 3).

Table 4 Fruit quality at 56 days after inoculation

Treatment	Number/plant	Fresh weight (g)	Length (cm)	Diameter (cm)	Fruit flesh thickness (mm)
Control	4.10 <sup>b</sup> ± 1.10	42.0 <sup>b</sup> ± 2.76	3.19 ± 0.24	3.78 <sup>b</sup> ± 0.34	3.34 <sup>b</sup> ± 0.17
BT02	6.50 <sup>a</sup> ± 1.35 (36.9%)	53.8 <sup>a</sup> ± 4.71 (21.9%)	3.47 ± 0.46	4.45 <sup>a</sup> ± 0.38 (15.0%)	4.38 <sup>a</sup> ± 0.47 (23.7%)

Note: Numbers with the same superscript indicate non-significant statistical differences ( $\alpha = 0.05$ ).



Figure 2 Tomato plants at 28 days after inoculation with *Streptomyces rochei* BT02  
CT: control; BT02: *Streptomyces rochei* BT02.

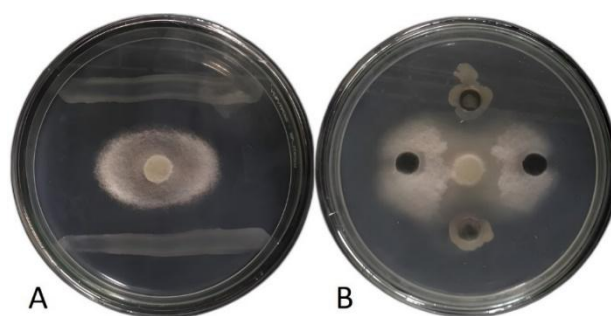


Figure 3 Inhibitory ability against *Fusarium solani* BT02 at 6 days after inoculation  
Dual culture method (A) and agar well diffusion method (B).

Under greenhouse conditions at 28 DAI, following pre- and post-incubation of actinomycetes with fungal infection, disease incidence varied between 22.0% and 36.0%. The corresponding AUDPC values were 511 and 686, indicative of control effectiveness at 41.6% and 21.6%, respectively (Table 5, Figure 4).

Under the experimental conditions, inoculation with *S. rochei* BT02 hampered the

growth of the tested fungi due to the production of diverse secondary metabolites with biological activities including chitinase, cellulase, and  $\beta$ -1,3-glucanase (Nguyen *et al.* 2023). These hydrolases degrade fungal cell walls and inhibit hyphal growth (Woith *et al.* 2021), while bacterial-produced antimicrobial substances also impede the growth of pathogens (El-Sharkawy & Abdelrazik 2022).

Table 5 Disease index (%), area under the disease progress curve (AUDPC), and control efficacy (%) of tomato root rot disease caused by *Fusarium solani* BT02 under greenhouse conditions

Treatment	Height (cm)	Root length (cm)	Disease incidence (%)	Disease index (%)			AUDPC	Control efficacy (%)
	28 DAI	28 DAI	28 DAI	14 DAI	21 DAI	28 DAI	28 DAI	28 DAI
Control	64.5 <sup>ab</sup> ±2.72	23.4 <sup>b</sup> ±0.34	0.00	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>		-
<i>F. solani</i>	52.7 <sup>c</sup> ±2.60	20.1 <sup>c</sup> ±2.04	100.0	32.0 <sup>a</sup>	60.0 <sup>a</sup>	60.0 <sup>a</sup>	875 <sup>b</sup> ±133	-
BT02	66.4 <sup>a</sup> ±4.71	26.7 <sup>a</sup> ±1.02	0.00	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>		-
BT02- <i>F. solani</i>	64.1 <sup>ab</sup> ±4.59	25.5 <sup>a</sup> ±1.91	100.0	24.0 <sup>b</sup>	26.0 <sup>cb</sup>	22.0 <sup>cb</sup>	511 <sup>b</sup> ±109	41.6
<i>F. solani</i> -BT02	61.1 <sup>b</sup> ±2.80	23.0 <sup>b</sup> ±1.34	100.0	30.0 <sup>b</sup>	36.0 <sup>cb</sup>	36.0 <sup>b</sup>	686 <sup>b</sup> ±167	21.6

Note: Numbers with the same superscript in the same column indicate non-significant statistical differences ( $\alpha = 0.01$ ).  
AUDPC: area under the disease progress curve; DAI: days after inoculation.



Figure 4 Efficiency to control root rot disease of the BT02 strain  
+Fs: Inoculated with *Fusarium solani*; CT: Control; BT02: Inoculated with BT02; BT02-Fs: Inoculated with BT02 24 hours before *Fusarium solani* (Fs); Fs-BT02: Inoculated with BT02 24 hours after *Fusarium solani* (Fs).

The application of *S. rochei* BT02 to tomatoes enhanced plant growth through the production of plant growth regulators, siderophores, phosphate solubilization, and resistance to pathogenic fungi by bioactive metabolites including hydrolases and antibiotics. *S. rochei* is categorized as a microorganism with a biosafety level of 2/4, in accordance with Directive 90/679/EEC of the European Community on biosafety. Our findings support the agricultural use of *S. rochei* BT02 as a PGPR strain, highlighting the exploration of both known and unknown secondary metabolites produced by this bacterial strain. This study primarily examined the potential effects of *S. rochei* BT02 on crop plants. However, further research is necessary to elucidate its functions, mechanisms of action, and interactions with plants and other microorganisms in the soil environment.

## CONCLUSIONS

*Streptomyces rochei* BT02 showed promise as a candidate for sustainable agriculture, displaying dual functionality by enhancing plant growth through mechanisms such as IAA production, phosphate solubilization, and siderophore production, while also suppressing the pathogenic fungus *Fusarium solani*. These multifaceted actions established strain BT02 as a potent biofertilizer and biocontrol agent, underscoring its efficacy for sustainable agriculture. Future research should focus on understanding the molecular mechanisms of *Streptomyces rochei* BT02 and explore its benefits by refining application methods on various crops, thereby significantly expanding the utility and contribution of this strain to promote sustainable agricultural practices.

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