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## Integrating Phosphorus Solubilization and Fungal Pathogens Biocontrol: *Streptomyces* spp. as a Dual-Solution for Sustainable Tomato Production in Egypt

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### ABSTRACT

Phosphorus (P) deficiency and fungal plant diseases critically limit tomato productivity in Egypt's P-deficient soils. This study investigated the dual potential of indigenous *Streptomyces* spp. to enhance P bioavailability and suppress fungal diseases. Seventeen actinobacterial isolates from tomato rhizospheres were screened for P-solubilization and antifungal activity. *Streptomyces* sp. BK5 demonstrated superior rock phosphate (RP) solubilization (phosphate solubilization index: 1.77) via acidification, releasing 130 mg mL<sup>-1</sup> P at 4 g/L RP. Pot trials revealed that the BK5 isolate inoculation significantly increased tomato fresh weight (5.65 vs. 4.89 g control) and tissue P concentration (0.20% vs. 0.18% control), particularly with rock phosphate (RP), highlighting microbial-mediated P mobilization. Factorial ANOVA confirmed P application rate ( $\leq 75\%$  recommended dose) as the primary growth driver, while the isolate BK5 enhanced P assimilation without affecting dry biomass. In addition to the positive effect to solubilize P, Confrontation experiment was assessed. Results of the *in vitro* experiment revealed that *Streptomyces* sp. BK5 inhibited *Fusarium oxysporum* (27%), *F. solani* (32%), *Alternaria solani* (53%), and *Rhizoctonia solani* (45%) through antifungal metabolites. Detached leaf assays validated the isolate BK5's crude extract suppressed pathogen growth by 58-67%. These findings underscore *Streptomyces* sp. BK5's dual agrobiotechnological role in sustainable P management and broad-spectrum disease suppression, advocating integrated biofertilizer-biocontrol strategies to reduce reliance on synthetic inputs, optimize tomato yields, and advance eco-friendly agriculture in P-stressed ecosystems.

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### INTRODUCTION

Phosphorus (P) is a critical macronutrient for plant growth because of its role in transfer in the form of Adenosine Tri-Phosphate (ATP), photosynthesis, and biosynthesis of nucleic acids and membranes (de Mello Prado, 2021; Khan et al., 2023). In agriculture, phosphorus fertilizers such as superphosphate and rock phosphate are widely used to address soil P deficiencies, though their efficacy is limited by solubility-rock phosphate- or cost and environmental concerns-superphosphate- (Tian et al., 2021). Additionally, P plays a dual role in plant disease resistance as it enhances resistance against pathogens like *Fusarium* wilt in tomato (Kiralý, 1976) and citrus Huanglongbing (HLB) (Zhao et al., 2013), while increasing susceptibility in crops like tobacco to mosaic viruses (Kiralý, 1976) or sugarcane to rust (Huber & Graham, 1999). Its effectiveness is context-dependent; for instance, P promotes root growth in corn, mitigating root rot in P-deficient soils (Huber & Graham, 1999), and foliar sprays reduce powdery mildews in cucumber and rust in maize (Reuveni et al., 1998; Katan, 2009). Soil pH critically influences outcomes, as seen in tomato, where *Fusarium* wilt worsened at

pH 6.0 but improved at pH 7.0–7.5 (Katan, 2009). P deficiency also exacerbates disease severity, as demonstrated in HLB-affected citrus, where supplementation restored yields (Zhao et al., 2013). Overall, P's impact hinges on crop-pathogen interactions, soil conditions (e.g., pH, P availability), and application methods, necessitating tailored management to balance agronomic benefits with sustainability (Davis et al., 1976; Mayee, 1983; Adebitan, 1996; Reuveni et al., 1998, 2000; Kirkegaard et al., 1999; Mousa & El-Sayed, 2016).

Phosphorus availability is enhanced through the solubilization of insoluble phosphate compounds by the soil microorganisms Actinomycetes, particularly *Streptomyces* spp., which produce organic acids and enzymes. This process offers a sustainable strategy to improve fertilizer efficiency and soil health (Soumare et al., 2021; Sathya et al., 2017). *Streptomyces* spp., exhibit dual agrobiotechnological significance. Beyond P solubilization, they serve as potent biocontrol agents against soil-borne pathogens like *Fusarium* spp. (Gopalakrishnan et al., 2011), *Rhizoctonia solani* (Goudjal et al., 2014), and *Sclerotium* spp. (Jacob et al., 2018). Their biocontrol mechanisms include antibiotic synthesis, volatile organic compounds

(VOCs) that induce systemic acquired resistance (SAR) in plants (Abbasi et al., 2019), hyperparasitism (Chen et al., 2016), hydrolytic enzyme production e.g., chitinases (Hoster et al., 2005), and competitive substrate utilization. Additionally, they mitigate abiotic/biotic stresses via low-molecular-weight inhibitors (e.g., ammonia) and biocidal metabolites (Sathya et al., 2017). These traits underscore their role in reducing synthetic pesticide reliance while boosting crop productivity (Soumare et al., 2021; Sathya et al. 2017).

In Egypt, tomato (*Solanum lycopersicum* L.) is a strategic crop, contributing 7.9 million tons annually (32 % of vegetable cultivated area; FAO, 2019). However, productivity is hindered by two key challenges: inefficient phosphorus utilization and Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL), which disrupts xylem function and nutrient uptake (Srinivas et al., 2019). Addressing these requires integrated approaches. Optimizing microbial interventions (e.g., P-solubilizing actinomycetes) and cost-effective fertilizers like rock phosphate (Dias et al., 2009) can enhance phosphorus availability. Concurrently, deploying biocontrol agents (e.g., *Streptomyces* spp.) and chemical elicitors to combat FOL, supported by studies on plant defense gene expression (e.g., PR proteins) and proteomic responses (Chakraborty et al., 2017), offers a sustainable disease management strategy. The objective of the present study is to: 1) isolate, characterize, and exploit Actinomycetes from the rhizosphere of new reclaimed soils as sustainable agricultural tools. 2) identify strains with dual capabilities in phosphate solubilization, plant growth promotion, and pathogen suppression, ultimately contributing to eco-friendly strategies for enhancing tomato productivity in semi-arid regions.

## MATERIALS AND METHODS

### Sample collection and bacterial isolation

Microorganisms were isolated from 20 soil samples (5–10 cm depth) collected from the rhizosphere of healthy tomato plants across diverse locations in newly reclaimed areas of Borg El-Arab, Alexandria, Egypt. Each sample comprised three replicates, stored in sterile polybags at 4°C until analysis. Samples underwent separate physicochemical and microbiological processing. For microbial isolation, 1 g of soil was serially diluted to  $10^{-6}$ , and 100 µL from the  $10^{-5}$  dilution was spread onto starch nitrate agar (Starch, 10.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g;  $\text{K}_2\text{HPO}_4$ , 1.0 g; NaCl, 0.5 g;  $\text{CaCO}_3$ , 2.0 g;  $\text{KNO}_3$ , 2.0 g;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.01 g; agar, 20.0 g; distilled water, 1000 mL) plates. These dishes were incubated at 30°C for three weeks to monitor actinomycetes growth, identified via macroscopic/microscopic traits (Chaudhary et al., 2013; Kekuda et al., 2012). Pure cultures were

preserved in modified Glucose Malt agar slants at 4°C for future studies.

### Characterization of isolated bacteria

Cultural characteristics of the organism were analyzed by culturing it on oatmeal agar, glycerol asparagine agar, yeast extract-malt extract agar, inorganic salt starch agar, and starch casein agar for 14 days at 28°C. Morphological traits, including aerial hyphae structure, spore chain arrangement, spore surface, mycelia coloration (aerial and substrate), and diffusible pigment production, were assessed after 7 days of growth on ISP-4 medium using light microscopy. These observations followed the standardized methodology of Shirling & Gottlieb (1966).

### Screening of efficient phosphate solubilizing bacteria

#### - Source of rock phosphate

The rock phosphate sample used in this study was kindly provided by EL-Waha Mining Company, Cairo, Egypt. A chemical analysis of the rock phosphate was conducted, and the composition was determined as follows: moisture content of 1.7%, total phosphate ( $\text{P}_2\text{O}_5$ ) of 27.6%, calcium carbonate ( $\text{CaCO}_3$ ) of 12.8%, silica ( $\text{SiO}_2$ ) of 10%, iron and aluminum oxides ( $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ ) of 2%, chlorides ( $\text{Cl}^-$ ) of 0.06%, and magnesium oxide ( $\text{MgO}$ ) of 0.4%.

#### - Screening procedure

During the primary screening phase, bacterial isolates were evaluated for phosphate-solubilizing activity using a semi-quantitative agar spot assay on Pikovskaya's (PVK) agar medium supplemented with rock phosphate (RP; 3 g/L) as the sole phosphorus source. Plates were inoculated and incubated at 30°C for 14 days. The phosphate solubilization index (PSI) was subsequently calculated using the formula proposed by Mohamed et al. (2019):

$$\text{PSI} = [(\text{CD} - \text{HZ}) / \text{CD}] \times 100,$$

where CD = Colony diameter and HZ = Halo zone.

All trials were conducted in triplicate to validate reproducibility.

#### - Quantitative estimation of solubilized P

To optimize phosphate solubilization parameters, experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of Pikovskaya's (PVK) broth supplemented with rock phosphate (RP; 2 - 6 g/L) as the sole phosphorus source. The medium was inoculated with potent isolate spore suspensions (1 % or 2 % v/v), and initial pH levels were adjusted using 1N NaOH or HCl. Three RP concentrations (2, 4, and 6 g/L) were tested to assess their impact on solubilization efficiency. Cultures were incubated at 30°C with agitation (170 rpm) for 6–10 days, depending on experimental variables. Post-incubation, solubilized phosphorus was quantified spectrophotometrically

at 880 nm according to the standard curve via the molybdenum blue assay, while final pH values were measured potentiometrically. Uninoculated PVK broth served as the negative control, and all treatments were performed in triplicate to ensure reproducibility.

#### ***In vitro* plant growth promotor**

The plant growth-promoting potential (PGP) isolated actinobacteria was systematically evaluated through assays for siderophore, hydrogen cyanide (HCN), and indole-3-acetic acid (IAA) production. Siderophore biosynthesis was assessed via the Chrome Azurol S (CAS) agar assay (Schwyn & Neilands, 1987), with yellow-orange halo formation around colonies after 5 days at  $28 \pm 2^\circ\text{C}$  indicating iron-chelating activity. HCN synthesis was detected using a picric acid-based method (von Rohr et al., 2009): isolates streaked on glycine-amended tryptic soy agar released cyanogenic compounds, reacting with alkaline picrate-soaked filter paper to produce a reddish-brown chromogen after 7 days. IAA production was determined by culturing isolates in King's B broth, followed by centrifugation and reaction of supernatants with Salkowski reagent (Gordon & Weber, 1951); a pink chromogen developed within 25 min confirmed auxin synthesis. All assays incorporated standardized incubation conditions ( $28 \pm 2^\circ\text{C}$ , agitation where applicable) and triplicate experimental designs to ensure reproducibility, collectively enabling robust screening of microbial strains for agriculturally relevant traits.

#### **Screening assays for antimicrobial activity**

The antagonistic potential of the isolated actinobacteria was evaluated against four phytopathogenic fungi (*Fusarium solani*, *F. oxysporum*, *Alternaria solani* and *Rhizoctonia solani*) using an *in vitro* dual-culture assay as described by Khamna et al., (2009). The phytopathogenic fungi were obtained from Plant Pathology Culture Collection - Alexandria University and were preserved on potato dextrose agar (PDA) at room temperature. Five-day-old mycelial plugs (6 mm diameter) of each test fungal pathogen were inoculated onto the center of fresh PDA plates. Concurrently, 7-day-old actinomycete colonies, pre-cultured on yeast malt extract agar (YME) at  $28^\circ\text{C}$ , were aseptically transferred as 6 mm discs to opposing positions 3 cm from the fungal inoculum. Control assays excluded actinomycete inoculation. Plates were incubated at  $28 \pm 2^\circ\text{C}$  for 14 days, and fungal growth inhibition was quantified using the formula:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100,$$

where C = radial growth (mm) in control plates and T = radial growth (mm) in dual-culture plates. All trials were conducted in triplicate to validate reproducibility.

#### **Pot Experiment**

A pot experiment was conducted in a split-plot design with three replicates to evaluate the potent actinobacteria as a bio-fertilizer in phosphorus-deficient soil for tomato cultivation. Main plots comprised two phosphorus sources (superphosphate and rock phosphate), while subplots tested graded phosphorus application rates (0, 25, 50, 75 and 100 % of recommended dose) with or without bio-inoculum. Each pot (2 kg soil) received uniform nitrogen (ammonium nitrate) and potassium (potassium sulfate) inputs. Bio-inoculum ( $10 \text{ mL}$ ;  $3 \times 10^5 \text{ CFU mL}^{-1}$ ) was applied to seedlings at cultivation initiation and 14 days post-transplant. Plants were harvested after 50 days for fresh weight (FW) measurement, followed by desiccation at  $75^\circ\text{C}$  for 48 h. Total phosphorus in dried, homogenized plant tissue (0.5 g) was quantified via nitric acid-hydrogen peroxide digestion and spectrophotometric analysis (Olsen & Sommers, 1982).

#### **Preparation of crude extracts from actinomycete**

Metabolite production was achieved through submerged fermentation in nutrient broth. A mother inoculum was prepared by adding 5 mL of freshly grown, potent actinomycete culture to 45 mL of nutrient broth, followed by incubation at  $37^\circ\text{C}$  with shaking (120 rpm) for 3 days. For large-scale production, 1 L of sterile nutrient broth was inoculated with 5% (v/v) of this mother inoculum and incubated under similar conditions ( $37^\circ\text{C}$ , 140 rpm) for 12–14 days. The fermented broth was then centrifuged at 8000 rpm for 20 minutes to harvest metabolites.

#### **Evaluation of plant pathogen suppression ability**

A detached leaf assay was performed on 3-week-old tomato plants using the first and second true leaves, adapted from Akbar et al., (2018) with modifications. Leaves were sterilized, blotted dry, and placed on moist sterile paper towels in Petri dishes. Three treatments were applied: (1) 2% LB in water (negative control), (2) 2% LB + pathogenic fungal plug, and (3) 2% crude extract + fungal plug. Pathogens tested (*Fusarium solani*, *F. oxysporum*, *Alternaria solani*, *Rhizoctonia solani*) were inoculated as 5 mm agar plugs from 5-day-old cultures. Plates were incubated at  $25^\circ\text{C}$ , and necrotic lesions were measured 5 days post-inoculation. Data was analyzed using a *t*-test, with six leaflets per treatment and three experimental replicates.

#### **Data analysis**

The *in vitro* experiments were analyzed as complete block design (CBD) with three replications. The pot experiment was analyzed as split plot where the main plots comprised two phosphorus sources (superphosphate, rock phosphate), while subplots tested graded phosphorus application rates (0 - 100 % of recommended dose) with or without bio-inoculum. The main plots and treatments were randomly distributed in the

subplots. All analyses were carried out using Proc Mixed SAS software package (ver. 9.4, SAS Institute Inc., Cary, NC, USA 2013).

## RESULTS AND DISCUSSION

### Morphological characterization of isolated Actinomycetes

Seventeen isolates exhibiting *Streptomyces*-like morphology (branched mycelium, spore-bearing hyphae) were isolated from the rhizosphere of healthy tomato plants across diverse locations in newly reclaimed areas of Borg El-Arab, Alexandria, Egypt. Phenotypic analysis categorized these into seven morphotypes (BK1–BK17) presented in Table 1, revealing taxonomic and functional diversity. Groups 1 (BK7, BK8, BK11, BK13), 4 (BK10, BK14), 5 (BK3, BK6), and 7 (BK5) produced brown, orange, or yellow-brown pigments, suggesting secondary metabolite biosynthesis (e.g., melanin, antibiotics). In contrast, Groups 2 (BK4, BK9, BK12, BK15) and 3 (BK1, BK2, BK16) lacked pigments, with Group 2 displaying spiral sporophores linked to hydrolytic enzyme potential. Unique traits included Group 6's (BK17) greenish-grey aerial mycelium and Group 7's smooth

cylindrical spores, aligning with antibiotic-producing taxa. Pigment absence in Groups 2–3 may reflect ecological prioritization of non-pigmented adaptations (e.g., enzyme secretion).

### Screening of Phosphate Solubilizing Bacteria

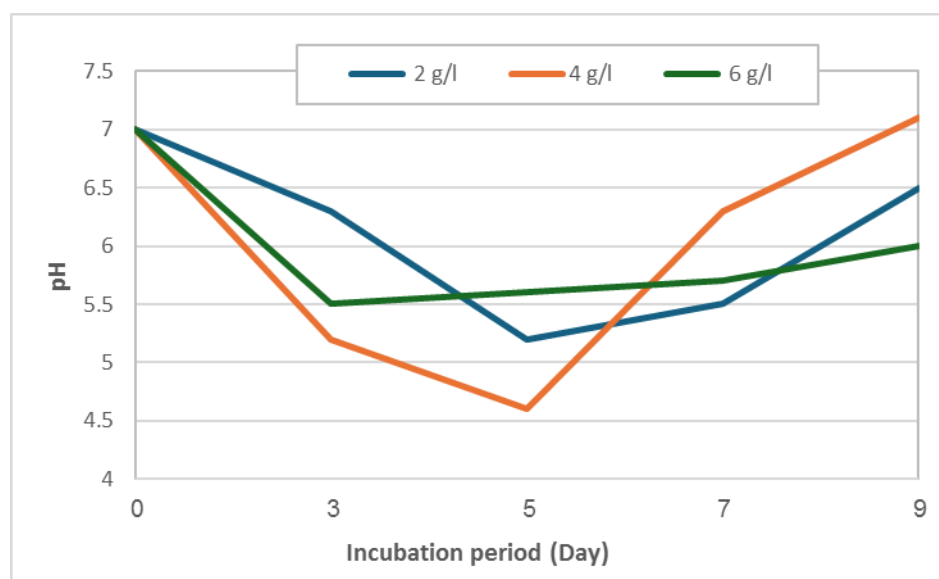
Seventeen actinobacterial isolates were screened for *in vitro* phosphate solubilization potential using PVK agar medium supplemented with rock phosphate (RP) as the sole phosphorus source. Six isolates (35.3%) demonstrated solubilization activity, evidenced by distinct halo zones around colonies, with phosphate solubilization indices (PSI) ranging from 1.17 to 1.75. Isolate *Streptomyces* sp. BK5 exhibited the highest PSI (1.77). Based on this preliminary assessment, the isolate BK5 was selected for subsequent investigations due to its superior solubilization capacity.

### Effect of *Streptomyces* on pH

Data presented in Fig. 1 showed the effect of *Streptomyces* sp. BK5 growth on pH acidification. Initial pH measurements (Day 0) confirmed a neutral baseline (pH 7.0) across all treatments.

**Table 1: Phenotypic characteristics of isolated actinobacterial strains**

Aerial mycelium	Color of substrate mycelium	Diffusible pigment	Shape of Sporophores	Isolates (codes)
White	Yellow	Brown	Retinaculiaperti	BK7, BK8, BK11 & BK13
White	Yellow	None	Spira	BK4, BK9, BK12&BK15
Grey	Yellow	None	Spira, hairy	BK1, BK2 & BK16
Grey	Cream	Brown	Rectiflexibilis	BK10 & BK14
Creamish	Brown-red	Orange	Spiny spore	BK3 & BK6
Greenish grey	Brown	Brown	Smooth, spira	BK17
Grey	yellow - brown	Yellow-brown	Smooth, cylindrical	BK5



**Figure 1: pH modulation dynamics mediated by *Streptomyces* sp. *in vitro* under graded rock phosphate (RP) concentrations.**

By Day 3, a concentration-dependent acidification trend was observed: pH declined to 6.3 (2 g/L RP), 5.2 (4 g/L RP), and 5.5 (6 g/L RP), correlating with enhanced microbial organic acid synthesis. These observations were in accordance with the results of Vyas & Gulati (2009). Progressive acidification continued through Day 5, reaching pH 5.2 (2 g/L RP), 4.6 (4 g/L RP), and 5.6 (6 g/L RP), confirming *Streptomyces*-mediated proton excretion as a driver of rock phosphate solubilization. However, by Day 7, pH rebounded in the 4 g/L RP (6.3) and 6 g/L RP (5.7) treatments, suggesting metabolic equilibrium or buffering capacity counteracting acidification. By Day 9, the 4 g/L RP treatment exceeded initial pH (7.1), while the 6 g/L RP treatment stabilized at pH 6.0, indicative of metabolic shifts or substrate saturation. These results were in agreement with results obtained by Parastesh et al., (2019). These biphasic pH dynamics - initial acidification followed by partial recovery - highlight *Streptomyces*'s role in modulating environmental pH through transient organic acid production during phosphate solubilization. The observed concentration-dependent trends underscore the interplay between microbial activity, substrate availability, and geochemical equilibria in phosphorus mobilization processes.

#### Effect of *Streptomyces* in solubilizing rock phosphate

Figure 2 illustrates the effect of *Streptomyces* sp. BK5 in solubilizing different concentrations of rock

phosphate. Initial phosphate solubilization (Day 0) across all treatments exhibited minimal mobilization, indicative of lag-phase microbial acclimation and delayed organic acid synthesis by *Streptomyces* spp. (Rodríguez et al., 2006). By Day 1, solubilization increased (2 g/L: 11 mg mL<sup>-1</sup>; 4 g/L: 16 mg mL<sup>-1</sup>; 6 g/L: 12 mg mL<sup>-1</sup>), coinciding with early-phase organic acid excretion (e.g., citric, oxalic acids) critical for Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> dissolution (Tian et al., 2021). A pronounced solubilization peak occurred at Day 5, particularly at 4 g/L (130 mg mL<sup>-1</sup>), with 6 g/L and 2 g/L reaching 69 mg mL<sup>-1</sup> and 64 mg mL<sup>-1</sup>, respectively, reflecting a log-phase microbial activity and optimal acidogenesis under intermediate substrate availability (Afrida et al., 2022). This aligns with phosphorus-induced microbial growth stimulation, enhancing acid-mediated solubilization (Oteino et al., 2015).

Post-peak declines (Day 7: 2 g/L: 33 mg mL<sup>-1</sup>; 4 g/L: 38 mg mL<sup>-1</sup>; 6 g/L: 18 mg mL<sup>-1</sup>; Day 9: 2 g/L: 31 mg mL<sup>-1</sup>; 4 g/L: 29 mg mL<sup>-1</sup>; 6 g/L: 13 mg mL<sup>-1</sup>) suggest substrate depletion, catabolite repression, or nutrient-limitation stress, compounded by potential acidification-induced microbial community shifts (Parastesh et al., 2019). Maximal efficiency at 4 g/L rock phosphate underscores a biostimulatory threshold balancing bioavailable phosphorus and microbial metabolic capacity.

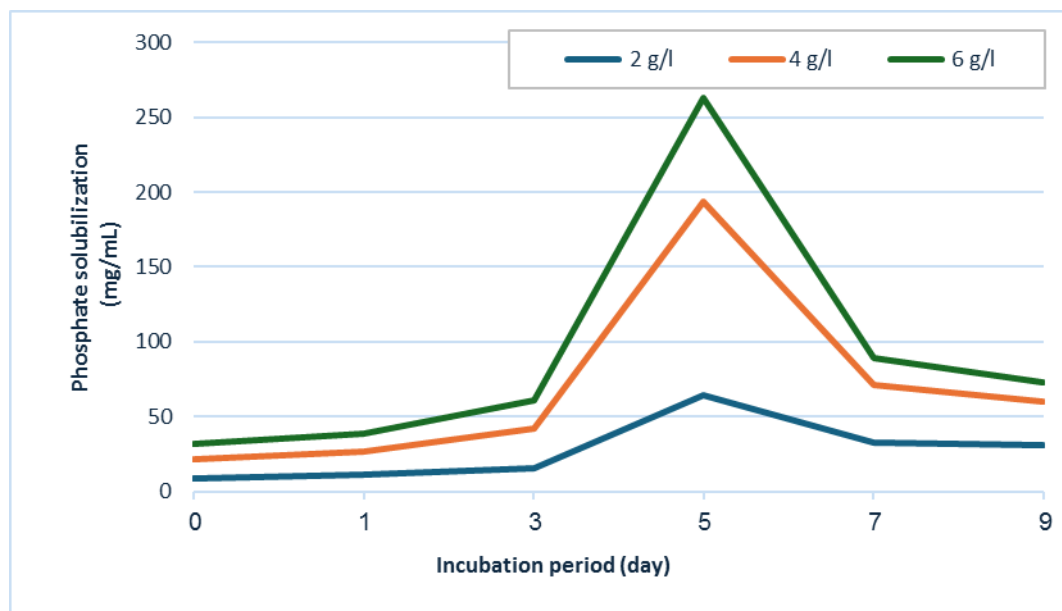


Figure 2: Phosphorus solubilization dynamics in *Streptomyces* sp. BK5 cultures under graded rock phosphate (RP) concentrations.

**Pot experiment:**

A factorial ANOVA assessed phosphorus source (rock/superphosphate), application rate (0–100 %), and *Streptomyces* inoculation effects on tomato growth (fresh/dry weight) and tissue phosphorus concentration. Results in Table 2 highlighted microbial-enhanced phosphorus bioavailability and dose-dependent growth optimization, informing sustainable fertilizer strategies.

A factorial ANOVA elucidated the effects of phosphorus source (superphosphate vs. rock phosphate), application rate (0–100 % recommended dose), and *Streptomyces* sp. inoculation on tomato growth and phosphorus assimilation. Phosphorus source exhibited no significant main effect on fresh weight (FW), dry weight (DW), or tissue phosphorus concentration (P %) ( $p > 0.05$ ), indicating negligible influence on plant growth or nutrient uptake. In contrast, phosphorus levels exerted a highly significant dose-dependent enhancement of all parameters ( $p <$

0.001), with FW and DW increasing by 337% and 300%, respectively, from 0 % to 100 % P supplementation, and P% rising from 0.16 (control) to 0.23 (100% P).

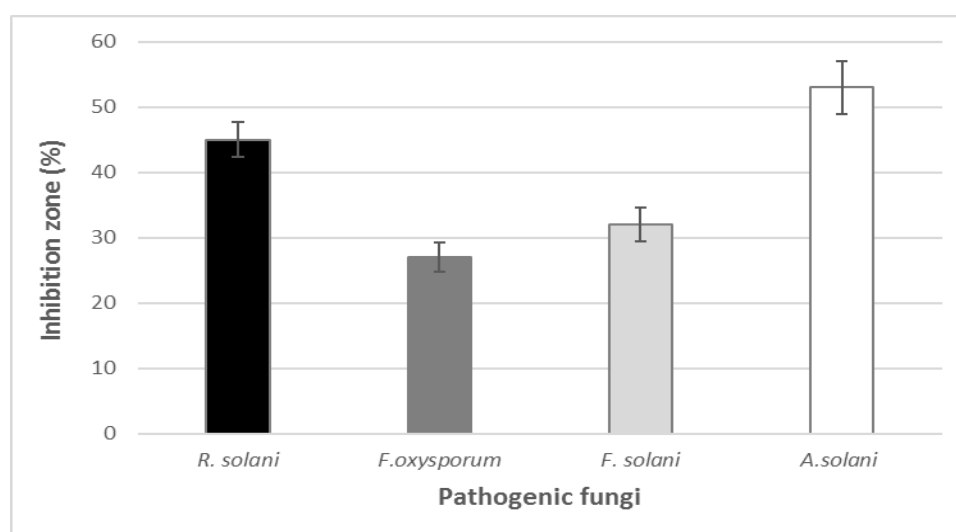
A synergistic interaction between phosphorus source and level ( $p < 0.05$ ) was observed for P%, suggesting differential phosphorus bioavailability between superphosphate and rock phosphate at higher application rates (75–100 %). *Streptomyces* inoculation significantly enhanced FW ( $p < 0.05$ ) and P% ( $p < 0.001$ ) but not DW ( $p > 0.05$ ), underscoring its role in microbial-mediated phosphorus mobilization rather than direct biomass accumulation. Higher-order interactions (phosphorus source  $\times$  *Streptomyces*, phosphorus level  $\times$  *Streptomyces*, and three-way interaction) were non-significant ( $p > 0.05$ ), indicating microbial effects were consistent across phosphorus sources and levels, with no compounded influence from combined factors.

**Table 2: ANOVA of Source-Level-Microbe Interactions. Mean squares and levels of significance of tomato growth, phosphorus concentration (P %) and phosphorus uptake (P uptake) of the tomato plant.**

Source of variance	DF	Mean squares		
		Fresh weight (g)	Dry weight (g)	P %
Phosphorus types (Pt)	1	5.22 ns	0.17 ns	0.0002 ns
Phosphorus levels (Pl)	3	52.20 ***	1.49 ***	0.004 ***
Pt * Pl	3	0.88 ns	0.10 ns	0.001*
<i>Streptomyces</i> (S)	1	4.45 *	0.04 ns	0.003 ***
Pt * S	1	0.14 ns	0.01 ns	0.0002 ns
Pl * S	3	0.92 ns	0.03 ns	0.0002 ns
Pt * Pl * S	3	0.24 ns	0.02 ns	0.00003 ns

\*Significant at 0.05 level of probability, \*\* Significant at 0.01 level of probability,

\*\*\* Significant at 0.001 level of probability and ns: Non-Significant.



**Figure 3: Antifungal activity of *Streptomyces* sp. strain BK5 by dual culture method against four phytopathogenic tomato plants.**

These findings advocate for optimized phosphorus dosing ( $\leq 75\%$  recommended rate) to maximize growth while minimizing environmental risks, coupled with *Streptomyces* spp. BK5 inoculation to enhance phosphorus bioavailability, particularly for sparingly soluble rock phosphate. Future research should prioritize field-scale validation of microbial consortia and soil-microbe interactions to amplify phosphorus use efficiency in sustainable agroecosystems.

The study demonstrated that phosphorus application rate exerted a significant main effect ( $p < 0.001$ ) on tomato growth and phosphorus assimilation, while phosphorus source (superphosphate vs. rock phosphate) showed no significant impact ( $p > 0.05$ ) (Hellal et al., 2019). *Streptomyces* sp. BK5 inoculation significantly enhanced fresh weight ( $p < 0.05$ ) and tissue phosphorus concentration ( $p < 0.001$ ) but had no effect on dry weight ( $p > 0.05$ ), indicating its role in enhancing phosphorus bioavailability rather than direct biomass accumulation (Rajguru & Bhatt, 2022). This bio-solubilization effect was particularly pronounced with rock phosphate, a sparingly soluble P source, where microbial activity facilitated phosphate mobilization via organic acid synthesis (Bünemann et al., 2008). The absence of a significant DW response to *Streptomyces* sp. BK5 inoculation underscores the need to investigate microbial impacts on carbon partitioning and long-term biomass dynamics. Future research should explore synergies between *Streptomyces* and other soil microbiota, as well as field-scale validation of integrated P management strategies combining microbial inoculants with optimized P dosing (Battini et al., 2017). Such approaches could reduce reliance on chemical fertilizers while mitigating environmental risks, such as eutrophication from P leaching (Khan et al., 2023).

#### Optimizing Phosphorus Supplementation in Tomato

Tomato plant exhibited a dose-dependent response to phosphorus supplementation across all measured parameters (Table 3). Fresh weight increased from 1.74 g at 0% P (control) to 7.60 g at

100 % P, with significant differentiation ( $p < 0.05$ ) between treatments (LSD = 0.73 g). Notably, FW plateaued at  $\geq 75\%$  P (7.56–7.60 g), indicating saturation beyond this threshold. Dry weight mirrored this trend, rising from 0.35 g (control) to 1.40 g (100% P; LSD = 0.22 g), reflecting proportional biomass accumulation. Tissue phosphorus concentration (P%) increased linearly from 0.16 (control) to 0.21 (75–100 % P; LSD = 0.009), underscoring enhanced P assimilation with elevated supplementation.

These results demonstrated that P application  $\leq 75\%$  of recommended rates maximized growth and nutrient uptake while avoiding inefficiencies associated with excess fertilization. The saturation effect at higher doses aligns with phosphorus adsorption saturation in soil matrices, beyond which additional P fails to improve productivity (Khan et al., 2023). To mitigate environmental risks (e.g., eutrophication from P leaching) and optimize resource use, precision P management is critical. Integrating slow-release fertilizers or microbial inoculants (e.g., *Streptomyces* spp.) could enhance P bioavailability from organic pools, reducing dependency on conventional inputs (Montes-Montes et al., 2024).

#### *Streptomyces* - Mediated Phosphorus Solubilization

Plants inoculated with *Streptomyces* exhibited a statistically significant increase in fresh weight (5.65 g vs. 4.89 g;  $p < 0.05$ , LSD = 0.52 g) and phosphorus concentration (0.20 % vs. 0.18 %; LSD = 0.02) as presented in Table (4), likely due to the genus's ability to solubilize soil phosphorus and produce phytohormones like auxins and cytokinins that enhance nutrient uptake and root development (Sousa et al., 2016; Chakraborty et al., 2017). However, while dry weight was marginally higher in treated plants (0.93 g vs. 0.86 g), the difference was not statistically significant (LSD = 0.09 g), suggesting that *Streptomyces* may promote transient water retention or early growth stimulation rather than sustained biomass accumulation (Rafique et al., 2017).

**Table 3: Phosphorus Application Rate Modulates Tomato Growth and P Uptake.**

Phosphorus levels	Tomato growth (gm)		P Uptake (P %)
	FW	DW	
Control	1.74* d	0.35 d	0.16 d
25%	5.2 c	0.74 c	0.18 c
50%	6.70 b	1.05 b	0.19 b
75%	7.56 a	1.37 a	0.21 a
100%	7.60 a	1.40 a	0.21 a
LSD 0.05	0.73	0.22	0.009

\*Values within columns followed by different lowercase letters differ significantly ( $p < 0.05$ ) based on LSD analysis. Data are average of three replicates each.

These findings underscore the potential of *Streptomyces* to improve phosphorus bioavailability and fresh biomass, though further research is needed to clarify its limited impact on dry matter and optimize its agricultural application.

#### Interaction between phosphorus types and phosphorus levels

Table(5) delineated tissue phosphorus concentration in tomato leaves under factorial combinations of phosphorus source [RP vs. SP] and application rate (0–100 % recommended dose). Phosphorus assimilation efficiency was significantly modulated by both source and dosage, with SP exhibiting superior solubility-driven bioavailability compared to RP. Under RP treatment, P % increased marginally from 0.1773 (0 % P) to 0.2124 (75–100 % P), reflecting limited solubilization without microbial mediation. In contrast, SP-treated plants achieved higher P % (0.1729 at 0 % P; 0.2326 at 100 % P), consistent with its water-soluble  $\text{PO}_4^{3-}$  form. Statistical analysis revealed a significant source  $\times$  level interaction ( $p < 0.05$ ), with divergent P % between RP and SP at higher doses (75–100 %). Phosphorus level exerted a stronger main effect ( $p < 0.001$ ) than source ( $p < 0.05$ ), aligning with dose-dependent uptake kinetics. At 75–100 % P, RP and SP achieved comparable P % (RP: 0.2124; SP: 0.2326), though SP retained a solubilization advantage ( $\Delta\text{P} \%=0.02$ ), underscoring its efficacy in high-input systems. The observed plateau in P% at  $\geq 75$  % application underscores soil adsorption saturation and diminishing returns. RP's lower bioavailability stems from its  $\text{Ca}_3(\text{PO}_4)_2$  composition, necessitating microbial acidogenesis (e.g., *Streptomyces* spp.) for solubilization

(Bünemann et al., 2008). Integrating RP with bio-solubilizing inoculants could mitigate SP dependency, enhancing P use efficiency in low-P soils while reducing eutrophication risks from leaching (Hellal et al., 2019; Rajguru & Bhatt, 2022). These findings advocate for precision P management, balancing immediate bioavailability (SP) with sustainable alternatives (RP + microbes). Future research should prioritize field-scale validation of microbial synergies and soil-P dynamics to advance eco-efficient agroecosystems.

#### Characterization of Plant Growth Promotion and Antimicrobial Activities

Based on the results obtained by quantitative assay in liquid medium, the strain *Streptomyces* sp. BK5 was selected for further characterization as other plant growth promotion activities and antimicrobial ability. Quantitative analysis revealed that *Streptomyces* sp. BK5 was able to produce siderophores up to  $15.19 \pm 1.01$   $\mu\text{g}$  as well as to synthesize IAA in liquid medium with and without tryptophan ( $1.46 \pm 0.03$  and  $6.81 \pm 0.38$   $\mu\text{g/L}$ , respectively). Interestingly, *Streptomyces* sp. BK5 exerted consistent antimicrobial activity against four different tomato fungal pathogens such as *Fusarium oxysporum*, *F. solani*, *Alternaria solani*, and *Rhizoctonia solani* (Fig. 3). Hyphal growth of *A. solani* was inhibited 53% in the presence of the potent actinomycete isolate. Growth inhibition of *R. solani* was recorded to be 45%. It was relatively effective against *F. oxysporum* and *F. solani* with 27% and 32% reduction in hyphal growth respectively.

**Table 4: Impact of *Streptomyces* isolate BK5 Treatment on tomato plant biomass and phosphorus content**

Treatment	Plant biomass (g)		Phosphorus Content
	FW	DW	P %
<i>Streptomyces</i> sp. BK5	5.65* a	0.93 a	0.2 a
Control	4.89 b	0.86 a	0.18 b
LSD 0.05	0.52	0.09	0.02

\*Values within columns followed by different lowercase letters differ significantly ( $p < 0.05$ ) based on LSD analysis. Data are average of three replicates each.

**Table 5: Interactive Effects of Phosphorus Source and Application Level on Leaf Phosphorus Concentration (%) in Tomato plants.**

Source of P	Levels of P				
	Control	25%	50%	75%	100%
Rock phosphate	0.1773 Ab	0.18 Ab	0.2024 Aa	0.2124 Ba	0.2123 Ba
Super phosphate	0.1729 Abc	0.18 Ab	0.1849 Ab	0.2326 Aa	0.2326 Aa
L.S.D. 0.05	0.012				

\*Means followed by different small letter(s) within the same row or different capital letter(s) within the same column are significantly different according to the L.S.D. Test at 0.05 level of probability. Data are the average of three replicates each.



**Table 6: *In vitro* biocontrol effect of cell free crude extract of strain BK5 on detached leaves challenged with pathogenic fungi.**

Treatment	Diameter of lesions developed from pathogenic fungal growth on leaves <sup>a</sup>			
	<i>A. solani</i>	<i>F. oxysporum</i>	<i>F. solani</i>	<i>R. solani</i>
CK	12±2*	12±1	13±1	17±2
CFs	4±1	5±2	5±1	6±1

<sup>a</sup>: Detached leaves were challenged with 0.5 cm diameter pathogenic fungal plugs and further treated with cell-free crude extract (CFs) of strain BK5 for 5 days, and (CK) is the control treatment.

\*Data are the average of three replicates each. The diameter of fungal growth was measured in mm.

Data in Table (6) demonstrated that cell-free crude extract (CFs) from bacterial strain BK5 significantly inhibits the growth of four pathogenic fungi (*Alternaria solani*, *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani*) on detached leaves under controlled conditions. The CFs of *Streptomyces* sp. BK5 significantly suppressed the growth of four phytopathogenic fungi (*Alternaria solani*, *F. oxysporum*, *F. solani*, and *Rhizoctonia solani*) on detached leaves, reducing fungal colony diameters by 58 - 67 % compared to untreated controls. This aligns with the well-documented role of *Streptomyces* in producing extracellular bioactive metabolites, such as polyketides, chitinases, and siderophores, which disrupt fungal cell integrity and nutrient acquisition (Sousa et al., 2016; Olanrewaju & Babalola, 2019). For instance, *Streptomyces* spp. are known to inhibit *R. solani* via secreted hydrolytic enzymes and antibiotics like actinomycin, consistent with the 67% suppression observed for *R. solani* in this study (Zhang et al., 2020; Zhong et al., 2023). Similarly, the inhibition of *Fusarium* spp. by BK5's CFs mirrors findings by Gohel et al. (2006), where *Streptomyces* cell-free extracts reduced *F. oxysporum* growth by >50 % through synergistic activity of antifungal volatiles and secondary metabolites. The broad-spectrum efficacy of BK5's extract parallels report of *Streptomyces griseoviridis* and *S. lydicus* suppressing diverse pathogens via extracellular compounds (Trejo-Estrada et al., 1998; Rajguru & Bhatt, 2022). However, *in vitro* antifungal activity of *Streptomyces* extracts may not fully predict field performance due to environmental instability or host-microbe interactions, underscoring the need for in planta validation and compound characterization.

## CONCLUSION

This study identifies *Streptomyces* sp. BK5, a rhizosphere isolate from arid soils, as a sustainable solution for tomato cultivation in phosphorus - deficient regions. The strain enhances P bioavailability by solubilizing rock phosphate via organic acids and suppresses pathogens (*Fusarium oxysporum*, *Rhizoctonia solani*) through antifungal metabolites. Optimal P application at ≤75% of recommended doses maximized tomato growth

while mitigating environmental risks like eutrophication. *Streptomyces* sp. BK5's multifunctionality - siderophore production, auxin synthesis, and disease suppression - reduces reliance on synthetic fertilizers and pesticides. Recommendations include integrating *Streptomyces* sp. BK5 with reduced P inputs for cost-effective, eco-friendly farming, particularly in calcareous soils. Policymakers should incentivize biofertilizer adoption through subsidies and education, while the future researchers should be focused on validation of BK5's efficacy in field trials and characterize its bioactive compounds for commercial use. Farmer training on microbial applications and precision P management is essential to scale this approach. This strategy bridges agronomic productivity with ecological sustainability, addressing food security challenges in phosphorus-scarce, climate-vulnerable regions.

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## الملخص العربي

### الأثر المزدوج لإذابة الفوسفور والمكافحة الحيوية على مسببات الأمراض الفطرية: بكتيريا الستربتوميسيس كحل ثنائي لإنتاج الطماطم المستدام في مصر

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يُعد نقص الفوسفور (P) والأمراض الفطرية من العوامل المحددة لإنتاجية نباتات الطماطم في التربة المصرية الفقيرة في عنصر الفوسفور. وتهدف هذه الدراسة إلى استكشاف الدور المزدوج لسلالات محلية من بكتيريا الستربتوميسيس (*Streptomyces* spp.) في تعزيز التوافر الحيوي للفوسفور ومكافحة الأمراض الفطرية. تم عزل ١٧ عزلة من الأكتينوبكتيريا من محيط جذور الطماطم ودراسة قدرتها على إذابة الفوسفور ومقاومة بعض الفطريات المسببة لأمراض النبات. أظهرت عزلة الستربتوميسيس BK5 تفوقاً في إذابة فوسفات الصخور (مؤشر الإذابة: ١٠.٧٧) عبر إنتاج الأحماض، مع إطلاق ١٣٠ ملجم/مل من الفوسفور عند استخدام ٤ جم/لتر من فوسفات الصخور. وفي تجارب الأصص، أدى تلقيح العزلة BK5 إلى زيادة معنوية في الوزن الطازج للطماطم (٥.٦٥ مقابل ٤.٨٩ جم في معاملة الكنترول) وتركيز الفوسفور في الأنسجة (٠.٢٠٪ مقابل ٠.١٨٪ في معاملة الكنترول)، خاصة مع استخدام فوسفات الصخور، مما يؤكد دور الميكروبات في إتاحة الفوسفور. أكد تحليل التباين العاملي (ANOVA) أن معدل إضافة الفوسفور (≥٧٥٪ من الجرعة الموصى بها) كان هو العامل الرئيسي المحفز لنمو نباتات الطماطم، بينما عززت العزلة BK5 امتصاص الفوسفور دون تأثير على الوزن الجاف للنباتات المعاملة. بالإضافة إلى تأثيرها الإيجابي في إذابة الفوسفور، تم تقييم فعالية هذه العزلة في تجارب المواجهة المباشرة. أظهرت نتائج الاختبارات المعملية أن العزلة BK5 قد تثبطت نمو الفطريات الممرضة: (*Fusarium oxysporum* (27%)، *solani* (32%)، *Alternaria solani* (53%)، و *Rhizoctonia solani* (45%). أكدت تجارب الأوراق المقطوعة أن المستخلص الخام للعزلة BK5 قد قلل نمو مسببات الأمراض النباتية موضع الدراسة بنسبة ٥٨-٦٧٪. تسلط هذه النتائج الضوء على الدور الزراعي-التكنولوجي المزدوج للعزلة BK5 في الإدارة المستدامة للفوسفور ومكافحة الأمراض النباتية واسعة الانتشار، مما يدعم تبني استراتيجيات متكاملة تجمع بين التسميد الحيوي والمكافحة الحيوية لتقليل الاعتماد على المدخلات الكيماوية، وتحسين إنتاج الطماطم، وتعزيز الزراعة المستدامة في النظم البيئية المتأثرة بنقص الفوسفور.