The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
21(1) S (1) 535-554,2026 www.thebioscan.com

"Rhizosphere-Derived *Stenotrophomonas maltophilia* enhances nutrient mobilization and suppresses tomato phytopathogens."

Abhijit B. Shinde[1], Sandip S. Gare[2]*

[1]Department of Microbiology, Deshbhakt Anandrao Balwantrao Naik Arts and Science College, Chikhali, Dist.- Sangli 415 408 M.S., India.

[2]* Department of Microbiology, Vishwasrao Naik Arts, Commerce and Baba Naik Science Mahavidyalaya, Shirala Dist.-Sangli 415 408 M.S., India.

Corresponding author email: sandipgare@gmail.com

DOI: https://doi.org/10.63001/tbs.2026.v21.i01.S.I(1).pp535-554

KEYWORDS:

PGPR; *Stenotrophomonas maltophilia*; tomato rhizosphere; phosphate solubilization; biological control; sustainable agriculture

Received on: 01-01-2026

Accepted on: 16-02-2026

Published on: 28-02-2026

ABSTRACT

The rhizosphere represents a dynamic ecological niche enriched with beneficial microorganisms capable of enhancing plant growth and suppressing soil-borne pathogens. The present study aimed to isolate, characterize, and evaluate plant growth–promoting and antagonistic rhizobacteria associated with tomato (*Solanum lycopersicum* L.) cultivated in the Shirala region of Maharashtra, India. Physicochemical analysis of rhizosphere soils revealed near-neutral pH (6.92 ± 0.13), moderate moisture content (21.70 ± 1.02%), and sufficient organic carbon (0.83 ± 0.06%), creating favourable conditions for microbial activity. High bacterial populations ranging from 1.23×10^7 to 9.7×10^7 CFU g^{-1} soil were recorded, indicating a biologically active rhizosphere. Eight morphologically distinct bacterial isolates were screened for plant growth–promoting traits, including phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore secretion, ammonia production, and hydrogen cyanide (HCN) production. Among them, isolate ASN exhibited the highest qualitative and quantitative phosphate solubilization (186.4 µg mL^{-1}), maximum IAA production (42.8 µg mL^{-1}), strong siderophore activity, and robust ammonia and HCN production. Dual culture assays demonstrated significant antagonistic activity of isolate ASN against major tomato phytopathogens (*Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Alternaria solani*), with inhibition exceeding 68% across all pathogens. One-way ANOVA confirmed statistically significant differences among isolates ($p < 0.001$). Morphological, biochemical, and 16S rRNA gene sequencing analysis identified isolate ASN as *Stenotrophomonas maltophilia* with an accession number LC920483. Overall, the study highlights *Stenotrophomonas maltophilia* as a multifunctional plant growth–promoting rhizobacterium with strong biocontrol potential, suggesting its promising application as a sustainable bioinoculant for enhancing tomato productivity and disease management.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops cultivated across India, including the agriculturally active region of Shirala in Maharashtra. The crop plays a significant role in farmer income and nutritional security due to its high content of vitamins, antioxidants, and essential minerals (FAO, 2022). However, tomato production in this region is increasingly affected by soil-borne pathogens, declining soil fertility, and intensive chemical input practices. Diseases such as wilt, root rot, damping-off, and leaf spot frequently occur in local fields, leading to reduced yield and compromised fruit quality (Agrios, 2005; Singh *et al.,* 2018). Continuous reliance on synthetic fertilizers and pesticides has further contributed to environmental degradation, microbial imbalance in soils, and the emergence of resistant plant pathogens (Glick, 2012).

In recent years, sustainable agricultural practices have gained importance as eco-friendly alternatives to conventional farming systems. Among these approaches, plant growth–promoting rhizobacteria (PGPR) have emerged as effective biological tools for improving crop productivity and soil health. These beneficial microorganisms enhance plant growth through multiple mechanisms including phosphate solubilization, production of phytohormones such as indole-3-acetic acid (IAA), improved nutrient uptake, and stimulation of root development (Kloepper *et al.,* 2004; Vessey, 2003). In addition to growth promotion, many PGPR possess antagonistic properties against phytopathogens through the production of antimicrobial metabolites, siderophores, and hydrolytic enzymes, as well as competition for nutrients and ecological niches in the rhizosphere (Compant *et al.,* 2005).

Stenotrophomonas maltophilia is a rhizosphere-associated bacterium that has recently attracted attention for its multifunctional agricultural potential. It is widely distributed in soil and plant root environments and exhibits remarkable metabolic adaptability (Ryan *et al*., 2009). Several studies have reported its ability to promote plant growth by solubilizing essential nutrients and producing phytohormones (Berg *et al.,* 2005; Alavi *et al.,* 2013). Furthermore, the bacterium demonstrates strong antagonistic activity against a range of fungal and bacterial plant pathogens through antibiotic production, siderophore-mediated iron competition, and secretion of lytic enzymes such as chitinases and proteases (Kai *et al.,* 2010).

Despite these promising attributes, limited research has been conducted on the plant growth–promoting and antagonistic properties of *Stenotrophomonas maltophilia* under the specific agro-climatic conditions of the Shirala region. Local soil characteristics, cropping practices, and microbial diversity can significantly influence the effectiveness of beneficial bacteria. Therefore, region-specific characterization is essential for developing efficient and reliable bioinoculant formulations for sustainable tomato cultivation.

The present study aims to characterize the plant growth–promoting traits and antagonistic potential of *Stenotrophomonas maltophilia* isolated from tomato rhizosphere soils of the Shirala region of Maharashtra. The research focuses on evaluating key plant growth–promoting attributes along with its inhibitory activity against selected tomato pathogens, with the objective of exploring its suitability as an eco-friendly alternative for enhancing crop productivity and disease management.

Materials and Methods

Study area and soil sampling

Rhizosphere soil samples were collected from tomato (*Solanum lycopersicum* L.) fields in Shirala, Sangli district, Maharashtra, India (semi-arid zone). The region experiences a monsoon-influenced climate with mean annual temperatures of 22–35°C and rainfall of 600–750 mm. Sampling was performed during the vegetative stage (45–60 days after transplantation). Five fields with uniform agronomic practices and no recent microbial inoculant application were selected. From each field, rhizosphere soil firmly adhering to the roots of five randomly selected healthy plants was aseptically collected following a zigzag sampling pattern and pooled to form composite samples (Anderson & Ingram, 1993). Samples were transported and processed within 24 h. Air-dried and sieved (2 mm) soil samples were analyzed for pH using a 1:2.5 soil–water suspension method (Jackson, 1973), moisture content by the gravimetric method (Gardner, 1986), and organic carbon by the Walkley–Black wet oxidation method (Walkley & Black, 1934).

Isolation of rhizosphere bacteria

Rhizosphere bacteria were isolated using the serial dilution and spread plate technique under aseptic conditions. One gram of homogenized soil was suspended in 9 mL sterile physiological saline (0.85% NaCl) and vortexed for 5 min to obtain a uniform microbial suspension (Cappuccino & Sherman, 2014). The suspension was serially diluted up to 10^{-8}, and 100 µL aliquots from appropriate dilutions were spread onto nutrient agar plates (Holt *et al*., 1994). Plates were incubated at 30 ± 2 °C for 24–48 h in triplicate. Colonies were counted from plates containing 30–300 colonies, and total viable counts were expressed as CFU g^{-1} of dry soil using the standard plate count method (Tortora *et al*., 2019). Morphologically distinct colonies were purified by repeated streaking (Madigan *et al*., 2018) and preserved on nutrient agar slants at 4 °C. Representative isolates were selected for further screening of plant growth–promoting and antagonistic activities.

Phosphate solubilization assay

Qualitative phosphate solubilization

Qualitative phosphate solubilization was assessed on Pikovskaya's agar medium supplemented with tricalcium phosphate as an insoluble phosphorus source (Pikovskaya, 1948). Fresh bacterial isolates were spot-inoculated onto agar plates and incubated at 30 ± 2 °C for 5–7 days. The appearance of a clear halo zone surrounding colonies indicated phosphate solubilization. Colony diameter and halo zone diameter were measured using a digital caliper. The phosphate solubilization index (PSI) was calculated following Edi-Premono *et al.* (1996). All observations were performed in triplicate, and mean values were recorded.

Quantitative phosphate solubilization

Quantitative estimation of soluble phosphate was conducted in Pikovskaya's broth supplemented with tricalcium phosphate as described by Pikovskaya's (1948). Each bacterial isolate was inoculated into 50 mL of broth and incubated at 30°C for 7 days under shaking conditions (120 rpm). After incubation, cultures were centrifuged at 10,000 rpm for 10 min to remove bacterial cells and insoluble residues. The supernatant was collected, and soluble phosphate content was determined using the molybdenum blue colorimetric method (Murphy &

Riley, 1962). Briefly, 1 mL of culture supernatant was mixed with ammonium molybdate reagent followed by ascorbic acid reducer to develop a blue-coloured complex, and absorbance was measured at 820 nm using a UV–visible spectrophotometer. The concentration of soluble phosphate was calculated from a standard curve prepared using potassium dihydrogen phosphate (KH_2PO_4) and expressed as µg mL^{-1}. All assays were conducted in triplicate, and results were expressed as mean ± standard deviation.

Indole-3-Acetic acid (IAA) production assay

Indole-3-acetic acid (IAA) production was quantified using the Salkowski colorimetric method (Gordon & Weber, 1951; Patten & Glick, 2002). Bacterial isolates were inoculated (1% v/v) into Luria–Bertani broth supplemented with 0.1% L-tryptophan and incubated at 30 ± 2 °C for 48 h under shaking (120 rpm). Cultures were centrifuged at 10,000 rpm for 10 min, and the supernatant was used for analysis. For color development, 1 mL of supernatant was mixed with 2 mL Salkowski's reagent (35% perchloric acid with 0.5 M $FeCl_3$) and incubated in the dark for 30 min. The pink coloration formed was measured at 530 nm using a UV–visible spectrophotometer. IAA concentration was determined from a standard curve (10–100 µg mL^{-1}) and expressed as mean ± standard deviation of triplicates.

Siderophore production assay

Siderophore production was qualitatively evaluated using the Chrome Azurol S (CAS) agar assay (Schwyn & Neilands, 1987). CAS medium, containing the iron–dye complex (CAS–Fe^{3+}–HDTMA), exhibits a characteristic blue color. Fresh bacterial cultures (5 µL) were spot-inoculated onto CAS agar plates and incubated at 30 ± 2 °C for 48–72 h. Siderophore production was indicated by a visible color change from blue to orange/yellow around colonies due to iron chelation by secreted siderophores. The halo diameter was measured using a digital caliper to estimate production intensity. All assays were performed in triplicate with uninoculated plates as controls. Isolates producing prominent halos were considered strong siderophore producers and selected for further characterization.

Ammonia Production Assay

Ammonia production was evaluated in peptone water broth following Cappuccino and Sherman (2014). Bacterial isolates (1% v/v) were inoculated into 10 mL sterile peptone water and incubated at 30 ± 2 °C for 48 h under static conditions. Uninoculated broth served as the control. After incubation, 0.5 mL Nessler's reagent was added to each tube. Development of a yellow to brown coloration indicated ammonia production due to degradation of nitrogenous compounds. The intensity was recorded semi-quantitatively as weak (+), moderate (++), or strong (+++). All assays were performed in triplicate.

Hydrogen cyanide (HCN) production assay

Hydrogen cyanide (HCN) production was assessed on nutrient agar supplemented with 4.4 g L^{-1} glycine following Bakker and Schippers (1987). Bacterial isolates were streaked onto the agar surface. A sterile Whatman No. 1 filter paper strip soaked in picric acid solution (0.5% picric acid in 2% sodium carbonate) was placed in the lid without touching the medium. Plates were sealed and incubated at 30 ± 2 °C for 48–72 h. HCN production was indicated by a color change of the filter paper from yellow to brown or reddish-brown, reflecting cyanide release. Uninoculated plates served as controls. All assays were performed in triplicate and results were recorded qualitatively based on color intensity.

Primary screening of antagonistic rhizosphere bacteria

Primary screening of rhizosphere bacterial isolates for antagonistic activity against *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Alternaria solani* was performed using cross-streak and dual culture assays (Dennis & Webster, 1971; Skidmore & Dickinson, 1976). In the cross-streak method, bacterial isolates were streaked centrally on PDA plates and incubated at 30 ± 2 °C for 24 h. Fungal pathogens were then streaked perpendicular to the bacterial line and incubated at 28 ± 2 °C for 3–5 days. Visible suppression of fungal growth indicated antagonistic activity. Promising isolates were further evaluated by dual culture assay. A 5 mm fungal mycelial disc was placed at the center of PDA plates, and bacterial isolates were streaked ~2.5 cm away. Control plates contained only the fungus. After incubation at 28 ± 2 °C for 5–7 days, radial fungal growth was measured and percentage inhibition was calculated. Each experiment was conducted in triplicate. Bacterial isolates exhibiting ≥50% inhibition were considered strongly antagonistic and selected for further characterization.

Selection of efficient isolate for morphological and biochemical characterization

Following primary screening, bacterial isolates were ranked based on cumulative performance in plant growth–promoting traits and antifungal activity. Isolates showing high phosphate solubilization, significant IAA production, siderophore secretion, ammonia production, and strong pathogen inhibition were considered potential PGPR and selected using a quantitative scoring system. Selected isolates were characterized for colony morphology on nutrient agar (24–48 h at 30 ± 2 °C), Gram reaction, and cell morphology. Biochemical tests including catalase, oxidase, citrate utilization, indole, methyl red, Voges–Proskauer, carbohydrate fermentation, and nitrate reduction were performed for preliminary phenotypic identification prior to molecular analysis.

Molecular identification by 16S rRNA gene sequencing

The potential bacterial isolates were identified at a molecular level by 16S rRNA gene sequencing. Genomic DNA was isolated from selected bacterial isolates and purified through column-based purification steps. The 16S rRNA gene was amplified by polymerase chain reaction (PCR). Successfully amplified products were purified and subjected to Sanger sequencing. Obtained nucleotide sequences were edited using BioEdit software and compared with reference sequences available in the NCBI GenBank database using the BLASTn algorithm for taxonomic identification (Altschul *et al*., 1990). Sequences showing ≥97% similarity were considered reliable species-level matches. Phylogenetic analysis was performed using MEGA X software (Kumar *et al*., 2018). Multiple sequence alignment was conducted using the ClustalW algorithm, and a phylogenetic tree was constructed using the neighbour-joining method with 1,000 bootstrap replications to assess evolutionary relationships (Saitou & Nei, 1987).

Statistical analysis

All experiments were conducted in triplicate, and data were expressed as mean ± standard deviation. Statistical significance among treatments was evaluated using one-way analysis of variance (ANOVA) to determine differences among bacterial isolates for various plant growth–promoting and antagonistic traits. Statistical significance was considered at $p < 0.05$, following standard biostatistical procedures (Gomez & Gomez, 1984; Montgomery, 2017).

Results and discussion:

The physicochemical characteristics of tomato rhizosphere soils from five fields are summarized in **Table 1**. Soil pH ranged from 6.8 to 7.1 (6.92 ± 0.13), indicating near-neutral conditions favorable for nutrient availability and microbial activity. Moisture content varied between 20.5% and 23.1% (21.70 ± 1.02%), reflecting the high water-holding capacity of Vertisols and supporting active microbial metabolism. Organic carbon ranged from 0.74% to 0.89% (0.83 ± 0.06%), suggesting moderate organic matter availability essential for sustaining rhizosphere microbial populations.

Overall, the near-neutral pH, adequate moisture, and moderate organic carbon content provided conducive conditions for microbial colonization and functional activity, which may have supported the observed plant growth–promoting and biocontrol potential of the isolated bacteria, as soil physicochemical properties strongly influence rhizosphere microbial composition and nutrient dynamics (Maciel-Rodríguez *et al*., 2025).

The population density of cultivable rhizosphere bacteria from tomato fields in the Shirala region is shown in **Table 2**. Bacterial counts ranged from 1.23×10^7 to 9.7×10^7 CFU g^{-1} soil, indicating a highly active rhizosphere microbial community. Fields 3 and 5 recorded comparatively higher populations and were categorized as very high abundance, whereas Fields 1, 2, and 4 showed high but relatively lower densities. The overall mean population was $4.44 \times 10^7 \pm 3.73 \times 10^7$ CFU g^{-1}. High bacterial abundance reflects a nutrient-enriched rhizosphere influenced by root exudates that stimulate microbial colonization and activity. Such dense microbial populations contribute to nutrient cycling, pathogen suppression, and beneficial plant–microbe interactions. Variations among fields likely reflect differences in soil physicochemical characteristics and local agronomic conditions (Rout & Southworth, 2023).

The colony morphology of the bacterial isolates is summarized in **Table 3.** Considerable variation was observed in colony colour, shape, margin, elevation, surface texture, and size. Most isolates (ASN, ASN2, ASN5, ASN7) produced circular, smooth colonies with entire margins and were grouped under Group I (1.6–2.5 mm). Group II isolates (ASN1, ASN4, ASN6) exhibited irregular to circular colonies with undulate margins and rough or mucoid textures (2.9–3.5 mm). ASN3 (Group III) was morphologically distinct, forming filamentous, lobate, dry colonies with the largest diameter (4.0 mm). Overall, the morphological diversity indicates phenotypic heterogeneity among the isolates, supporting their selection for further plant growth–promoting and antagonistic evaluation (Glick, 2020; Santoyo *et al*., 2021).

The phosphate solubilization assay demonstrated considerable variation among rhizosphere bacterial isolates, with PSI values ranging from 1.63 to 4.72 **(Table 4)**. Isolate ASN exhibited the highest solubilization efficiency (PSI 4.72), followed by ASN5, ASN2, and ASN7, which were categorized as strong solubilizers. Moderate activity was observed in isolates ASN1, ASN4, and ASN6, while isolate ASN3 showed weak solubilization potential. The superior performance of isolate ASN indicates efficient secretion of organic acids capable of converting insoluble tricalcium phosphate into plant-available forms, a key mechanism of phosphate-solubilizing bacteria (Alori *et al.*, 2017; Sharma *et al.*, 2022). Such variability highlights functional diversity within the tomato rhizosphere microbiome, contributing to enhanced phosphorus availability and nutrient cycling (Backer *et al.*, 2018; Santoyo *et al.*, 2021).

Quantitative estimation of soluble phosphate in Pikovskaya's broth showed marked variation among isolates **(Table 5; Figure 1)**, with phosphate release ranging from 64.5 to 186.4 µg mL^{-1}. Isolate ASN recorded the highest solubilization (186.4 ± 6.8 µg mL^{-1}), followed by ASN5, ASN7, and ASN2, which demonstrated high efficiency. The isolates ASN1, ASN4, and ASN6 showed moderate activity, whereas isolate ASN3 exhibited the lowest phosphate release (64.5 ± 4.0 µg mL^{-1}). The superior performance of isolate ASN likely reflects enhanced organic acid production and proton-mediated solubilization mechanisms that facilitate dissolution of insoluble phosphate. The strong agreement between qualitative PSI values and quantitative broth results confirms the reliability of the screening approach. Overall, the findings highlight ASN as a promising phosphate-solubilizing bacterium with potential application in sustainable tomato cultivation, as efficient PGPR improve phosphorus availability and reduce dependence on chemical fertilizers (Alori *et al.*, 2017; Backer *et al.*, 2018; Santoyo *et al.*, 2021; Delgado-Baquerizo *et al.*, 2020).

Quantitative estimation of IAA production revealed substantial variation among isolates **(Table 6),** with concentrations ranging from 12.5 to 42.8 µg mL^{-1}. The isolate ASN produced the highest IAA level (42.8 ± 2.1 µg mL^{-1}), followed by ASN5, ASN2, and ASN7, all categorized as high producers. The isolates ASN1, ASN4, and ASN6 showed moderate production, whereas isolate ASN3 exhibited low IAA synthesis. High IAA production suggests efficient tryptophan-dependent auxin biosynthesis pathways, which can enhance root elongation, lateral root formation, and nutrient uptake. The presence of multiple high IAA-producing isolates indicates a functionally active tomato rhizosphere capable of influencing plant growth and development. The observed variation reflects metabolic diversity among isolates and supports the selection of high-producing strains for potential bioinoculant development (Glick, 2014; Spaepen & Vanderleyden, 2011; Backer *et al.*, 2018).

The CAS agar assay showed clear variation in siderophore production among isolates **(Table 7),** with SPI values ranging from 1.53 to 3.88. The isolates ASN, ASN2, ASN5, and ASN7 exhibited strong siderophore activity (SPI ≥ 3.5), while isolates ASN1, ASN4, and ASN6 showed moderate production. The isolate ASN3 displayed weak iron-chelating ability.

High SPI values indicate efficient iron acquisition under limited conditions, providing a competitive advantage in the rhizosphere. Siderophore-producing bacteria enhance iron availability to plants and restrict pathogen access to iron, contributing to plant growth promotion and biocontrol. The concurrence of strong siderophore production with high phosphate solubilization and IAA production in several isolates highlights their multifunctional plant growth–

promoting potential. Overall, the observed variability reflects functional diversity within the tomato rhizosphere and supports the selection of efficient strains for sustainable crop improvement (Kramer *et al*., 2020; Backer *et al*., 2018; Santoyo *et al*., 2021).

The ammonia production assay revealed variation in nitrogenous metabolite release among isolates (**Table 8**). The isolates ASN, ASN2, ASN5, and ASN7 showed strong ammonia production (+++), isolates ASN1, ASN4, and ASN6 exhibited moderate activity (++), while isolate ASN3 displayed weak production (+). Strong ammonia-producing isolates may contribute to nitrogen mineralization, thereby enhancing nitrogen availability in the rhizosphere and supporting plant growth (Glick, 2012; Hayat *et al*., 2010). The high activity observed in isolate ASN further confirms its multifunctional plant growth–promoting potential alongside phosphate solubilization, IAA production, and siderophore secretion, traits commonly associated with effective rhizobacteria (Ahmad *et al*., 2008; Vessey, 2003). Overall, the variability in ammonia production reflects functional diversity within the tomato rhizosphere microbial community and indicates potential for improved nitrogen cycling and plant nutrition (Richardson et al., 2009).

The qualitative HCN assay showed clear differences among isolates based on picric acid filter paper color changes **(Table 9).** The isolates ASN, ASN2, ASN5, and ASN7 exhibited strong HCN production (+++), indicated by dark to reddish-brown coloration. The isolates ASN1, ASN4, and ASN6 showed moderate production (++), while isolate ASN3 did not show HCN production. Hydrogen cyanide is a volatile metabolite that suppresses soil-borne pathogens by inhibiting respiratory enzymes. The presence of several strong HCN-producing isolates highlights the biocontrol potential of the tomato rhizosphere in the Shirala region. The absence of HCN activity in isolate ASN3 further emphasizes functional diversity within the microbial community. When combined with traits such as phosphate solubilization, siderophore production, and IAA synthesis, HCN production enhances the overall antagonistic and plant growth–promoting capacity of selected isolates (Bakker & Schippers, 1987; Rijavec & Lapanje, 2016; Backer *et al*., 2018; Santoyo *et al*., 2021). The antagonistic activity of rhizosphere bacterial isolates against major tomato phytopathogens was evaluated using the dual culture assay **(Table 10; Figure 2).**

The superior performance of isolate ASN aligns with its strong plant growth–promoting traits (phosphate solubilization, IAA production, siderophore secretion, ammonia, and HCN production), suggesting a synergistic mechanism involving nutrient competition, antibiosis, and enzymatic inhibition of pathogens. Overall, the results highlight the tomato rhizosphere of Shirala region as a rich source of multifunctional PGPR with promising biocontrol potential and suitability for sustainable bioinoculant development (Backer *et al*., 2018; Santoyo *et al*., 2021; Compant *et al*., 2020; Keswani *et al*., 2019).

One-way ANOVA revealed highly significant differences ($p < 0.001$) in antagonistic activity among rhizosphere bacterial isolates against all tested tomato phytopathogens **(Table 11).** The high F-values for *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Alternaria solani* indicate strong isolate-dependent variation in fungal growth inhibition. These results confirm that antagonistic performance varied significantly among isolates. Isolates ASN, ASN2, ASN5, and ASN7 demonstrated statistically superior inhibition, while isolates ASN1, ASN4, and ASN6 showed moderate inhibition, and ASN3 exhibited weak inhibition. The significant between-isolate variation validates the reliability of the dual culture assay and supports the selection of high-performing strains. Overall, the statistical analysis strengthens the conclusion that isolate ASN possesses robust and consistent biocontrol potential, highlighting its suitability as a promising PGPR candidate for sustainable tomato disease management (Backer *et al*., 2018; Compant *et al*., 2020; Santoyo *et al*., 2021).

The morphological and biochemical characteristics of isolate ASN are presented in **Table 12.** The isolate ASN was Gram-negative, motile, rod-shaped bacterium occurring predominantly as single cells with active flagellar movement, typical of many rhizosphere-associated plant growth–promoting bacteria. The biochemical profile showed positive reactions for catalase activity, citrate utilization, Voges–Proskauer test, glucose and sucrose fermentation and nitrate reduction, while oxidase, indole, methyl red, and lactose fermentation tests were negative **(Table 12).** This combination of morphological and biochemical characteristics is consistent with typical features reported for *Stenotrophomonas maltophilia*. The oxidase-negative and catalase-positive reactions, along with citrate utilization and motility, are key diagnostic traits supporting its preliminary identification. Overall, the biochemical profile suggests that the isolate ASN might be *Stenotrophomonas maltophilia*, a metabolically versatile bacterium well recognized for its adaptability in soil environments and strong biocontrol potential against a broad range of phytopathogens (Ryan *et al*., 2009; Berg & Martinez, 2015; Santoyo *et al*., 2021). Further molecular identification was carried out by 16S rRNA gene sequencing for precise taxonomic confirmation. The neighbour-joining phylogenetic tree based on 16S rRNA gene sequences **(Figure 3)** showed that isolate ASN clustered closely with reference strains of *Stenotrophomonas maltophilia*. Other *Stenotrophomonas* species formed adjacent but distinct clades, clearly separating isolate ASN from closely related taxa and confirming its species-level identity. The obtained 16S rRNA gene sequence of isolates ASN was submitted to the DNA Data Bank of Japan as *Stenotrophomonas maltophilia* ASN with an accession number LC920483.

The molecular identification corroborates earlier morphological and biochemical characteristics of isolate ASN (Gram-negative, catalase-positive, oxidase-negative), consistent with reported profiles of *S. maltophilia* (Ryan *et al*., 2009; Berg & Martinez, 2015). Members of this species are well documented for their plant growth–promoting and biocontrol capabilities, including siderophore production, phytohormone synthesis, and antagonistic metabolite secretion (Santoyo *et al*., 2021). Overall, the combined phenotypic and genotypic evidence confirms isolate ASN as *Stenotrophomonas maltophilia* and highlights its strong potential as a multifunctional PGPR for sustainable tomato cultivation.

Table 1: Physicochemical properties of tomato rhizosphere soils from Shirala region

Parameter	Field 1	Field 2	Field 3	Field 4	Field 5	Mean ± SD
Soil pH	7.0	6.8	7.1	6.9	6.8	6.92 ± 0.13
Moisture content (%)	20.5	22.2	21.8	20.9	23.1	21.70 ± 1.02
Organic carbon (%)	0.74	0.84	0.89	0.81	0.87	0.83 ± 0.06

Table 2: Population density of rhizosphere bacteria isolated from tomato fields of Shirala region

Field Site	Bacterial Population (CFU g^{-1} soil)	Microbial Abundance Level
Field 1	1.51×10^7	High
Field 2	1.23×10^7	High
Field 3	9.7×10^7	Very high
Field 4	1.45×10^7	High
Field 5	8.4×10^7	Very high
Mean ± SD	**$4.44 \times 10^7 \pm 3.73 \times 10^7$**	—

Table 3: Colony morphology of representative rhizosphere bacterial isolates selected for screening

Isolate Code	Colony Colour	Shape	Margin	Elevation	Surface Texture	Size (mm)	Preliminary Group
ASN	Cream	Circular	Entire	Convex	Smooth	2.5	Group I
ASN1	White	Irregular	Undulate	Flat	Rough	3.1	Group II
ASN2	Yellow	Circular	Entire	Raised	Smooth	1.8	Group I
ASN3	Pale cream	Filamentous	Lobate	Flat	Dry	4.0	Group III
ASN4	Off-white	Circular	Entire	Convex	Mucoid	2.9	Group II
ASN5	Light yellow	Circular	Entire	Raised	Smooth	2.1	Group I
ASN6	Cream	Irregular	Undulate	Convex	Rough	3.5	Group II
ASN7	White	Circular	Entire	Flat	Smooth	1.6	Group I

Table 4: Qualitative phosphate solubilization activity of rhizosphere bacterial isolates

Isolate Code	Colony Diameter (mm)	Halo Zone Diameter (mm)	PSI Value	Phosphate Solubilization Ability
ASN	2.5	9.3	4.72	Strong
ASN1	3.1	5.4	2.74	Moderate
ASN2	1.8	4.9	3.72	Strong
ASN3	4.0	2.5	1.63	Weak
ASN4	2.9	5.2	2.79	Moderate
ASN5	2.1	6.0	3.86	Strong
ASN6	3.5	4.3	2.23	Moderate
ASN7	1.6	3.74	3.34	Strong

Table 5: Quantitative phosphate solubilization by rhizosphere bacterial isolates in Pikovskaya's broth

Isolate Code	Soluble Phosphate Released (µg mL^{-1} ± SD)	Solubilization Efficiency
ASN	186.4 ± 6.8	Very high
ASN1	112.3 ± 5.2	Moderate
ASN2	158.7 ± 6.1	High
ASN3	64.5 ± 4.0	Low
ASN4	118.9 ± 5.5	Moderate
ASN5	172.6 ± 6.3	High
ASN6	104.8 ± 5.0	Moderate
ASN7	165.2 ± 6.0	High

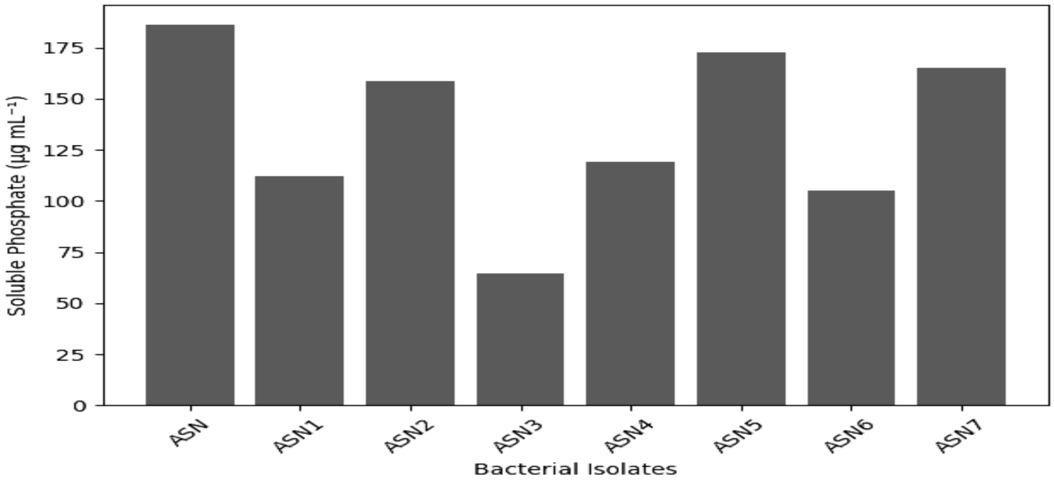

Figure 1: Quantitative phosphate solubilization by rhizosphere bacterial isolates

Table 6: Indole-3-acetic acid (IAA) production by rhizosphere bacterial isolates in LB broth supplemented with L-tryptophan

Isolate Code	IAA Produced ($\mu g\ mL^{-1} \pm SD$)	IAA Production Level
ASN	42.8 ± 2.1	High
ASN1	26.4 ± 1.8	Moderate
ASN2	38.6 ± 2.0	High
ASN3	12.5 ± 1.2	Low
ASN4	29.8 ± 1.6	Moderate
ASN5	40.2 ± 2.3	High
ASN6	24.1 ± 1.7	Moderate
ASN7	36.9 ± 1.9	High

Table 7: Siderophore production by rhizosphere bacterial isolates on CAS agar

Isolate Code	Colony Diameter (mm)	Orange Halo Diameter (mm)	SPI Value	Siderophore Production Ability
ASN	2.5	7.2	3.88	Strong
ASN1	3.1	4.6	2.48	Moderate
ASN2	1.8	4.50	3.50	Strong
ASN3	4.0	2.1	1.53	Weak
ASN4	2.9	4.9	2.69	Moderate
ASN5	2.5	6.8	3.72	Strong
ASN6	3.5	4.2	2.20	Moderate
ASN7	1.6	4.50	3.81	Strong

Table 8: Ammonia production by rhizosphere bacterial isolates in peptone water

Isolate Code	Color Intensity After Nessler's Reagent	Ammonia Production Score	Ammonia Production Ability
ASN	Deep brown	+++	Strong
ASN1	Yellow-brown	++	Moderate
ASN2	Brown	+++	Strong
ASN3	Pale yellow	+	Weak
ASN4	Yellow-brown	++	Moderate
ASN5	Brown	+++	Strong
ASN6	Yellow-brown	++	Moderate
ASN7	Brown	+++	Strong

Table 9: Hydrogen cyanide (HCN) production by rhizosphere bacterial isolates on glycine-supplemented nutrient agar.

Isolate Code	Filter Paper Color Change	HCN Production Score	HCN Production Ability
ASN	Reddish-brown	+++	Strong
ASN1	Light brown	++	Moderate
ASN2	Dark brown	+++	Strong
ASN3	No change (yellow)	–	Negative
ASN4	Light brown	++	Moderate
ASN5	Dark brown	+++	Strong
ASN6	Light brown	++	Moderate
ASN7	Dark brown	+++	Strong

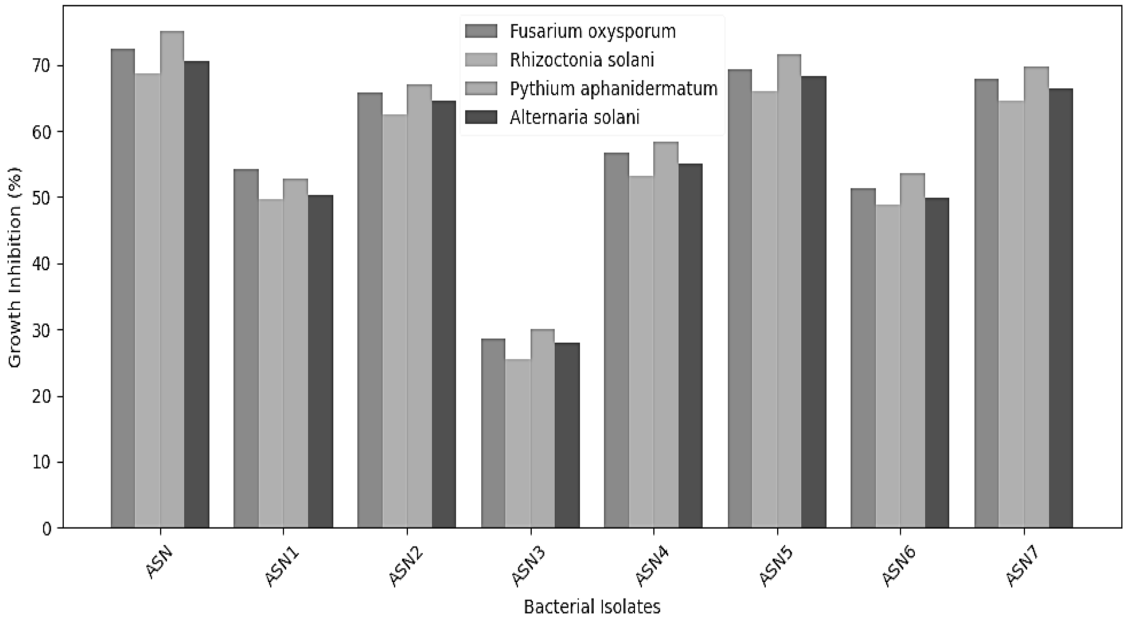

Figure 2: Bar graph showing antagonistic activity of all isolates against the four tomato phytopathogens

Table 10: Antagonistic activity of rhizosphere bacterial isolates against major tomato phytopathogens (dual culture assay)

Isolate Code	**% Inhibition of *Fusarium oxysporum***	**% Inhibition of *Rhizoctonia solani***	**% Inhibition of *Pythium aphanidermatum***	**% Inhibition of *Alternaria solani***	**Antagonistic Strength**
ASN	72.4 ± 2.1	68.7 ± 1.9	75.2 ± 2.4	70.6 ± 2.0	Strong
ASN1	54.3 ± 1.8	49.6 ± 2.0	52.8 ± 1.7	50.2 ± 1.9	Moderate
ASN2	65.8 ± 2.0	62.4 ± 1.8	67.1 ± 2.2	64.5 ± 1.9	Strong
ASN3	28.6 ± 1.5	25.4 ± 1.3	30.1 ± 1.6	27.9 ± 1.4	Weak
ASN4	56.7 ± 1.9	53.2 ± 1.7	58.4 ± 2.0	55.1 ± 1.8	Moderate
ASN5	69.3 ± 2.2	66.1 ± 2.0	71.5 ± 2.3	68.2 ± 2.1	Strong
ASN6	51.4 ± 1.6	48.8 ± 1.5	53.7 ± 1.8	49.9 ± 1.6	Moderate
ASN7	67.9 ± 2.1	64.5 ± 1.9	69.8 ± 2.0	66.4 ± 1.8	Strong

Table 11: One-way ANOVA summary for antagonistic activity of rhizosphere bacterial isolates against tomato phytopathogens

Pathogen	**Source of Variation**	**Sum of Squares (SS)**	**Degrees of Freedom (df)**	**Mean Square (MS)**	**F-value**	**p-value**
Fusarium oxysporum	Between isolates	2594.36	7	370.62	142.85	< 0.001
	Within isolates	72.66	16	4.54	—	—
	Total	2667.02	23	—	—	—
Rhizoctonia solani	Between isolates	2411.18	7	344.45	135.27	< 0.001
	Within isolates	75.48	16	4.72	—	—
	Total	2486.66	23	—	—	—
Pythium aphanidermatum	Between isolates	2783.90	7	397.70	151.92	< 0.001
	Within isolates	83.75	16	5.23	—	—
	Total	2867.65	23	—	—	—
Alternaria solani	Between isolates	2526.44	7	360.92	139.64	< 0.001
	Within isolates	79.16	16	4.95	—	—
	Total	2605.60	23	—	—	—

Table 12: Morphological and biochemical characteristics of rhizosphere bacterial isolate ASN

Test / Character	Result
Gram nature	Gram-negative
Motility	Motile
Catalase test	Positive
Oxidase test	Negative
Citrate utilization	Positive
Indole production	Negative
Methyl red (MR) test	Negative
Voges–Proskauer (VP) test	Positive
Glucose fermentation	Positive
Lactose fermentation	Negative
Sucrose fermentation	Positive
Nitrate reduction	Positive

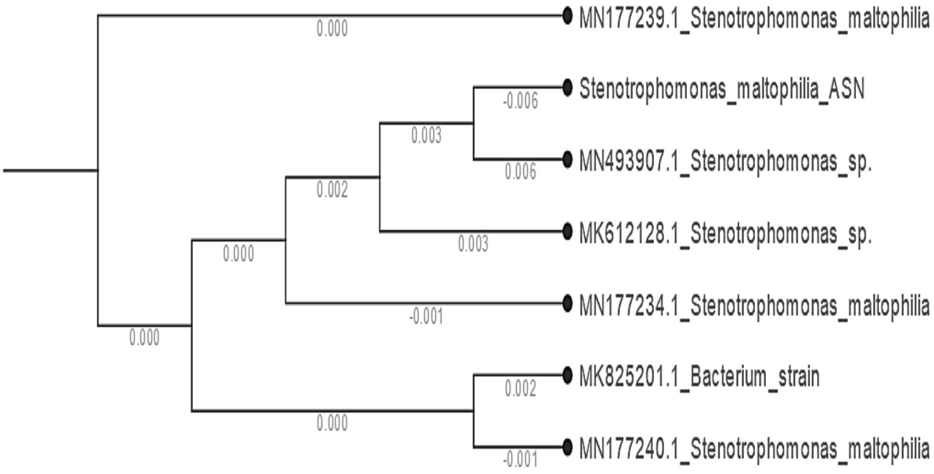

Figure 3: Phylogenetic analysis of isolate ASN

CONCLUSION

The present study demonstrated that tomato rhizosphere soils of the Shirala region harbor a diverse and functionally active bacterial community under favorable physicochemical conditions. Among the isolates, ASN consistently exhibited superior plant growth–promoting and biocontrol traits, including high phosphate solubilization, significant IAA production, strong siderophore secretion, ammonia and HCN production, and broad-spectrum antagonistic activity against major tomato phytopathogens. Statistical analysis confirmed significant variation in antagonistic performance, with isolate ASN emerging as the most effective strain. Morphological, biochemical, and 16S rRNA gene sequencing analysis conclusively identified the isolate ASN as *Stenotrophomonas maltophilia*. Overall, the multifunctional characteristics and strong antagonistic potential of the isolate *Stenotrophomonas maltophilia* highlight its promise as a sustainable bioinoculant candidate for enhancing tomato growth and managing soil-borne diseases under eco-friendly agricultural systems.

Acknowledgement

The authors sincerely thank the Principals of V. N. B. N. and D. A. B. N. College for their valuable support and for providing the necessary facilities to carry out this research work.

REFERENCES

- Agrios, G. N. (2005). *Plant pathology* (5th ed.). Elsevier Academic Press.
- Ahmad, F., Ahmad, I., & Khan, M. S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. *Microbiological Research, 163*(2), 173–181. https://doi.org/10.1016/j.micres.2006.04.00.
- Alavi, P., Müller, H., Cardinale, M., Zachow, C., & Berg, G. (2013). The beneficial rhizosphere bacterium *Stenotrophomonas maltophilia* promotes plant growth and suppresses fungal pathogens. *Applied Soil Ecology, 72*, 13–20. https://doi.org/10.1016/j.apsoil.2013.05.006.
- Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology, 8*, 971. https://doi.org/10.3389/fmicb.2017.00971.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
- Anderson, J. M., & Ingram, J. S. I. (1993). *Tropical soil biology and fertility: A handbook of methods* (2nd ed.). CAB International.
- Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D. L. (2018). Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science, 9*, 1473.

https://doi.org/10.3389/fpls.2018.01473.

- Bakker, A. W., & Schippers, B. (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth stimulation. *Soil Biology and Biochemistry, 19*(4), 451–457. https://doi.org/10.1016/0038-0717(87)90062-6.
- Berg, G., & Martinez, J. L. (2015). Friends or foes: Can we make a distinction between beneficial and harmful strains of the *Stenotrophomonas maltophilia* complex? *Frontiers in Microbiology, 6*, 241. https://doi.org/10.3389/fmicb.2015.00241.
- Berg, G., Eberl, L., & Hartmann, A. (2005). The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. *Environmental Microbiology, 7*(11), 1673–1685. https://doi.org/10.1111/j.1462-2920.2005.00891.x.
- Cappuccino, J. G., & Sherman, N. (2014). *Microbiology: A laboratory manual* (10th ed.). Pearson.
- Compant, S., Duffy, B., Nowak, J., Clément, C., & Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms, and future prospects. *Applied and Environmental Microbiology, 71*(9), 4951–4959. https://doi.org/10.1128/AEM.71.9.4951-4959.2005.
- Compant, S., Samad, A., Faist, H., & Sessitsch, A. (2020). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research, 19*, 29–37. https://doi.org/10.1016/j.jare.2019.03.004.
- Delgado-Baquerizo, M., Oliverio, A. M., Brewer, T. E., et al. (2020). A global atlas of the dominant bacteria found in soil. *Science, 359*(6373), 320–325. https://doi.org/10.1126/science.aap9516.
- Dennis, C., & Webster, J. (1971). Antagonistic properties of species-groups of *Trichoderma*. I. Production of non-volatile antibiotics. *Transactions of the British Mycological Society, 57*(1), 25–39. https://doi.org/10.1016/S0007-1536(71)80077-3.
- Edi-Premono, M., Moawad, A. M., & Vlek, P. L. G. (1996). Effect of phosphate-solubilizing bacteria on phosphate uptake and growth of maize. *Agronomy Journal, 88*(4), 617–623. https://doi.org/10.2134/agronj1996.00021962008800040016x.
- FAO. (2022). *FAOSTAT statistical database*. Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat.
- Gardner, W. H. (1986). Water content. In A. Klute (Ed.), *Methods of soil analysis: Part 1—Physical and mineralogical methods* (2nd ed., pp. 493–544). ASA and SSSA.

- Glick, B. R. (2012). Plant growth-promoting bacteria: Mechanisms and applications. *Scientifica, 2012*, 963401. https://doi.org/10.6064/2012/963401.
- Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiological Research, 169*(1), 30–39. https://doi.org/10.1016/j.micres.2013.09.009.
- Glick, B. R. (2020). *Beneficial plant-bacterial interactions*. Springer.
- Gomez, K. A., & Gomez, A. A. (1984). *Statistical procedures for agricultural research* (2nd ed.). John Wiley & Sons.
- Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiology, 26*(1), 192–195. https://doi.org/10.1104/pp.26.1.192.
- Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: A review. *Annals of Microbiology, 60*(4), 579–598. https://doi.org/10.1007/s13213-010-0117-1.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., & Williams, S. T. (1994). *Bergey's manual of determinative bacteriology* (9th ed.). Williams & Wilkins.
- Jackson, M. L. (1973). *Soil chemical analysis*. Prentice Hall of India.
- Kai, M., Effmert, U., Berg, G., & Piechulla, B. (2010). Volatiles of bacterial antagonists inhibit mycelial growth of the plant pathogen *Rhizoctonia solani*. *Archives of Microbiology, 192*(10), 803–811. https://doi.org/10.1007/s00203-010-0610-4.
- Keswani, C., Singh, H. B., García-Estrada, C., Caradus, J., He, Y. W., Mezaache-Aichour, S., & Glare, T. R. (2019). Antimicrobial secondary metabolites from agriculturally important fungi as next-generation pesticides. *Applied Microbiology and Biotechnology, 103*, 9287–9301. https://doi.org/10.1007/s00253-019-10192-3.
- Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology, 94*(11), 1259–1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259.
- Kramer, J., Özkaya, Ö., & Kümmerli, R. (2020). Bacterial siderophores in community and host interactions. *Nature Reviews Microbiology, 18*, 152–163. https://doi.org/10.1038/s41579-019-0284-4.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

- Maciel-Rodríguez, F., et al. (2025). Influence of soil physicochemical properties on rhizosphere microbial communities. *Soil Biology & Biochemistry.*
- Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M., & Stahl, D. A. (2018). *Brock biology of microorganisms* (15th ed.). Pearson.
- Montgomery, D. C. (2017). *Design and analysis of experiments* (9th ed.). John Wiley & Sons.
- Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta, 27*, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.
- Patten, C. L., & Glick, B. R. (2002). Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Applied and Environmental Microbiology, 68*(8), 3795–3801. https://doi.org/10.1128/AEM.68.8.3795-3801.2002.
- Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya, 17*, 362–370.
- Richardson, A. E., Barea, J. M., McNeill, A. M., & Prigent-Combaret, C. (2009). Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil, 321*(1–2), 305–339. https://doi.org/10.1007/s11104-009-9895-2
- Rijavec, T., & Lapanje, A. (2016). Hydrogen cyanide in the rhizosphere: Not suppressing plant pathogens, but rather regulating availability of phosphate. *Frontiers in Microbiology, 7*, 1785. https://doi.org/10.3389/fmicb.2016.01785.
- Rout, M. E., & Southworth, D. (2023). The rhizosphere microbiome and plant health. *Current Opinion in Microbiology, 64*, 102–110. https://doi.org/10.1016/j.mib.2023.102110.
- Ryan, R. P., Monchy, S., Cardinale, M., et al. (2009). The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology, 7*(7), 514–525. https://doi.org/10.1038/nrmicro2163.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454.
- Santoyo, G., Guzmán-Guzmán, P., Parra-Cota, F. I., Santos-Villalobos, S. D. L., Orozco-Mosqueda, M. D. C., & Glick, B. R. (2021). Plant growth stimulation by rhizobacteria. *Microbiological Research, 252*, 126867. https://doi.org/10.1016/j.micres.2021.126867.
- Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination

of siderophores. *Analytical Biochemistry, 160*(1), 47–56. https://doi.org/10.1016/0003-2697(87)90612-9.

- Sharma, S. B., Sayyed, R. Z., Trivedi, M. H., & Gobi, T. A. (2022). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency. *3 Biotech, 12*, 33. https://doi.org/10.1007/s13205-021-03098-4.
- Singh, D., Sharma, R. R., & Singh, R. (2018). Diseases of tomato and their management. *Indian Phytopathology, 71*, 167–178.
- Skidmore, A. M., & Dickinson, C. H. (1976). Colony interactions and hyphal interference between septate soil fungi. *Transactions of the British Mycological Society, 66*(1), 57–64. https://doi.org/10.1016/S0007-1536(76)80092-5
- Spaepen, S., & Vanderleyden, J. (2011). Auxin and plant–microbe interactions. *Cold Spring Harbor Perspectives in Biology, 3*(4), a001438. https://doi.org/10.1101/cshperspect.a001438.
- Tortora, G. J., Funke, B. R., & Case, C. L. (2019). *Microbiology: An introduction* (13th ed.). Pearson.
- Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil, 255*, 571–586. https://doi.org/10.1023/A:1026037216893
- Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter. *Soil Science, 37*(1), 29–38. https://doi.org/10.1097/00010694-193401000-00003.