Journal of Applied and Natural Science
17(1), 320 - 330 (2025)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Potential of *Stenotrophomonas rhizophila* as plant growth promoting rhizobacterium to improve the growth of mustard crop

A. Philomena Joy Lindsey
Division of Food Processing Technology, School of Engineering and Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore-641114 (Tamil Nadu) India
S. Murugan
Division of Biotechnology, School of Engineering and Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore-641114 (Tamil Nadu) India
J. Veninstine Vivik
Division of Mathematics, School of Sciences, Arts and Media, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore-641114 (Tamil Nadu) India
D. Gershom
Division of Food Processing Technology, School of Engineering and Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore-641114 (Tamil Nadu) India
R. Emilin Renitta*
Division of Food Processing Technology, School of Engineering and Technology, Karunya Institute of Technology and Sciences, Karunya Nagar, Coimbatore-641114 (Tamil Nadu) India

*Corresponding author. E-mail: emilinrenitta@gmail.com

Article Info
https://doi.org/10.31018/jans.v17i1.6068
Received: August 14, 2024
Revised: March 01, 2025
Accepted: March 05, 2025

How to Cite
Lindsey, A. P. J. *et al.* (2025). Potential of *Stenotrophomonas rhizophila* as plant growth promoting rhizobacterium to improve the growth of mustard crop. *Journal of Applied and Natural Science*, 17(1), 320 - 330. https://doi.org/10.31018/jans.v17i1.6068

Abstract
Plant Growth-Promoting Rhizobacteria (PGPR) enhances soil quality and enriches soil fertility. *Stenotrophomonas rhizophila* is one such bacteria that enhances plant growth, especially in saline soil. The indirect role of this bacterium has been identified in Integrated Pest Management wherein Indian mustard has been utilized as a pest trap crop in cauliflower fields. The present study aims to enhance the growth of mustard plants with the periodic application of *S. rhizophila*. Increasing the population of this bacterium in the soil is also expected to enrich soil fertility and ensure protection from pests in cauliflower fields. Thirteen bacteria (S_1 to S_{13}) were isolated from soil samples collected near the root nodules of cauliflower plants from different agricultural fields. Bacterial isolate S3 was identified as *S. rhizophila* through biochemical tests and 16s rRNA sequencing. Four treatments (T_1 to T_4) were selected for a 28-day pot study by varying the soil condition (sterile, non-sterile) and biopriming of mustard seeds with the inoculum of *S. rhizophila*. Once in every 4 days, 0.5 % of the inoculum was added to the pots (T_1, T_3) to identify changes in the growth of mustard plantlets. A high bacterial load of $2.97*10^8$ in the T3 pot was observed comparatively. The obtained results were also statistically significant (*ie.,* $P < 0.05$) regarding total plant height, chlorophyll content, and microbial load. Hence, it was found that *S. rhizophila* can effectively influence the growth of mustard plants thereby encouraging a pest-free environment in cauliflower fields.

Keywords: *Stenotrophomonas rhizophila*, Plant Growth Promoting Rhizobacteria, 16s rRNA sequencing, Mustard

INTRODUCTION

Agriculture significantly contributes to the global economy and its impact on a country's economy is reflected in the rising demand for food and related commodities. Consequently, additional pressure to increase the production is thrusted upon the agricultural sector. To sustain the production rate in the agricultural field, losses encountered must be mitigated. Several methods involving the application of chemical fertilizers, pesticides and biological formulations are being adopted to prevent the invasion of pathogenic microbes and pests. Application of PGPR (Plant Growth Promoting Rhizobacteria) is an effective approach that can significantly increase plant growth and yield. These bacteria protect the plant roots by secreting antimicrobial compounds, induce systemic resistance that could suppress the activity of phytopathogenic bacteria and fungi and facili-

This work is licensed under Attribution-Non Commercial 4.0 International (CC BY-NC 4.0). © : Author (s). Publishing rights @ ANSF.

tate the plant to adapt biotic and abiotic stress (Compant *et al.* 2010; Zhou *et al.* 2021).

Imadi *et al.* (2010) studied the compatibility of plant to the varying saline condition of the soil and reported that salinity has a vigorous effect on land fertility that could eventually lead to economic loss. It was also stated that the increased release of salt ions owing to several anthropogenic activities and reduction in the amount of salt that is leaching out from the rhizosphere in drought regions resulted in the decline of land fertility. Moreover, salinity reduces crop yield, pigmentation, photosynthetic rate, water uptake, and plant growth rate and increases the senescence rate. Mustafa and Akhtar (2019) and Yuvaraj *et al.* (2021) recommended a few strategies to monitor and reduce soil salinity, including periodical monitoring of soil salinity, following appropriate irrigation practices, mulching, crop rotation, replacement of top layer of soil, grafting, planting more trees, deep ploughing, leaching, the addition of nutrients to the soil, phytoremediation and microbial remediation.

Stenotrophomonas rhizophila is reported to survive in high-saline soil, which makes it suitable for enhancing crop yield (Egamberdieva *et al.* 2011). This bacterium also has antifungal properties that aid it in fighting soil-borne phytopathogens (Wolf *et al.* 2002). The antifungal compounds, identified as volatile organic compounds such as terpenes, furans and sulfur-containing compounds, were able to inhibit the growth of fungal phytopathogens *Alternaria alternata* and *Botrytis cinerea* (Raio *et al.* 2023). It is also documented by Imparato (2022) and Schmidt *et al.* (2012) that the bacterium possesses the ability to eliminate deleterious fungal phytopathogens in the rhizosphere of tomato and sweet pepper plants. Additionally, the algicidal activity of *S. rhizophila* was observed in strains isolated from mangrove (*Kandelia candel*) grown in the coastal wetlands and seawater collected from dinoflagellate bloom (Yin *et al.* 2018; Zhang *et al.* 2021). Trehalose utilization, production of glutathione S-transferase enzyme and cold shock proteins are the characteristic features of *S. rhizophila* that describe their plant growth-promoting ability. It also possesses phytodegradation properties (Pinski *et al.* 2020). This research focuses on identifying the plant growth ability of *S. rhizophila* over mustard seedlings. The feasibility of engaging *S. rhizophila* with the naturally present beneficial bacteria is highlighted in this study.

MATERIALS AND METHODS

Sample collection and isolation of Rhizobacteria

Soil sample, especially near the root of a healthy cauliflower plant, was collected from an agricultural field in Theni district, Tamil Nadu, India. The collected soil was properly sealed in a polyethylene bag and stored for further analysis. Rhizobacteria was isolated by spread plate technique using crystal violet pectate (CVP) media prepared according to Helias *et al.* (2022). The media consisted of crystal violet mix ($CaCl_2.2H_2O$- 0.204 g, tryptone- 0.2 g, trisodium citrate- 1 g, $NaNO_3$- 0.4 g, agar-agar- 3 g, crystal violet (1% aqueous solution)- 0.3 mL) and pectin mix (5M NaOH- 0.4 mL, pectin- 3.6 g). Pectin mix was prepared and heated to achieve homogenization of pectin. Both the mixes were sterilized at 121°C, 15 psi for 20 minutes, mixed (pH 7) and poured onto petri dishes. 0.1 mL of serially diluted soil sample was spread onto each plate and the plates were incubated at 30°C for 48 to 72 h. CFU per gram of soil was calculated and colonies were identified based on morphology and sub-cultured in nutrient broth (Himedia) for further analysis (Khan *et al.* 2018).

Biochemical characterization of Rhizobacteria

Based on the morphological variation of bacterial colonies that resembles *Enterobacteria*, thirteen unique bacteria were selected from the incubated petri plates and were assigned numbers from 1 to 13 *ie.,* S_1 to S_{13} ('S' sample) based on the order of sample collection and isolation. Then, each colony was sub cultured in sterile nutrient broth for biochemical analysis. Cell morphology of all the isolated bacteria were observed by Gram staining test (Beveridge, 2001). Growth of each bacterial isolate in Yeast extract Glucose Calcium carbonate medium (YGC), Eosin Methylene Blue (EMB), ability to withstand high salt (5% NaCl) and high temperature (37°C) were checked. Tests including starch hydrolysis, KOH (Potassium Hydroxide) solubility, acid production (Methyl red test), ammonia production (Urease test), production of tryptophanase enzyme (Indole test), gas production through fermentation of sugars (Triple Sugar Iron test) and utilization of citrate as sole carbon (Citrate utilization test) were also experimented (Kado, 2006, Sharafi *et al.* 2010, Ashmawy *et al.* 2015; Adegoke *et al.* 2017, Amoli *et al.* 2017, Ragavi *et al.* 2019, Ashmawy *et al.* 2020; Velmurugan *et al.* 2021, Milek and Lamkiewicz, 2022; Said *et al.* 2023).

Molecular characterization of the Rhizobacterium by 16s rRNA sequencing

Isolation of bacterial DNA

The selected bacterium was subjected to DNA isolation to facilitate sequence identification through bioinformatic tools. DNA lysis buffer, neutralization buffer, RNase enzyme was added to the bacterial cells and incubated at 65˚C for 30 minutes. The mixture was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was added to the chloroform-isoamyl alcohol mixture and centrifuged again. Subsequently, the binding buffer was added to the supernatant and centrifuged. The obtained pellet was then added with washing buffer I, II, and elution buffer and centrifuged. The pellet was collected, and the DNA concentration was measured by

agarose gel electrophoresis (Robe *et al.* 2003; Ghatak *et al.* 2013).

DNA amplification by PCR and sequencing

Polymerase Chain Reaction (PCR) was employed to amplify the isolated DNA using the enzyme Taq DNA polymerase. This enzyme uses oligonucleotides as primer to generate an extended region of double-stranded DNA. Using 2X Taq buffer, dNTPs, magnesium chloride, the isolated DNA was amplified at different temperatures following the standard PCR protocol (Kyule *et al.* 2022; Reyes-Castillo *et al.* 2019). PCR product was purified and sequenced using 16s rRNA universal primers. Finally, the obtained sequence was purified and precipitated using ethanol and eluted by electrophoresis.

Phylogenetic analysis of the DNA sequence

The National Center for Biotechnology Information (NCBI) blast similarity search tool was utilized to identify the obtained 16s rRNA sequence. Subsequently, multiple sequence alignment was checked to identify sequence similarity using the program MUSCLE 3.7 (MUltiple Sequence Comparison by Log-Expectation). To construct the phylogenetic tree of the aligned sequence, PhyML 3.0 aLRT program was selected and the results were meticulously documented in reference to the protocol followed by Edgar (2004), Dereeper *et al.* (2008), Kumar and Manjunatha (2015).

Application of *Stenotrophomonas rhizophila* as the growth-promoting agent of mustard plants

Preparation of treatment pots and mustard seed inoculum

A total of 4 pots were chosen for the study, and each was filled with 10 kg of loamy soil collected from an irrigated region. Soil in two pots was subjected to dry heat sterilization at 160°C for 3 hours, while the other two were left non-sterile (Trevors, 1996; Zhou *et al.,* 2014). Mustard seeds were disinfected by soaking for 5 minutes in 5 % sodium hypochlorite and washed thoroughly with sterile distilled water until the disinfectant solution was completely washed off (Perez-Garcia *et al.* 2023). The treatments included sterile soil with treated seeds (T_1), sterile soil with untreated seeds (T_2), non-sterile soil with treated seeds (T_3), non-sterile soil with untreated seeds (T_4). Treatments were selected, as mentioned in Zhou *et al.* (2014). Treated seeds were previously inoculated with *S. rhizophila*. This is referred to as seed inoculum and was prepared by adding 200 µl of *S. rhizophila* (OD_{600}= 1.0) to 200 mL of sterile nutrient broth (Himedia) and incubated at 30°C in an orbital shaker incubator for 24 hours. Biomass was collected after centrifugation at 5000 rpm for 20 minutes. Using PBS (Phosphate Buffer Saline), the collected biomass was washed thrice to eliminate the growth medium. Ten gram of disinfected mustard seeds were mixed with 10% starch solution to impart an adhesive nature on the seed surface by shaking in an orbital shaker at 100 rpm for 30 minutes. This was added to the washed biomass and mixed well with the help of an orbital shaker at 30°C for 30 minutes. The treated seeds were then air-dried for 30 minutes in a sterile chamber and immediately sowed in the pre-labelled pot.

Growth analysis of mustard plants

Mustard seed growth was observed once every 4 days until 28 days. Total plant height, shoot height, root height, and total plant weight were measured periodically. Absolute growth rate and relative growth rate for each treatment were calculated using the following formulae,

$$\text{Absolute Growth Rate} = (\text{W2} - \text{W1})/(\text{t2} - \text{t1}) \quad \text{Eq. 1}$$

$$\text{Relative Growth Rate} = \ln\left(\frac{\text{W2}}{\text{W1}}\right)/(\text{t2} - \text{t1}) \quad \text{Eq. 2}$$

Where (t2-t1) is the difference in time (days) between two observation period. W1 is the dry weight of the plant at time t1, and W2 is the dry weight of the plant at time t2 (García *et al.* 2006).

Determination of chlorophyll content in young mustard leaves

Chlorophyll content of mustard leaves was determined regarding the protocol mentioned by Perez-Patricio *et al.* (2018). Fresh mustard leaves (0.5g) were collected and macerated in a mortar and pestle. To this, acetone (99%) and ethanol were added in 2:1 ratio and stirred for 1 minute to make it into a homogenised mixture. The content was transferred to a test tube covered with aluminium foil and incubated for 30 minutes in a dark environment at refrigeration, followed by centrifugation at 2000 rpm for 10 minutes. The supernatant was collected and covered with aluminium foil to ensure that the extract was placed in dark condition and added with 5mL of acetone-ethanol mixture (2:1). After 1 minute of stirring, the optical density of the sample was measured at 663 nm and 645 nm in a UV spectrophotometer using acetone ethanol mixture as control. Readings were recorded for all samples and the chlorophyll content was calculated according to the following formula mentioned by Shakeel *et al.* (2019)

$$\text{Chlorophyll a}\left(\frac{\text{mg}}{\text{g}}\right) = ((12.7 * \text{A663}) - (2.69 * \text{A645}))/(\frac{V}{W*1000}) \quad \text{Eq. 3}$$

$$\text{Chlorophyll b}\left(\frac{\text{mg}}{\text{g}}\right) = ((22.9 * \text{A645}) - (4.68 * \text{A663})/(\frac{V}{W*1000}) \quad \text{Eq. 4}$$

$$\text{Total Chlorophyll Content} = \text{Chlorophyll a} + \text{Chlorophyll b} \quad \text{Eq. 5}$$

Where V is the total volume and W is the weight of sample

Enumeration of bacteria in treatment pots

Bacterial load of each treatment pot was determined by the total plate count method from the 0th day till the 28th day. One gram of soil sample was collected from each pot and subjected to serial dilution. From each dilution, 100 µl of the sample was transferred to sterile nutrient agar plates (Himedia) and uniformly spread with an L-rod. After 24 h incubation at 30°C, the observed bacterial colonies were counted using a digital colony counter to determine Colony forming unit (CFU) per gram (Mushtaq *et al.* 2023).

Statistical Analysis

The documented data was statistically analysed using a two-way ANOVA (Analysis of Variance) in the R programming language and the least significant difference between the treatments was computed with a significance level of $p < 0.05$.

RESULTS

Biochemical characterization of isolated rhizobacteria

Thirteen bacterial isolates (S_1 to S_{13}) were identified and subjected to biochemical characterization. Among them, the isolate S_3 exhibited properties similar to *Stenotrophomonas rhizophila.* This bacterium (S_3) was identified to have Gram-negative rod-shaped morphology and exhibited no growth in YGC agar but was observed to grow actively in EMB agar. It survived under high salt conditions (5% NaCl) but could not sustain its growth at 37°C. Negative results were observed for starch hydrolysis, KOH solubility, methyl red, triple sugar iron and urease test while positive results were reported for indole and citrate utilization tests. Biochemical test results of all the 13 bacterial isolates are reported in Table 1.

16S rRNA sequencing and phylogenetic analysis of isolated DNA

The obtained biochemical result of S_3 bacterium conferred with *S. rhizophila.* Sequence identification by 16S rRNA sequencing protocol confirmed S_3 bacterium as *S. rhizophila.* Moreover, BLASTn similarity search tool resulted in 100% query for the obtained sequence conforming its identity as *S. rhizophila.* A phylogenetic tree was obtained using PhyML software. This tree helps to understand the evolutionary connection of *S. rhizophila* (Fig. 1) (Kumar and Manjunatha, 2015).

Application of *Stenotrophomonas rhizophila* as growth promoting agent of mustard plants

Characteristics of cultivable soil

The collected soil was reported to be suitable for irrigation and was found to be 7.5 years old. The texture of the soil was identified as clay loam, non-calcareous, with a bulk density of 1.11 g/cc. The pH of the soil was neutral (pH 7) and possess electrical conductivity of 0.16 dSm-^{1}. The organic carbon present in the soil is quite high (1.19%), low nitrogen content (175.6 Kg/ha), high phosphorus content (26.2 Kg/ha) and high potassium content (672 Kg/ha).

Treatment of mustard seeds and saplings with *S. rhizophila*

Perfectly washed biomass (1g) of *S. rhizophila* was mixed with starch treated mustard seeds in a shaker incubator at room temperature for 30 minutes and dried thoroughly before sowing into the pots. Plants in T_1 and T_3 were watered with 100 mL of sterile distilled water containing biomass suspension (0.5 g) every four days. Shoot height, root height and total height of mustard plants were measured every four days and the data is represented graphically (Figs 2-4). Total weight of a mustard plant was also checked periodically using a chamber hood precision weighing balance (Fig. 5).

Absolute Growth Rate (AGR) of a plant is the measurement of plant growth per unit of time, while Relative Growth Rate (RGR) is the change in mass accumulation with respect to the initial stage of plant growth (Ghule *et al.* 2013). AGR, RGR, and Chlorophyll content for all treatments were calculated and are represented graphically (Figs 6-8).

Enumeration of bacteria in treatment pots containing mustard plants

Periodical microbial enumeration in soil samples collected from each treatment pot is mentioned graphically in Fig. 9. It was observed that the microbial load gradually increased.

DISCUSSION

Stenotrophomonas rhizophila was identified as Gram-negative, rod-shaped bacteria. This is similar to the study by Ozsahin *et al.* (2014) and Elhosieny *et al.* (2023) who successfully isolated this bacterium and identified its morphology. Among the 13 isolated bacteria (S_1 to S_{13}), S_3 has been identified as *S. rhizophila* through 16s rRNA sequencing. The phylogenetic tree revealed that this bacterium shares a common ancestor with *S. maltophilia*, a Gamma proteobacterium which was mentioned in Ryan *et al.* (2009). Ability of the isolated bacterium to sustain its growth at 5% NaCl in nutrient agar supplemented with excess salt confers its PGPR property. The major property of PGPR lies in its capability to grow in saline soils and was previously documented by Roder *et al.* (2005), Egamberdieva *et al.* (2011), and Alexander *et al.* (2020). In the present study, the isolated bacterium did not exhibit its growth at 37°C. In another study, *S. maltophilia* was reported to grow at 37°C despite being the closely related spe-

Table 1. Biochemical test results of the isolated Rhizobacteria exhibiting the morphological characteristics and inherent property ("S" bacterial isolate, "+" positive test result, "-" negative test result)

Isolate No.	Gram staining	Growth				Starch Hydrolysis	KOH solubility	Methyl Red Test	Urease Test	Indole Test	TSI Test	Citrate utilization
		EMB	YGC	5% NaCl	37°C							
S_1	-	-	-	-	-	-	-	-	+	+	+	-
S_2	-	+	-	-	+	+	+	-	-	+	+	+
S_3	-	+	-	+	-	-	+	-	-	+	+	+
S_4	-	+	-	-	+	+	+	-	-	+	+	+
S_5	-	+	-	-	+	+	+	-	-	+	+	+
S_6	-	+	-	+	+	+	+	+	-	+	+	+
S_7	-	+	-	+	+	+	+	+	+	-	+	+
S_8	-	+	+	+	+	+	+	-	-	+	+	+
S_9	-	+	-	+	+	+	+	-	+	+	+	+
S_{10}	-	+	-	+	+	+	+	-	+	+	+	+
S_{11}	-	+	+	+	+	+	+	+	+	-	+	+
S_{12}	-	+	+	+	+	+	-	+	+	-	+	+
S_{13}	-	+	+	+	+	+	+	+	-	-	+	+

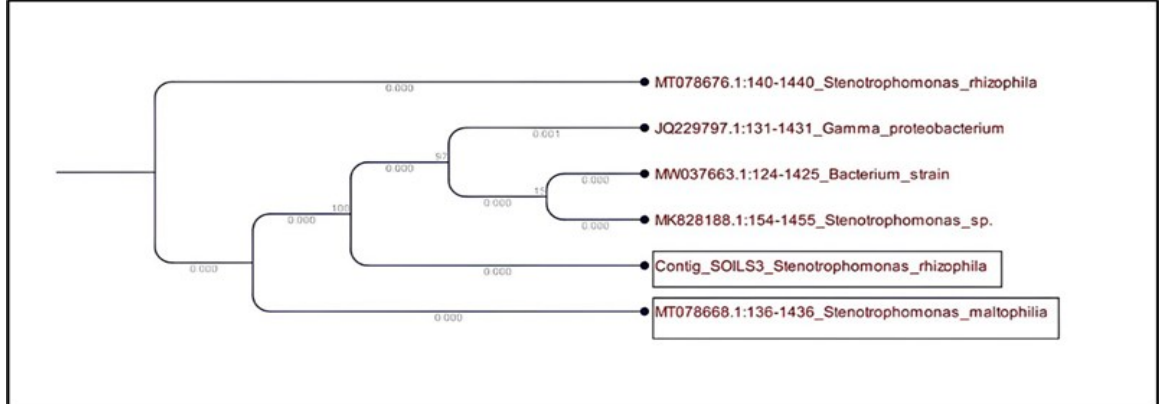


Fig. 1. *Phylogenetic tree of the isolated S. rhizophila demonstrating its ancestral origin from the Stenotrophomonas sp. and its close relationship with Stenotrophomonas maltophilia*

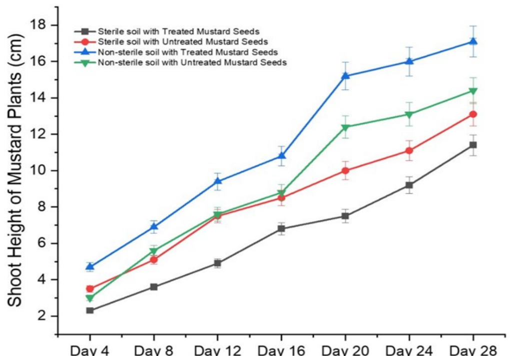


Fig. 2. *Shoot height of mustard plants grown in sterile and non-sterile soil*

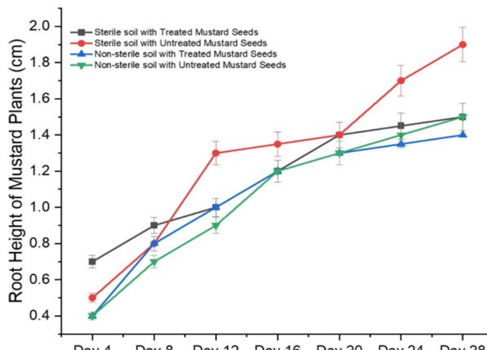


Fig. 3. *Root height of mustard plants grown in sterile and non-sterile soil*

cies of *S. rhizophila*. This difference is due to the absence of heat shock genes and other virulence factors in *S. rhizophila* as documented by Alavi *et al.* (2014). In the present study, bacterial isolate 'S_3' conferred with the biochemical test results of *Stenotrophomonas* sp. reported in Wolf *et al.* (2002). Biochemical test results conferred that the isolated bacterium can effectively utilize glucose, sucrose, lactose and citrate but was unable to hydrolyze starch, secrete acetic and other acids. Lebrazi *et al.* (2020) investigated the role of tryptophan in IAA production by the rhizospheric bacteria *Phyllobacterium sp., Bacillus sp., Agrobacterium sp.,* and *Rhizobium sp.* isolated from root nodules. It was reported that IAA production by these bacteria plays a key role in stimulating the growth of *Acacia cyanophylla* seedlings. Indole test helps to identify the presence of tryptophan, a precursor of IAA (Indole acetic acid) production. IAA is an auxin that enhances plant growth and development and is the indicator of the PGPR property of a bacterium. The enzyme tryptophanase in

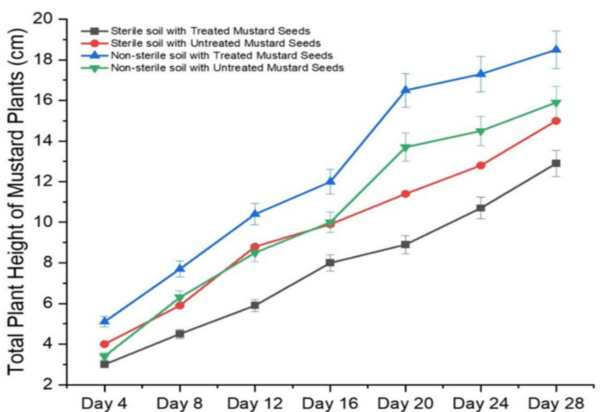


Fig. 4. *Total plant height of mustard plants grown in sterile and non-sterile soil*

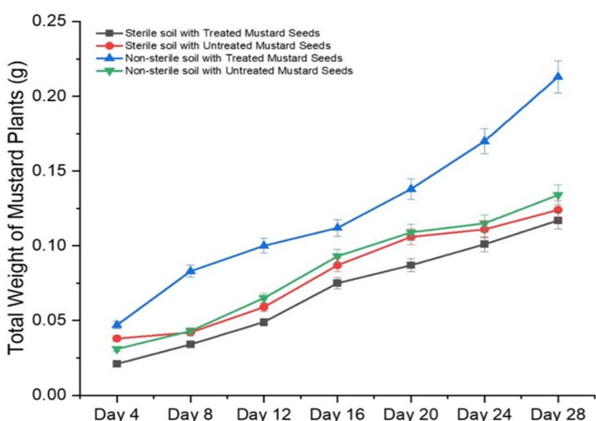


Fig. 5. *Total weight of mustard plants grown in sterile and non-sterile soil*

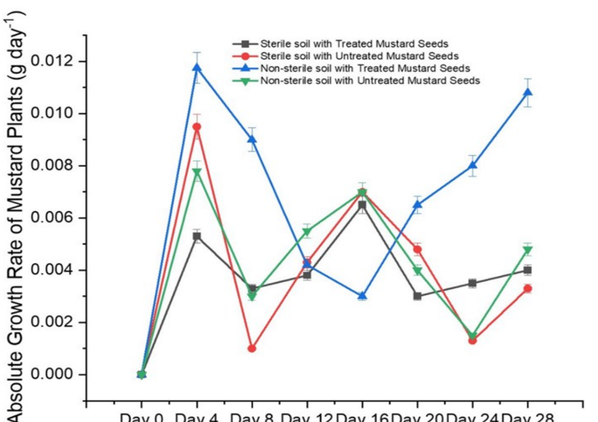


Fig. 6. *Absolute Growth Rate of mustard plants grown in sterile and non-sterile soil*

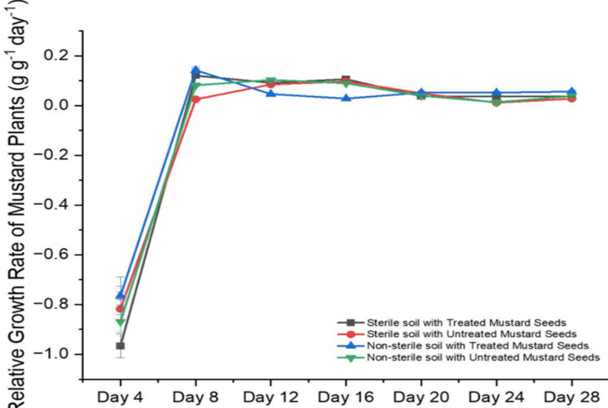


Fig. 7. *Relative Growth Rate of mustard plants grown in sterile and non-sterile soil*

S. rhizophila converts tryptophan into indole, which was observed through green ring formation upon adding Kovac's reagent. This confirms the PGPR property of *S. rhizophila.*

Indian mustard was identified as a trap crop in cauliflower, cabbage, Chinese cabbage and broccoli fields and their efficacy in controlling pests was studied as a part of insect-pest management by Charleston and Kfir (2000) and George *et al.* (2009). As it was determined to improve mustard plant growth, the seeds were initially treated with *S. rhizophila* by seed biopriming. Biopriming involves the application of a binding agent to bind the bacterium onto the seed. This method is reported to be eco-friendly and has the potential to improve growth and yield by Mahmood *et al.* (2016); Rocha *et al.* (2019); Chakraborti *et al.* (2022) and Fiodor *et al.* (2023). In this study, the starch solution was prepared in a ratio of 1:10 to bind *S. rhizophila* onto the surface of mustard seeds as a means of biopriming. This process aided adherence of the bacterial inoculum onto the surface of seeds through proper mixing and drying at room temperature.

In this study, the growth of mustard plants was expected to increase after the periodical application of *S. rhizophila*. Inoculation of seeds with PGPR, biopriming and the periodic application of *S. rhizophila* in treatment pots positively enhanced the growth of mustard plantlets in treatment pots T_3 and T_4. Plantlets in T_4 treatment pots showed increased growth than T_2 and T_1 but were not greater than T_3. Since, *S. rhizophila* was not added to T_4 pot, a reduction in the growth of mustard plantlets was observed, whereas the utilization of sterile soil in T_1 and T_2 treatment pots affected the growth of mustard plantlets. Amongst the treatment pots, plantlets in treatment pot T_3 exhibited healthy shoot and root growth as PGPR-inoculated seeds were sowed in the pot. This result is similar to the study by Sharma *et al.* (2018), which reported enhanced growth of mustard plants in PGPR-inoculated seeds compared to uninoculated mustard seeds. Though T_1 and T_2 pots were filled with sterile soil, the growth of mustard plants was comparatively higher in T_3 and T_4 pots in terms of total plant height, shoot height and weight. Therefore, soil condition was identified as a factor of concern in this study. Similarly, Gholami *et al.* (2009) and (Appanna, 2007) reported an increase in the plant height and weight of maize plants treated with *Azospirillum lipoferum,* which is attributed to the synthesis of indole 3-acetic acid

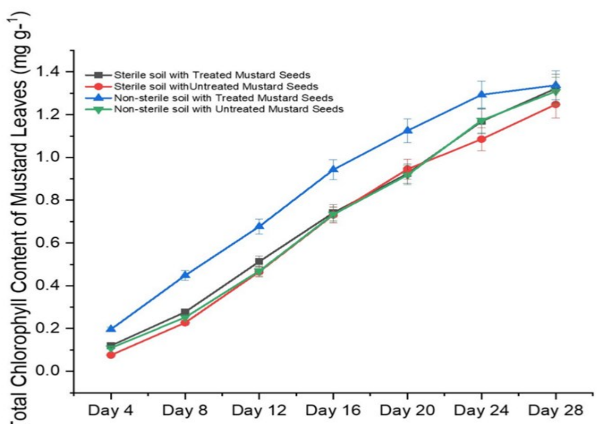


Fig. 8. *Total chlorophyll content of mustard leaves collected from the treatment pots*

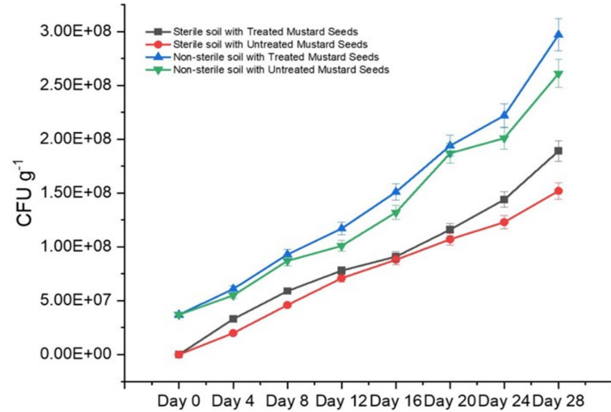
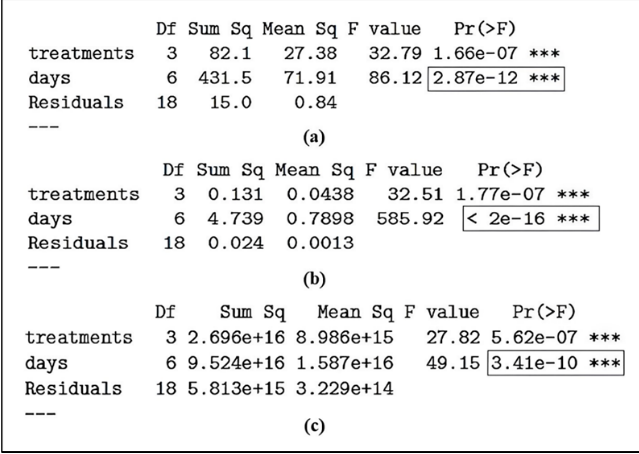


Fig. 9. *Microbial load of soil collected from treatment pots with mustard plants*

(IAA) and other auxins. Earlier, Khalid *et al.* (2004) also observed an increase in auxin synthesis by PGPR in non-sterile soil with a positive impact on the growth and yield of wheat crops. The competitive ability of PGPR to survive in the presence of other microbes could also have enhanced wheat plant growth. On the contrary, Ding *et al.* (2023) observed decreased microbial load in sterile soil. Though the macronutrient content remained the same, the diversity of microorganisms was found to be depleted, and it was reflected in the rate of crop growth. Nezarat and Gholami (2009) also noticed stimulating effects on leaf surface area, dry weight of leaf, and shoot in maize crops grown in non-sterile soil. It is inferred from these studies that growth was comparatively better in non-sterile soil with PGPR than in sterile soil. The present study also exhibited commendable growth of mustard plantlets in T_3 pot. This could also be reasoned as the synergistic activity of *S. rhizophila* with other beneficial bacteria present in the non-sterile soil.

The relative growth of a plant, in terms of height, weight, and time, defines the growth rate of a plant. AGR and RGR are important in identifying the growth model of mustard plants (Tessmer *et al.* 2013). Absolute growth rate defines the total growth of a plant per unit time while the relative growth rate defines the rate of plant growth per unit dry matter (Ghule *et al.* 2013). In the present study, the RGR of mustard plantlets exhibited a monomolecular growth pattern (Fig. 7). it follows a first-order growth pattern. This type of growth pattern is also called the Mitscherlich model and is a non-linear growth type. AGR was reported to increase faster during the initial plant growth stage and decrease later for this type of plant growth. Paine et al. (2011) observed this type of growth who documented the *Cerastium diffusum* and *Holcus lanatus* growth model. In the present study, the AGR of plantlets in T_3 pot was comparatively high compared to other treatment pots (Fig. 6) and the results demonstrated the monomolecular growth pattern. Earlier, Lowry and Smith (2018) stated an increase in the RGR of cover plants that effectively enhanced the suppression of weed plants. This was due to increased mass accumulation in the agricultural field. Carbon has been reported as the prime nutrient for mass accumulation, and it induces heightened growth of shoots and roots in plants. The present study observed higher shoot growth of mustard seeds in a monomolecular pattern.

Previously, Khan *et al.* (2023) observed an increase in chlorophyll content after the application of the PGPR strains *Pseudomonas fluorescens* and *Azotobacter chroococcum*. In another study, *Pseudomonas fluorescens* and *Bacillus subtilis* were able to indirectly increase chlorophyll content and reduce the intensity of Turnip Mosaic Virus in the Indian mustard plant (Diyansah *et al.* 2013). Metal ions also influence chlorophyll content in the soil. Metal toxicity in soil hinders plant growth due to the interaction of certain metal ions with the enzymes responsible for respiration and photosynthesis. Pal *et al.* (2019) introduced IAA-producing



	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
treatments	3	82.1	27.38	32.79	1.66e-07	***
days	6	431.5	71.91	86.12	2.87e-12	***
Residuals	18	15.0	0.84			

(a)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
treatments	3	0.131	0.0438	32.51	1.77e-07	***
days	6	4.739	0.7898	585.92	< 2e-16	***
Residuals	18	0.024	0.0013			

(b)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
treatments	3	2.696e+16	8.986e+15	27.82	5.62e-07	***
days	6	9.524e+16	1.587e+16	49.15	3.41e-10	***
Residuals	18	5.813e+15	3.229e+14			

(c)

Fig, 10. *Two-way ANOVA results between treatments and (a) total plant height (b) total chlorophyll content (c) microbial load during the 28-day study. P value obtained for each study was less than 0.05 and the data is highlighted for each statistical analysis*

PGPR bacteria *Lysinibacillus varians* and *Pseudomonas putida* that effectively improved the growth of mustard plants in soil contaminated with cadmium. Meanwhile, chlorophyll content was also observed to increase after PGPR application on mustard plants. The present study exhibited increased chlorophyll content of mustard plantlets in the T_3 pot compared with other treatment pots. This is on par with the growth rate results, which also influences the chlorophyll content. It was evident that the change in chlorophyll content of a plant indicates its response to PGPR application. The chlorophyll content of mustard leaves in the present study was comparatively low in treatment pots that did not receive *S. rhizophila* application (Fig. 8).

Moreover, the population of bacteria in the T_3 pot exhibited a gradual increase with the periodic addition of *S. rhizophila*, and it was relatively lower in other treatments (Fig. 9). As PGPR possesses the ability to suppress soil-borne pathogens and enhance plant growth, the increased bacterial load observed in this study was anticipated as *S. rhizophila* and other naturally available PGPR in the soil. Earlier, Ortiz-Castro *et al.* (2009) and Koza *et al.* (2022) reviewed and reported the availability of various PGPR in soil and its role in enhancing plant growth. The proposed research also demonstrated the same. The present research validates the hypothesis that soil and seed treatment has an observable effect on plant height, chlorophyll content in mustard leaves, and total microbial count during the 28-day study. Based on the statistical analysis of the observed data, we found a significant difference between the treatments in terms of total plant height, chlorophyll content, and microbial load. The statistical results are depicted in Fig. 10. It was evident through this study that *S. rhizophila* can effectively enhance the growth of mustard plants in synergy with other naturally available PGPR in soil.

Conclusion

The plant growth-promoting ability of *S. rhizophila* is elucidated through this study. Among the four treatments, *i.e.,* treatment pot containing sterile soil with treated seeds (T_1), sterile soil with untreated seeds (T_2), non-sterile soil with treated seeds (T_3), non-sterile soil with untreated seeds (T_4), treatment pot T_4 exhibited increased growth of mustard plantlets. The addition of 0.5% of *S. rhizophila* at four-day intervals further attributed to the growth rate of mustard plants. It is also observed that the possible synergistic activity of *S. rhizophila* with other natural microbiota in the T_3 pot has enriched the growth of mustard plantlets. It is recommended that *S. rhizophila* be integrated into biofertilizer formulations, particularly to hasten the growth of mustard seedlings and other *Brassica* sp.

ACKNOWLEDGEMENTS

The author(s) are thankful to Karunya Institute of Technology and Sciences, Coimbatore and the University Grants Commission (UGC) for providing the necessary support for successfully completing this research.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Adegoke, A. A., Stenstrom, T. A., & Okoh, A. I. (2017). *Stenotrophomonas maltophilia* as an Emerging Ubiquitous Pathogen: Looking Beyond Contemporary Antibiotic Therapy. *Frontiers in Microbiology*, 8, 02276. https://doi.org/10.3389/fmicb.2017.02276
2. Alavi, P., Starcher, M. R., Thallinger, G. G., Zachow, C., Muller, H., & Berg, G. (2014). *Stenotrophomonas* comparative genomics reveals genes and functions that differentiate beneficial and pathogenic bacteria. *BMC Genomics*, 15(1), 482. https://doi.org/10.1186/1471-2164-15-482
3. Alexander, A., Singh, V. K., & Mishra, A. (2020). Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 Induces Salt Tolerance by Modulating Physiology and Biochemical Activities of *Arachis hypogaea*. *Frontiers in Microbiology*, 11(568289). https://doi.org/10.3389/fmicb.2020.568289
4. Amoli, R. I., Nowroozi, J., Sabokbar, A., & Rajabniya, R. (2017). Isolation of *Stenotrophomonas maltophilia* from clinical samples: An investigation of patterns motility and production of melanin pigment. *Asian Pacific Journal of Tropical Biomedicine*, 7(9), 826–830. https://doi.org/10.1016/j.apjtb.2017.08.012
5. Appanna, V. (2007). Efficacy of Phosphate Solubilizing Bacteria Isolated from Vertisols on Growth and Yield Parameters of Sorghum. *Research Journal of Microbiology*, 2(7), 550–559.
6. Ashmawy, N. A., Behiry, S. I., Al-Huqail, A. A., Ali, H. M., & Mohamed. (2020). Bioactivity of Selected Phenolic Acids and Hexane Extracts from *Bougainvilla spectabilis* and *Citharexylum spinosum* on the Growth of *Pectobacterium carotovorum* and *Dickeya solani* Bacteria: An Opportunity to Save the Environment. *Processes*, 8(4), 482–482. https://doi.org/10.3390/pr8040482
7. Ashmawy, N. A., Jadalla, N. M., Shoeib, A. A., & El-Bebany, A. F. (2015). Identification and Genetic Characterization of Pectobacterium spp. and Related Enterobacteriaceae Causing Potato Soft Rot Diseases in Egypt. *Journal of Pure and Applied Microbiology*, *9*(3), 1847–1858.
8. Beveridge, T. V. (2001). Use of the Gram stain in microbiology. *Biotechnic & Histochemistry*, 76(3), 111–118.
9. Chakraborti, S., Bera, K., Sadhukhan, S., & Dutta, P. (2022). Bio-priming of seeds: Plant stress management and its underlying cellular, biochemical and molecular mechanisms. *Plant Stress*, 3, 100052. https://doi.org/10.1016/j.stress.2021.100052
10. Charleston, D. S., & Kfir, R. (2000). The possibility of using Indian mustard, *Brassica juncea*, as a trap crop for the

diamondback moth, *Plutella xylostella*, in South Africa. *Crop Protection*, 19(7), 455–460. https://doi.org/10.1016/s0261-2194(00)00037-5

11. Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, 42 (5), 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024
12. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F. ., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M., & Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 36, W465–W469. https://doi.org/10.1093/nar/gkn180
13. Ding, J. Y. M., Ho, L. S., Ibrahim, J., Teh, C. K., & Goh, K. M. (2023). Impact of sterilization and chemical fertilizer on the microbiota of oil palm seedlings. *Frontiers in Microbiology*, 14(1091755). https://doi.org/10.3389/fmicb.2023.1091755
14. Diyansah, B., Aini, L. Q., & Hadiastono, T. (2013). The effect of PGPR (Plant Growth Promoting Rhizobacteria) *Pseudomonas fluorescens* and *Bacillus subtilis* on Leaf Mustard Plant (Brassica juncea L.) Infected by TuMV (Turnip Mosaic Virus). *Journal of Tropical Plant Protection*, 1(1), 30–38.
15. Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, 32(5), 1792–1797. https://doi.org/10.1093/nar/gkh340
16. Egamberdieva, D., Kucharova, Z., Davranov, K., Berg, G., Makarova, N., Azarova, T., Chebotar, V., Tikhonovich, I., Kamilova, F., Validov, S. Z., & Lugtenberg, B. (2011). Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. *Biology and Fertility of Soils*, 47(2), 197–205. https://doi.org/10.1007/s00374-010-0523-3
17. Elhosieny, A. A. E., Zayed, M. S., Selim, S., & Aziz, N. H. A. (2023). *Stenotrophomonas rhizophila* a Novel Plant-Associated Bacterium with Distinguished PGPRs Properties. *Arab Universities Journal of Agricultural Sciences*, 31 (1), 41–50. https://doi.org/10.21608/ajs.2023.159562.1493
18. Fiodor, A., Ajijah, N., Dziewit, L., & Pranaw, K. (2023). Biopriming of seed with plant growth-promoting bacteria for improved germination and seedling growth. *Frontiers in Microbiology*, 14, 1142966. https://doi.org/10.3389/fmicb.2023.1142966
19. García, A. L., Franco, J. A., Nicolás, N., & Vicente, R. M. (2006). Influence of Amino Acids in the Hydroponic Medium on the Growth of Tomato Plants. *Journal of Plant Nutrition*, 29(12), 2093–2104. https://doi.org/10.1080/01904160600972183
20. George, D. R., Collier, R., & Port, G. (2009). Testing and improving the effectiveness of trap crops for management of the diamondback moth *Plutella xylostella* (L.): a laboratory-based study. *Pest Management Science*, 65(11), 1219–1227. https://doi.org/10.1002/ps.1813
21. Ghatak, S., Muthukumaran, R. B., & Nachimuthu, S. K. (2013). A Simple Method of Genomic DNA Extraction from Human Samples for PCR-RFLP Analysis. *Journal of Biomolecular Techniques*, 24(4), 224–231. https://doi.org/10.7171/jbt.13-2404-001
22. Gholami, A., Shahsavani, S., & Nezarat, S. (2009). The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination, Seedling Growth and Yield of Maize. *World Academy of Science, Engineering and Technology*, 49, 19–24.
23. Ghule, P. L., Dahiphale, V. V., Jadhav, J. D., & Palve, D. K. (2013). Absolute growth rate, relative growth rate, net assimilation rate as influenced on dry matter weight of Bt cotton. *International Research Journal of Agricultural Economics and Statistics*, 4(1), 42–46.
24. Helias, V., Hamon, P., Huchet, E., Wolf, J. V. D., & Andrivon, D. (2011). Two new effective semiselective crystal violet pectate media for isolation of *Pectobacterium* and *Dickeya*. *Plant Pathology*, 61(2), 339–345. https://doi.org/10.1111/j.1365-3059.2011.02508.x
25. Imadi, S. R., Shah, S. W., Kazi, A. G., Azooz, M. M., & Ahmad, P. (2010). Phytoremediation of Saline Soils for Sustainable Agricultural Productivity. In M. Ashraf, M. Ozturk, & M. S. A. Ahmad (Eds.), *Plant Adaptation and Phytoremediation* (pp. 455–468). Springer Dordrecht. https://doi.org/10.1016/b978-0-12-803158-2.00018-7
26. Imparato, V. M. (2022). *Stenotrophomonas rhizophila Chitinase Variation and its Role in Suppressing Fusarium oxysporum Pathogenesis in Tomato* (pp. 1–8). https://www.proquest.com/dissertations-theses/em-stenotrophomonas-rhizophila-chitinase/docview/2734632892/se-2
27. Kado, C. I. (2006). Erwinia and Related Genera. *The Prokaryotes*, 6, 443–450. https://doi.org/10.1007/0-387-30746-x_15
28. Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96(3), 473–480. https://doi.org/10.1046/j.1365-2672.2003.02161.x
29. Khan, F., Inamul, H., Nimatullah, Tariq, S., Muhammad, F., Ohia, C., & Tauseef, A. (2018). Isolation, Characterisation and Identification of Plant Growth Promoting Rhizobacteria from Cauliflower (*Brassica oleracea*). *Archives of Basic and Applied Medicine*, 6(1), 55–60.
30. Khan, V., Umar, S., & Iqbal, N. (2023). Palliating Salt Stress in Mustard through Plant-Growth-Promoting Rhizobacteria: Regulation of Secondary Metabolites, Osmolytes, Antioxidative Enzymes and Stress Ethylene. *Plants*, 12(4), 705. https://doi.org/10.3390/plants12040705
31. Koza, N. A., Adedayo, A. A., Babalola, O. O., & Kappo, A. P. (2022). Microorganisms in Plant Growth and Development: Roles in Abiotic Stress Tolerance and Secondary Metabolites Secretion. *Microorganisms*, 10(8), 1528. https://doi.org/10.3390/microorganisms10081528
32. Kumar, S. V. P., & Manjunatha, B. K. (2015). Studies on hydrocarbon degradation by the bacterial isolate *Stenotrophomonas rhizophila* (PM-1) from oil spilled regions of Western Ghats of Karnataka. *Science, Technology and Arts Research Journal*, 4(3), 139–144. https://doi.org/10.4314/star.v4i3.21
33. Kyule, D. N., Maingi, J. M., Njeru, E. M., & Nyamache, A. K. (2022). Molecular Characterization and Diversity of Bacteria Isolated from Fish and Fish Products Retailed in Kenyan Markets. *International Journal of Food Science*, 2022(2379323), 1–12. https://doi.org/10.1155/2022/2379323
34. Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi,

M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. *Journal of Genetic Engineering and Biotechnology*, 18(1). https://doi.org/10.1186/s43141-020-00090-2

35. Lowry, C. I., & Smith, R. G. (2018). Weed Control Through Crop Plant Manipulations. In K. Jabran & B. S. Chauhan (Eds.), *Non-Chemical Weed Control* (pp. 73–96). Academic Press. https://doi.org/10.1016/B978-0-12-809881-3.00005-X
36. Mahmood, A., Turgay, O. C., Farooq, M., & Hayat, R. (2016). Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS Microbiology Ecology*, 92 (fiw112). https://doi.org/10.1093/femsec/fiw112
37. Milek, J., & Lamkiewicz, J. (2022). The starch hydrolysis by α-amylase Bacillus spp.: an estimation of the optimum temperatures, the activation and deactivation energies. *Journal of Thermal Analysis and Calorimetry*, 147, 14459–14466. https://doi.org/10.1007/s10973-022-11738-1
38. Mushtaq, H., Ganai, B. A., & Jehangir, A. (2023). Exploring soil bacterial diversity in different micro-vegetational habitats of Dachigam National Park in North-western Himalaya. *Scientific Reports*, 13(3090). https://doi.org/10.1038/s41598-023-30187-w
39. Mustafa, G., & Akhtar, M. S. (2019). Crops and Methods to Control Soil Salinity. In M. S. Akhtar (Ed.), *Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches* (pp. 237–251). Springer. https://doi.org/10.1007/978-981-13-8805-7_11
40. Nezarat, S., & Gholami, A. (2009). Screening Plant Growth Promoting Rhizobacteria for Improving Seed Germination, Seedling Growth and Yield of Maize. *Pakistan Journal of Biological Sciences*, 12(1), 26–32. https://doi.org/10.3923/pjbs.2009.26.32
41. Ortiz-Castro, R., Contreras-Cornejo, H. A., Macías-Rodriguez, L., & López-Bucio, J. (2009). The role of microbial signals in plant growth and development. *Plant Signaling & Behavior*, 4(8), 701–712. https://doi.org/10.4161/psb.4.8.9047
42. Ozsahin, E., Sezen, K., Demir, I., & Demirbag, Z. (2014). Bacterial isolates from *Palomena prasine* (Hemiptera: Pentatomidae) include potential microbial control agents. *Biocontrol Science and Technology*, 24(9), 1039–1051. https://doi.org/10.1080/09583157.2014.918584
43. Paine, C. E. T., Marthews, T. R., Vogt, D. R., Purves, D., Rees, M., Hector, A., & Turnbull, L. A. (2011). How to fit nonlinear plant growth models and calculate growth rates: an update for ecologists. *Methods in Ecology and Evolution*, 3(2), 245–256. https://doi.org/10.1111/j.2041-210x.2011.00155.x
44. Pal, A. K., Mandal, S., & Sengupta, C. (2019). Exploitation of IAA Producing PGPR on mustard (Brassica nigra L.) seedling growth under cadmium stress condition in comparison with exogenous IAA application. *Plant Science Today*, 6(1), 22–30. https://doi.org/10.14719/pst.2019.6.1.440
45. Perez-Garcia, L.-A., Sáenz-Mata, J., Fortis-Hernandez, M., Navarro-Munoz, C. E., Palacio-Rodríguez, R., & Preciado-Rangel, P. (2023). Plant-Growth-Promoting Rhizobacteria Improve Germination and Bioactive Compounds in Cucumber Seedlings. *Agronomy*, 13(2), 315. https://doi.org/10.3390/agronomy13020315
46. Perez-Patricio, M., Camas-Anzueto, J., Sanchez-Alegria, A., Aguilar-González, A., Gutiérrez-Miceli, F., Escobar-Gómez, E., Voisin, Y., Rios-Rojas, C., & Grajales-Coutino, R. (2018). Optical Method for Estimating the Chlorophyll Contents in Plant Leaves. *Sensors*, 18(2), 650. https://doi.org/10.3390/s18020650
47. Pinski, A., Zur, J., Hasterok, R., & Hupert-Kocurek, K. (2020). Comparative Genomics of *Stenotrophomonas maltophilia* and *Stenotrophomonas rhizophila* Revealed Characteristic Features of Both Species. *International Journal of Molecular Sciences*, 21(14), 4922. https://doi.org/10.3390/ijms21144922
48. Ragavi, G., Muthamilan, M., Nakkeeran, S., Kumaravadivel, N., Sivakumar, U., & Suganthi, A. (2019). Molecular Detection of the Causative Agent of Soft Rot (*Pectobacterium carotovorum* subsp *carotovorum*) in Banana (*Musa* sp.). *International Journal of Current Microbiology and Applied Sciences*, 8(11), 1854–1868. https://doi.org/10.20546/ijcmas.2019.811.218
49. Raio, A., Brilli, F., Neri, L., Baraldi, R., Orlando, F., Pugliesi, C., Chen, X., & Baccelli, I. (2023). *Stenotrophomonas rhizophila* Ep2.2 inhibits growth of *Botrytis cinerea* through the emission of volatile organic compounds, restricts leaf infection and primes defense genes. *Frontiers in Plant Science*, 14(1235669). https://doi.org/10.3389/fpls.2023.1235669
50. Reyes-Castillo, A., Gerding, M., Oyarzúa, P., Zagal, E., Gerding, J., & Fischer, S. (2019). Plant growth-promoting rhizobacteria able to improve NPK availability: selection, identification and effects on tomato growth. *Chilean Journal of Agricultural Research*, 79(3), 473–485. https://doi.org/10.4067/s0718-58392019000300473
51. Robe, P., Nalin, R., Capellano, C., Vogel, T. M., & Simonet, P. (2003). Extraction of DNA from soil. *European Journal of Soil Biology*, 39(4), 183–190. https://doi.org/10.1016/s1164-5563(03)00033-5
52. Rocha, I., Ma, Y., Souza-Alonso, P., Vosátka, M., Freitas, H., & Oliveira, R. S. (2019). Seed Coating: A Tool for Delivering Beneficial Microbes to Agricultural Crops. *Frontiers in Plant Science*, 10, 1357. https://doi.org/10.3389/fpls.2019.01357
53. Roder, A., Hoffmann, E., Hagemann, M., & Berg, G. (2005). Synthesis of the compatible solutes glucosylglycerol and trehalose by salt-stressed cells of *Stenotrophomonas* strains. *FEMS Microbiology Letters*, 243(1), 219–226. https://doi.org/10.1016/j.femsle.2004.12.005
54. Ryan, R. P., Monchy, S., Cardinale, M., Taghavi, S., Crossman, L., Avison, M. B., Berg, G., van der Lelie, D., & Dow, J. M. (2009). The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nature Reviews Microbiology*, 7(7), 514–525. https://doi.org/10.1038/nrmicro2163
55. Said, M. S., Tirthani, E., & Lesho, E. (2023). *Stenotrophomonas Maltophilia.* PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK572123/
56. Schmidt, C. S., Alavi, M., Cardinale, M., Muller, H., & Berg, G. (2012). *Stenotrophomonas rhizophila* DSM14405T promotes plant growth probably by altering fungal communities in the rhizosphere. *Biology and Fertility of Soils*, 48(8), 947–960. https://doi.org/10.1007/s00374-012-0688-z

57. Shakeel, A., Khan, A. A., & Ahmad, G. (2019). The potential of thermal power plant fly ash to promote the growth of Indian mustard (*Brassica juncea*) in agricultural soils. *SN Applied Sciences*, 1(4), 375. https://doi.org/10.1007/s42452-019-0404-9
58. Sharafi, S. M., Rasooli, I., & Beheshti-Maal, K. (2010). Isolation, characterization and optimization of indigenous acetic acid bacteria and evaluation of their preservation methods. *Iranian Journal of Microbiology*, 2(1), 38–45.
59. Sharma, R., Sindhu, S., & Sindhu, S. S. (2018). Bioinoculation of Mustard (*Brassica juncea* L.) with Beneficial Rhizobacteria: A Sustainable Alternative to Improve Crop Growth. *International Journal of Current Microbiology and Applied Sciences*, 7(5), 1375–1386. https://doi.org/10.20546/ijcmas.2018.705.163
60. Tessmer, O. L., Jiao, Y., Cruz, J. A., Kramer, D. M., & Chen, J. (2013). Functional approach to high-throughput plant growth analysis. *BMC Systems Biology*, 7(S6), S17. https://doi.org/10.1186/1752-0509-7-s6-s17
61. Trevors, J. T. (1996). Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods*, *26*(1-2), 53–59. https://doi.org/10.1016/0167-7012(96)00843-3
62. Velmurugan, S., Anokhe, A., & Kalia, V. (2021). Biochemical Characterisation of Starch Hydrolysing Bacteria. *AgriCos E-Newsletter*, 2(11), 63–65.
63. Wolf, A., Fritze, A., Hagemann, M., & Berg, G. (2002). *Stenotrophomonas rhizophila* sp. nov., a novel plant-associated bacterium with antifungal properties. *International Journal of Systematic and Evolutionary Microbiology*, 52(6), 1937–1944. https://doi.org/10.1099/00207713-52-6-1937
64. Yin, P., Zhang, Q., Zhu, J., Wu, G., Yin, S., Ma, Z., & Zhou, J. (2018). The data of genomic and phenotypic profiles of the N-acyl homoserine lactone-producing algicidal bacterium Stenotrophomonas rhizophila GA1. *Data in Brief*, 21, 966–971. https://doi.org/10.1016/j.dib.2018.10.051
65. Yuvaraj, M., Bose, K. S. C., Elavarasi, P., & Tawfik, E. (2021). Soil Salinity and Its Management. In R. S. Meena & R. Datta (Eds.), *Soil Moisture Importance*. IntechOpen. 10.5772/intechopen.93329
66. Zhang, Q., Wang, Y., & Zhou, J. (2021). Complete Genome Sequence of *Stenotrophomonas rhizophila* KC1, a Quorum Sensing–Producing Algicidal Bacterium Isolated from Mangrove *Kandelia candel*. *Molecular Plant-Microbe Interactions*, 34(7), 857–861. https://doi.org/10.1094/mpmi-12-20-0346-a
67. Zhou, L., Song, C., Li, Z., & Kuipers, O. P. (2021). Antimicrobial activity screening of rhizosphere soil bacteria from tomato and genome-based analysis of their antimicrobial biosynthetic potential. *BMC Genomics*, 22(1), 29. https://doi.org/10.1186/s12864-020-07346-8
68. Zhou, W., Qin, S., Lyu, D., & Zhang, P. (2014). Soil sterilisation and plant growth-promoting rhizobacteria promote root respiration and growth of sweet cherry rootstocks. *Archives of Agronomy and Soil Science*, 61(3), 361–370. https://doi.org/10.1080/03650340.2014.935346