**Running Title:** *Stenotrophomonas rhizophila* and Integrated Pest Management

**Evaluation of Plant Growth Promoting Activity of *Stenotrophomonas rhizophila* for the Growth Enhancement of Mustard Seedlings**

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**Novelty statement**

*Stenotrophomonas rhizophila* has been exploited for its PGPR property to enhance the growth of mustard seedlings. The observed results substantially ensure the growth promoting activity of mustard seedlings in synergistic activity with the natural microbiota in soil. Since mustard plants serve as trap crops in cauliflower fields, growth enhancement of mustard makes them a better choice for Integrated Pest Management.

**Abstract**

Indian mustard has been utilized as a trap crop in Integrated Pest Management strategies for cauliflower plants. Growth, yield and soil fertility of these trap crops can be enriched by the application of Plant Growth Promoting Rhizobacteria (PGPR) in the cultivable soil. PGPRs naturally inhabit agricultural soil and are predominately found in association with root nodules of plants. By increasing their presence in soil, plant growth can be enhanced, particularly under conditions of salt stress, through the production of substances like glucosyl glycerol and trehalose. *Stenotrophomonas rhizophila* is a notable known for its growth promoting properties. In this study, *S. rhizophila* was isolated from soil samples collected from a cauliflower field and identified through the 16s rRNA protocol. The BLASTn sequence similarity search tool yielded a total score of 2403 for the identified partial sequence with 100% query cover, indicating its identity with *S. rhizophila* in the NCBI database. Phylogenetic analysis using PhyML revealed *S. rhizophila* to be an evolutionary descendant of Stenotrophomonas sp. and closely related to *S. maltophilia.* The growth-promoting ability of *S. rhizophila* was assessed through a 28-day observation period using four treatment pots (T1- T4) sown with mustard seeds. Results indicated enhanced growth in the T3 pot, which received regular applications of *S. rhizophila*. Given that mustard plants serve as trap crop in cauliflower, broccoli and cabbage field, it is recommended to enrich cultivable soil with this bacterium to enhance the growth of mustard plants for improved pest management, suggesting its potential application as a bio-fertilizer.

**Keywords:** *Stenotrophomonas rhizophila*, PGPR, 16s rRNA sequencing, phylogeny, mustard, pot study

**Introduction**

 Agriculture significantly contributes to the global economy and its impact over a country’s economy is reflected through the rising demand and supply of food and related commodities. Consequently, additional pressure to increase the production is thrusted upon agricultural sector. To sustain the rate of production in 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 which can significantly increase the growth and yield of plant. These bacteria protect the plant roots by secreting antimicrobial compounds, induce systemic resistance that could suppress the activity of phytopathogenic bacteria and fungi and facilitate the plant to adapt biotic and abiotic stress (Compant *et al.* 2010; Zhou *et al.* 2021).

 The PGPR activity of *Arthrobacter, Variovorax, Azosprillum, Alcaligenes, Enterobacter, Bradyrhizobium, Burkholderia, Serratia, Azobacter, Klebsiella, Mesorhizobium, Rhodococcus, Streptomyces, Flavobacterium, Acinetobacter, Bacillus, Micrococcus, Pseudomonas, Arthrobacter, Stenotrophomonas* were reported earlier (Kumar *et al.* 2012; Odoh 2017, Singh and Jha 2017; Singh 2018, Singh *et al.* 2020; Alexander *et al.* 2020). The compatibility of soil and crop can be defined by the saline nature of the soil. Imadi *et al.* (2010) addressed the effect of soil salinity stating that it could vigorously affect land fertility eventually leading to economic loss. This is due to the increased release of salt ions into the soil through several anthropogenic activities and reduced leaching out of salt from the rhizosphere because of insufficient rain. Salinity reduced crop yield, pigmentation, photosynthetic rate, water uptake and growth rate and increased the rate of senescence. 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, addition of nutrients to the soil, phytoremediation and microbial remediation are the strategies recommended to reduce soil salinity (Mustafa and Akhtar, 2019; Yuvaraj *et al.* 2021).

 *Stenotrophomonas rhizophila* is reported to survive in high saline soil worthwhile enhancing crop yield (Egamberdieva *et al.* 2011). This bacterium also has antifungal properties which aid them in fighting against 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 was 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. Although the closely related *S. maltophilia* is reported to contain the same properties, it was keyed out by Brooke (2012) as an emerging global opportunistic pathogen. 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. 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 which was prepared in accordance to Helias *et al.* (2022). The media consisted of crystal violet mix (CaCl2.2H2O- 0.204 g, tryptone- 0.2 g, trisodium citrate- 1 g, NaNO3- 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. Both mixes were then 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**

  The isolated bacteria were subjected to various biochemical tests to identify its morphology and biochemical characteristics. 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 along with starch hydrolysis, KOH solubility, acid production (Methyl red test), ammonia production (Urease test), production of tryptophanase enzyme (Indole test), gas production of fermentation of sugars (Triple Sugar Iron test) and utilization of citrate as sole carbon (Citrate utilization test) (Ashmawy *et al.* 2015; Ashmawy *et al.* 2020).

**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. Initially, 24h bacterial cells were mixed with 500 μl of DNA lysis buffer, 500 μl of neutralization buffer and 4 μl of RNase enzyme. This mixture was incubated at 65˚C in a thermostatic water bath for 30 minutes followed by centrifugation at 10,000 rpm for 10 minutes. Afterward, 600 μl of chloroform isoamyl alcohol was added to the collected supernatant and centrifuged for 10 minutes at 10,000 rpm. Subsequently, 600 μl of binding buffer was added and incubated for 5 minutes. The pellet obtained after a 2-minute centrifugation at 10,000 rpm was discarded, and washing buffers I and II were sequentially added, followed by centrifugation. Finally, elution buffer was added and centrifuged to obtain the DNA pellet. The isolated DNA was eluted by agarose gel electrophoresis and the concentration was measured (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, 0.4mM dNTPs, 3.2mM MgCl2 and 0.02% bromophenol blue, the isolated DNA was amplified at different temperatures in accordance with the standard PCR protocol (Kyule *et al.* 2022; Reyes-Castillo *et al.* 2019). PCR product was purified, and single-pass sequencing was performed 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 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. To construct the phylogenetic tree of the aligned sequence, PhyML 3.0 aLRT program was selected and the results were meticulously documented (Edgar, 2004; Dereeper *et al.* 2008; Kumar and Manjunatha, 2015).

**Application of *Stenotrophomonas rhizophila* as 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 were filled with 10 kg of loamy soil collected from an irrigated region. Soil in two pots were subjected to dry heat sterilization at 160°C for 3 hours while the other two pots 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 is completely washed off (Perez-Garcia *et al.* 2023). The treatments included sterile soil with treated seeds (T1), sterile soil with untreated seeds (T2), non-sterile soil with treated seeds (T3), non-sterile soil with untreated seeds (T4) (Zhou *et al.* 2014). Treated seeds were previously inoculated with *S. rhizophila*. This is referred as seed inoculum and was prepared by adding 200 µl of *S. rhizophila* (OD600= 1.0)to 200 mL of sterile nutrient broth (Himedia) and incubated at 30°C in an orbital shaker incubator for 24 hours. Centrifugation at 5000 rpm for 20 minutes aids in biolass collection. Using PBS (Phosphate Buffer Saline), the collected biomass was washed thrice to eliminate the growth medium completely. 10g of disinfected mustard seeds were mixed with 10% starch solution to impart adhesive nature on seed surface by shaking in an orbital shaker at 100 rpm for 30 minutes. This was added with 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**

 Growth of mustard seeds were observed once in every 4 days until 28 days. Total plant height, shoot height, root height, total plant weight was measured regularly. Absolute growth rate and relative growth rate for each treatment was calculated sung the following formula,

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 (Garcia *et al.* 2006).

**Determination of chlorophyll content in young mustard leaves**

Chlorophyll content of mustard leaves were determined with reference to 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 refrigerated condition followed by centrifugation at 2000 rpm for 10 minutes. The supernatant was collected and closed with aluminium foil to retain dark condition and added with 5mL of acetone ethanol mixture (2:1). After 1 minute of stirring, 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 in Shakeel *et al.* (2019)

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 total plate count method beginning 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 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).

**Results**

**Biochemical characterization of isolated rhizobacteria**

The isolated bacterium was identified as Gram-negative rods and it tested negative in YGC medium, positive in EMB medium. It possesses the ability to survive under high salt condition (5% NaCl) but cannot withstand at 37°C. Negative result was observed for starch hydrolysis, KOH solubility, methyl red, triple sugar iron and urease test while positive result was reported for indole and citrate utilization test. Biochemical test results of all the 13 bacterial isolates are reported in the table below (Table 1).

**16S rRNA sequencing and phylogenetic analysis of isolated DNA**

 The obtained biochemical results of S3 bacterium was similar to *Stenotrophomonas rhizophila.* Sequence identification by 16S rRNA sequencing protocol confirmed S3 bacterium as *Stenotrophomonas rhizophila.* BLASTn similarity search tool resulted in 100% query for the obtained sequence conforming its identity as *S. rhizophila.* 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 *S. 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 sapling 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. The treatments are mentioned in table 2. Plants in T1 and T3 were watered with 100mL of sterile distilled water containing biomass suspension (0.5 g) every four days. Total plant height, shoot height, root height was measured every four days and the data is represented graphically in Fig. 2, 3 & 4. Plant weight 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 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 and RGR for all treatments were calculated and represented graphically (Fig. 6 & 7). Chlorophyll content was also determined periodically and the values are tabulated (Table 3).

**Enumeration of bacteria in treatment pots containing mustard plants**

Periodical microbial enumeration in soil samples collected from each treatment pots is mentioned graphically in Fig. 8. It is observed from the graph that the microbial load has been gradually increasing.

**Discussion**

 *Stenotrophomonas rhizophila* was identified as Gram-negative, rod-shaped bacteria (Ozsahin *et al.* 2014; Elhosieny *et al.* 2023). Among the 13 isolated bacteria, S3 has been identified as *Stenotrophomonas rhizophila* through 16s rRNA sequencing. The phylogenetic tree revealed that this bacterium shares a common ancestor with *S. maltophilia*, a Gamma proteobacterium (Ryan *et al.* 2009). The ability of this bacterium to retain its growth at 5% NaCl in a growth medium confers its PGPR property (Roder *et al.* 2005; Egamberdieva *et al.* 2011; Alexander *et al.* 2020). At 37°C, *S. rhizophila* did not exhibit growth while S. maltophilia is reported to grow as it is an opportunistic human pathogen. This difference is due to the absence of heat shock genes and other virulence factors in *S. rhizophila* (Alavi *et al.* 2014). Bacterial isolate ‘S3’ conferred with the biochemical test results of *Stenotrophomonas* sp. reported in Wolf *et al.* (2002). The biochemical test results conferred that the bacterium can effectively utilize glucose, sucrose, lactose and citrate but was unable to hydrolyze starch, secrete acetic and other acids (Lehman 2005; Kado 2006; Lal and Cheeptham, 2007; Sharafi *et al.* 2010; Brink, 2010; Adegoke *et al.* 2017; Amoli *et al.* 2017; Ragavi *et al.* 2019; Velmurugan *et al.* 2021; Milek and Lamkiewicz, 2022; Said *et al.* 2023). Indole test helps to identify the presence of tryptophan which is 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 *S. rhizophila* converts tryptophan into indole which was observed through green color ring formation upon the addition of Kovac’s reagent. This confirms the PGPR property of *S. rhizophila* (MacWilliams, 2009; Lebrazi *et al.* 2020).

 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. This method is reported to be eco-friendly and has the potential to improve growth and yield (Chakraborti *et al.* 2022). Biopriming involves the application of a binding agent to bind the bacterium onto the seed. In this study, the starch solution was prepared in a ratio of 1:10 to bind *S. rhizophila* onto the surface of mustard seeds such that the bio-primed seeds were able to retain the inoculum after proper mixing and drying at room temperature (Mahmood *et al.* 2016; Rocha *et al.* 2019; Fiodor *et al.* 2023).

 The growth of mustard plants was expected to increase after the periodic application of *S. rhizophila*. Inoculation of seeds with PGPR and their periodic application in treatment pots positively enhanced growth in pots T3 and T4. Plants in T4 treatment pots showed heightened growth compared to T2 and T1 but were not greater than T3. The lack of *S. rhizophila* in T4 pots exhibited reduced growth while sterilization affected T1 and T2 treatment pots. Sharma *et al.* (2018) reported enhanced growth of mustard plants in PGPR-inoculated seeds than in uninoculated mustard seeds. Though T1 and T2 pots were filled with sterile soil, the growth of mustard plants was comparatively higher in T3 and T4 pots in terms of total plant height, shoot height and weight. Therefore, the condition of the soil is identified as a concerning factor in this study. Similarly, Gholami *et al.* (2009) 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 (IAA) and other auxins (Appanna, 2007). Earlier, Khalid *et al.* (2004) observed an increase in auxin synthesis by PGPR in non-sterile soil with a positive impact over the growth and yield of wheat crops. The competitive ability of PGPR to survive in the presence of other microbes could also have enabled enhanced growth of wheat plants. 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 deplete, and it was reflected over the rate of crop growth. Nezarat and Gholami (2009) also noticed stimulating effects over leaf surface area, dry weight of leaf and shoot in maize crop grown in non-sterile soil. The results were comparatively better than in sterile soil. The growth of mustard plants in T3 pot is also conceived to be the synergistic activity of *S. rhizophila* with other beneficial bacteria present in non-sterile soil. In this pot study, the increase in plant height and weight in non-sterile treated pots is reasoned as the microbial diversity which could have synergistically acted with *S. rhizophila* to enhance plant growth.

 AGR and RGR are important in identifying the growth model of mustard plants (Tessmer *et al.* 2013). The relative growth of a plant concerning height, weight, and time defines the growth rate of a plant. 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). RGR of mustard seeds exhibited a monomolecular growth pattern (Fig. 7) *ie.,* it follows first-order growth pattern. This type of growth pattern is also called the Mitscherlich model and is a non-linear growth type. For this type of plant growth, AGR was reported to increase faster during the initial stage of plant growth and to decrease later (Paine *et al.* 2011). In the present study, AGR of the T3 pot was comparatively high when compared with other treatment pots (Fig. 6) and the results adherer to the monomolecular growth pattern. Lowry and Smith (2018) stated that an increase in RGR aids in enhanced weed suppression due to the increased mass accumulation. Carbon has been reported as the prime nutrient for mass accumulation and it induces heightened growth of shoot and root of a plant. We observed heightened shoot growth of mustard seeds in a monomolecular pattern.

 The chlorophyll content of T3 was determined to be high among other treatments. This is on par with the results of the growth rate. Khan *et al.* (2023) observed an increase in chlorophyll content after the application of PGPR strains *Pseudomonas fluorescens* and *Azotobacter chroococcum*. In another study, *Pseudomonas fluorescens* and *Bacillus subtilis* were able to increase chlorophyll content and reduce the intensity of Turnip Mosaic Virus in the Indian mustard plant (Diyansah *et al.* 2013). Metal toxicity in soil hinders plant growth due to its interaction with the enzymes responsible for respiration and photosynthesis. Introduction of IAA-producing *Lysinibacillus* *varians* and *Pseudomonas putida* improved the vigor of mustard plants in soil contaminated with cadmium. Meanwhile, chlorophyll content was also observed to increase after PGPR application on mustard plants (Pal *et al.* 2019). It is 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 plants with no *S. rhizophila* application (Table 3). Hence, it is necessitated to enhance the population of PGPR in soil cultivated with Indian mustard plants. Moreover, the population of bacteria in T3 demonstrates a gradual increase with periodic addition of *S. rhizophila* and it was relatively less 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 is anticipated as PGPR and *S. rhizophila* (Ortiz-Castro *et al.* 2009; Koza *et al.* 2022). Through this research, it is evident that *S. rhizophila* can enhance growth of mustard plantlets in synergism with other naturally available PGPR in soil.

**Conclusion**

The plant growth-promoting ability of *S. rhizophila* is demonstrated through this study. Among the four treatments, non-sterile soil with treated mustard seeds projected increased growth comparatively. The addition of *S. rhizophila* every four days further attributed to the growth rate of mustard plantlets. Through this research, it is recommended to add *S. rhizophila* in biofertilizer preparations, especially to hasten the growth of mustard seedlings and other *Brassica* sp.

**Acknowledgment**

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

**Author Contribution**

RER planned the experiments, APJL and DG executed the research work, APJL interpreted the results with illustration and SM edited the manuscript.

**Conflict of interest**

The authors declare that they have no potential conflicts of interest.

**Funding**

This research was funded by the University Grants Commission (UGC), a central government funding agency for NET (National Eligibility Test) qualified candidates in India. [Award number- 1543/(NET-JULY 2018)]

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author.

**Ethics Approval**

Not applicable to this paper.

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**Fig. 1**: Phylogenetic tree of the isolated *S. rhizophila*

**Fig. 2:** Comparison of total plant height between treatments of mustard seedlings

**Fig. 3:** Comparison of shoot height between treatments of mustard seedlings

 **Fig. 4:** Comparison of root height between treatments of mustard seedlings

**Fig. 5:** Comparison of total plant weight between treatments of mustard seedlings

**Fig. 6**: Absolute Growth Rate of mustard seedlings during the 28 days growth period in all the treatment pots

**Fig. 7:** Relative Growth Rate of mustard seedlings during the 28 days growth period in all the treatment pots

**Fig. 8:** Microbial load of soil collected from treatment pots with mustard seedlings

**Table 1:** Biochemical test results of the isolated Rhizobacteria

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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** |
| S1 | - | - | - | - | - | - | - | - | + | + | + | - |
| S2 | - | + | - | - | + | + | + | - | - | + | + | + |
| S3 | - | + | - | + | - | - | + | - | - | + | + | + |
| S4 | - | + | - | - | + | + | + | - | - | + | + | + |
| S5 | - | + | - | - | + | + | + | - | - | + | + | + |
| S6 | - | + | - | + | + | + | + | + | - | + | + | + |
| S7 | - | + | - | + | + | + | + | + | + | - | + | + |
| S8 | - | + | + | + | + | + | + | - | - | + | + | + |
| S9 | - | + | - | + | + | + | + | - | + | + | + | + |
| S10 | - | + | - | + | + | + | + | - | + | + | + | + |
| S11 | - | + | + | + | + | + | + | + | + | - | + | + |
| S12 | - | + | + | + | + | + | - | + | + | - | + | + |
| S13 | - | + | + | + | + | + | + | + | - | - | + | + |

**Table 2:** Selected treatments for pot study

|  |  |  |
| --- | --- | --- |
| **Treatment No.** | **Soil Sterility** | **Nature of Seed Sowed** |
| T1 | Sterile | Treated with *S. rhizophila* |
| T2 | Sterile | Untreated |
| T3 | Non-sterile | Treated with *S. rhizophila* |
| T4 | Non-sterile | Untreated |

**Table 3:** Chlorophyll content of mustard leaves in each treatment pot

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Chlorophyll a (mg/g)** | **Chlorophyll b (mg/g)** | **Total Chlorophyll (mg/g)** |
| **Days** | **T1** | **T2** | **T3** | **T4** | **T1** | **T2** | **T3** | **T4** | **T1** | **T2** | **T3** | **T4** |
| 4 | 0.038 | 0.028 | 0.043 | 0.031 | 0.083 | 0.048 | 0.153 | 0.078 | 0.120 | 0.076 | 0.196 | 0.109 |
| 8 | 0.054 | 0.045 | 0.098 | 0.066 | 0.223 | 0.183 | 0.349 | 0.186 | 0.277 | 0.227 | 0.448 | 0.252 |
| 12 | 0.127 | 0.123 | 0.154 | 0.124 | 0.386 | 0.342 | 0.522 | 0.345 | 0.513 | 0.465 | 0.677 | 0.469 |
| 16 | 0.184 | 0.159 | 0.239 | 0.198 | 0.558 | 0.572 | 0.704 | 0.535 | 0.742 | 0.731 | 0.943 | 0.733 |
| 20 | 0.231 | 0.205 | 0.325 | 0.261 | 0.694 | 0.741 | 0.799 | 0.657 | 0.925 | 0.945 | 1.125 | 0.918 |
| 24 | 0.329 | 0.267 | 0.399 | 0.334 | 0.839 | 0.818 | 0.894 | 0.839 | 1.169 | 1.085 | 1.293 | 1.174 |
| 28 | 0.459 | 0.428 | 0.489 | 0.454 | 0.864 | 0.820 | 0.854 | 0.855 | 1.322 | 1.248 | 1.337 | 1.309 |