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Determination of Novel Plant Growth Promoting Bacteria *Stenotrophomonas maltophilia* Isolated from Rhizospheric Soils of South Gujarat Forest, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Forest regions have good weather conditions for agricultural practices. Crop growth and productivity are affected by the presence of novel bio organisms in these areas. The present study sought to identify the location of the forest rhizosphere soil of some field crops. Screening procedures were used to assess their growth capabilities and evaluate their potential as PGPB. Various features of PGPB, like phosphate and potassium solubilization, nitrogen fixation, indole-3-acetic acid (IAA) production, ammonia (NH_3) synthesis, siderophore generation, and hydrogen cyanide (HCN) synthesis, were analyzed morphologically, biochemically, and physiologically during the screening process. One isolate out of the nine only produced good results for every attribute studied that promoted plant development. By sequencing their 16S rRNA, the promising isolates were

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determined to be *Stenotrophomonas maltophilia*. To assess the efficacy of PGPB and its liquid and powder formulation as a biofertilizer, a field trial had been conducted. In comparison to the uninoculated control, all tested inoculants considerably enhanced the yield components of several crops, the amount of N and P that plants could absorb from the soil, the activity of some soil enzymes, and the overall number of bacteria. The application of *Stenotrophomonas maltophilia* as a PGPR demonstrated significant promise in addressing the obstacles associated with sustainable agriculture in a variety of environmental circumstances.

Keywords: PGPB; soil enzymes; 16S rRNA sequencing; Stenotrophomonas maltophilia.

1. INTRODUCTION

Plant growth-promoting rhizobacteria (PGPR) are bacteria that inhabit the plant root surface and enhance plant growth [1]. The use of these microorganisms can diminish reliance on artificial pesticides and fertilizers, thus fostering sustainable agriculture [2]. PGPR "can boost plant growth either directly or indirectly. Direct plant growth promotion involves mechanisms that supply plants with nitrogen (N), phosphorus (P), iron (Fe), and IAA, whereas indirect promotion involves the suppression of phytopathogens through antimicrobial metabolites as well as extracellular enzymes [3,4]. A multitude" of advantageous microorganisms, that includes bacteria as well as fungi, synthesize "antibiotics as secondary metabolites that impede the growth as well as proliferation of phytopathogens by altering their cell walls, membranes, or metabolic pathways. Beneficial bacteria can compete with phytopathogens for vital nutrients in the" root zone. Plants exhibit heightened resistance to pathogens that induce systemic resistance. The proliferation and dissemination of infections can be halted by breaking the cell walls. The cell can undergo hydrolysis due to the secretion of extracellular proteases and lipases [5,6].

Gram-negative *Stenotrophomonas* species are linked to a variety of environments, including both plant and animal hosts [7]. Furthermore, this bacterium is global and omnipresent, existing in a wide range of environmental habitats, including harsh ones. It is fundamentally associated with the rhizosphere of plants, where it predominantly facilitates the elemental cycling of nitrogen and sulphur. It decomposes complex molecules and contaminants, promoting the growth and health of plants [8,9].

The advantages of the genus *Stenotrophomonas* for plant systems have been documented by numerous researchers. The potential utility of *Stenotrophomonas* bacteria as efficient bio inoculants for enhancing plant development and controlling a number of food crop diseases is prompting more research into this bacterium. Biotechnological interest in this area is expanding [10]. It also has the capability to protect itself from bacteria and fungi that induce plant diseases, endure biotic as well as abiotic stress, and demonstrate anti-quorum sensing as well as anti-biofilm capabilities [10]. *S. rhizophilia*, closely related, presents a viable alternative for biotechnological uses without compromising human health [8].

In the accumulation zone of the Dediyapada forests in the Bharuch district of Ankleshwar, Gujarat, India, a new strain of bacteria was discovered. We carried out tests for plant growth capacity, 16s rRNA sequencing analysis [11], and morphological and biochemical characterisation of the *Stenotrophomonas maltophilia* strain [12].

2. MATERIALS AND METHODS

2.1 Site Description

Soil samples had been collected in the winter months from the Dediyapada jungle in South Gujarat (Dediyapada: 21.60452°N, 73.74450°E). Samples of soil were taken at a depth of 4 inches from the root zones of the soils. The adherent soil sample was shipped in less than 24 hours to Arihant Bio Science (India) Pvt Ltd.'s microbiological lab in Ankleshwar, Gujarat, India, where it was kept at a regulated 4°C. Despite this, it was found that the soil samples taken from the jungle environment had a pH of 7.3.

2.2 Isolation of Bacteria

A forest plant's rhizosphere yielded a bacterium that was isolated and cultured for 48 hours at 37°C, pH 7, and standard nutritional agar media. The bacterial isolate was further described by its biochemical properties (Bergey's Manual of Systematic Bacteriology), morphological characteristics (Gram's staining), and cultural conditions.

2.3 Morphological and Microscopic Observation

The strain was cultivated and purified on various specialized media, such as Jensen's medium, nutritional medium, manual CRYEM medium, Azotobacter specific medium, PKVK medium, and Aleksandrow medium, at 37 ± 2 °C for 48–72 hours in a BOD incubator. By using the plate streak approach, a single colony was produced. Under a microscope, the single colony's morphological features were examined.

Gram's staining for microscopic identification of bacterial isolates Each clean glass slide received a thick layer of strain, which was then heat dried for a short while by passing over a burner. Following the addition of one to three drops of crystal violet stain, the smear was allowed to rest at room temperature for one minute. After that, a mild tap water wash was used to get rid of the crystal violet stains. After applying one to three drops of gram's iodine, the smear was left to incubate for thirty to sixty seconds at room temperature. The smear was then gently cleaned with tap water after being decolored with Gram's decolourizer and counter-stained with one to three drops of safranin. It was then incubated for thirty to sixty seconds at room temperature. After an overnight period at room temperature, the slide was examined under a light microscope.

2.4 Biochemical Characterization

The strain was screened for extra cellular enzymes such as lipase, oxidase, protease [13], nitrate reductase [14], starch hydrolysis [15], amylase [13], urease, cellulose [13], methyl red, Voges proskauer, gelatin hydrolysis, casein, hydrogen sulphide, citrate utilization [15] and catalase [16] was carried out in accordance with the guidelines [17]. According to the procedures described by Liu, Tian et al. [18], the BIOLOG system verified each and every biochemical test.

2.5 Molecular Identification and Phylogeny

The phenol-chloroform approach was utilized to recover the whole genomic DNA from the bacterium. Primer 27F/1492R was used to amplify a portion of the 16S rRNA gene [19]. The isolated strain's 16S rRNA gene sequence was found using BLAST alignment, which was deduced from the NCBI public database (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and entered into the NCBI GenBank database. The study's homologous 16S rRNA gene sequences were obtained using NCBI BLAST searches. The evolutionary distances are measured in base substitutions per site and had been calculated by employing the Maximum Composite Likelihood technique. Eleven nucleotide sequences were examined. The 1st, 2nd, 3rd and noncoding codon locations had been examined. All positions with incomplete data and gap were removed. The completed dataset had a total of 758 locations. Evolutionary analyses were conducted in MEGA6 [20,21]. All of the nucleotide sequences produced from this research were added to GenBank (NCBI) with the GeneBank accession number PQ192266.

2.6 Plant Growth Promoting Traits

The recently isolated strain was evaluated in relation to several traits that promote plant growth, including the production of indole acetic acid [22], phosphate [23], potassium [24], nitrogen fixation [25], zinc [26], organic acid [12], ammonia [27], HCN [28], siderophore [29], and ACC deaminase [12,30,31].

3. RESULTS AND DISCUSSION

3.1 Isolation and Biochemical Characterization of Bacteria

PGPR promotes plant development directly or indirectly by colonizing plant roots and lowering the presence of harmful bacteria. The rhizosphere is an ecological niche in which plant roots and soil microorganisms coexist and exchange nutrients. Thus, rhizobacteria help to improve soil fertility and sustainability. In this research, we investigated bacterial ecology in jungle plant rhizosphere. Soil samples were gathered from several fields in Dediyapada forest. So many isolates were obtained using the serial dilution procedure. To evaluate the plant growth promoting ability of the isolated strain, we streaked the plates in different specific mediums such as Nitrogen agar medium, Jensen agar medium, CRYEM agar medium, Aleksandrow agar medium, PKVK agar medium, GYC agar medium, Nutrient agar medium, and Calcium agar medium, as shown in Fig. 1. Positive growth on all of the agar mediums listed above indicates that the isolated strain is capable of fixing atmospheric nitrogen, and solubilizing potassium, phosphate, and calcium.

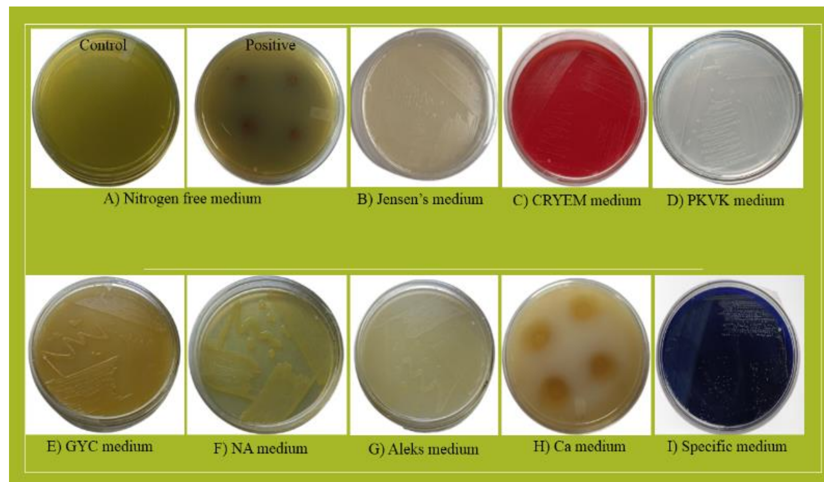

Fig. 1. primary screening for plant growth promoting traits A) Nitrogen free agar medium, B) Jensen agar medium, C) CRYEM agar medium, D) PKVK agar medium, E) GYC agar medium, F) Nutrient agar medium, G) Aleksandrow agar medium, H) Calcium agar medium, I) Species-specific medium

Table 1. Biochemical characterization of bacteria

Sr. No.	Biochemical Test	Strain
1	Gram's stain	Negative
2	Amylase production test	+
3	Protease production test	+
4	Lipase production test	+
5	Chitinase production test	+

Table 1 presents the results of various biochemical tests performed in vitro conditions. The result shows that the isolated strain is gram's negative and shows positive results towards different enzyme production tests.

3.2 Molecular Identification Based on 16S rRNA Gene Sequence

16S rRNA gene sequencing was used to identify the organisms based on their appearance, biochemical properties, and microscopy. Based on an identity criterion of at least 97% for the 16S rRNA gene sequencing result, it was concluded that the strain IAM 12423 isolate was closely related to the *Stenotrophomonas maltophilia* strain, with 99.21% ANI. Table 2 shows the identification of bacterial strains using 16S rRNA gene sequences.

The gene sequences of the isolated bacterial strain were submitted to NCBI GenBank under the accession code PQ192266. A phylogenetic tree of the isolated strain and its relatives was created (Fig. 2).

Fig. 2 shows a phylogenetic tree of strains among Stenotrophomonas species and related taxa. The "Neighbour-Joining approach was used to infer evolutionary history. The bootstrap consensus tree constructed from 500 repetitions [32] is used to illustrate the taxa's evolutionary history. Branches associated with partitions occurring in fewer than 50% of bootstrap replicates are collapsed. The percentage of duplicate trees in which the relevant taxa clustered together during the bootstrap test (500 repetitions) is given next to the branches [32]. The evolutionary distances had been calculated by employing the Maximum Composite Likelihood technique and are shown as the number of base substitutions per site [33]. This" study comprised eleven nucleotide sequences. All not known positions have been eliminated from each sequence pair employing the pairwise deletion method. The final dataset comprised 762 locations. MEGA11 was employed to do evolutionary analysis [34]. The findings are consistent with previous research and indicate that the genus Stenotrophomonas has the ability to promote plant development.

Table 2. Identification of bacteria based on 16S rRNA gene sequencing

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident	Acc. Len	Accession
Stenotrophomonas maltophilia strain IAM 12423 16S ribosomal RNA, partial sequence	*Stenotrophomonas maltophilia*	1369	1369	100%	0	99.21	1538	NR_041577.1
Stenotrophomonas maltophilia strain NBRC 14161 16S ribosomal RNA, partial sequence	*Stenotrophomonas maltophilia*	1365	1365	100%	0	99.08	1470	NR_113648.1
Stenotrophomonas maltophilia strain LMG 958 16S ribosomal RNA, partial sequence	*Stenotrophomonas maltophilia*	1365	1365	100%	0	99.08	1500	NR_119220.1
Stenotrophomonas maltophilia strain ATCC 13637 16S ribosomal RNA, partial sequence	*Stenotrophomonas maltophilia*	1363	1363	100%	0	99.08	1467	NR_112030.1
Stenotrophomonas pavanii strain LMG 25348 16S ribosomal RNA, partial sequence	*Stenotrophomonas pavanii*	1349	1349	100%	0	98.68	1497	NR_118008.1
Stenotrophomonas pavanii strain ICB 89 16S ribosomal RNA, partial sequence	*Stenotrophomonas pavanii*	1347	1347	100%	0	98.68	1483	NR_116793.1
Stenotrophomonas maltophilia strain ATCC 19861 16S ribosomal RNA, partial sequence	*Stenotrophomonas maltophilia*	1341	1341	100%	0	98.55	1517	NR_040804.1
[Pseudomonas] hibiscicola strain ATCC 19867 16S ribosomal RNA, partial sequence	*[Pseudomonas] hibiscicola*	1341	1341	100%	0	98.42	1519	NR_024709.1
Stenotrophomonas geniculata ATCC 19374 = JCM 13324 16S ribosomal RNA, partial sequence	*Stenotrophomonas geniculata* ATCC 19374 = JCM 13324	1319	1319	100%	0	97.89	1497	NR_024708.1
Stenotrophomonas cyclobalanopsidis strain TPQG1-4 16S ribosomal RNA, partial sequence	*Stenotrophomonas cyclobalanopsidis*	1301	1301	99%	0	97.63	1439	NR_180613.1

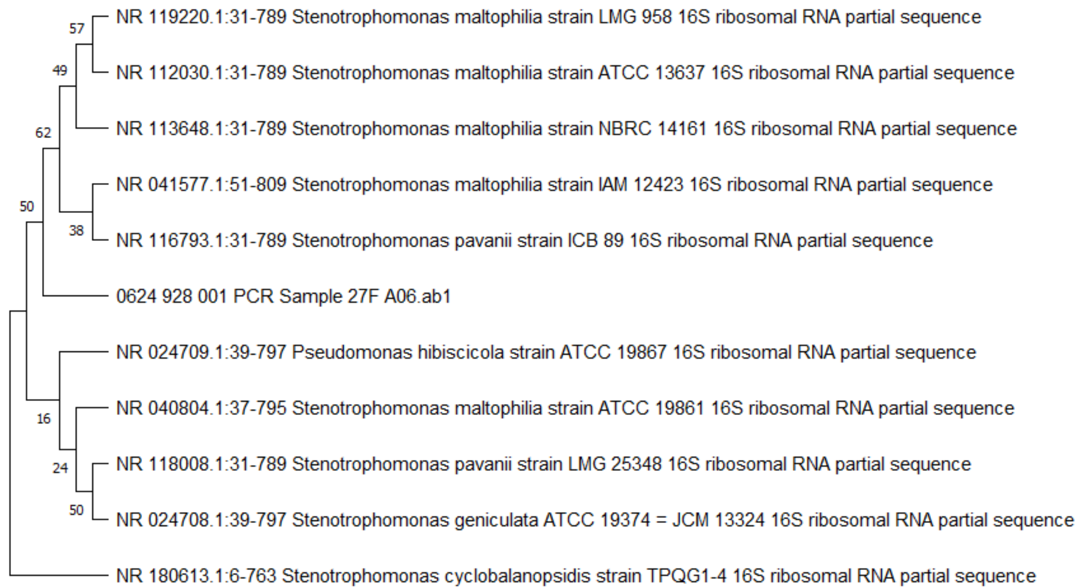

Fig. 2. Phylogenetic tree of the isolated strain and its relatives

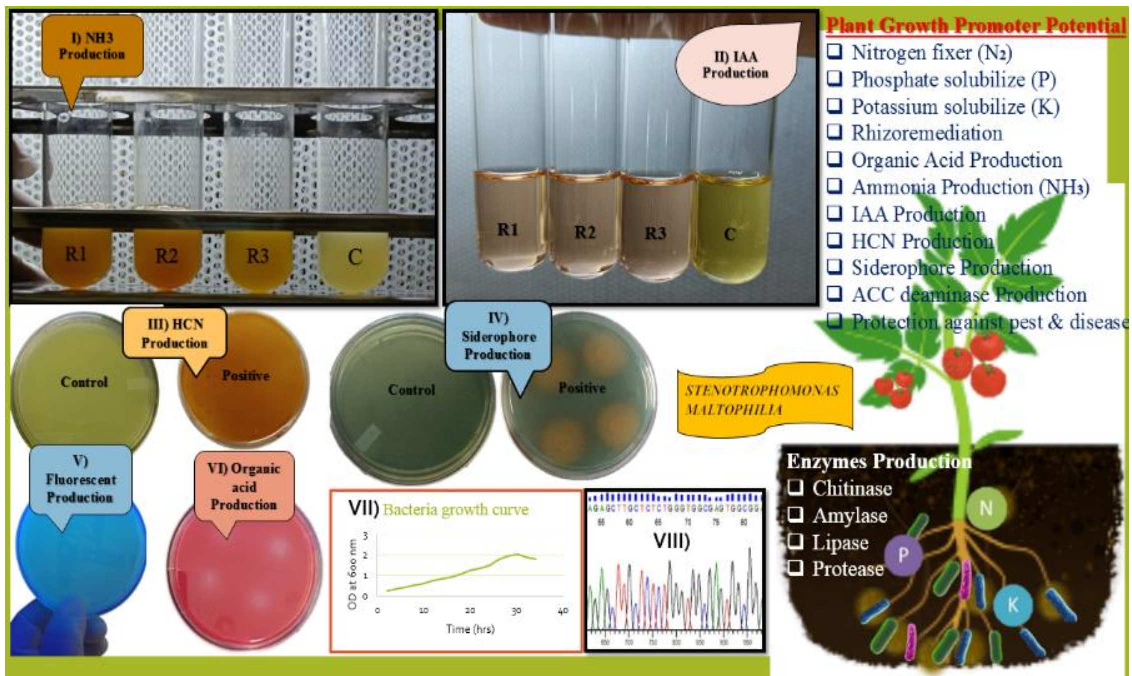

Fig. 3. PGP traits for the *Stenotrophomonas maltophilia* strain

3.3 Plant Growth-promoting Traits

Fig. 3 depicts the findings of PGP traits for the *Stenotrophomonas maltophilia* strain. *Stenotrophomonas maltophilia* strain produced ammonia (NH_3), IAA, hydrogen cyanide (HCN), siderophore, fluorescent, and organic acid. Masclaux-Daubresse, Daniel-Vedele et al. (2010) state that ammonia build-up in the soil may result in alkaline conditions that inhibit the growth of a number of plant diseases. Since ammonia production encourages the growth of roots, shoots, and biomass, which speeds up plant growth, it is crucial for the accumulation of nitrogen. The PGPB produces a lot of indole acetic acid because it facilitates nutrient absorption and root growth. Keswani, Singh et al. (2020) state that IAA, which is generated by

bacteria, is one of the most significant auxins in the regulation of plant vegetative development. By synthesizing some antibiotics or enzymes that break down cell walls, the production of hydrogen cyanide (HCN) as a secondary metabolite inhibits the growth of the majority of fungal phytopathogens, according to Kumar, Bahadur et al. (2015). Through its effect on the mobilization of elements from rock-forming minerals and its indirect promotion of plant development, hydrogen cyanide also improved the availability of nutrients. According to research, some plant growth-promoting bacteria (PGPB) may remove iron from the soil by producing low-molecular-weight compounds (400–1500 Da) [35]. Siderophores are chemicals that bind to iron and enhance their bioavailability in plants [36]. Good bacteria in the soil and on plants produce siderophores, which are a crucial biological control mechanism because they prevent detrimental plant diseases from getting iron supplies by out-competing them [37].

Fig. 3. Shows the Plant growth-promoting activities like I) NH_3 production II) IAA production III) HCN production IV) Siderophore production V) Fluorescent production VI) Organic acid production VII) Bacterial growth curve VIII) PCR of strain.

Diverse plant growth-promoting bacteria (PGPB) frequently have numerous growth-stimulating characteristics, each demonstrating distinct activities under varying environmental and soil conditions. [38]. Phosphate and zinc solubilization, together with IAA, HCN, and exopolysaccharide production, are but a subset of the several characteristics exhibited by PGPB that facilitate plant growth [39]. The research conducted by Zhao, Ding et al. [40] indicates that *Stenotrophomonas* strains exhibit remarkable environmental adaptability and have great promise for use as plant growth-promoting bacteria. Alexander, Singh et al. (2019) suggest that *S. maltophilia* requires more investigation as a PGPR for crop growth in N_2 deficient environments. *Stenotrophomonas maltophilia* AVP 27 is a potential plant growth-promoting rhizobacteria with a wide range of pathways, according to Kumar and Audipudi [41]. Our isolated strain may be used in the field to enhance plant development and replace nutrients since it exhibits several actions that promote plant growth [42-45].

4. CONCLUSION

We isolated *Stenotrophomonas maltophilia* from the rhizospheric soils of south Gujarat forest for this study. 16s rRNA sequencing and bioinformatics analysis were used to confirm the isolated species. The ability of the isolated species to develop plants was examined *In vitro* using a variety of biochemical assays and plant growth activators. The findings of this investigation suggests that the isolated strain in the present study should be evaluated for its capacity to promote plant growth in unsterilized soil as well as in the field. Although beneficial rhizobacteria have a well-established effect on improving plant development, more research is still needed to fully understand the physiological and molecular processes underlying these microbes' improved utilization. However, mechanistic studies involving PGPR and plants are still required to explore the mechanisms by which PGPR promotes plant growth.

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1. QuillBot free version for editing of manuscript

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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