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Plant Growth Promoting Traits of Indigenous Root-associated Bacteria of *Santalum album* L. from Bankura and Burdwan Forest Divisions, West Bengal

Gargi Das¹, Shabnam Rahman², Jagatpati Tah³, Atiskumar Chattopadhyay^{4*} and Paltu Kumar Dhal^{1*}

¹Department of Life Science and Biotechnology, Jadavpur University, Kolkata – 700032, India.

²Department of Zoology, MrinaliniDattaMahavidyapith, Birati, Kolkata – 700051, West Bengal, India.

³Department of Botany, The University of Burdwan, Burdwan, 713104, West Bengal, India.

⁴Department of Allied Health Science, Brainware University, Kolkata, West Bengal 700125

*Corresponding Author: paltuk.dhal@jadavpuruniversity.in, anwesh.chattopadhyay@gmail.com

Corresponding author: Dr. Paltu Kumar Dhal

Assistant Professor

Department of Life Science and Biotechnology

Jadavpur University, Kolkata - 700 032

Mobile: + 91 9732695969

Tel: + 91 33 2457-2992(O)

Email: paltuk.dhal@jadavpuruniversity.in

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Abstract

Santalum album L., one of the most valuable tropical tree species, is predominantly distributed in the southern regions of India, with few reports of its cultivation in West Bengal. The slow-growing, hemiparasitic nature, unique climatic condition for cultivation, and illicit trafficking lead to 80% decline of its natural population over the past three decades. In this study, the morphologically distinct 16 bacteria isolated from the rhizosphere (R=6) and endosphere (E=10) of sandalwood plants collected from Bankura and Burdwan Forest Divisions in West Bengal were taxonomically identified and investigated for their various plant growth promotion (PGP) abilities: production of indole acetic acid, polysaccharide, siderophore, 1-aminocyclopropane-1-carboxylate deaminase, nitrogen-fixing and phosphate solubilization. The bacterial isolates belonged to phylum Bacillota, Actinomycetota, Pseudomonadota and Bacteroidota and 81% exhibited at least three tested PGP traits. *Stutzerimonas* sp. E3 is reported as a novel PGP endophyte that was positive for all the PGP attributes and may act as a biomarker for sandalwood in these regions. This is the first effort to gain knowledge about the sandalwood-bacterial association and also these indigenous beneficial bacterial strains may be utilized in developing biofertilizers for sandalwood, especially in climatic-constrained, dry regions of the Bankura and Burdwan Forest Divisions of West Bengal.

Keywords: Sandalwood, endophyte, rhizosphere, plant growth promotion.

Introduction

Santalum album L., native to the Indian subcontinent, belongs to the family Santalaceae and is the most valuable tropical evergreen tree species predominantly distributed in Tamil Nadu, Karnataka, and Kerala (Chitra and Jijeesh 2021). The heartwood containing 1.5–5% of the strong-specific fragrance of the oil is widely used for carving, pharmaceutical, and cosmetic purposes and holds significant socio-economic importance (Ratnaningrum and Indrioko 2015). *S.album*, also known as East Indian sandalwood, prefers rising temperatures and reduced rainfall, indicating its climate-restricted cultivation under dry conditions, There was no published literature on its existence in West Bengal until 2015 (Mishra et al., 2018; Ratnaningrum and Indrioko 2015). Over the past three decades, an 80% decline in the Indian sandalwood population due to illegal trading, insufficient regeneration, epidemic of spike diseases, and geographical limitations is outstripping the global demand of about 5000–6000 t year⁻¹ and subsequently categorized it as threatened species in the International Union for Conservation of Nature (IUCN) Red List, 1998 (Chitra and Jijeesh 2021).

S. album is a slow-growing hemiparasite unable to absorb water and mineral nutrients required for its growth directly from the soil due to a lack of root hairs, relying primarily on host plants (Mishra et al., 2018). The secondary host is needed to improve sandalwood's growth, survival, and yield, ultimately limiting its natural population. Failure of previous attempts at artificial regeneration of *Santalum* species is a poor silvicultural understanding of its obligate hemiparasitic nature (Mishra et al., 2018). Therefore, scientists are undertaking more comprehensive inter-disciplinary research collaboration to overcome the limitations of sandalwood-host association and geographical and climatic limitations.

Recent advancements in the field of sustainable agriculture where scientists are using beneficial bacteria that produce metabolites or enzymes such as indole 3-acetic acid (IAA), ACC (1-aminocyclopropane-1-carboxylate) deaminase, siderophore, fixes nitrogen, solubilize phosphate efficient in plant growth promotion (PGP) and combat biotic and abiotic stress of host plants has illuminated a modern approach for proper exploitation of these plant growth-promoting bacteria (PGPB) in sandalwood cultivation where they can survive without any host plant association (Pradhan et al., 2014). However, as far as we know, such attempts on sandalwood are very few (Chitra and Jijeesh., 2021; Pradhan et al., 2014). Therefore, this will be the first study to extensively investigate the PGP functionality of bacteria associated with sandalwood.

To develop sandalwood-specific efficient biofertilizer based on indigenous microorganisms (IMO) technology, the first step is (i) the isolation of the rhizospheric and endophytic bacteria

from the rhizosphere and endosphere of *Santalum album*, respectively, and (ii) screening of competent endophytic bacteria with PGP traits, which is the aim of our present investigation.

2. Materials and methods

2.1. Site description and sample collection

For the present study, three field sites (Site 1, Site2, and Site 3) in Bankura and Burdwan Forest Divisions in West Bengal were selected for soil and plant sample collection in September 2019 (**Table 1**).

Root-associated samples (S1A, S1B, S2 and S3) comprising intact sandalwood roots and their rhizospheric soil were randomly collected from respective field sites. Intact roots and their respective rhizosphere soil were dug out carefully during the plant growing season from the top 0-15-cm layer. All samples were packaged in sterile bags (Himedia) placed on ice and immediately brought back to the laboratory for further processing within 24 h.

2.2. Bacterial isolation and selection

2.2.1. Isolation of Rhizosphere bacteria

One gram of rhizosphere soil from each of the four root samples of *S.album* is separately suspended in 100 ml of sterile phosphate-buffered saline (PBS) and placed in a shaker incubator for 40 min at $28\pm 2^{\circ}\text{C}/200$ rpm. The solution was kept still for 30 min, and dilutions 10^{-4} - 10^{-6} were spread onto nutrient agar (NA) plates and incubated at $28\pm 2^{\circ}\text{C}$ for 24 h. The most prominent distinct colonies are isolated and preserved in glycerol stock at -80°C until further study.

2.2.2. Isolation of endophytic bacteria

The roots of *S.album* were surface sterilized by rinsing with tap water, then treated with 0.1% tween 20 solutions, exposed to 75% ethanol for about 2 min, followed by sodium hypochlorite (4% available chlorine) for 2 min and washing several times with sterile distilled water. Sterility was checked by plating 100 μl of the last washed water on NA plates and incubated at 30°C for 4 days. The surface sterilized roots were cut into 0.5 cm fragments and gently homogenized with sterile 0.1M PBS placed in B.O.D shaker incubator for 40 min at $28^{\circ}\text{C}/200$ rpm. The resulting homogenates were kept still for another 40 min, dilutions 10^{-4} - 10^{-6} were spread onto NA plates and incubated at $28\pm 2^{\circ}\text{C}$ for 24 h. The most prominent colonies are isolated and preserved in glycerol stock at -80°C until further study.

2.3. Phenotypic characterization of bacteria

The morphological and phenotypic properties of all the isolates were studied. The systematic assessment of colonies' appearance based on edge, shape, size, colour, elevation, surface and

thermogenesis was observed by the spread plate technique on NA plates and identified according to Bergey's Manual of Determinative Bacteriology (Bergey 1994).

2.4. Molecular Identification of Bacterial isolates

2.4.1. DNA Extraction, Polymerase Chain Reaction Amplification and Sequencing of 16S rRNA gene

According to the manufacturer's protocol, the genomic DNA of bacterial strains grown in NB media was extracted using a DNeasyUltraClean Microbial kit (Qiagen). Amplification of the 16S rRNA gene was done using bacterial-specific universal primers (Dhal et al., 2011). Finally, the purified, amplified PCR products were sent for sequencing at Eurofins Genomics India Pvt. Ltd.

Bacterial taxonomic affiliations were assigned based on the similarity search of sequences available at the NCBI database (<http://www.ncbi.nlm.nih.gov/>) using the BLAST search. A 98% sequence match threshold with query sequences was considered for phylogenetic analysis in MEGA (version 10.2.6) using a neighbour-joining method with 1000 bootstrap replicates (Tamura et al., 2012).

2.4.2. Nucleotide accession numbers

The nucleotide sequences were deposited at NCBI GenBank with accession numbers OR648232 to OR648247.

2.5. Screening for Plant growth-promoting properties of bacterial isolates

All the isolated strains were analysed for their plant growth-promoting abilities, which include direct mechanisms like phosphate solubilization, nitrogen fixation, production of ACC deaminase, IAA, and polysaccharide, as well as indirect mechanisms involving the formation of siderophores.

2.5.1 Siderophore detection

The medium described by (Schwyn and Neilands 1987) was used to screen for siderophore production. Briefly, overnight-grown cultures in NB were streaked on blue dye Chrome Azurol S (CAS) agar plates. Bacterial isolates producing an orange halo zone around the colony after five days of incubation at $28 \pm 2^\circ\text{C}$ were considered siderophore-producing strains.

2.5.2. Determination of N₂ fixation activity

Nitrogen-fixing bacteria were tested by growing the bacteria on nitrogen free base (Nfb) agar medium. The colonies that fix nitrogen alkalize the culture medium and turns the media blue because of ammonia production (Tang et al., 2020).

2.5.3. IAA production

Bacterial isolates were cultured overnight supplemented with 100µg/ml or without adding L-tryptophan in NB and incubated at 28±2°C for 48 h. Cell-free supernatant was obtained by centrifugation at 8,000× g for 10 min. 2 ml of supernatant was mixed vigorously with two drops of orthophosphoric acid; 4 ml of Salkowski's reagent (0.1ml of FeCl₃ in 50 of 35% perchloric acid) and kept in the dark for 30 min. Development of a pink colour indicated IAA production and the absorbance was measured at 535 nm (Benizri et al., 1998).

2.5.4. Solubilization of phosphate

Phosphate-solubilizing bacterial colonies were isolated on Pikovskaya agar media. The plates were incubated at 30°C for five days, and the clearing zone around colonies was considered positive for phosphate solubilization (Pikovskaya 1948).

2.5.5. ACC utilization

The ability of isolates to produce ACC deaminase was screened on minimal media containing ACC as its sole nitrogen source, as described in (Glick 1995). Growth of isolates on ACC-supplemented plates were taken as ACC deaminase producers.

2.5.6. Polysaccharide production

The ability of the bacterial isolates to produce polysaccharides was determined by using overnight-grown cultures in NB and streaking them on nutrient agar plates supplemented with 5% sucrose (Bueno & Garcia-Cruz 2006). Growth of isolates indicates a positive test for polysaccharide production.

3. Results and Discussion

The plant-associated microbial community, including endophyte and rhizosphere bacteria, has increased the yield of crops as well as induced disease resistance and stress tolerance, thereby being utilized as an efficient alternatives to chemical-based solutions for higher crop production (Hossain et al., 2023). Studies reporting the utilization of those bacteria for host plant growth promotion through increased nutrient acquisition and modulation of plant physio-biochemical constituents have been reported in various cereals, fruits, vegetables and medicinal plants (Hossain et al., 2023; Tang et al., 2020). However, the commercial application of PGPB from sandalwood in sustainable agriculture represents an unexplored scenario. The *S.album* owing to its morpho-physiological seed dormancy; the unique climatic condition for cultivation; soil limiting factors such as nutrients, water availability in the root zone, soil depth, and soil structure; socio-economic and technical requirements primarily restricts its abundance in India (Debta et al., 2023; Sarmah 2022).

To date, there are only two reports on using polyethylene glycol and manganese sulfate as priming agents for improving germination and seedling growth of sandalwood (Debta et al.,

2023; Jijeesh et al., 2022). However, with the recent advancement in agro-technology, priming with beneficial microbes is becoming a potentially prominent technique that not only overcomes germination constraints but also promotes desired attributes in crop growth (Sarkar et al., 2021). Two of the reports from Uttarakhand and Odisha utilized PGP rhizospheric bacteria to observe their effect on sandalwood seedlings (Parkash et al., 2022; Pradhan et al., 2014). Another two studies from Karanataka, examined a combination of bacterial treatments as biofertilizers to study the growth performance of *S.album* seedlings (Arade et al., 2020; Muthu-Kumar et al., 2023). A study from Kerala examined impact of biopriming with *Pseudomonas fluorescens* on sandalwood seed germination and performance of seedling (Chitra and Jijeesh 2021). However, those studies have been carried out either using host non-specific bacteria or indigenous rhizosphere bacteria whose efficacy has not been extensively tested, thereby restricting their utilization as efficient biofertilizers in real agricultural fields.

This study, therefore, aims to our scientific knowledge on isolation, identification, and characterization of bacterial isolates from the sandalwood rhizosphere and root-endosphere cultivated in the Burdwan and Bankura districts of West Bengal, highlighting the similarities and/or differences attributed to their taxonomy and traits of beneficial plants. Besides, this will be the first attempt to uncover sandalwood-microbe interaction in the state of West Bengal (a new geographical area with favourable soil and climatic conditions with respect to sandalwood cultivation).

3.1. Isolation of endophytic and rhizospheric bacteria

A total of 47 bacterial isolates were isolated from the rhizosphere (22; 46.81%) and surface-sterilized roots (25; 53.19%) of *S. album* collected from four sampling field sites of Bankura and Burdwan Forest Divisions in West Bengal. Based on their unique morphological characterization, a total of sixteen (six rhizosphere and ten endophytes) bacterial isolates were selected for further investigation.

3.2. Identification of bacterial strains based on 16S rRNA gene sequences

The molecular identification of those bacterial isolates was ascertained by 16S rRNA gene sequencing followed by phylogenetic lineage analysis (**Figure 1**).

The result from NCBI-BLAST showed that tested isolates have substantial similarity (97–100%) with culturable bacteria and are located in four major clusters affiliated with *Bacillota*, *Actinomycetota*, *Pseudomonadota* and *Bacteroidota* (**Table 2**). The most abundant group of isolates was affiliated with the phylum *Bacillota*, comprising two different clads representing families *Bacillaceae* and *Staphylococcaceae*, accounting for 43.75% of all bacterial isolates. Isolates R17, E20 and E18 were found to be *Bacillus* species having

maximum similarity with *Bacillus tropicus* (GenBank MK467571) and *Bacillus albus* (GenBank MK993477), isolated from the rhizosphere of mango and aloe vera plants, respectively with high bootstrap value. E4 was identified as a *Priestia species showing maximum similarity with Priestiaaryabhattai* (OQ295975), reported as a plant growth-promoting endophytic bacteria isolated from tea leaves. Under the clad of family *Staphylococcaceae*, R3 showed maximum similarity with *Micrococcus* sp. (KR109041), isolated from the air samples, while strains R7 and E19 represented maximum similarity with *Staphylococcus* species (MN581178, LC481680 respectively) which characterized bacterial community in a closed habitat during human occupation and high fertility clay soil associated with wheat.

Under the family *Microbacteriaceae*, E5 had 100% sequence similarity with the type strain *Micrococcus luteus* (GenBank MZ595691), isolated from the root tissue of a medicinal plant grown in a desert environment. Other strains, E11 and E2, fell in the same clad in the phylogenetic tree and have close similarity with *Microbacterium* species (MN923313) isolated from river sediment and halophytic plants, respectively.

Under phylum *Pseudomonadota*, there are a total of four clads of family taxa. Under the family *Burkholderiaceae*, both the bacterial strains R22 and E17 showed a strong relation with *Burkholderiacepacia* (OR563792), isolated from the rhizosphere of moso bamboo having phosphate-solubilizing ability having high bootstrap value. Under the family *Pseudomonadaceae*, E3 showed the highest similarity with *Stutzerimonasstutzeri* (MN093397), isolated from the rhizosphere of the aloe vera plant. Under the family *Yersiniaceae*, R20 showed maximum similarity with *Serratianematodiphila* (OL660656), reported as rhizobacteria isolated from *Terminalia* trees. Under the family *Enterobacteriaceae*, R9 showed a strong relation with *Enterobacterkobei* (MN691917), isolated from rice paddy soil.

Under the clad, *Bacteroidota* falls in the family *Weeksellaceae*, where strain E8 had 100% bootstrap similarity with *Chryseobacterium* species.

3.3. Bacterial isolates screened for plant growth-promoting traits

The isolated sixteen bacterial strains were screened for their plant growth potential such as phosphate solubilization, production of polysaccharide, IAA, siderophore, ACC deaminase, and nitrogen fixation (**Table 3**).

IAA is an important phytohormone that plays a key role in regulation of plant growth by increasing the rate of xylem and enhancing lateral and adventitious root formation (Brescia et al., 2023). In the present study, 50% of the isolated bacteria (R=2; E=6) belonging to *Micrococcus*, *Enterobacter*, *Microbacterium*, *Stutzerimonas*, *Priestia*, *Micrococcus*,

Chryseobacterium and *Staphylococcus* genera can produce IAA. Among them, *Micrococcus* sp. E5, *Enterobacter* sp. R9 and *Macrococcus* sp. R3 produced highest IAA 114 ppm, 101 ppm, and 24.5 ppm IAA respectively. Previous studies also reported them as IAA producers consisting of rhizobacteria associated with bean, rice and flowering plants or endophytic bacteria isolated from other plants like millet, wheat, and lentil (Brescia et al., 2023; Flores-duarte et al., 2022; Kumar et al., 2012; Manjunatha et al., 2019; Shahid et al., 2022).

The PGPB are versatile organisms that convert insoluble phosphates to orthophosphate, through the production of organic acids and acid phosphatases for increasing plant yields (Emami et al., 2019). About 37.5 % (R=4; E=2) dominated by *Burkholderia*, *Macrococcus*, *Enterobacter*, *Serratia* and *Stutzerimonas* solubilized phosphate. Some recent studies have also reported them as phosphate solubilizing microorganisms (Islam et al., 2013; Kumar et al., 2012; Manjunatha et al., 2019).

Nitrogen is an essential nutrient for the growth and development of plants and its fixation by PGPB is one of the main mechanisms by which plants benefit from the microbial association (Moustaine et al., 2017). In this study, a large number of R (n=6) and E (n=7) isolates were able to fix nitrogen dominated by *Bacillus*, *Burkholderia* and followed by *Macrococcus*, *Staphylococcus*, *Enterobacter*, *Serratia*, *Microbacterium*, *Stutzerimonas*, *Priestia* and *Micrococcus*. This is consistent with previous observations that diverse groups of bacteria were found to exhibit nitrogen fixation ability (Flores-duarte et al., 2022; Moustaine et al., 2017; Siddikee et al., 2010).

ACC deaminase synthesized by PGPB can significantly reduce plant ethylene levels by degrading its precursor ACC, into α -ketobutyrate and ammonia, and thus help plants to withstand biotic or abiotic stress (Islam et al., 2013). About 75% of the isolates tested positive for ACC deaminase production, mainly dominated by *Bacillus*, *Burkholderia* and followed by *Macrococcus*, *Staphylococcus*, *Enterobacter*, *Serratia*, *Stutzerimonas*, *Priestia* and *Micrococcus*. As previously reported, that bacteria belonging to the genera of *Bacillus* and *Enterobacter* had had the highest ACC deaminase activity, is in line with this study (Kuźniaret al., 2019). Other studies who have found these bacterial genera as the most efficient ACC deaminase producers are (Brescia et al., 2023; Islam et al., 2013; Kumar et al., 2012; Manjunatha et al., 2019; Shahid et al., 2022; Siddikee et al., 2010).

Siderophore production also influences plant growth by efficiently scavenging available iron (Fe³⁺) and making it available to plants (Islam et al., 2013). In addition to iron acquisition, they also limit iron availability to pathogens and inhibit their growth (Islam et al., 2013). In this study, the production of siderophore was detected by orange halos around colonies in CAS-agar medium exhibited by four bacterial strains (R=2, E=2) belonging to *Serratia*,

Burkholderia and *Stutzerimonas*. Previous studies have also demonstrated the benefits of siderophore-producing bacteria for plant growth and bioremediation of soils (Gil et al., 2023; Islam et al., 2013).

All the isolates were positive for polysaccharide production. Other studies that observed polysaccharide production by various bacterial genera are (Franchi et al., 2017; Tsegaye et al., 2019).

Plant growth promotion by beneficial microorganisms is a well-established and complex phenomenon often achieved by multiple PGP traits exhibited by plant-associated bacteria (rhizosphere and endophyte). In our study, 81% of the isolates showed at least three tested PGP traits and *Stutzerimonas* sp. E3 is reported as a novel PGP endophyte that was positive for all the PGP attributes. Bacteria with several PGP characteristics can benefit plants by accelerating seed germination, promoting plant development, inducing stress tolerance and suppressing disease. Besides, it has already been reported that an effective biofertilizer strain isolated from one region may not perform similarly in other soil or climatic conditions (Duffy et al., 1997). Thus, our isolated indigenous bacterial strains associated with sandalwood could act as biomarkers for sandalwood cultivation in West Bengal, which has not yet been explored. Consequently, these PGPBs could play a significant role in improving nutrient uptake and plant growth if applied as biofertilizers, and also may help overcome the parasitic nature of sandalwood.

4. Conclusions

This study successfully isolated 16 efficient and beneficial rhizome and endo-bacterial communities from the soil and root of sandalwood, respectively. Among them, 81% of the isolates exhibited a minimum of three tested PGP traits and *Stutzerimonas* sp. E3 is reported as a novel PGP endophyte that was positive for all the attributes. Furthermore, the multiple PGP traits of selected bacterial isolates, which are unique to the sampling sites, can be represented as biomarkers for sandalwood. These functional indigeneous bacterial strains could potentially exploited for the development of biofertilizers capable of enhancing soil fertility and plant growth through beneficial interactions.

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Competing interests

The authors declare that there are no conflicts of interest.

References

1. Chitra, P., &Jijeesh, C. M. (2021). Biopriming of seeds with plant growth promoting bacteria *Pseudomonas fluorescens* for better germination and seedling vigour of the East Indian sandalwood. *New Forests*, 52(5), 829–841. <https://doi.org/10.1007/s11056-020-09823-0>.
2. Ratnaningrum, Y. W. N., &Indrioko, S. (2015). Response of Flowering and Seed Production of Sandalwood (*Santalum Album* Linn.,Santalaceae) to Climate Changes. *Procedia Environmental Sciences*, 28(Sustain 2014), 665–675. <https://doi.org/10.1016/j.proenv.2015.07.078>.
3. Mishra, B., Chakraborty, S., Sandhya, M. C., &Viswanath, S. (2018). Sandalwood farming in India: problems and prospects. *Indian Journal of Tropical Biodiversity*, 26(1), 1–12.
4. Pradhan, A., Baisakh, B., & Mishra, B. B. (2014). Plant growth characteristics of bacteria isolated from rhizosphere region of *Santalum album*. *J Pure ApplMicrobiol*, 8(6), 4245–5054.
5. Bergey, D. H. (1994).*Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.
6. Dhal, P. K., Islam, E., Kazy, S. K., &Sar, P. (2011). Culture-independent molecular analysis of bacterial diversity in uranium-ore/-mine waste-contaminated and non-contaminated sites from uranium mines. *3 Biotech*, 1, 261-272.
7. Tamura, T., Matsuzawa, T., Oji, S., Ichikawa, N., Hosoyama, A., Katsumata, H., ...& Fujita, N. (2012). A genome sequence-based approach to taxonomy of the genus *Nocardia*. *Antonie Van Leeuwenhoek*, 102, 481-491.
8. Schwyn, B., &Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 160(1), 47–56.
9. Tang A, Haruna AO, Majid NMA, Jalloh MB. Potential PGPR Properties of Cellulolytic, Nitrogen-Fixing, Phosphate-Solubilizing Bacteria in Rehabilitated Tropical Forest Soil. *Microorganisms*. 2020 Mar 20;8(3):442. doi: 10.3390/microorganisms8030442.
10. Benizri, E., Courtade, A., Picard, C., &Guckert, A. (1998).Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens*M. 3.1. *Soil Biology and Biochemistry*, 30(10-11), 1481-1484.
11. Pikovskaya, R. I., (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*. 17: 362-370.

12. Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*, 41(2), 109-117.
13. Bueno, S. M., & Garcia-Cruz, C. H. (2006). Optimization of polysaccharides production by bacteria isolated from soil. *Brazilian Journal of Microbiology*, 37, 296-301.
14. Hossain, A., Hassan, Z., Sohag, M. H., & Khan, M. (2023). Impact of the endophytic and rhizospheric bacteria on crop development: prospects for advancing climate-smart agriculture. *Journal of Crop Science and Biotechnology*, 26(4), 405–431. <https://doi.org/10.1007/s12892-023-00195-3>.
15. Debta, H., Kunhamu, T. K., Petrík, P., Fleischer, P., & Jisha, K. C. (2023). Effect of Hydropriming and Osmopriming on the Germination and Seedling Vigor of the East Indian Sandalwood (*Santalum album* L.). *Forests*, 14(6), 1–14. <https://doi.org/10.3390/f14061076>
16. Sarmah, D. (2022). *Down, but Not Out: Sandal in Karnataka*. Notion Press.
17. Jijeesh, C. M., Chitra, P., Hrideek, T. K., Santhoshkumar, A. V., & Kunhamu, T. K. (2022). Nutripriming of Seeds with Manganese Sulphate for Better Germination and Seedling Vigour in the East Indian Sandalwood (*Santalum album* L.). *Journal of Plant Growth Regulation*, 41(3), 1004–1012. <https://doi.org/10.1007/s00344-021-10359-6>
18. Sarkar, D., Singh, S., Parihar, M., & Rakshit, A. (2021). Seed bio-priming with microbial inoculants: A tailored approach towards improved crop performance, nutritional security, and agricultural sustainability for smallholder farmers. *Current Research in Environmental Sustainability*, 3, 100093.
19. Parkash, V., Hunney, K., & Singh, H. (2022). Effect of elevated carbon dioxide on growth and development of *Santalum album* L. seedlings inoculated with plant growth promoting microorganisms in Open Top Chambers. *International Journal of Environment, Agriculture and Biotechnology*, 7, 5.
20. Arade, S., Khannam, A., & T.S, R. (2020). Influence of Biofertilizers on In-Vitro Raised Plantlets of *Santalum album*(Linn.) for Better Growth and Survival under Nursery Conditions. *International Journal of Biotech Trends and Technology*, 10(01), 10–14. <https://doi.org/10.14445/22490183/ijbtt-v10i1p602>
21. Muthu-Kumar, A., Sandhya, G. M., & Karthikeyan, A. (2023). Evaluation of Bio-Fertiliser (Bio-Inoculant) Consortia and Their Effect on Plant Growth Performance of Sandalwood (*Santalum Album*) Seedlings. *Journal of Tropical Forest Science*, 35(3), 311–321. <https://doi.org/10.26525/jtfs2023.35.3.311>

22. Brescia, F., Sillo, F., Balestrini, R., Sbrana, C., &Zampieri, E. (2023). Characterization of endophytic bacteria isolated from root nodules of lentil in intercropping with durum wheat. *Current Research in Microbial Sciences*, 100205.
23. Flores-duarte, N. J., Mateos-naranjo, E., Redondo-g, S., Rodriguez-llorente, I. D., & Navarro-torre, S. (2022). *Role of Nodulation-Enhancing Rhizobacteria in the Promotion of Medicago sativa Development in Nutrient-Poor Soils*.
24. Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., Negi, S., & others. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent Research in Science and Technology*, 4(1), 1–5.
25. Manjunatha, B. S., Paul, S., Aggarwal, C., Bandeppa, S., Govindasamy, V., Dukare, A. S., Rathi, M. S., Satyavathi, C. T., & Annapurna, K. (2019). Diversity and Tissue Preference of Osmotolerant Bacterial Endophytes Associated with Pearl Millet Genotypes Having Differential Drought Susceptibilities. *Microbial Ecology*, 77(3), 676–688. <https://doi.org/10.1007/s00248-018-1257-2>
26. Shahid, M., Zeyad, M. T., Syed, A., Singh, U. B., Mohamed, A., Bahkali, A. H., Elgorban, A. M., &Pichtel, J. (2022). Stress-Tolerant Endophytic Isolate Priestiaaryabhatai BPR-9 Modulates Physio-Biochemical Mechanisms in Wheat (*Triticumaestivum* L.) for Enhanced Salt Tolerance. *International Journal of Environmental Research and Public Health*, 19(17). <https://doi.org/10.3390/ijerph191710883>.
27. Emami, S., Alikhani, H. A., Pourbabaei, A. A., Etesami, H., Sarmadian, F., &Motessharezadeh, B. (2019). Effect of rhizospheric and endophytic bacteria with multiple plant growth promoting traits on wheat growth. *Environmental Science and Pollution Research*, 26(19), 19804–19813. <https://doi.org/10.1007/s11356-019-05284-x>
28. Islam, M. R., Sultana, T., Joe, M. M., Yim, W., Cho, J. C., &Sa, T. (2013). Nitrogen-fixing bacteria with multiple plant growth-promoting activities enhance growth of tomato and red pepper. *Journal of Basic Microbiology*, 53(12), 1004–1015. <https://doi.org/10.1002/jobm.201200141>
29. Moustaine, M., Elkahkahi, R., Benbouazza, A., Benkirane, R., &Achbani, E. H. (2017). Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (*Solanumlycopersicum* L.) and characterization for direct PGP abilities in Morocco. *International Journal of Environment, Agriculture and Biotechnology*, 2(2), 238708.

30. Siddikee, M. A., Chauhan, P. S., Anandham, R., Han, G. H., & Sa, T. (2010). Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. *Journal of Microbiology and Biotechnology*, 20(11), 1577–1584. <https://doi.org/10.4014/jmb.1007.07011>.
31. Kuźniar, A., Włodarczyk, K., & Wolińska, A. (2019). Agricultural and other biotechnological applications resulting from trophic plant-endophyte interactions. *Agronomy*, 9(12), 779.
32. Gil, T., Teixeira, R., Sousa, A., d'OliveiraPalmeiro, M. A., Cruz Coimbra de Matos, A., Niza Costa, M., Ferrer, M. V., Rodrigues dos Santos, A. S., SequeroLópez, C., RebeloRomão, I., & Vílchez, J. I. (2023). Isolation and Characterization of Culturable Osmotolerant Microbiota in Hypersaline and Hypergypsic Soils as New Treatment for Osmotic Stress in Plants. *Soil Systems*, 7(4), 86. <https://doi.org/10.3390/soilsystems7040086>
33. Franchi, E., Rolli, E., Marasco, R., Agazzi, G., Borin, S., Cosmina, P., Pedron, F., Rosellini, I., Barbafieri, M., & Petruzzelli, G. (2017). Phytoremediation of a multi contaminated soil: mercury and arsenic phytoextraction assisted by mobilizing agent and plant growth promoting bacteria. *Journal of Soils and Sediments*, 17(5), 1224–1236. <https://doi.org/10.1007/s11368-015-1346-5>.
34. Tsegaye, Z., Gizaw, B., Tefera, G., Feleke, A., Chaniyalew, S., Alemu, T., & Assefa, F. (2019). Isolation and biochemical characterization of Plant Growth Promoting (PGP) bacteria colonizing the rhizosphere of Tef crop during the seedling stage. *Journal of Plant Science and Phytopathology*, 3(1), 13–27.
35. Duffy, B. K., Ownley, B. H., & Weller, D. M. (1997). Soil chemical and physical properties associated with suppression of take-all of wheat by trichoderma koningii. *Phytopathology*, 87(11), 1118–1124. <https://doi.org/10.1094/PHYTO.1997.87.11.1118>.

Figures

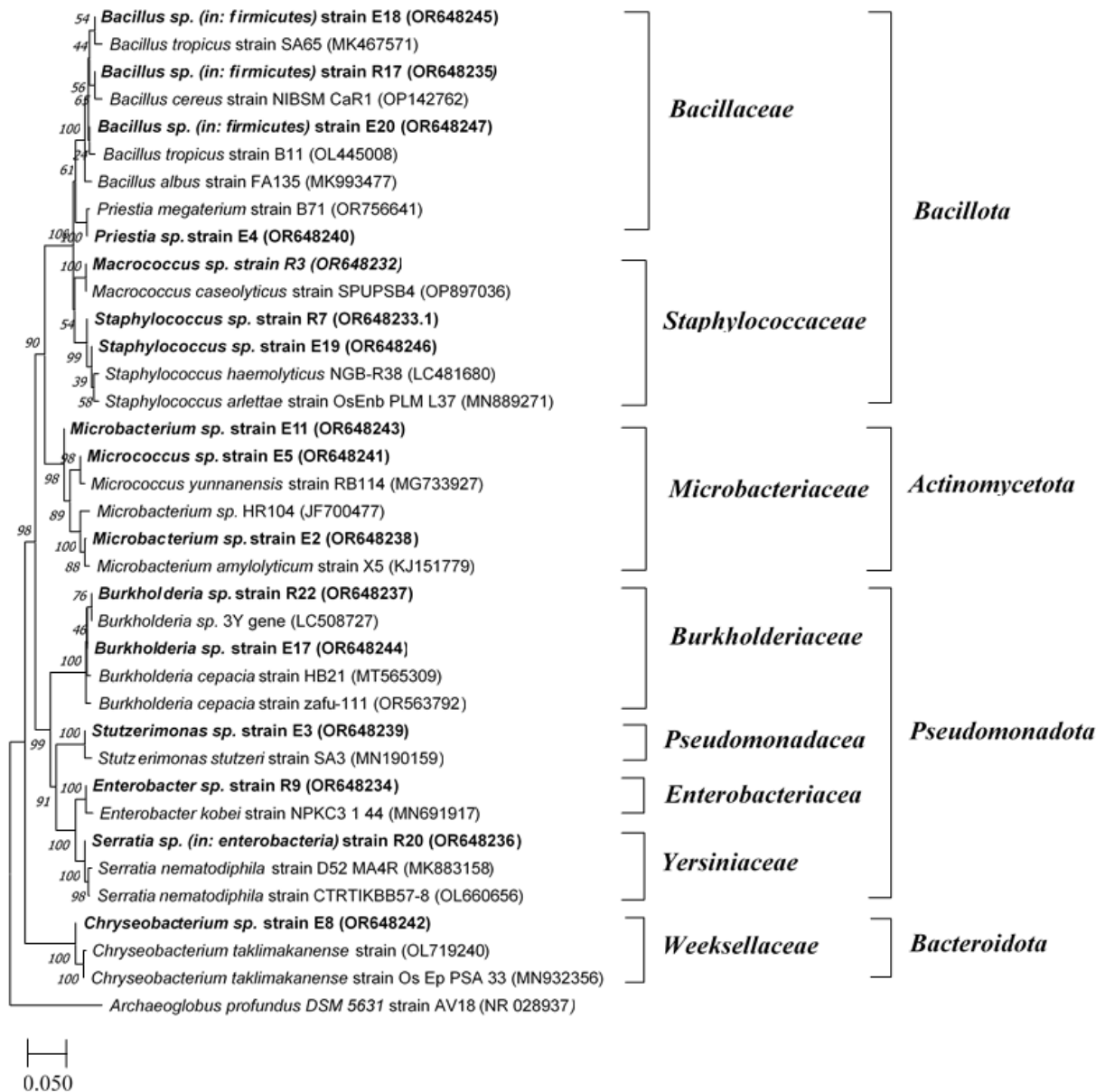


Figure 1: Phylogenetic affiliation of the isolated bacterial strains: The phylogenetic tree of 16S rRNA gene sequence of isolated bacterial strains was constructed in MEGA11 by neighbor-joining method and 1000 bootstraps values. The outgroup is *Archaeoglobus profundus*. Sequences represented in boldface font are derived from this study

Table 1: Details of the collected samples: Sandalwood plants and rhizospheric soil samples were collected from three different forest ranges in the Burdwan and Bankura districts of

West Bengal, India. Different numbers of rhizospheric and endophytic bacteria isolated from the studied sites are also listed.

Site No.	Site Id	Location	GPS Coordinates	Sample type	Bacterial type	
					Rhizosphere	Endophyte
Site 1	S1A	Ausgram Range Office Campus; Burdwan, West Bengal	N23°30'47.266" E87°39'22.517	Whole plant with roots and soil excavated	9	9
	S1B			Sucker root associated soil	3	5
Site 2	S2	Khandari; Burdwan, West Bengal	N23°25'59" E87°32'26"	Whole plant	5	7

Table 2: Taxonomic affiliation of the 16S rRNA gene sequences of isolated bacterial strains based on NCBI-BLAST similarity search. The sequences were searched for similar sequences from the NCBI database with a minimum threshold of 98% similarity.

Table 3: Plant growth promotion abilities of the selected bacterial isolates. “+” sign indicates presence of activity and “-” sign represents no activity by qualitative estimation. IAA: indole acetic acid, Polysaccharide, Phosphate: Phosphate solubilizing, Nitrogen:

Sl. no	Bacterial Type	Strains Id	GenBank accession	Taxonomic affiliation				
				Phylum	Class	Order	Family	Genus
1	Rhizosphere	R3	OR64823 2	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Macrococcus</i>
2		R7	OR64823 3	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Staphylococcus</i>
3		R9	OR64823 4	<i>Pseudomonadota</i>	<i>Gammaproteobacteria</i>	<i>Enterobacterales</i>	<i>Enterobacteriaceae</i>	<i>Enterobacter</i>
4		R17	OR64823 5	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>
5		R20	OR64823 6	<i>Pseudomonadota</i>	<i>Gammaproteobacteria</i>	<i>Enterobacterales</i>	<i>Yersiniaceae</i>	<i>Serratia</i>
6		R22	OR64823 7	<i>Pseudomonadota</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>
7	Endophyte	E2	OR64823 8	<i>Actinomycetota</i>	<i>Actinomycetes</i>	<i>Micrococcales</i>	<i>Microbacteriaceae</i>	<i>Microbacterium</i>
8		E3	OR64823 9	<i>Pseudomonadota</i>	<i>Gammaproteobacteria</i>	<i>Pseudomonadales</i>	<i>Pseudomonadaceae</i>	<i>Stutzerimonas</i>
9		E4	OR64824 0	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Priestia</i>
10		E5	OR64824 1	<i>Actinomycetota</i>	<i>Actinomycetes</i>	<i>Micrococcales</i>	<i>Micrococcaceae</i>	<i>Micrococcus</i>
11		E8	OR64824 2	<i>Bacteroidota</i>	<i>Flavobacteriia</i>	<i>Flavobacteriales</i>	<i>Weeksellaceae</i>	<i>Chryseobacterium</i>
12		E11	OR64824 3	<i>Actinomycetota</i>	<i>Actinomycetes</i>	<i>Micrococcales</i>	<i>Microbacteriaceae</i>	<i>Microbacterium</i>
13		E17	OR64824 4	<i>Pseudomonadota</i>	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>
14		E18	OR64824 5	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>
15		E19	OR64824 6	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Staphylococcaceae</i>	<i>Staphylococcus</i>
16		E20	OR64824 7	<i>Bacillota</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>

Nitrogen fixation, ACC:ACC (1-aminocyclopropane-1-carboxylate) deaminase, Fe: siderophore producing isolates respectively.

Sample	Bacterial Type	Taxonomic affiliation	IAA (ppm)	Polysaccharide	Phosphate	Nitrogen	ACC	Fe
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S1A	Rhizosphere	<i>Macrococcus</i> sp. R3	24.5	+	+	+	+	-
S1A		<i>Staphylococcus</i> sp. R7	0.0	+	-	+	+	-
S1A		<i>Enterobactersp.</i> R9	101.0	+	+	+	+	-
S2		<i>Bacillus</i> sp. R17	0.0	+	-	+	+	-
S3		<i>Serratia</i> sp. R20	0.0	+	+	+	+	+
S3		<i>Burkholderia</i> sp. R22	0.0	+	+	+	+	+
S1A	Endophyte	<i>Microbacterium</i> sp. E2	21.4	+	-	+	-	-
S1A		<i>Stutzerimonas</i> sp. E3	17.6	+	+	+	+	+
S1A		<i>Priestia</i> sp. E4	18.7	+	-	+	+	-
S1A		<i>Micrococcus</i> sp. E5	114.0	+	-	+	+	-
S1A		<i>Chryseobacterium</i> sp. E8	14.1	+	-	-	-	-
S1B		<i>Microbacterium</i> sp. E11	0.0	+	-	-	-	-
S2		<i>Burkholderia</i> sp. E17	0.0	+	+	+	+	+
S2		<i>Bacillus</i> sp. E18	0.0	+	-	+	+	-
S2		<i>Staphylococcus</i> sp. E19	20.1	+	-	-	-	-
S2		<i>Bacillus</i> sp. E20	0.0	+	-	+	+	-