Short Research Article

Studies on the Impact of Microbial Consortia towards Enhancing the Growth of Red Sanders

ABSTRACT

The use of plant growth-promoting microorganisms, either alone or combined, as bioinoculants presents an economical and eco-friendly alternative to chemical fertilizers. This method provides a comprehensive solution for enhancing plant growth and yield. This study aimed to develop robust microbial consortia possessing plant growth-promoting capabilities and evaluate their effectiveness in enhancing tree seedling growth. Six bacterial strains were collected and screened for specific traits linked to promoting plant growth. Selected strains were then assessed for compatibility with each other, resulting in the formation of bacterial consortia. These consortia demonstrated diverse plant growth-promoting properties, including the production of the plant growth hormone indole acetic acid and the synthesis of exopolysaccharides. Consequently, these bacterial consortia exhibit significant potential as bioinoculants, offering a promising approach to enhancing plant growth and productivity in an environmentally friendly manner. This contributes to the progress of efforts aimed at promoting seedling growth

~~Keywords :~~Keywords:*Plant growth promoting Rhizobacteria,Microbial consortia, Red ~~sanders, Seedling~~sanders, Seedling*

1. INTRODUCTION

The rhizosphere, a dynamic soil environment shaped by living plant roots and a diverse community of microorganisms and fauna, serves as a habitat for Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal (AM) fungi. These microorganisms play a vital role in enhancing plant growth by performing functions such as nitrogen fixation and phosphorus solubilization. Moreover, they stimulate root development by producing metabolites like indole acetic acid (IAA) and other growth hormones.

PGPR and AM fungi offer environmentally friendly, renewable, and cost-effective alternatives to chemical fertilizers, reducing pollution. Utilizing these beneficial microorganisms as bio-inoculants presents an efficient and sustainable method to boost plant growth. The documented positive effects and potential of various beneficial microorganisms in agriculture underscore their importance in promoting eco-friendly and sustainable farming practices (Subba Rao, 1993; Dash and Gupta, 2011; Brahmaprakash and Sahu, 2012).

The effective utilization of bio-inoculants for trees not only brings economic benefits but also plays a crucial role in preserving soil fertility and sustainability in natural soil ecosystems. However, successful implementation relies on identifying the most suitable bio-inoculants. Due to limited information on the application of AM fungi, Azospirillum,Azotobacter, and Phosphobacteria on forest tree species, particularly economically significant fast-growing native tree species, this study aims to investigate the efficacy of these bio-inoculants in enhancing the growth of Sandal & Red Sandal plants in the nursery

2. MATERIAL AND METHODS

Rhizospheric soil samples were collected from the Red Sanders plantations located at the Forest College & Research Institute in Mettupalayam, India, for the isolation of PGPR. To maintain soil integrity and prevent

moisture loss, the samples were stored in plastic bottles or bags with loose ties to ensure adequate aeration. This method was adopted as per the protocol outlined by Modi et al. (2017).

Isolation & characterization of potential plant growth promoting rhizobacteria

PGPR isolates were isolated from the rhizospheric soil sample by serial dilution and spread plate method using King's B 25 medium at 28ºC (Singh & Lal, 2016).

Colony morphology and pigment production

Colony morphology (form, elevation, margin, shape & surface) and the production of pigment was checked on King's B agar at 28± 2ºC after 24 to 48 hours (Kumar et al., 2020).

Characterization of selected isolates for various PGP attributes

Phosphate solubilization

The ability of bacteria to solubilize phosphorus was tested on Pikovskaya's agar plates. Each bacterial culture was spot inoculated in the centre of Pikovskaya's agar plates containing tricalcium phosphate as insoluble phosphate source (Kumar et al., 2020). The plates were incubated at 28°C for 5-7 days and halozone development around the bacterial growth was observed.

Indole acetic acid production

Indole acetic acid produced by bacteria was determined as described by Brick et al. Bacterial cultures were grown in NB amended with tryptophan (100µg/ml) at 30°C for 48 hrs on shaker (120 rpm). The cultures were centrifuged at 3000 rpm for 30 minutes. The supernatant (2 ml) was mixed with two drops of o-phosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M FeCl3 solution) and incubated for 25 mins at room temperature in dark. Development of pink color indicate IAA production. The intensity of pink color was read at 530 nm spectrophotometrically and the amount of IAA produced was extrapolated from the standard curve.

Biochemical characterization of PGPR bacteria

Different biochemical parameters were performed to identify bacterial isolates. Preliminarily, Gram's staining technique, motility, Indole production test, Methyl red (MR) and Voges-Proskauer's (VP) test & Ammonia production tests were performed and identified according to Bergeys manual of determinative Bacteriology

Estimation of AM Colonization.

The mixed soil and roots samples of each plant species were packed in polyethylene bags, ~~labeled~~labeled, and brought to the laboratory. The soil samples were air-dried at room temperature. Roots were washed to remove soil particles, preserved with FAA. For colonization measurement, roots were cleared in 10% (w/v) KOH and placed in a water bath (90◦C) for 20–30 min. The cooled root samples were then washed with water and stained with 0.5% (w/v) acid fuchsin. Fifty root fragments for each sample (ca. 1 cm long) were mounted on slides in a polyvinyl alcohol solution and examined for the presence of AM structures at 100–400x magnification with an Olympus BX50 microscope for the presence of AM structures. The percentage of root colonization was calculated using the following formula:

$$\text{Root colonization (\%)} = \frac{\text{Number of arbuscular mycorrhiza positive segments}}{\text{Total number of segments studied}} \times 100\%$$

AM Fungus Spore Quantification and Identification. Three aliquots of soil (20 g) were obtained for every plant species. AM fungal spores were extracted from the soil samples by wet sieving and sucrose density gradient centrifugation. Spores were counted under a dissecting microscope, and spore densities (SD) were expressed as the number of spores per 100 g of soil.

3. RESULTS AND DISCUSSION

Table 1. Microbial population in Rhizosphere and Non-Rhizosphere of Red sanders trees

Microorganism	Red sanders	
	Rhizosphere soil (cfu's/gram of soil)	Non-Rhizosphere soil (cfu's/gram of soil)
Bacteria	$49x10^6$	$14.33x10^4$
Fungi	$22x10^4$	$9x10^3$
Actinomycetes	$12x10^5$	$8x10^3$

Mean of Three Replications

A total of 6 strains were identified as promising bacterial isolates. They were subjected to Gram's staining for determining cell morphology and gram reaction, colony morphology and biochemical characterization for carbon source utilization as per the procedure of Bergey's manual of determinative bacteriology for preliminary identification (Bergey et al. 1994).

Table 2. Morphological Characteristics of the dominant isolates isolated from the Rhizosphere Soils of Red sanders trees

Colony	Size	Colony form	~~Colour~~Color	margin	Optical nature	Elevation	Surface	Gram
A1	Pinpoint	Rhizoid	Cream	Entire	Opaque	Convex	Glistering with rough	Gram -ve short rod
A2	Pequeña	Round	Pale	Entire	Opaque	Convex	Glistering	Gram – ve short

Comment [Revisor1]: Define unit by mm or by name

Comment [Revisor2]: missing brightness, color, texture

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	1mm		yellow					rod
A3	Pinpoint <1 mm	Punctiform	Cream	Entire	Opaque	Convex	Smooth	Gram -ve short rod

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Characterization of bacterial isolates

The morphological characteristics of the bacterial isolates varied widely as shown in (Table). All the isolates produced round to rhizoid shaped colonies, the elevation were convex, had entire margin with the colour ranging from creanm to yellow. Microscopic observations were performed to investigate the ~~some~~same characteristics of the isolates such as cell shape, Gram reaction and motility (Table3). All the isolates ~~were ,cell~~were, cell shape was mostly rod and majority were Gram negative in ~~reaction~~reaction.

Table ~~3 .~~3. Growth promotion activity of the dominant PGPR isolates

Isolate no	IAA	Phosphate ~~solubilisation~~solubilization
A1	+	+
A2	+	+
A3	-	-

+ indicates Production &~~Solubilisation~~Solubilization

- Indicates absence

Table 4. Biochemical Characterization of ~~dominant PGPR~~dominant PGPR Isolates

Isolate no	Gram Test	IAA	Methyl Red	VP test	Ammonia Production
A1	-	+	+	+	+
A2	-	+	+	+	+
A3	-	-	-	-	-

+ indicates Production

-Indicates absence and Gram Negative

Table .5. Quantification and characterization of AM Fungal spores as spore density in Rhizosphere & Non-Rhizosphere soils of red sanders

Comment [Revisor3]: The characteristics of the mycorrhizae and photographs of them under microscopy are not shown.

Sample	No of Spores	Rhizosphere soil	Non-Rhizosphere soil	~~Charecters~~Characters of spores
Red sanders	AM fungi	450 / 50 g of soil	245 / 50 g of soil	Sore found single, Yellow in ~~colour~~color, Globose

The study result found AMF spores in the rhizosphere soil of sandal& Red sanders The AMF spore density ranged from 39.5-89.5 (spores/50 g soil), with an average of 525 spores/50 g soil & 450 spores/50 g soil in the Rhizosphere region. The spores were spherical to Oval in shape & Brown in colour

Table ~~.~~6. Growth of ~~Red~~red sanders on inoculation with dominant endophyte and AM Fungi

Treatment	Shoot length(cm)	Root Length(cm)	No of Leaves / Plant
T1	15.9	8.7	5
T2	17.4	9.6	6
T3	18.3	10.1	6
T4	20.1	11.1	8
Control	15.1	8.3	5
Mean	17.2	9.56	6
SED	0.3841	0.1609	0.8563
CD(P=05)	0.8872	0.3717	1.9782

T1= 20 g of dominant PGPR isolate

T2 =20 g of dominant PGPR isolate + 20 g Phosphobacteria

T3 = 20 g of dominant PGPR isolate + 20 g AM

T4=20 g of dominant PGPR isolate + 20 g Phosphobacteria+ 20 g AM

Screening of bacteria for plant growth promotion activity through bioassay Data regarding shoot/root length and number of leaves are shown in Table 6-. The inoculation of bacterial isolates in showed positive response on growth. Maximum shoot length (20.1 cm) was observed in treatment 4 and showed significant difference statistically from control. The root length, and number of leaves / plant was also showed higher response upon inoculation as compared to control. The improvement in plant physical parameters may be the result of production of different plant growth regulators and siderophores

4. CONCLUSION

~~The prosperity or setback of a plantation can be anticipated based on the quality of the maintained nursery. Consequently, to establish a thriving plantation, it is imperative to cultivate high-quality elite seedlings.~~

~~The production of robust and superior planting stock holds immense significance in meeting the plantation targets set by state forest departments, wood-based industries, and tree growers.~~ In the current study, an experiment was undertaken to assess the effectiveness of various bio-inoculants, namely Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal (AM) fungi, both individually and in combinations, on enhancing the growth of economically important fast-growing tree species.

The data clearly indicate that all the bio-inoculants significantly improved seedling growth in the nursery. The dual application of bio-inoculants demonstrated superior results compared to single inoculations, suggesting a synergistic effect. Therefore, the inoculation of nursery seedlings with carefully selected bio-inoculants shows promise in enhancing seedling quality, paving the way for improved plantation success.

REFERENCES

Arade S, Khannam A & Rathore ~~TS..~~TS. Influence of ~~biofertilisers~~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* 2020, 10: 10–14

Asif M, Mughal AH, Bisma R, Mehdi Z, Saima S, Ajaz M. & Sidique S.. Application of different strains of biofertilisers for raising quality forest nursery. *International Journal of Current Microbiology and Applied Sciences* 2018 ,7: 3680–3686

Bhattacharyya, P. N. and Jha, D. ~~K.,.~~K., Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 2012, 28(4), pp.1327-1350

Furtak K, Gajda AM..Activity and variety of soil microorganisms depending on the diversity of the soil tillage system. *Sustainability of agroecosystems*.; 2018, 45.

Kalaigandhi V, Kannapiran E, Harimuraleedharan AM, Sivakumar T, Arasu VT. Azotobacter population in rhizosphere and non-rhizosphere sediments of tondi coast. ~~2010 .,*Int*~~2010, Int *J Biol Technol*.;1:63-65.

Kumar, V., Sharma, ~~N.~~N., and Kansal, ~~S.,~~S., Characterization of Potential PGPR's Isolated from Rhizosphere of Wheat from Trans-Himalayas and their Efficacy on seed germination and growth promotion of wheat under net house conditions. 2019, *Journal of Plant Development Sciences* Vol, 11(3), pp.121-131.

Modi, K., Patel, ~~P.~~P., and Parmar, ~~K.,~~K., Isolation, ~~screening~~screening, and characterization of PGPR from rhizosphere of rice. 2017, *Int J Pure Appl Biosci*, 5, pp.264-270.

Muthu Kumar A & Sandhya ~~GM.~~GM. Synergistic potential of microorganisms (bio-fertiliser) on growth performance of Melia dubia, *Ecology, Environment and Conservation*. 2022. ~~*28 :*~~*28*: S352–S359.

Pavan ~~KP.~~KP. Influence of bio-inoculants on the growth of Albizia lebbeck in nursery condition. *Research Journal of Agricultural Sciences*2011, 2: 265–268.

Pikovskaya, R. ~~I.,~~I., Mobilization of phosphorus in soil in connection with vital activity of some microbial species. ~~1948,*Mikrobiologiya*~~1948,*Mikrobiologiya*, 17, pp.362- 370.

Saharan, B. ~~S.~~S., and Nehra, ~~V.,~~V., Plant growth promoting rhizobacteria: a critical review. ~~2011,*Life*~~2011, *Life Sci Med Res*, 21(1), p.30.

Thomson, B.D., Hardy, G.E.S., Malajczuk, N. *et al.* . The survival and development of inoculant ectomycorrhizal fungi on roots of outplanted *Eucalyptus globulus* Labill. ~~1996,*Plant*~~1996, *Plant Soil* 178, 247–253

Verma, M., Mishra, J. and Arora, N. ~~K.,~~K., Plant growth-promoting rhizobacteria: diversity and applications. 2019, *In Environmental biotechnology: for sustainable future* Springer, Singapore. (pp. 129-173).