



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
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NAAS Rating (2026): 5.20
www.agronomyjournals.com
2026; SP-9(6): 276-286
Received: 20-03-2026
Accepted: 24-04-2026

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Exploration of culturable microbes for their functionalities in rhizospheric soil of Cotton-Sesame cropping system

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DOI: <https://www.doi.org/10.33545/2618060X.2026.v9.i6Sd.5821>

Abstract

Cotton (*Gossypium spp.*) is a major commercial crop with significant economic importance, but its productivity is often constrained by declining soil fertility and excessive dependence on chemical inputs. In this context, the present study aimed to isolate, characterize, and evaluate rhizospheric microorganisms for their plant growth-promoting (PGP) potential in a cotton-sesame cropping system.

The present study aimed to isolate, characterize, and evaluate rhizospheric microorganisms for their plant growth-promoting (PGP) potential in a cotton-sesame cropping system. A total of 47 microbial isolates, including bacteria, actinomycetes, and fungi, were obtained from rhizospheric soils of RARS, Jagtial. Based on pathogenicity screening, fungal isolates were excluded, and 39 bacterial and actinomycete isolates were subjected to detailed morphological and functional characterization. These isolates exhibited diverse PGP traits, including phosphate, potassium, and zinc solubilization, along with the production of indole-3-acetic acid (IAA), siderophores, ammonia, and hydrogen cyanide (HCN).

Among these, 18 isolates exhibited phosphate solubilization (6.5-20.8 mm), 9 isolates showed potassium solubilization (8-16.8 mm), and 23 isolates demonstrated zinc solubilization (4-25.5 mm). Additionally, 34 isolates produced indole-3-acetic acid (IAA) in the range of 0.0628 to 1.0999 mg ml⁻¹. Further screening revealed that 19 isolates produced siderophores, 25 isolates produced ammonia, and 15 isolates showed positive hydrogen cyanide (HCN) production. Significant variations ($p < 0.05$) in the isolates solubilization and PGPR capacities were validated by statistical analysis. Five promising isolates were shortlisted and further evaluated for compatibility using the cross-streak method, which identified three compatible isolates (CSRB 16, CSRB 21, and CSRA

34) for consortium development. Molecular identification through 16S rRNA sequencing revealed their affiliation to *Sinorhizobium meliloti*, *Halobacillus dabanensis*, and *Streptomyces rameus*.

The study demonstrates that the combined application of microbial consortia with FYM and reduced chemical fertilizers significantly enhances crop growth, nutrient availability, and soil biological activity. This integrated approach offers a sustainable and eco-friendly strategy for improving productivity and soil health in cotton-sesame cropping systems.

Keywords: Cotton, sesame, microbial consortium, pgpr, actinomycetes, soil fertility, integrated nutrient management

1. Introduction

Cotton (*Gossypium spp.*) plays a pivotal role in India's agrarian landscape, providing livelihood to nearly 6.5 million farmers and serving as a crucial raw material for the textile sector. Beyond fibre, cotton seed is an important source of edible oil, livestock feed, and nutritional products. Nevertheless, cotton production in India is projected to decline by 7.98

% during the 2024-25 season, reaching a seven-year low of 299.26 lakh bales (170 kg each), as reported by the Cotton Association of India (2024). This decline has been largely attributed to reduced sowing area, erratic climatic conditions, and severe infestation of pink bollworm. Concurrently, the national average yield has dropped to 429 kg ha⁻¹, representing nearly a 24% reduction from 2013-14 levels. These trends highlight the urgent need for sustainable strategies to stabilize cotton productivity.

Culturable rhizospheric microorganisms are known to enhance nutrient uptake, synthesize phytohormones, and protect plants against soil-borne diseases.

Therefore, isolation and functional characterization of such microbes can facilitate the development of eco-friendly bioinoculants and reduce dependence on chemical fertilizers and pesticides. The composition and activity of rhizosphere microbial communities are governed by multiple biotic and abiotic factors and play a key role in plant growth and stress tolerance through modulation of nutrient acquisition (Berendsen *et al.*, 2012)^[3].

Despite their importance, research on the rhizosphere microbiota of cotton remains relatively limited. Only a few studies have explored the diversity and structure of microbial communities associated with cotton roots. For instance, Zhang *et al.* (2017)^[26] examined actinobacterial communities in the rhizosphere of transgenic and non-transgenic cotton using molecular approaches and reported no significant differences between the genotypes. Such findings underscore the need for broader investigations focusing on functional microbial groups and their ecological roles under different cropping systems and environmental conditions.

In contrast, sesame (*Sesamum indicum* L.) is a traditionally rainfed oilseed crop valued for its high drought tolerance and ability to thrive in nutrient-poor soils. Sesame is widely cultivated in marginal environments and contributes significantly to climate-resilient agriculture. According to the Indian Oilseeds and Produce Export Promotion Council (IOPEPC, 2023), sesame production in India during 2023-24 was estimated at 1.46 lakh tonnes with an average productivity of 238 kg ha⁻¹. These characteristics make sesame highly suitable for inclusion in diversified cropping systems, particularly in regions prone to rainfall variability and low soil fertility.

Integration of sesame with cotton through intercropping or rotational sequences offers multiple agro-ecological advantages. The deep and fibrous root system of sesame improves soil porosity, enhances aggregate stability, and promotes better aeration and water infiltration. Such improvements in soil physical properties create favourable microhabitats for beneficial microorganisms and strengthen root microbe interactions in the succeeding cotton crop. Moreover, the temporal and spatial complementarity between cotton and sesame enhances resource-use efficiency, nutrient recycling, thereby contributing to long-term soil fertility and system productivity.

Rhizosphere microbial richness is often shaped by selective pressures imposed by plant roots. Root exudates favour beneficial microorganisms while suppressing less competitive taxa, resulting in dynamic shifts between rhizosphere and bulk soil communities. Long-term monocropping of cotton may further narrow microbial diversity, whereas diversified systems can promote adaptive and functionally efficient microbial populations. Root-mediated selection and enrichment processes have been well documented in agricultural soils (Qiao *et al.*, 2017). These complex microbial dynamics highlight the importance of identifying stable and beneficial microbial consortia capable of performing under varying soil and climatic conditions.

Despite these advances, cotton cultivation in semi-arid regions of India continues to depend heavily on external inputs, resulting in soil nutrient mining and declining biological health. Continuous cotton monocropping under intensive fertilization reduces microbial functional diversity and weakens soil structural stability (Larkin, 2015)^[14]. These problems are particularly acute in light-textured rainfed soils with rapid organic matter turnover (Lal, 2015)^[13]. Cropping system

diversification through cotton-sesame integration has emerged as a promising ecological strategy to address these challenges (Venter *et al.*, 2016)^[25]. However, location-specific information on culturable, multifunctional microbes adapted to the cotton-sesame rhizosphere remains scarce, especially under Indian agro-ecological conditions.

In view of these gaps, the present investigation was undertaken to explore culturable rhizospheric microbes associated with the cotton-sesame cropping system, with emphasis on their plant growth-promoting functional traits and their potential to enhance soil fertility and cotton productivity.

2. Materials and Methods

2.1 Soil Sample Collection

Soil samples were collected from Cotton-Sesame cropping system, an experimental field at RARS, Jagtial, Telangana. The samples were collected near the rhizosphere region at 30 cm depth (Cotton) and 20 cm depth (Sesame) kept in sterilized polyethylene covers and brought to the laboratory.

2.2 Isolation of different microbes from rhizosphere by using different media

For the isolation of bacteria, fungi and actinomycetes, 1 gm of each soil sample was diluted to 10-fold in 9 ml of sterile normal saline (0.85%). Dilutions of 10⁻⁶, 10⁻⁷, 10⁻⁸ were used for isolation of bacteria while dilutions of 10⁻⁴ and 10⁻⁵ for isolation of fungi and 10⁻³, 10⁻⁴, 10⁻⁵ for isolation of actinomycetes were used. 0.1ml of respective dilutions were spread on to Nutrient Agar medium (NA), MacConkey Agar (MAC), Mannitol-Yolk-Polymyxin Agar (MYP), Potato Dextrose Agar medium (PDA) or Martin 's Rose Bengal Agar medium (RBA) and KenKnight's medium (KM) for isolation of bacteria, fungi and actinomycetes present in rhizosphere soil. These media plates were incubated at 28 ± 2 °C for 24 - 48 hours, 25 ± 2 °C for 3 - 4 days, 28 ± 2 °C for 7-10 days for isolation of bacteria, fungus and actinomycetes respectively.

2.3 Purification and maintenance of different microbial isolates

2.3.1 Streak Plate Method

A total of 47 varying isolates were obtained of which 39 were purified after morphological studies. Purification of different bacterial isolates was done by quadrant streak plate method by using suitable medium and growth conditions. A portion of the well-isolated colonies were taken and streaked on a suitable medium under aseptic conditions and incubated at 30 °C for 5 to 7 days and then store at 4 °C for further use.

2.3.2 Maintenance of the Isolates

The purified isolates were preserved for further analysis in nutrient agar and ken Knights's agar slants. The 20% glycerol stocks were also prepared for the preservation of cultures.

2.4 Morphological characterization of culturable microbial isolates

2.4.1 Characterization of the isolates

The isolates were identified based on morphological, cultural, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt *et al.*, 1984)^[10].

2.4.2 Colony Morphology

Morphological characteristics of the colony of each isolate was examined by growth of the isolates on their respective medium

and incubated them for 5 to 7 days. Cultural characterization of isolates were observed by different characteristics of colonies such as shape, size, colour, appearance of the colony, surface, pigmentation etc., were recorded.

2.4.3 Cultural and Morphological Characterization

All the isolates were studied for colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

2.4.4 Gram's Staining

A drop of sterile distilled water was placed in the centre of a glass slide. A loopful of freshly grown culture was taken and mixed with water on the slide, spread into a thin smear by using a sterile inoculation loop. The smear was air-dried and heat-fixed near the flame. Two drops of crystal violet solution was added to the smear and kept aside for 30 sec, after that washed gently with wash bottle. Then the slide was flooded with gram's Iodine solution for 30 sec and drained out with water followed by flooding with a 95 per cent decolourizer for 10 to 15 sec. After that, washed with water. The smear was loaded with safranin solution for 30 sec. The slide was washed gently with wash bottle and dried with the blotting paper. Then the slide was examined under a microscope at 100 X power with oil immersion and the data was recorded.

2.4.5 Enumeration of microbes in soil samples

Enumeration of bacteria, fungi and actinomycetes was done on Nutrient agar medium, Rose Bengal agar medium and Actinomycetes agar medium respectively, by using serial dilution spread plate technique. The media were prepared and sterilized in an autoclave at 15 psi pressure and 121° C temperature for 20 minutes.

Serial dilutions (up to 10%) were made by pipetting 1 ml of the soil suspension into 9 ml of sterile water blank. Finally, 0.1 ml aliquot of the diluted soil suspension was uniformly spread with the help of sterilized spreader on solidified petri plates having respective medium. The petri plates were incubated for 1 to 3 days at $28 \pm 2^\circ \text{C}$ in an inverted position. After incubation, the number of colonies appeared on medium plates were counted, averaged and multiplied by the dilution factor to find the number of microbial colonies per gram of soil sample.

$$\text{Total microbial population (CFU/gm)} = \frac{\text{Total number of colonies}}{\text{Dilution factor} \times \text{Volume of the sample}}$$

2.4.6 Pathogenicity testing of fungal isolates

Fungal isolates obtained from the rhizosphere of cotton and sesame crops were evaluated for their pathogenic potential using blood agar medium, which is widely employed to assess hemolytic activity as an indicator of virulence. Hemolysis on blood agar reflects the ability of microorganisms to produce extracellular enzymes and toxins that can lyse red blood cells, thereby serving as a preliminary marker for pathogenicity (Forbes *et al.*, 2016).

The inoculated plates were incubated at $28 \pm 2^\circ \text{C}$ for 48-72 hours under aerobic conditions. Plates were observed periodically for growth and hemolytic activity. After incubation, plates were examined for the presence and type of hemolysis around fungal colonies. Complete lysis of red blood cells, resulting in a clear zone around the colony indicates β -hemolysis (beta hemolysis), which is considered a strong indicator of potential pathogenicity due to the production of extracellular

lytic enzymes and toxins by the isolate, thereby supporting its possible role as a non-beneficial or pathogenic microorganism in the present study.

2.5 *Invitro* Screening of the isolates for different functionalities

The pure isolates were screened for plant growth-promoting activities.

2.5.1 Phosphate Solubilization

Isolates were tested for phosphate solubilization by spot inoculation of pure isolates on Pikovskaya's agar media plates aseptically. The plates were incubated for 6 - 8 days at 30°C and the zone of phosphate solubilization (mm) formed around the grown colonies was recorded (Pikovskaya, 1948)^[17].

2.5.2 Potassium Solubilization

Potassium solubilisation was assayed by using Aleksandrov's agar medium. The isolates were spot inoculated on media plates under aseptic conditions and incubated at 30°C for 7- 8 days. The clear zone diameter around the colonies were measured (Prajapati and Modi, 2012)^[17].

2.5.3 Zinc Solubilization

Tris minimal agar containing 0.1% of insoluble zinc compounds such as Zinc Oxide (ZnO) was used for Zinc solubilisation studies. The isolates were spot inoculated on media plates and incubated at 30°C for 3-4 days. The diameter around the colonies were measured (Saravanan *et al.*, 2003)^[21].

2.5.4 Hydrogen cyanide Production (HCN)

The HCN production was tested by the method of Bakker and Schippers (1987)^[1] with few modifications. Medium plates i.e., modified nutrient agar media were prepared by adding 4.4 g of glycine per litre separately. Loop full of culture of each isolate was streaked on respective media plates.

Then a disc of Whatman's no.1 filter paper of the diameter equal to the Petri plate, impregnated with an alkaline picric acid solution (0.2 per cent picric acid (w/v) in 1 per cent sodium carbonate) was placed in the upper surface of the inoculated Petri plates under aseptic condition and wrapped it with a thin film to prevent HCN gas escape for accurate results. However, the control plate remains uninoculated.

The plates were incubated upside down at 30°C for 6 to 7 days. The plates were observed for the discolouration of filter paper from yellow to orange - light brown, moderate or strong reddish brown indicates microbial production of cyanide and was taken as positive for HCN production.

2.5.5 Siderophores Production

Production of siderophores were analysed by the method given by Schwyn and Neilands, (1987) with few modifications. Aqueous Ferric chloride solution was used for qualitative estimation of siderophore production by the isolates. 0.06 g of Chrome Azurol Sulfonate (CAS) was dissolved in 5 ml of double-distilled water (solution I) and mixed with 9ml of solution II containing 0.0027 g of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 10 ml of 10 mM HCl. This was added to

0.073 g of hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 ml of double-distilled water. The dark blue colour CAS reagent was autoclaved for 15 min. and the king's B media was prepared separately then added CAS reagent slowly to the media, which gives blue colour to the media. The isolates were spot inoculated on the media under aseptic conditions and incubated for 3 to 4 days. Observed the orange colour zones

around the colonies on the plates which were considered positive for siderophore production. Then, the diameter of colour zone formation around the colonies were measured.

2.5.6 Ammonia Production

Ammonia production was done by using peptone water. Peptone water was prepared in test tubes with 10 ml volume and sterilized by using an autoclave. Inoculate the culture in each tube and kept for incubation for 2-3 days. After the incubation period centrifuge the culture at 10,000 rpm for 15 min at 4 °C. Supernatant was collected and added the Nessler's reagent and observed for colour change. Moreover, the development of yellow to brown or reddish brown colour in the supernatant was taken as positive for ammonia production and no colour change (remains pale yellow) is considered as negative for ammonia production (Cappucino and Sherman, 1992)^[5].

2.5.7 Indole Acetic Acid (IAA)

IAA production was estimated by the spectrophotometric method given by Glickmann and Dessaux (1995)^[8]. The nutrient broth was prepared and autoclaved, which was supplemented with 5 mM of tryptophan. Cultures were inoculated in nutrient broth and incubated in the tubes for 4 to 6 days.

After the incubation period, centrifuge the tubes at 10,000 rpm for 20 min, at 4 °C. Take 1 ml of supernatant from each tube and two drops of orthophosphoric acid, followed by 4 ml of Salkowski reagent. Later, incubated the tubes for 25 mins, at room temperature. Afterwards readings were taken spectrophotometrically at 530 nm and by comparison with the standard curve, IAA concentration was determined from the concentration of bacterial samples, the control tubes concentration was subtracted.

A standard stock solution of IAA was prepared by taking 100 µg/ml of IAA in 50% ethanol. A standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l of standard IAA solution in test tubes. To this 2 ml of distilled water was added followed by 4 ml of Salkowski reagent. These tubes were incubated for 25 minutes at room temperature, after that optical density was measured at 530 nm and the standard graph was prepared.

2.6 Molecular Characterization of Selected Isolates

The 16S rRNA sequences were aligned using BioEdit and contigs were created using Cap3 Software. The sequences obtained for different bacterial isolates were searched against the sequences of 16S rRNA of bacterial isolates available in the Gene bank Nucleotide Database (<http://www.ncbi.nih.gov/blast>) and based on the maximum identity of the sequences, the identity of the isolated bacteria was determined.

The 16S rRNA sequences (Bioserve Biotechnologies, Hyderabad) were processed according to the bacterial identification protocol (BBIPL/RS/SOP-013). Genomic DNA was isolated using the in-house Bacterial DNA isolation kit, and the purity and concentration were verified using a Denovix DS-11 spectrophotometer. The 16S rDNA region was amplified with Emerald Amp GT PCR Master Mix, and the resulting amplicons were purified and sequenced bi-directionally using the ABI 3730 Genetic Analyzer. The forward and reverse reads were assembled into consensus sequences using Gene Tool software, and BLAST analysis was performed against the NCBI GenBank nucleotide database.

2.7 Data Analysis

The data generated from the laboratory experiments and pot culture experiments were subjected to statistical analysis by Completely Randomized Design (CRD). In order to assess the significant variations between the treatments, CRD was performed using a P- value of 0.05% as the critical threshold for determining statically significance by Gomez and Gomez (1984)^[9].

3. Results and Discussion

3.1 Morphological and cultural characterization of rhizosphere culturable microbial isolates

Isolations were carried out by using nutrient agar, potato dextrose agar, MacConkey agar (MAC agar) Mannitol Egg Yolk Polymyxin Agar (MYP agar) and kenknights agar medium.

Depending on the colony morphology and gram staining the individual isolates were selected. The isolated colonies were purified by quadrant streak plate method. Moreover, pure cultures were preserved in 20% glycerol stocks to study their biochemical characters. The isolates were named according to the crop or soil region from which it has collected such as CSRB (Cotton- Sesame rhizospheric bacteria), CSRA (Cotton-Sesame Rhizosphere Actinomycetes enlisted in the table. A total of 47 strains were isolated which comprises 32 CSRB isolates, 7 CSRA isolates and 8 CSRF isolates.

3.2 Enumeration of microbial population in the Soil

The highest population of microbial colonies was observed in the sample R3T3 (17 x 10³ CFU/ml) and R1T5 (17.6 x 10⁶ CFU/ml) followed by R4T4 (13.2 x 10³ CFU/ml), R4T4 (13 x 10⁴ CFU/ml) is on par with R2T4 (13 x 10⁴ CFU/ml) followed by R1T3 (10.3 x 10⁶ CFU/ml) when compared with the other samples. The least number of colonies were observed in R2T5 (1.6 x 10⁶ CFU/ml), R1T5 (1.0 x 10⁸ CFU/ml).

3.2.1 Pathogenicity Screening of Fungal Isolates

The pathogenicity screening of fungal isolates using blood agar revealed hemolytic activity in all tested isolates, indicating the production of extracellular cytolytic factors. Hemolysis is a well-recognized indicator of pathogenic potential and has been previously reported in several soil-borne and rhizospheric fungi (Kumar *et al.*, 2013; Larone, 2018)^[12, 15]. The occurrence of hemolytic activity suggests that these fungal isolates may pose risks if applied as bioinoculants in agricultural systems.

The elimination of fungal isolates based on pathogenicity screening ensured biosafety and enhanced the credibility of the study. By restricting further analyses to non-pathogenic bacteria and actinomycetes, the present investigation confirms to internationally accepted standards for microbial biofertilizer development and sustainable agricultural applications. Similar approaches have been adopted by earlier researchers, who excluded pathogenic fungal isolates prior to functional characterization to prevent adverse effects on plants and non-target organisms (Berg *et al.*, 2005; Vassilev *et al.*, 2015)^[4, 24]. Based on the pathogenicity test results, fungal isolates were excluded, and cultural characterization was performed only for bacterial and actinomycetes isolates.

Table 1: Morphological and Cultural Characterization of the Rhizosphere Isolates

Isolate name	Gram reaction	Pigmentation	Shape of the cell	Colony EDG	Elevation	Surface of the colony
CSRB 1	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 2	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 3	Positive	White	Rods	Regular	Convex	Smooth, dry, Penetrating colonies
CSRB 4	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 5	Positive	Orange	Cocci	Regular	Convex	Smooth, superficial colonies
CSRB 6	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 7	Positive	Orange	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 8	Positive	White	Cocci	Regular	Convex	Smooth, superficial colonies
CSRB 9	Positive	White	Rods	Regular	Convex	Rough, penetrating colonies
CSRB 10	Negative	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 11	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 12	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 13	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 14	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 15	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 16	Negative	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 17	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 18	Positive	Creamy white	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 19	Negative	Creamy white	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 20	Positive	Creamy white	Rods	Regular	Convex	Smooth, penetrating colonies
CSRB 21	Positive	Orange	Rods	Regular	Convex	Smooth, penetrating colonies
CSRB 22	Positive	Creamy white	Short rods	Regular	Convex	Rough, penetrating colonies
CSRB 23	Positive	Creamy white	Rods	Regular	Convex	Rough, penetrating colonies
CSRB 24	Negative	Creamy white	Rods	Regular	Convex	Rough, penetrating colonies
CSRB 25	Positive	White	Rods	Regular	Convex	Smooth, superficial colonies
CSRB 26	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 27	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 28	Positive	White	Short rods	Regular	Convex	Rough, penetrating colonies
CSRB 29	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 30	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 31	Positive	White	Short rods	Regular	Convex	Smooth, superficial colonies
CSRB 32	Positive	Pink	Rods	Regular	Convex	Smooth, superficial colonies
CSRA 33	Positive	Creamy white	Rods	Irregular	Convex	Smooth, superficial colonies
CSRA 34	Positive	Dark brown	Rods	Irregular	Convex	Smooth, dry and superficial colonies
CSRA 35	Positive	Whitish brown	Rods	Irregular	Convex	Rough, dry and superficial colonies
CSRA 36	Positive	Whitish brown	Short rods	Irregular	Convex	Smooth, dry and penetrating colonies
CSRA 37	Positive	Creamy white	Rods	Irregular	Convex	Rough, dry and penetrating colonies
CSRA 38	Positive	Light green	Short rods	Irregular	Convex	Rough dry and superficial colonies
CSRA 39	Positive	Greenish white	Rods	Irregular	Convex	Rough dry and penetrating colonies

3.3 In-vitro screening of the isolates for their functionalities

3.3.1 Phosphate Solubilization

Among all the 39 isolates, a total of 18 isolates has shown the clear zones of solubilization around the colonies ranging from 6.5 mm to 20.8 mm are mentioned in the table 2, the highest solubilization has shown by CSRA 35 (20.8 mm), followed by CSRB 21 (19 mm), CSRA 18 (15.5 mm), CSRB 32 (15 mm), CSRA 34 (14 mm), CSRB 26 (13.8 mm), CSRB 25 (13 mm), CSRB 28 (12 mm), CSRB 16 (10 mm) which is on par with CSRA 38 CSRB (10 mm), CSRA 39 (10 mm) followed by CSRB (9 mm), CSRB (10 mm) followed by CSRB 7 (9 mm), CSRB17 (8.6 mm), CSRB 9 (8 mm), CSRB 8 (7 mm), CSRB 27 (6.8 mm), CSRB 10 (6 mm) and the least has shown by CSRB 15 (5 mm). In the present study, the formation of clear halo zones surrounding microbial colonies due to the secretion of organic acids such as citric, gluconic, lactic, malic, oxalic, propionic, and succinic acids, which lower the pH of the medium and release bound phosphate by chelating metal ions.

3.3.2 Potassium Solubilization

Among the 39 isolates screened for potassium solubilization, a total of 09 isolates has shown the clear zone of solubilization ranging from 8mm to 16.8 mm are shown in the table 4.5. the

isolates which showed highest zone is CSRA 35 (16.8 mm), followed by CSRB (16 mm), CSRB 16 (15 mm), CSRA 37 and CSRA 39 shown similar zones of solubilization (14 mm) followed by CSRB 21 and CSRA 34 shown similar zones (10 mm), CSRB 12 (9.8 mm) and the least was shown by CSRB 18 (8 mm). The clear solubilization zones around the microbial colonies is mainly attributed to the production of organic acids such as citric, oxalic, tartaric, gluconic, and α -ketogluconic acids, which acidify the surrounding medium and facilitate the release of potassium ions from insoluble silicate minerals through acidolysis, chelation, and exchange reactions.

3.3.3 Zinc Solubilization

A total of 23 isolates has shown clear zone of solubilization around the colonies which ranges from 4 mm to 25.5 mm. The isolates with highest clear zone of solubilization was reported in CSRB 28 (25.5mm), followed by CSRB 25 (23 mm). Moreover, the zone of solubilization for the isolates CSRB 22 (19 mm), CSRB 16 which is on par with CSRB 21 (18 mm) followed by CSRB 29 (17 mm), CSRB 27 (16 mm), and CSRB 23, CSRB 24, CSRB 26 ranges between 13 mm to 15.5 mm, which is followed by CSRB 8, CSRB 10, CSRB 12, CSRB 19, CSRB 18, CSRB 32, CSRA 34 which ranges between 9 mm to 12 mm, also followed by CSRB 4, CSRB 9, CSRB 14,

CSRB15, CSRB 30 ranges between 6 mm to 8 mm whereas the least was shown by CSRB 5 (5 mm).

In the present study, the development of clear solubilization halos around microbial colonies on Tris-minimal agar is due to the production of organic acids, which acidify the medium and chelate zinc cations from insoluble compounds like zinc oxide, zinc carbonate, and zinc phosphate, thereby converting them into soluble forms available for plant uptake.

3.3.4 HCN Production

All the 39 isolates were spread on the nutrient agar plates which was supplemented with glycine separately, after the incubation period the change in colour from yellow to brown colour was considered positive for HCN production are presented in the table 2. Out of all the isolates only 15 isolates CSRB 1, CSRB 6, CSRB 11, CSRB 12, CSRB 16, CSRB 18, CSRB 19, CSRB 21, CSRB 23, CSRB 24, CSRB 25, CSRB 26, CSRB 28, CSRB 32 and CSRA 34 has shown light brown to reddish brown colour.

In the present investigation, the development of a brownish coloration on picric acid-soaked filter paper strips placed inside culture plates indicated the production of hydrogen cyanide (HCN) by the isolates. HCN is a volatile secondary metabolite commonly produced by certain rhizobacteria, particularly *Pseudomonas* and *Bacillus* spp., through the oxidative decarboxylation of glycine gene.

3.3.5 Siderophore Production

A total of 39 isolates were screened for siderophore production among which 19 were shown positive which are mentioned in the table 2, which were developed orange yellow colour halo zones around the colonies among which 5 isolates with high (+++) CSRB 2, CSRB 3, CSRB 15, CSRB 16, CSRB 21, followed by 3 isolates with moderate (++) halo zone which includes CSRB 32, CSRA 34, CSRA 39 and the 11 isolates were with low (+) orange to yellow colour zone around the colonies includes CSRB 1, CSRB 4, CSRB 5, CSRB 6, CSRB 7, CSRB 8, CSRB 9, CSRB 11, CSRB 12, CSRB 14, CSRB 17 (Fig.4.10). The appearance of the colored zone is due to the removal of Fe^{3+} ions from the dye-iron complex by the microbial siderophores, resulting in a colour change from blue to orange (Schwyn and Neilands, 1987). Siderophores are low molecular weight, high-affinity iron-chelating compounds secreted by

microorganisms under iron-limited conditions to solubilize and transport Fe^{3+} from the surrounding environment.

3.3.6 Ammonia Production

All the 39 isolates were screened for ammonia production where only 25 isolates were shown brown colour after the addition of Nessler's reagent where the 11 isolates were shown high (+++) brown to yellow colour which includes CSRB 1, CSRB 9, CSRB 10, CSRB 16, CSRB 19, CSRB 22, CSRB 24, CSRA 34, CSRA 35, CSRB 36, CSRA 39 followed by 09 isolates that is CSRB 3, CSRB 8, CSRB 15, CSRB 17, CSRB 20, CSRB 21, CSRB 26, CSRB 29, CSRB 32 with moderate (++) production of ammonia and the 05 isolates has shown low (+) colour change which includes CSRB 2, CSRB 13, CSRB 18, CSRA 33, CSRA 37. The isolates has given high (+++), moderate (++) and low (+) which are presented in the table 2, shown the culture with colour change which were positive for ammonia production.

3.3.7 IAA Production

Among 39 isolates analysed for IAA production, a total of 34 isolates were found positive with IAA values ranging from 0.0628 to 1.0999 mg/ml, as presented in Table 2. Among the isolates, CSRB 10 recorded the highest IAA production (1.0999 mg/ml), followed by CSRB 7 (0.1754 mg/ml) and CSRB 6 (0.1652 mg/ml), which were on par with CSRB 22, CSRB 32, CSRB 28, CSRB 14, and CSRB 24, exhibited IAA values ranging between 0.1586 to 0.1439 mg/ml. These were followed by CSRB 16, CSRB 25, CSRB 27, CSRB 21, CSRB 11, and CSRB 8, has shown moderate IAA production ranging from 0.1363 to 0.1232 mg/ml. The isolates CSRB 26, CSRB 30, CSRB 29, CSRB 13, CSRB 4, CSRB 3, CSRB 15, CSRB 31, CSRA 33, CSRA 34, CSRB 5, CSRB 23, CSRB 9, and CSRA 35 recorded values between 0.1195 to 0.101 mg/ml. Further, isolates CSRB 18, CSRB 1, CSRB 20, CSRB 19, CSRB 12, CSRB 17, and CSRA 37 showed lower IAA production values ranging from 0.0926 to 0.0842 mg/ml. The least IAA producers were CSRA 38 (0.0917 mg/ml), CSRA 39 (0.0908 mg/ml), CSRB 2 (0.0833 mg/ml), and CSRA 36 (0.0628 mg/ml).

In the present investigation, isolates has shown visible pink coloration indicated positive IAA production, signifying their potential as phytohormone producers.

Table 2: *In-vitro* Screening of the Isolates for Plant Growth Promoting Activities

Isolate name	Phosphate Solubilization (mm)	Potassium Solubilization (mm)	Zinc Solubilization (mm)	HCN	Siderophore Production	Ammonia Production	IAA Production (mg/ml)
CSRB 1	-	-	-	++	+	+++	0.114
CSRB 2	-	-	-	-	+++	+	0.083
CSRB 3	-	-	-	-	+++	++	0.113
CSRB 4	-	-	8.0	-	+	-	-
CSRB 5	-	-	5.0	-	+	-	-
CSRB 6	-	-	-	+	+	-	0.165
CSRB 7	9.0	-	-	-	+	-	0.175
CSRB 8	7.0	-	12.0	-	+	++	0.123
CSRB 9	8.0	-	6.0	-	+	+++	0.108
CSRB 10	6.0	-	10.0	-	-	+++	0.158
CSRB 11	-	-	-	+++	+	-	0.132
CSRB 12	-	9.8	10.0	+	+	-	-
CSRB 13	-	-	-	-	-	+	-
CSRB 14	-	-	7.0	-	+	-	0.143
CSRB 15	5.0	16.0	8.0	-	+++	++	0.113

CSRB 16	10.0	15.0	18.0	++	+++	+++	0.136
CSRB 17	8.6	-	-	-	+	++	0.084
CSRB 18	15.5	8.0	12.0	++	-	+	0.121
CSRB 19	-	-	10.0	+	-	+++	0.092
CSRB 20	-	-	-	-	-	++	-
CSRB 21	19.0	10.0	18.0	++	+++	++	0.132
CSRB 22	18.0	-	19.0		-	+++	0.158
CSRB 23	-	-	13.0	++	-	-	0.101
CSRB 24	-	-	15.0	+++	-	+++	0.154
CSRB 25	13.0	-	23.0	+++	-	-	0.136
CSRB 26	13.8	-	15.5	+++	-	++	0.118
CSRB 27	6.8	-	16.0		-	-	-
CSRB 28	12.0	-	25.5	+	-	-	0.147
CSRB 29	-	-	17.0	-	-	++	0.127
CSRB 30	-	-	8.0	-	-	-	0.119
CSRB 31	-	-	-	-	-	-	-
CSRB 32	15.0	-	12.0	+	++	++	0.191
CSRA 33	-	-	-	-	-	+	0.116
CSRA 34	14.0	10.0	12.0	+	++	+++	0.112
CSRA 35	20.8	16.8	-	-	-	+++	0.110
CSRA 36	-	-	-	-	-	+++	-
CSRA 37	-	14.0	-	-	-	+	-
CSRA 38	10.0	14.0	-	-	++	-	0.091
CSRA 39	10.0	-	-	-	-	+++	0.090

*Values Mentioned in the Table are the Mean of three Replications

“+++”: High production “++”: Moderate production “+”: Low production “-”: Negative

3.6 Selection of Potential Strains for Consortia Preparation

Among the 39 rhizospheric isolates obtained from the Cotton - Sesame cropping system, 05 were initially shortlisted based on their biochemical characteristics and plant growth-promoting (PGP) activities, including phosphate, potassium, and zinc solubilization, as well as the production of indole-3-acetic acid (IAA), siderophores, ammonia, and hydrogen cyanide (HCN). These selected isolates were subsequently subjected to compatibility testing using the cross-streak method to assess their mutual interactions. The results revealed that certain combinations exhibited clear inhibition zones or restricted growth, indicating antagonistic interactions, whereas compatible isolates showed no inhibition zones and normal growth without interference. Based on these observations, three isolates, namely CSRB 16, CSRB 21, and CSRA 34, were found to be mutually compatible and were therefore selected for the development of a microbial consortium. The morphological and biochemical characteristics of these compatible isolates are presented in Table. 4.

Establishing compatibility among beneficial rhizobacteria and actinomycetes is essential for successful consortium performance, as it promotes synergistic interactions within the rhizosphere while preventing antagonistic effects. According to Santoyo *et al.* (2021) ^[20], a microbial consortium comprising *Pseudomonas fluorescens* ATCC 13525, *Bacillus subtilis* MTCC 441, and *Trichoderma harzianum* T-22 enhanced wheat shoot biomass by approximately 48% compared to individual inoculations (29% increase) under greenhouse conditions.

In cotton-based systems, similar beneficial effects of microbial consortia have been reported. Studies conducted by Triveni *et al.* (2018; 2020) ^[22, 23] on rhizospheric microbial consortia in cotton

demonstrated that the combined application of *Pseudomonas*, *Bacillus*, and actinomycete isolates significantly improved plant growth parameters, nutrient uptake, and soil enzyme activities compared to individual inoculations. Their findings indicated notable increases in plant height (20-30%), biomass accumulation (25-35%), and enhanced soil biological activity, highlighting the importance of selecting compatible multifunctional isolates for effective consortium development in cotton cropping systems.

3.7 Molecular Characterisation of the Potential Strains

Molecular identification of the selected isolates was carried out using 16S rRNA gene sequencing, a widely accepted method for bacterial taxonomy and phylogenetic analysis. The 16S rRNA is a structural component of the 30S small subunit of the prokaryotic ribosome and plays a crucial role in recognizing the Shine-Dalgarno sequence during translation initiation. This gene is preferred for microbial identification because of its conserved and variable regions, which evolve slowly enough to allow precise phylogenetic discrimination among bacterial taxa. In the present investigation, three isolates selected based on biochemical and compatibility assessments were subjected to amplification and sequencing of the 16S rRNA gene. The amplified fragment was approximately 1,500 - 1,550 base pairs in length, encompassing both conserved and hypervariable regions suitable for phylogenetic comparison. Primers complementary to the conserved ends of the gene were employed to obtain partial and near-complete sequences, while the variable regions were analyzed for taxonomic differentiation and evolutionary relationships among the isolates.

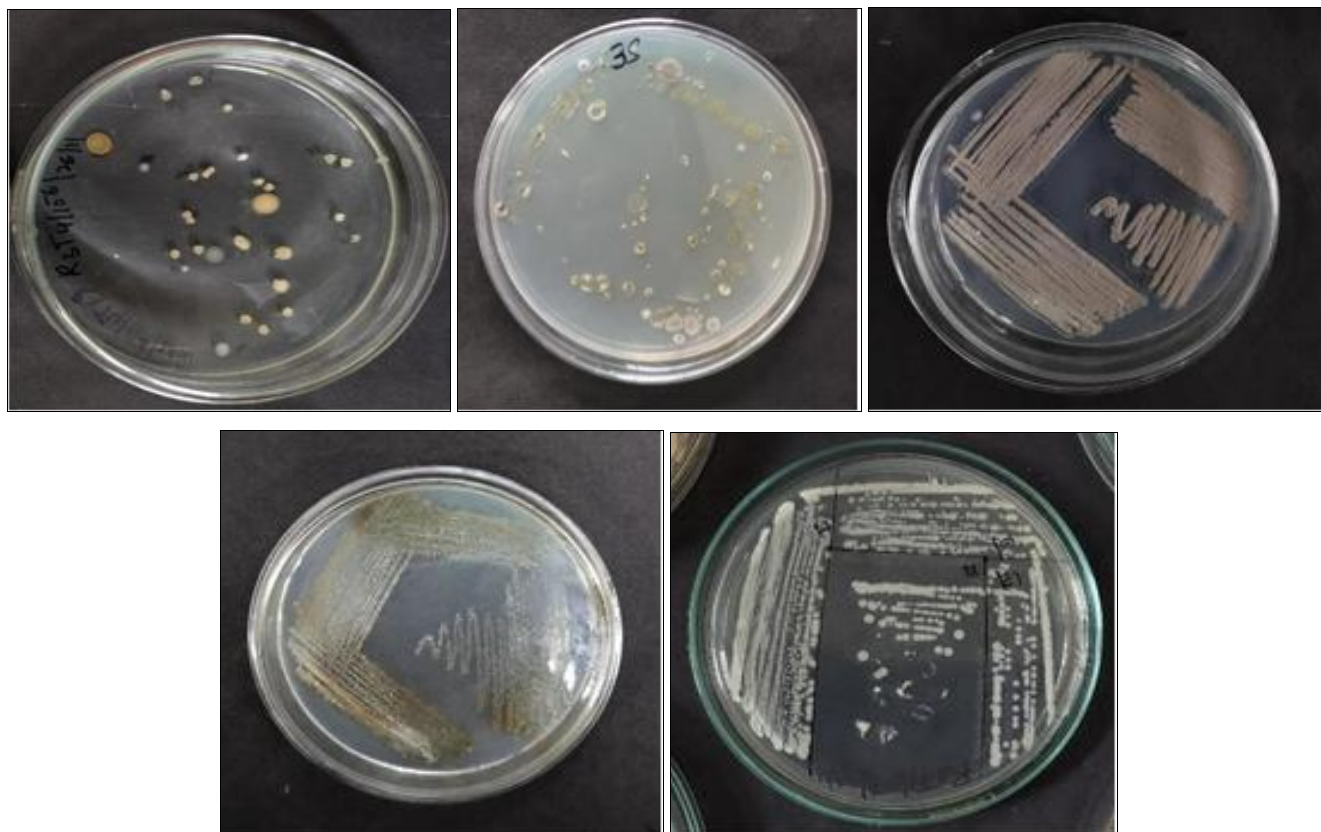


Fig 1: Microbial isolations and pure cultures of the isolates



Fig 2: Screening for Phosphate solubilization (Zone of hydrolysis)

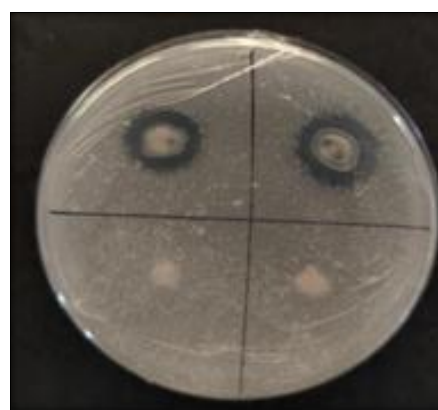


Fig 3: Screening for Potassium solubilization (Zone of hydrolysis)



Fig 4: Screening for Zinc solubilization (Zone of hydrolysis)



Fig 5: Screening for HCN production (orange to brown colour)

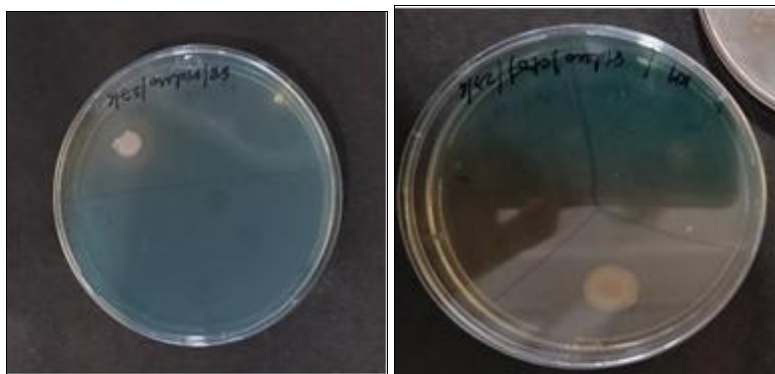


Fig 6: Screening for Siderophore production (Orange halo zones)

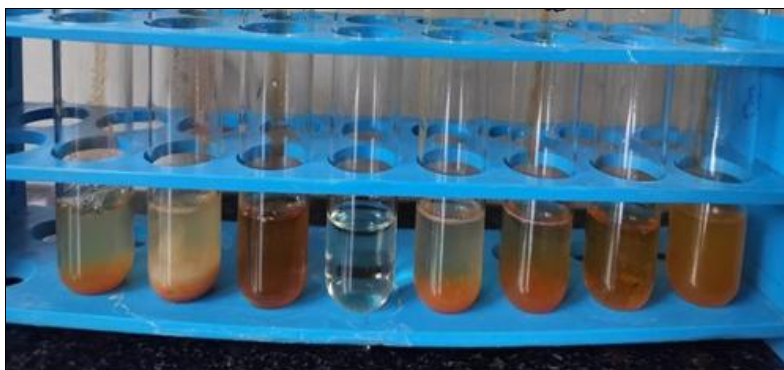


Fig 7: Screening for Ammonia production (Orange to brown colour)

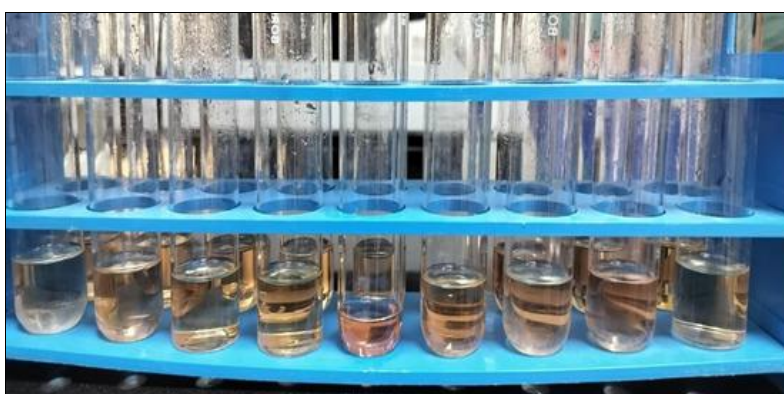


Fig 8: Screening of the isolates for IAA production by spectrophotometer

Table 3: Characteristics of the Potential Isolates Selected for Consortia Preparation

Characteristics	<i>Sinorhizobium meliloti</i>	<i>Halobacillus dabanensis</i>	<i>Streptomyces rameus</i>
P solubilization (in mm)	10.0	19.0	14.0
K solubilization (in mm)	15.0	10.0	12.0
Zn solubilization (in mm)	18.0	18.0	12.0
HCN production	+++	++	+
Siderophore production	++	++	+++
Ammonia production	+++	+++	+++
IAA Production (mg/ml)	0.136	0.132	0.112

Table 4: Molecular identification of the potential isolates by 16s rRNA sequencing

Isolate Name	Molecular Identification	Percent Similarity	Accession Number
CSRB 16	<i>Sinorhizobium meliloti</i>	98.87%	PX518210
CSRB 21	<i>Halobacillus dabanensis</i>	99.61%	PX518211
CSRA 34	<i>Streptomyces rameus</i>	99.48%	PX518212

3.8 Phylogentic tree of the isolates

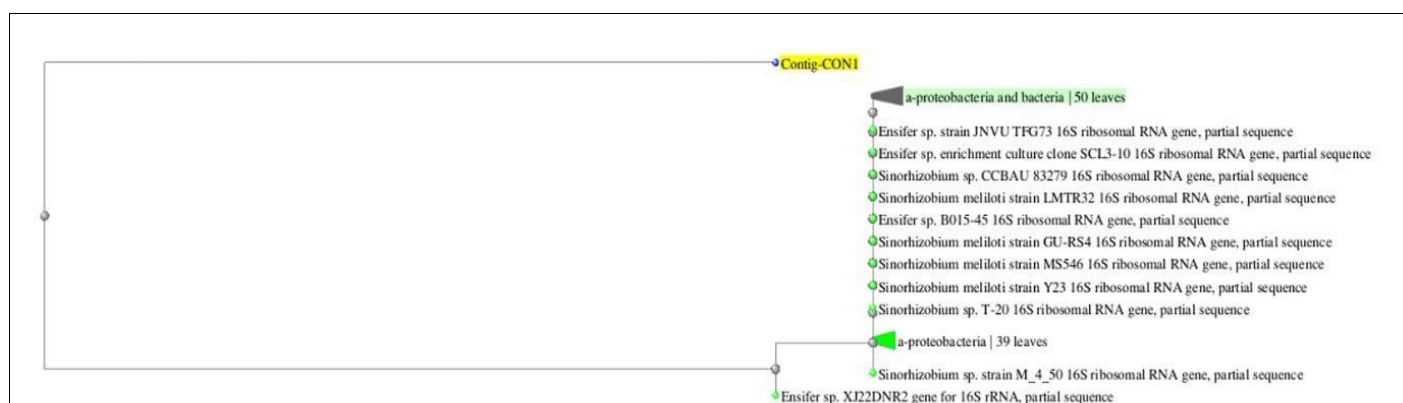


Fig 9: *Sinorhizobium meliloti*

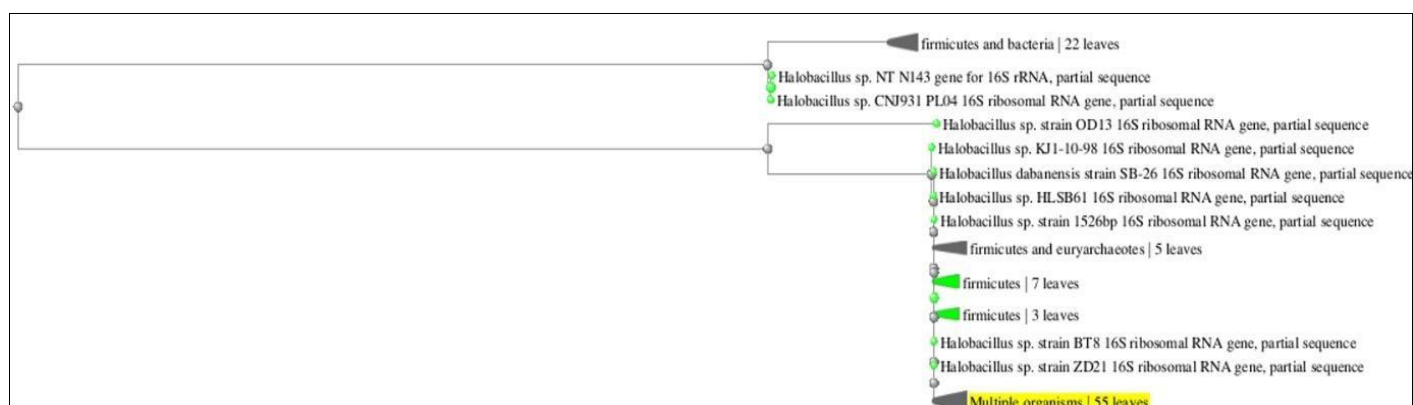


Fig 10: *Halobacillus dabanensis*

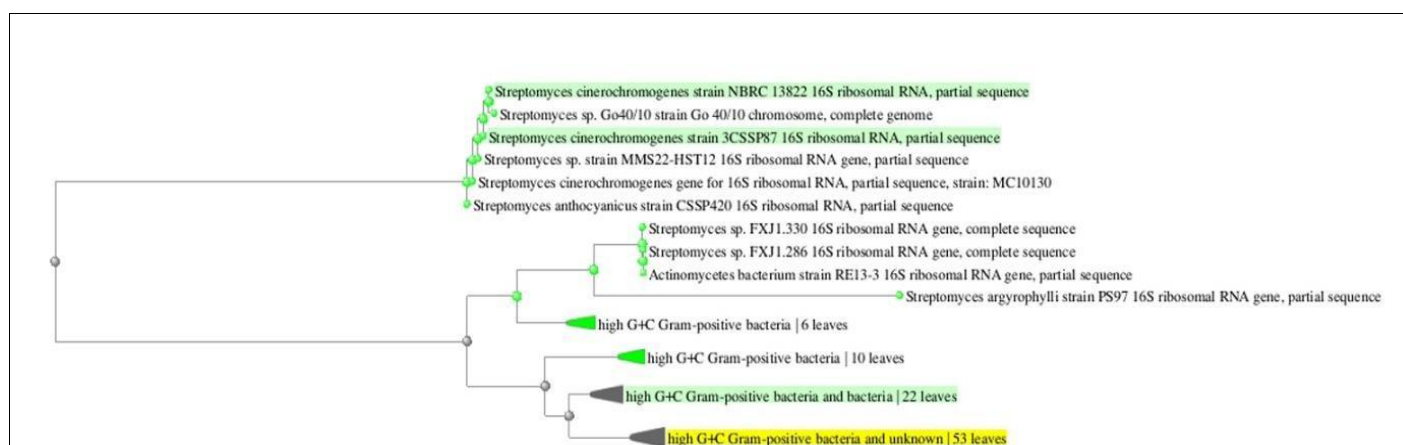


Fig 11: *Streptomyces rameus*

4. Conclusion

The present study demonstrated that the cotton-sesame rhizosphere harbors diverse and functionally efficient microorganisms with significant plant growth-promoting potential. A total of 47 isolates were obtained, among which 39 bacterial and actinomycete isolates exhibited multiple beneficial traits, including solubilization of phosphorus, potassium, and zinc, along with production of IAA, siderophores, ammonia, and HCN.

Based on their multifunctional efficiency, three compatible isolates (CSRB 16, CSRB 21, and CSRA 34) were selected and identified as *Sinorhizobium meliloti*, *Halobacillus dabanensis*, and *Streptomyces rameus* through 16S rRNA analysis. These isolates showed strong potential for improving nutrient

availability and plant growth through integrated NPK mobilization and PGP activities.

Overall, the findings highlight the potential of native rhizospheric microbes from cotton- sesame systems for developing effective microbial consortia, which can be utilized as sustainable bioinoculants to enhance soil fertility and crop productivity under low-input agricultural conditions.

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