Isolation, Identification and Molecular Characterization of Bacterial sp. from Different Agricultural Soil Samples in Dindigul District, Tamil Nadu.

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Abstract

This study revealed the isolation, identification, and molecular characterization of microorganisms that are present in agricultural soil habitat. The soil samples were collected fromdifferent sampling sites in Dindigul district, Tamil Nadu. The bacterial isolates CBPSS1, ONPSS2, RGPSS4, and CNPSS5 were found to be gram-positive *cocci*; LFPSS3 are gram positive *bacilli* Bacteria. The research project found that the species *Arthobacter sp., Staphylococcus sp., Bacillus sp., Micrococcus sp., and Streptococcus sp.* were present in the microbial isolated soil samples. The bacterial 16S rRNA gene was amplified by PCR. The resultant products were identified as 3 kb fragments.

Keywords: soil bacteria, isolation, agricultural soil bacteria, identification, genomic DNA isolation, 16S rDNA, PCR.

Introduction

Microorganisms constitute an important source of biodiversity in soils and form a necessary part of terrestrial ecosystems. They contribute to major biological functions such as nutrient and gas cycling, biogeochemical processes, and the decomposition and transformation of organic matter. Most of these bacteria are bioactive and survive in the top few inches of agricultural soils **(Foster JW and Woodruff HB, 2010).** Agriculture soil is a dynamic medium in which a large number of pathogenic and non-pathogenic bacterial and fungal floras live in close association. Microbes in the soil are the key to carbon and nitrogen recycling. Microorganisms create some useful compounds that are valuable to soil health and plant growth and play a significant role in nutritional chains that are an essential part of the biological balance of life on our planet **(Paul and Clerk,**

1966; Kummerer, 2004). Bacteria are some of the smallest and most rich microbes in the soil. In a single gram of soil, there can be billions of bacteria. There are an estimated 60,000 different bacteria species, most of which have yet to be even named, and each has its own particular roles and abilities. Most live in the top 10cm of soil where organic matter is present **(Greg Reid and Percy Wong, 2005).**

Materials and Methods Soil Sample Collection

Soil samples were collected from five sampling sites: Idayakottai, Ulagampatti, Thavasimadai, Nallampatty, and Chettiyapatty in Dindigul District in agricultural soil areas. The samples were subjected to isolation and analysis.

Soil pH Analysis

Soil pH was achieved by mixing soil with distilled water, and measurement was obtainedusing a pH-meter.

Isolation of Pure Bacterial Culture

After serial dilution, 100µl of diluted samples were transferred into nutrient agar plates and incubated at 37°C for 24 hours. Then a single colony was selected and streaked several times for a pure bacterial colony. A pure single colony was transferred into LB liquid medium for storage and further use.

Morphological and Biochemical Characterization of Bacteria

Morphological and biochemical tests were used for the specific identification of bacteria. Isolated bacteria were characterized by several morphological and biochemical tests, such as gram staining, motility, Indole, catalase, MR-VP broth, and starch hydrolysis tests.

Genomic DNA Isolation

Genomic DNA was extracted from LFPSS-3 and CNPSS-5 as described by **Sambrooket al. (2001).**

16S rDNA Sequence Determination

The bacterial 16S rRNA gene was amplified by PCR using the universal 16S rRNA primers: forward primer (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer (5'- GGT TACC TTG TTA CGA CTT-3'). PCR was carried out with a 50 µL reaction containing 1X PCR buffer with 0.6 mM MgCl2, 0.2 mM dNTP, Taq DNA

polymerase 1U, and 100 ng templateDNA using a Gene Amp PCR system 2700 (Applied Biosystems) with the following cycling conditions: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °Cfor 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min, and final extension at 72 °C for 5 min. A negative control without the DNA template was used for amplification along with the experiment. The PCR products were analyzed in 1.5% (w/v) agarose gel in 1X TAE buffer, stained with ethidium bromide (0.5 mg/mL), and observed under ultraviolet light before being subjected to further analysis.

Results

In this present study, fifty-five strains were isolated from five different sites (Sites A–18, B–13, C–11, -11, D - 8 and E–5 strains). Agricultural Soil samples pH ranges are 7.1, 6.8, 6.7, 7.3, and 6.6 identified (Table 1). The beginning identification of strains indicates that 19 isolates were gram-positive rod-shaped bacteria and 36 isolates were gram-positive *cocci*. From these, five bacterial isolates (CBPSS1, ONPSS2, LFPSS3, RGPSS4, and CNPSS5) were selected based on their nature. The strains (CBPSS1, ONPSS2, RGPSS4, and CNPSS5) were found to be gram- positive *cocci*, motile, and the stain LFPSS3 was found to be gram-positive rod, motile, respectively. Tables 2 and 3 show the detailed analysis of the morphological and biochemical characterization of five potential strains. *Arthobacter sp., Staphylococcus sp., Bacillus sp., Micrococcus sp., and Streptococcus sp.* are the species present in the soil samples. Successful isolation of genomic DNA from the LFPSS3 and CNPSS5 strains are done. The 16S rRNA gene was amplified by PCR using the universal primers. The amplified PCR products were confirmed,and a 3 kb fragments was identified. (Figure A).

Discussion

Karthik *et al.*, (2011) also reported the isolation, identification of microbes such as *Bacillus* species from agricultural waste dump soil. The isolation of various fungal, bacterial species showed that the agricultural soil is relatively rich in microbial flora. In agriculture process, soil microbes such as bacteria and fungi may play key roles in soil fertility and in the form of loss and gain in the production of grains, fruits, vegetables. Moreover, it also helps to maintain or develop the environment value and save natural resources. Most of the bacteria such as *Bacillusanthracis*, *B.subtilis*, and *S. aureus* and *S.*

epidermidis **(Amir and Pineau, 1998; Okoh *et al.*, 1999).** Many *Bacillus* species are recognized as plant-growth promoters, capable of promoting plant nutrient uptake, controlling phytopathogens, and producing phytohormones**(Tiwari S,*etal*., 2019).** *Bacillus* is a genus that belongs to the phylum Firmicutes, with diverse bacterial species that are Gram-positive, rod-shaped and spore formers **(Card S.,*et.al* 2016)**.In the present study, we have isolated and characterized, identified the *Arthobacter sp., Staphylococcus sp., Bacillus sp., Micrococcus sp., and Streptococcus sp.* The genomic DNA from the LFPSS3 and CNPSS5 strains are amplified by PCR produced a product is 3kb DNA fragment.

Conclusion

Our result also provides evidence for the presence of *Arthobacter sp., Staphylococcus sp., Bacillus sp., Micrococcus sp., and Streptococcus sp.* species isolated from agricultural soils. The result confirms that the PCR amplification produced a 3 kb DNA fragment. The future study is isolation, identification of potential bacterial candidates for bioremediation.

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TABLE 1: pH Value of the Soil Samples

SAMPLES	pH VALUES
CBPSS1	7.1
ONPSS2	6.8
LFPSS3	6.7
RGPSS4	7.3
CNPSS5	6.6

TABLE 2: Morphological Characterization of Bacterial Isolates

Characterization	CBPSS1	ONPSS2	LFPSS3	RGPSS4	CNPSS5
Colony size	1 mm	3 mm	0.5 mm	4 mm	1 mm
Colony shape	Round	Round	Circular	Circular	Circular
Colony color	White	Golden	White	Yellow	White gray
Colony count	48	70	52	56	64
Motility test	Motile	Motile	Motile	Motile	Motile
Gram nature	Gram +	Gram +	Gram +	Gram +	Gram +
Gram shape	*Cocci*	*Cocci*	*Rod*	*Cocci*	*Cocci*

TABLE 3: Biochemical Characterization of Bacterial Isolates

NAME OF THE TESTS	CBPSS1	ONPSS2	LFPSS3	RGPSS4	CNPSS5
Indole test	-VE	-VE	+VE	-VE	+VE
Catalase test	+VE	+VE	+VE	+VE	+VE
MR-VP test	-VE	+VE	-VE	+VE	-VE
Starch hydrolysisTest	+VE	-VE	+VE	+VE	-VE

Note: MR-VP= Methyl red and Voges-proskauer test, +VE=Positive, -VE=Negative.

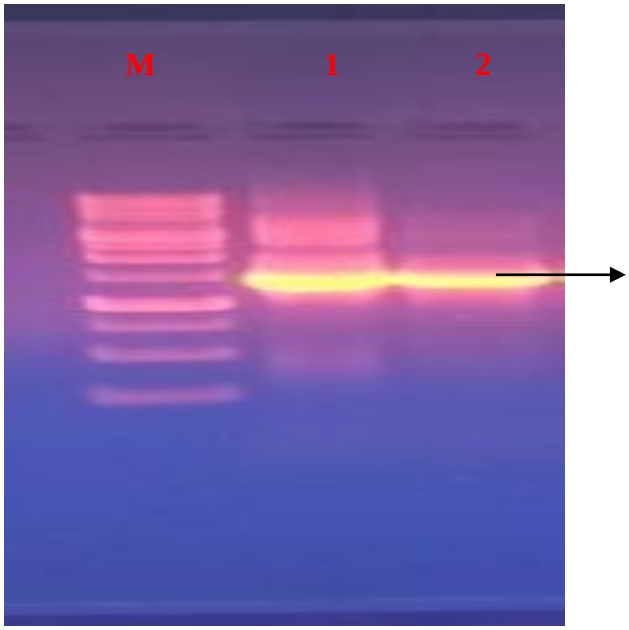

FIGURE: A - PCR Amplification of Bacterial Isolation

(Arrow represents the amplified product of optimized genomic DNA with 16S rRNA primers at 3 kb. M: Marker, Lane 1 isolated DNA from LFPSS3; Lane 2 isolated DNA from CNPSS5).

Note: CBPSS1 - Cluster Beans Plant Soil Sample 1, ONPSS2 - Onion Plant Soil Sample 2, LFPSS3-Lady's Finger Plant Soil Sample 4, RGPSS4 - Ridge Gourd Plant Soil Sample 4 andCNPSS5 - Corn Plant Soil Sample 5.