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ISOLATION, MOLECULAR IDENTIFICATION AND APPLICATION OF EXOGENOUS INDOLE ACETIC ACID (IAA) PRODUCING PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) FROM SOIL

ABDUL JALEEL.R[1], JADHEER AHSAN K.P [2], SWAGATO BHATTACHARJEE[3], S. MOHANA PRIYA[4], Dr.PRAVEENA[5]

[1,2,5]Mohammed Sathak Arts and Science College, University Of Madras, Tamil Nadu

[3]DBT-Rajiv Gandhi Centre for Biotechnology (RGCB)

[4]PSG College of Arts and Science, Coimbatore.

Abstract : Plant growth promoting rhizobacteria (PGPR) are often used as inoculants to promote the growth and yield of agricultural crops, however PGPR strain selection is extremely important. This bacteria colonies plant roots and promote plant growth through a variety of mechanisms including phytohormone production, improved water and nutrient uptake, improved nitrogen availability in the soil, production of ACC-deaminase for ethylene breakdown, phosphate solubilization, siderophore production, phytoremdiation, and phytopathogen defense. Microbial Auxin production is the primary component that promotes healthy plant growth and development. The ability of PGPR to produce phytohormones can be used to boost plant growth, reducing the need for chemical fertilizers and their harmful effects on the environment. The work in this study includes isolation, screening, molecular identification, and applying effective PGPR strains based on their ability to produce auxin in vitro. The soil samples were serially diluted, and the appropriate dilution was placed onto nutrient agar media. Invitro screening was performed on isolated colonies using the Salkowski reagent, which results in a pink coloring. The existence of IAA producers is indicated by the appearance of pink colour. This isolated organism was subjected to DNA isolation after passing morphological and biochemical tests. 16S PCR was used to amplify the isolated DNA. The bacteria was identified as Pseudomonas fluorescence after sequencing and analysing the amplified products with BLAST. When compared to other strains, Pseudomonas has a higher ability to produce auxin. In addition, a pot experiment in plants was carried out to test the influence of auxin (IAA) production by the isolated strain.

Keywords : PGPR , Indole acetic acid ,BLAST

1) INTRODUCTION

Crop yields must be increased urgently in order to meet the demands of providing food to the world's ever-increasing population. The use of chemical fertilizers and pesticides for diverse purposes has increased in tandem with the rate of population growth Although chemical fertilizers and pesticides produce good results, their disadvantages, such as pollution of vast water resources, loss of microorganisms, soil acidity, and reduction in soil fertility, are currently jeopardizing agricultural processes (Khin, 2012). In recent years, scientists have moved their attention to the potential of beneficial microbes, and the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has expanded in many parts of the world.

2) EXPERIMENTAL METHODS OR METHODOLOGY

Isolation and identification of exogenous IAA producing plant growth promoting rhizobacteria (PGPR) from agriculture soil and their application.

- **Sample collection**

At Kottakkal, Malappuram, soil samples were taken from the rhizosphere of the Mimosa pudica (Touch me not or thottavadi) plant. The intact root system was scraped out, and rhizospheric soil samples were carefully gathered in plastic

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bags and used for rhizosphere PGPR isolation.

- **Isolation of organism using specific media**

Subjected to serial dilution .For this , Two dilution test tubes (10^{-2} and 10^{-3}) were selected and performing spread plate.

- **Screening for IAA**

Quantitative & Qualitative Assay

- **Microscopical and Biochemical identification of bacteria**
- **Molecular identification**

Cell lysis, deactivation of cellular nucleases, and separation of the desired nucleic acids from cellular detritus are all required for the extraction and purification of DNA from biological material. Mechanical disruption, chemical treatment, and enzymatic digestion are all common lysis procedures.

3) RESULTS AND DISCUSSION

3.1 Quantitative Assay

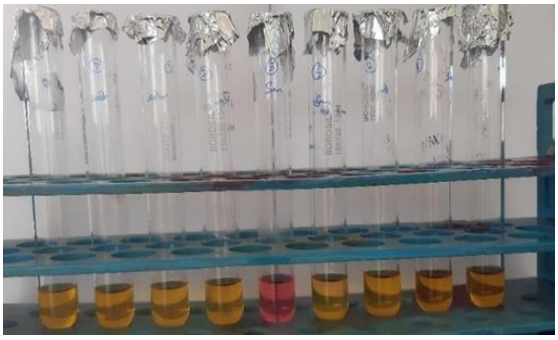

Fig1: The pink colour shows IAA producing culture without tryptophan

3.2 Pure Culturing Of Selected PGPR

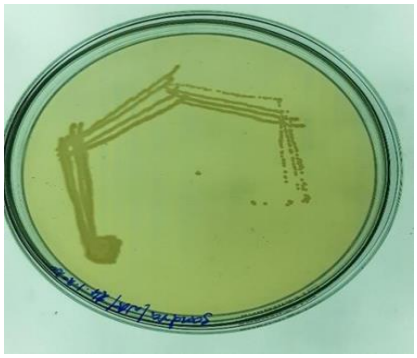

Fig 2: Quadrant streak of isolated colony

3.3 Gram's Staining

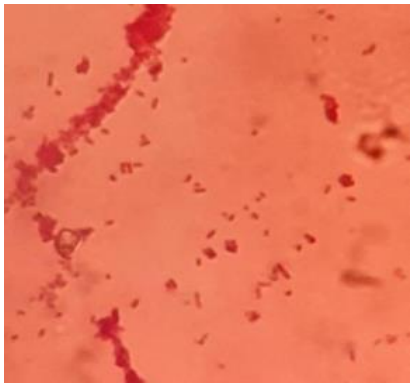

Fig3 : Gram negative rod shaped bacteria

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3.4 Biochemical Test

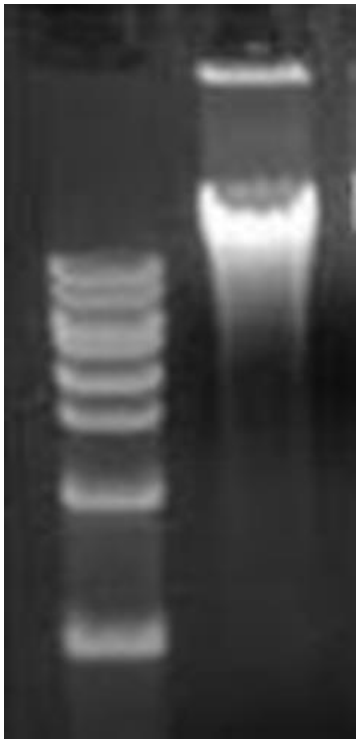
TEST	RESULT
INDOLE	Negative
MR	Positive
VP	Negative
Urease	Positive
OXIDASE	Positive
CATALASE	Positive
CITRATE	Positive

Fig.4Biochemical Test Result

3.5 Isolation Of Bacterial Genomic DNA

- Agarose Gell Electrophoresis (AGE)

LANE 1	Reference DNA
LANE 2	Isolated DNA



1 2

Fig5 :Bands observed under UV illumination

- Amplification Using 16S PCR

LANE 1	100bp MARKER
LANE 2	16 S GENE PCR P

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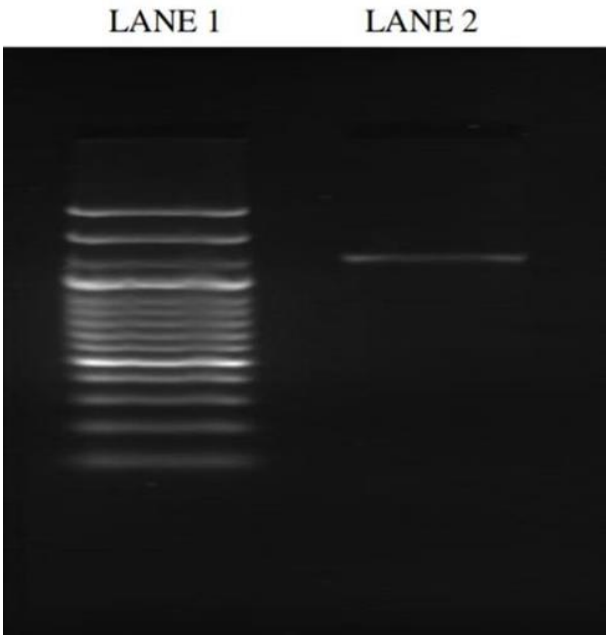

Fig6: Agarose Gel Electrophoresis of 16s ribosomal RNA gene

➢ BLAST

Blast was done and 99.88%similarity was noted with 16s rRNA gene of the organism*Pseudomonas fluorescens.*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas fluorescens strain FP23 16S ribosomal RNA gene, p	1580	1580	100%	0.0	99.88%	DQ201414.1
Pseudomonas sp. strain Fas14 16S ribosomal RNA gene, partial s	1574	1574	100%	0.0	99.77%	MH235971.1
Pseudomonas fluorescens strain VNS01 16S ribosomal RNA gene	1574	1574	99%	0.0	99.88%	KF758403.1
Pseudomonas fluorescens partial 16S rRNA gene, isolate s4	1570	1570	100%	0.0	99.65%	HF913576.1
Pseudomonas azotoformans partial 16S rRNA gene, isolate SW_H	1568	1568	100%	0.0	99.65%	LR722854.1
Pseudomonas sp. strain RGM_2656 16S ribosomal RNA gene, part	1568	1568	100%	0.0	99.65%	MN786797.1
Pseudomonas gessardii strain OBE3 16S ribosomal RNA gene, par	1568	1568	100%	0.0	99.65%	MN685265.1
Pseudomonas fluorescens strain ORTB3 16S ribosomal RNA gene	1568	1568	100%	0.0	99.65%	MN685247.1
Pseudomonas sp. strain 1JPC 16S ribosomal RNA gene, partial se	1568	1568	100%	0.0	99.65%	MN651328.1
Pseudomonas sp. CFSAN084952 chromosome, complete genome	1568	9413	100%	0.0	99.65%	CP045767.1
Pseudomonas azotoformans strain R-58 16S ribosomal RNA gene	1568	1568	100%	0.0	99.65%	MN560035.1
Pseudomonas paralactis strain CFH1-01 16S ribosomal RNA gene	1568	1568	100%	0.0	99.65%	MN559438.1
Pseudomonas costantinii strain P4M150 16S ribosomal RNA gene	1568	1568	100%	0.0	99.65%	MN421425.1
Pseudomonas costantinii strain P4M97 16S ribosomal RNA gene,	1568	1568	100%	0.0	99.65%	MN421413.1
Pseudomonas costantinii strain P4M92 16S ribosomal RNA gene,	1568	1568	100%	0.0	99.65%	MN421412.1
Pseudomonas costantinii strain P4M90 16S ribosomal RNA gene,	1568	1568	100%	0.0	99.65%	MN421411.1

Fig 7: Blast result

4) CONCLUSION

The results of this investigation clearly showed that the isolate tested could make IAA and, as a result, was classified as an IAA-producing rhizobacterium. P.fluorescens was the most recently isolated organism. This has the ability to create IAA, which was investigated on plants as part of this research. When compared to other species, Pseudomonas species are capable of creating more IAA. This research is important because it suggests that using PGPR as inoculants or bio fertilisers is an effective way to replace chemical fertilisers.

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