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Isolation and Molecular Characterization of Rhodococcus pyridinivorans and Its Efficacy on Plant Germination

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ABSTRACT:

In modern times exploiting beneficial microbes as biofertilizers is an intensifying area of research. The excess dosage of synthetic fertilizers has been applied since ages which affected the soil reproductive potential despite increases in yield. The diversity of microbes and the peculiarity of the crops also get scaled-down than usual because the organic content in the soil gets attenuated by repeated application of chemical fertilizers. To reclaim the potential nature of the soil, biological fertilizers or inoculants can be applied, which aids in the reconstruction of the soil's physical structure and microbial growth. In agriculture, germination of seeds is an essential process that determines the crop's potential for growth and productivity. Biofertilizers enrich the soil by converting the unusable form of nutrients to usable form by biological means, these are eco-friendly and cost-effective. In this study isolation of microorganisms from agricultural soils and culturing them in a culture medium by spread plate technique and confirming with biochemical tests, then from the consortium of organisms the less exploited organism was focused. The pigmented actinomycetes were selected for future research in which molecular characterization confirms the species as Rhodococcus pyridinivoran further optimization studies were performed in the outcomes revealed it grows well on pH 7 and temperature 25ºC and in the different combinations of the carbon and nitrogen source C3 & N2 (C3- Fructose & N2- Tryptone + NH4CL) shows better growth. R.pyridinivorans demonstrated notably higher rates of shoot and root elongation and germination in comparison to untreated control plants.

Keywords: Biofertilizer, Eco-Friendly, Microorganisms, Actinomycetes, Rhodococcus, plant germination.

1. Introduction

Bio-fertilizers are composed of viable microbial cells of distinct diversities, that can make the soil regain its viability by converting the unusable form of nutrients to usable form by the natural process. Its usages were limited to field crops in the past, but now it can be exploited for different crop varieties like fruit crops, medicinal crops, and also horticultural crops (Hazarika et al., 2007). In India, bio-fertilizer describes microbial diversities that assist the crops in acquiring their essential nutritional support by their accustomed biological activities, in other parts of the world bio-inoculants were being used. These are nothing but constructive microbes in an effective condition, when applied over the seed or rhizosphere it will improve the metabolism of plants via the uptake of essential nutrients and the synthesis of growth hormones (Brahmaprakash et al., 2012). The biological structure of the soil is affected due to the heavy reliance on inorganic fertilizer (Hazarika et al., 2007). Bio-fertilizer aids the plant against biotic stressors by improving its defense mechanisms, and also strengthening the roots from harmful pathogens and increasing its lifespan (Nosheen et al., 2021). Microbes incorporate and obtain necessary nutrients for plants, improve properties and attributes of the soil, and regulate the biochemical by-products, microbial inhibitors, botanical regulators, and distinct chemical messengers. Moreover, microorganisms expel bioenhancers which aids in improving biological functions and biochemical processes (Maitra et al., 2021).

Biofertilizers are formulated by utilizing a variety of microbiota and their associations with agricultural plants. Based on their characteristics and functions, they can be categorized in a variety of ways. Rhizobacteria that promote plant growth (PGPR) have long been the subject of research. At present the research findings demonstrated that the Nitrogen-fixing bacteria present within the plant (endophytic) may play a more vital role in restorative plant growth than rhizospheric bacteria because they avoid competing with those organisms and come into direct contact with plant tissues (Anubrata 2014**).** Among the fertilizers that provide other vital components to plants, those that supply nitrogen and phosphorus are considered to be significant. The different types of microbes that are employed in the production of biofertilizers are bacteria, cyanobacteria, and fungi (Thomas et al., 2019). A microbial consortium is a group of microbes living symbiotically and performing various physiological activities that are beneficial to plants in both growth stages and act as biocontrol agents. The consortium engages in competitiveness, antibiosis, mycoparasitism, and systemic resistance induction. Following the pathogen challenge, the consortium induces systemic resistance by activating the phenylpropanoid pathway and antioxidant enzyme activities, which results in the increase of proline, total phenolics, and pathogenesis-related (PR) proteins. A consortium fails to perform better if the microbes in the group are not compatible(kumar et al., 2016). The fact that multiple species can perform a variety of tasks in an ecosystem Such as retrieving the cultivable soil, soil vitality, vegetative expansion, and agronomic yield (Seenivasagan et al. 2021). In agriculture, germination of seeds is an essential process that determines the crop's potential for growth and productivity (Prasuna et al., 2024). Biofertilizers serve as an alternative to synthetic fertilizers because of their ecoconscious, economically viable, harmless, and simple-to-use properties and they even maintain the framework and ecological diversities of cultivable areas. The study focuses on the isolation of microorganisms from the agricultural soils and then focuses on a less exploited organism as a growth promoter from the consortium and then molecular characterization and optimization studies were performed for the selected organism.

2. Materials and method:

2.1 Isolation of Microorganisms from soil samples

The soil samples from different agricultural fields in the Udumalpet region were collected in sterilized bags and brought into the laboratory for the isolation of soil microorganisms. The collected soil samples were isolated by the spread plate technique in serial dilution method on a nutrient agar medium(Waksman 1927). The spread plate technique involves uniformly spreading a measured amount of a serially diluted sample of the original culture over the surface of the growing medium that has solidified. The soil samples collected are suspended in a 250-ml conical flask, and in nine milliliters of sterile distilled water, one gram of soil is suspended. After ample shaking, the sample was diluted. For bacterial enumeration (10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}) and (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}), dilutions were used for fungal isolates and incubated at 25℃ for 3 days. With a sterile 1 ml pipette, add each ml of each dilution to the plates. An inverted position was used to incubate the plates at 37℃ for three to seven days after the inoculum was evenly dispersed using the spread plate technique.

2.2 Microscopic identification of the selected organisms from soil:

Ten isolates were selected for microscopic identification. The isolated colonies were inoculated in a separate medium and incubated at 37℃ for 24 hours. After incubation, the isolated colonies were acquired for Gram staining using the standard protocol - Cappuccino Manual (Cappuccino et al., 1996).

2.3 Gram staining techniques:

Using a sterile loop, distribute a tiny volume of liquid culture uniformly across a sanitized glass slide. Let the film dry at room temperature gradually. Fix the dried film by driving it through the bunsen flame two or more times carefully for a brief time, avoiding direct flame exposure. Immerse the slide in crystal violet solution for about a minute. Rinse for five seconds with tap water before draining. The slide should be filled with Gram's Iodine solution, which will function as a mordant for a minute. Remove excess water by blotting the slide to avoid diluting the decolorization alcohol. Rinse the slide with tap water after 10 seconds of applying 95% alcohol. The slide should be stained for 30 seconds with the safranin solution before being washed with tap water. After exhausting gently dry it with blotting paper. All bacterial slides should be examined with the oil immersion lens. Gram-negative bacteria show as pink colonies, but gram-positive bacteria appear as purple colonies(Sharma et al., 2011).

2.4 Fungal identification:

Fungal propagules can be pigmented or seem colorless. The cytoplasm, hyaline mycelia, spores, and conidia were stained with lactophenol and cotton blue, respectively. The stained specimens were identified with a light microscope, and microphotographs were taken at magnifications ranging from 40X to 100X. Lactic acid protects fungal structures while phenol kills organisms, both of which are present in the (LPCB)LactoPhenol, Cotton Blue stain. Additionally, cellulose and chitin present in fungal cell walls are intensely stained by cotton blue(Raja et al., 2017).

2.5 Soil fertility assessment:

Soil pH, Electric Conductivity, Calcium, Magnesium, Sulfate, Chloride, Phosphorous, Total Organic Carbon, Nitrogen, Sodium, Potassium, Iron, Zinc, Manganese, and Copper were estimated as part of the soil fertility analysis (Devi et al., 2017).

2.6 Biochemical Confirmation tests:
Different biochemical tests such as Indole Production, Methyl red, Citrate utilization, Hydrogen Sulfide, Urease, Gelatin hydrolysis, Starch hydrolysis, Catalase, and Oxidase test were performed using standard protocol (Bergey 1994).

2.7 Optimization studies:
The optimization of the selected organisms was carried out based on distinct criteria, like optimizing the pH, temperature, carbon, and nitrogen source. The different pH is (5,6,7), temperature (25ºC, 30ºC, 37ºC), and Carbon sources include Glucose, Sucrose, Fructose, Sodium citrate, and Nitrogen sources including Peptone + Potassium nitrate, Urea + Ammonium dihydrogen phosphate, Trypton + Ammonium chloride, Beaf extract + Potassium nitrate. The growth was measured at regular intervals using a calorimeter and the Optical density was taken at 540nm (Engelhart-Straub et al., 2023).

2.8 16s sequence analysis
To ascertain the species of the selected organisms from the consortium, 16s rRNA analysis was performed. The Biobee Spin EXpure Microbial DNA isolation kit was used to isolate DNA from microbial sources. The isolates were identified molecularly by the process of amplifying and sequencing the 16S rRNA coding region. The protocol for extracting DNA from the isolates was adhered to by Van Soolingen (Van Soolingen et al. 1994). Primer 27F(5'AGAGTTTGATCTGGCTCAG3') & 1492R (5' TACGGTACCTTGTTACGACTT 3') are used in PCR to amplify specific cloned or genomic DNA sequences. This particular enzyme, Taq DNA polymerase, is supplied in 2X Taq buffer, 0.4mM dNTPs, 3.2mM MgCl2, and 0.02% bromophenol blue was the composition used in the Taq master mix. To 25 µL of PCR reaction solution (1.5 µL of Forward and Reverse primers, 5 µL of deionized water, and 12 µL of Taq Master Mix), add 5 µL of extracted DNA, by Utilizing the following heat cycling parameters while performing PCR. Purification and sequencing processes were carried out using AmpliTaq® DNA polymerase (FS enzyme) and ABI PRISM® BigDyeTM Terminator Cycle Sequencing Kits. The NCBI blast similarity search tool was used to blast the 16srRNA sequence. MUSCLE 3.7 was the program utilized for multiple sequence alignments (Edgar 2004). In conclusion, phylogenetic analysis was conducted using PhyML 3.0 aLRT software, with HKY85 serving as a replacement model (Talavera et al., 2007). To render trees, the application Tree Dyn 198.3 was utilized (Dereeper et al., 2008).

2.9 Evaluation of Plant Growth-Enhancing Characteristics:
A seed vigor assay was assessed for the plant growth-promoting potential of selected Actinomycetes isolates on Amaranthus viridis L. and Solanum lycopersicum L. through plate trials for ten days. The germination of surface-sterilized seeds was observed after being inoculated with broth made from overnight actinomycetes cultures (Raj Shekhar Sharma et al., 2024).

2.10 Maintenance of pure culture:
After being plated on nutrient agar medium and analyzed for colony features, the actinomycetes colonies exhibiting notable clear zones were sub-cultured on the nutrient agar slants, incubated for 24 hours at 37°C, and then stored at 4°C (Jaya Philip et al., 2024).

2.11. Genebank submission:
The study's generated nucleotide sequence data have been deposited with accession numbers **Contig_PSG OR418577** in the GenBank, EMBL, and DDBJ databases.
2.12 . Statistical analysis:

The average of the three replicates ± SD is used to express the data. To figure whether there were any statistically significant differences (P = 0.05) between the treatment means, a two-way (ANOVA) was performed. For all statistical studies, GraphPad Prism 10.2.220 was used (Vimal et al., 2019).

3. Results & Discussion:

A total of ten organisms were isolated and identified from the samples taken from various agricultural fields of Udumalpet. In this study of isolated colonies, bacteria were high in population, two fungal species and two actinomycetes were also identified. The isolated organisms and the morphological description of the isolated colonies are listed in (Table:1). Various biochemical tests were performed by following Bergey's standard procedure to confirm the isolated organisms from the sample and the test reports confirm the presence of organisms and the results are mentioned in (Table: 2). The soil parameters were checked while isolating organisms from the sample (Table: 3). This study was focused on isolating and identifying the less exploited organisms from the soil and employing them for plant growth as a promoter or as a bio-fertilizer and also for restoring the essential nutrients needed for the plant from the soil and retaining the soil fertility and productivity which has been low nowadays due to the excess usage of the synthetic fertilizer. Among the consortium of organisms, we have selected pigmented actinomycetes for future studies and carried out molecular characterization 16s r RNA for it. The species were chosen for their healthy growth, vibrant color, and characteristics, moreover to explore their features in plant growth-promoting activities, where the exploration of this species in this role is less. The 16s r RNA sequence reveals that based on the BLAST search in NCBI, the strain showed 98.91% identity with **Rhodococcus pyridinivoran** (fig. 1). The morphological identification showed it was a gram-positive rod-shaped, smooth round colony, antique pink to orangish pink in color.

The optimization studies of the isolated organisms were studied under different parameters such as evaluating the growth of strain under different pH (5, 6, and, 7), different temperatures (25ºC, 30ºC, 37ºC), and by adding different combinations of carbon and nitrogen sources. The results of these studies show that the strain responds positively to pH 7 and 25°C(Fig. 3)where its growth is higher than the other two. The carbon and nitrogen sources were given separately to the strain of five different combinations in that C3 & N2 (C3- Fructose & N2- Tryptone + NH4CL) showed better results. (Fig. 4 & 5).

3.1 Evaluation of Plant Growth-Enhancing Characteristics

A seed vigor assay was utilized initially to determine the plant germination and enhancing potential of specific actinomycetes isolates on Amaranthus viridis and Solanum lycopersicum using water medium. For these vitality assays, four distinct proportions (Rhodococcus pyridinivorans 1 ml, 2 ml, 3 ml, and 4 ml) of R.pyridinivoran were chosen. Periodically, readings were taken. pleasantly, 4ml (Rhodococcus pyridinivoran) concentrations of invivo plate assays showed the highest percentage of seed germination and elongation(Table-4 & Fig 6). In these trials, the organisms demonstrated notably higher rates of shoot and root elongation and germination in comparison to untreated control plants.

Actinomycetes have received much research due to their biotechnological significance in bioremediation and their high potential to break down a wide range of xenobiotics, including hazardous aromatic chemical compounds. We selected an organism from the genus Rhodococcus of the order Actinomycetales of the kingdom Bacteria for our investigation. According to the analysis's findings, the species was a pyridinivoran due to its high genome affinity.

Rhodococcus pyridinivorans, a new species, was first isolated from industrial effluent in Korea in 2000. It is an aerobic, rod-shaped, non-motile, non-sporulating mesophile that is gram-positive but is Gram-variable in older cultures. In recognition of its ability to break down pyridines, this species was given the name "Pyridinivorans" (pyridine-devouring). In the early stages of growth, the cells appear as rods and branched filaments before fragmenting into small rods or cocci. Colonies have elevated, opaque, somewhat wrinkled circular borders and range in shades from light orange to pink. The ideal pH range for growing is (7-8). It grows best between 30 and 37 °C; it also grows at 10 and 45 °C but not at 50 °C. Meso-diaminopimelic acid, arabinose, and galactose are found in the cell wall (Yoon et al. 2000). Experimented with a study on pyridinivorans and found that the dangerous naturally occurring endocrine-disrupting chemical (EDC), zearalenone, causes excessive estrogenic reactions in higher vertebrates, leading to reproductive abnormalities in both humans and agricultural animals (Kriszt et al. 2012). It is a nonsteroidal estrogenic mycotoxin that is generated on cereal grains by various Fusarium species. The non-pathogenic Rhodococcus pyridinivorans K408 strain we used in this study to construct a bacterial detoxification system demonstrated an 87.21% ZEA-degradation efficiency. There were no residual estrogenic effects as a result of ZEA biodegradation, which is a very effective biological instrument for eliminating ZEA's entire estrogenic effect (Yoon et al. 2000). As per the research, the recently discovered R. pyridinivorans strain PDB9T N-1 demonstrated the ability to effectively and completely degrade over 1600 mg l1 of phenol. It was discovered that the aromatic ring of the protocatechuate underwent cleavage at the third and fourth carbon bond position during the biodegradation of phenol by the actinomycetes species, with the oxalocrotonic acid pathway proceeding subsequently (Barik et al. 2021). In the investigation, a novel QQ (quorum quenching) strain of Rhodococcus pyridinivorans XN-36 was discovered that can efficiently breakdown AHL N-acyl homoserine lactone (AHL) and lessen the severity of illness in host plants. Without a lag phase, strain XN-36 was able to degrade AHL quickly; within 96 hours, over 85% of AHL (0.5 mmol•L1) had been broken down. These findings bring up new possibilities for the management of QS (Quorum sensing)-mediated bacterial illnesses and broaden the applicability of QQ strains as biocontrol agents (Zhou et al. 2022). An additional study revealed that activated sludge contaminated with many pesticides had a unique strain of Rhodococcus pyridinivorans Y6 that degrades pyrethroids. One of the most frequently applied pesticides, pyrethroids have unfavorable biological effects and are a severe hazard to both human health and natural systems. In less than 36 hours, the Y6 strain was able to metabolize many pyrethroid insecticides, including beta cypermethrin (50 mg•L1), fenvalerate, prallethrin, and D-cyphenothrin. The outcomes of the soil restoration process demonstrated that prallethrin could be successfully eliminated from the soil by the Y6 strain. They had no negative impacts on the variety and community structure of native microorganisms when they were introduced to the soil (Huang et al.2023). Rubber latex breakdown was carried out by employing a bacterial consortium in mineral salt media. The isolated Rhodococcus pyridinivorans strain (F5) performed better than the other isolated strains in the consortium. Combined with different strains, the F5 strain performs exceptionally well and breaks down rubber latex within 30 days (Nawong et al. 2018). In the study, Chinese blooming cabbage pot tests were conducted to determine whether phthalates (PAEs) were absorbed by the roots or the leaves and whether PAE accumulation by vegetables might be reduced by spraying a strain that degrades PAEs. The research demonstrated that Chinese kale cultivated in polyethylene greenhouses acquired excess PAEs from the environment contrary to cabbage grown externally in greenhouses. Underneath plastic greenhouses, the values of the leaf bioaccumulation factors were significantly higher (1.39–3.47 fold) than outside. Leaf surfaces can be sprayed with a bacterium species Rhodococcus pyridinivorans XB, which

breaks down PAE. The PAE-degrading strain effectively lowered PAEs by 12.9%–34.9% on the leaf epidermal layer. This study proved that PAE accumulation might be reduced by dousing plants grown in plastic greenhouses with a strain that breaks down PAE (Zeng et al. 2022). A study **was** conducted on the beneficial characteristics of endophytic bacteria in Simmondsia chinensis. Endophytes were isolated from the roots of jojoba plants(Simmondsia chinensis) and were 16s rDNA sequenced for identification. The isolated bacteria have the capability of promoting plant growth; Rhodococcus pyridinivorans and Oceanobacillus kimchi are reported as the best plant growth promoters (Perez-Rosales et al. 2017). The experiment was made to evaluate the enzyme activity of in vitro Simmondia chinensis when inoculated with endophytic bacteria. All inoculated endophytic bacterial strains were good at promoting vegetative development and root growth. All inoculated endophytic bacterial strains were good at promoting vegetative development and root growth. Plants were found to differ in their amounts of some enzymes. The strain of Rhodococcus pyridinivorans (JRR 22) that was inoculated exhibited the highest level of enzyme activity and was associated with a prospective enhancement in plant resilience toward disease invasion (Perez-Rosales et al. 2018).

4. Conclusion:

In the study, we isolated the organisms from agricultural fields, a total of ten organisms were isolated, selected one organism, was with molecular characterization and optimization studies. In the molecular characterization, 98% affinity confirms the species as **Rhodococcus pyridinivorans,** and the optimization studies showed that the **R.pyridinivorans** grows well in various pH, temperatures and various carbon and nitrogen sources especially in pH 7 & 25^{o}C and in C3 & N2 (C3- Fructose & N2- Tryptone + NH4CL). **R.pyridinivorans** demonstrated notably higher rates of shoot and root elongation and germination in comparison to untreated control plants. So it can be utilized as growth promoters. The selected organism was utilized previously for several bio-remediation processes, by breaking down heavy metals, and also for plant growth regulations. The future work will be based on evaluating and exploiting its potential as a growth promoter and converting it into value-added bioproducts which will be bio-friendly and cost-effective products that help the farmers in society.

Tables:

(Table -1) list of organisms isolated from the sample and its morphological features

Organisms	Microscopic shape	Strain	Macroscopic	Medium	Motility
Bacillus sp.1	Elongated rod	Gram-positive	Large irregular in shape	Nutrient agar medium	Non-motile
Bacillus sp. 2	Slightly curved	Gram-positive	Rough, opaque fussy white to yellow	Nutrient agar medium	Non-motile
Azotobacter sp.1	Short rods	Gram-negative	Small circular and translucent colonies	Jensen's medium	Motile

Azotobacter sp. 2	Oval to spherical Swollen rods	Gram-negative	Medium circular translucent colonies	Jensen's medium	Motile
Acetobacter.1	Elongated rod	Gram-negative	Smooth, rough spheroid to flattened aggregated with a pillowed surface	Acetobacter media	Motile with its microfibrils
Bacillus sp. 3	Rod-shaped	Gram-positive	Large irregular in shape	Nutrient agar medium	Non-motile
Alternaria sp.	Beak like elongated large spore with trasversly septated	Nil	Greyish olivaceous suede-like floccose	Rose – Bengal agar	Non-motile.
Aspergillus sp.	Thick round black spores.	Nil	Blue-green colonies with suede-like surfaces with dense felt of conidiophores	Nutrient agar	Non motile
Streptomycetes sp.	Filamentous rods	Gram-positive	Yellow pigmented small round colonies	Nutrient agar	Non-motile
Rhodococcus pyridinivorans	Rod shape	Gram-positive	Antique pink small round colonies	Nutrient agar	Non-motile

Organisms	catalase	oxidase	Starch hydrolysis	Gelatine hydrolysis	citrate	TSI	Indole	Methyl	nitrate	urease	H2S
Bacillus sp.1	+	+	+	+	+	+	+	_	+	_	+
Bacillus sp.2	+	+	+	+	+	+	+	_	+	_	+
Azotobacter sp. 1	–	+	–	–	+	+	–	–	+	–	–
Azotobacter sp.2	–	+	–	–	+	+	–	–	+	–	–
Acetobacter sp.	+	–	–	–	+	+	–	–	+	+	+
Bacillus	+	+	+	+	+	+	+	_	+	_	+

spp 3											
Streptomycetes sp.	+	–	–	+	–	–	–	–	–	+	+
Rhodococcus pyridinivorans	+	–	–	–	+	–	–	–	–	+	–

(Table: 2) **Biochemical characterization of the isolated organisms**

SAMPLE	pH	MOISTURE	N(mg/l)	P(mg/l)	K(mg/l)	Fe(ppm)	Mn(ppm)	Zn(ppm)	Cu(ppm)
RED	7.23	1.89	3.12	1.85	0.67	5.93	2.91	0.323	0.113
BLACK	6.99	13.64	3.68	2.2	1.23	6.02	5.73	0.558	0.200

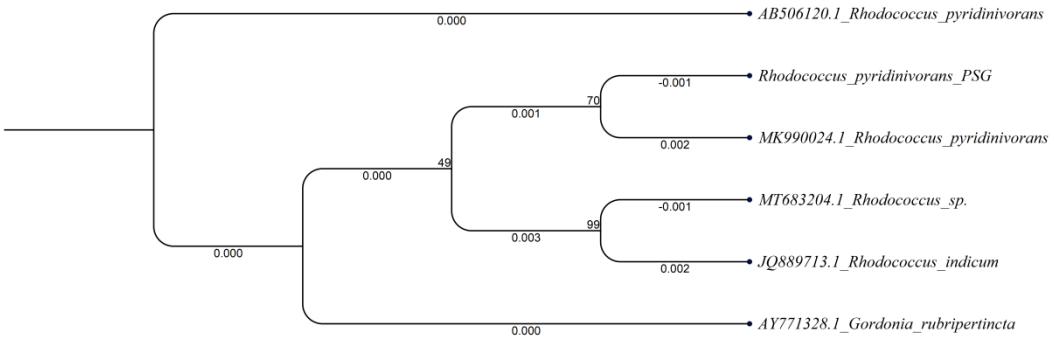
(Table:3) **Physio Chemical Properties of Soil**

(**Table 4)- Growth enhancement properties of R.pyridinivorans vs Control**

Source	No of seeds		Germination %		Elongation %	
	Plant 1	Plant 2	Plant1	Plant 2	Plant1	Plant 2
Control	20	20	4.5	2.5	1	1
RP.1ml	20	20	3.5	3	1.5	1.5
RP.2ml	20	20	4	3	2	1.5
RP.3ml	20	20	7.5	5	5	5
RP.4ml	20	20	9	6	5.5	6.5

Plant 1- Amaranthus viridis
Plant 2 -Solanum lycopersicum

(Figure -1) Phylogenetic tree based on the strain's 16s rRNA gene sequence



GCTGGCGGCGTGCTTAACACATGCAAGTCGAACGATGAAGCCCAGCTTGCTGGG
TGGATTAGTGGCGAACGGGTGAGTAACACGTGGGTGATCTGCCCTGCACTCTGG
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GTAATGGCCTACCAAGGCGACGACGGGTAGCCGGCCTGAGAGGGCGACCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTAGGGAGGCAGCAGTGGGGAATATT
GCAAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGACGGCCTTCG
GGTTGTAAACCTCTTCACCCATGACGAAGCGCAAGTGACGGTAGTGGGAGAAGA

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GTCCGGAATTACTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCGTCTGTGA
AATCCCGCAGCTCAACTGCGGGCTTGCAGGCGATACGGGCAGACTCGAGTACTG
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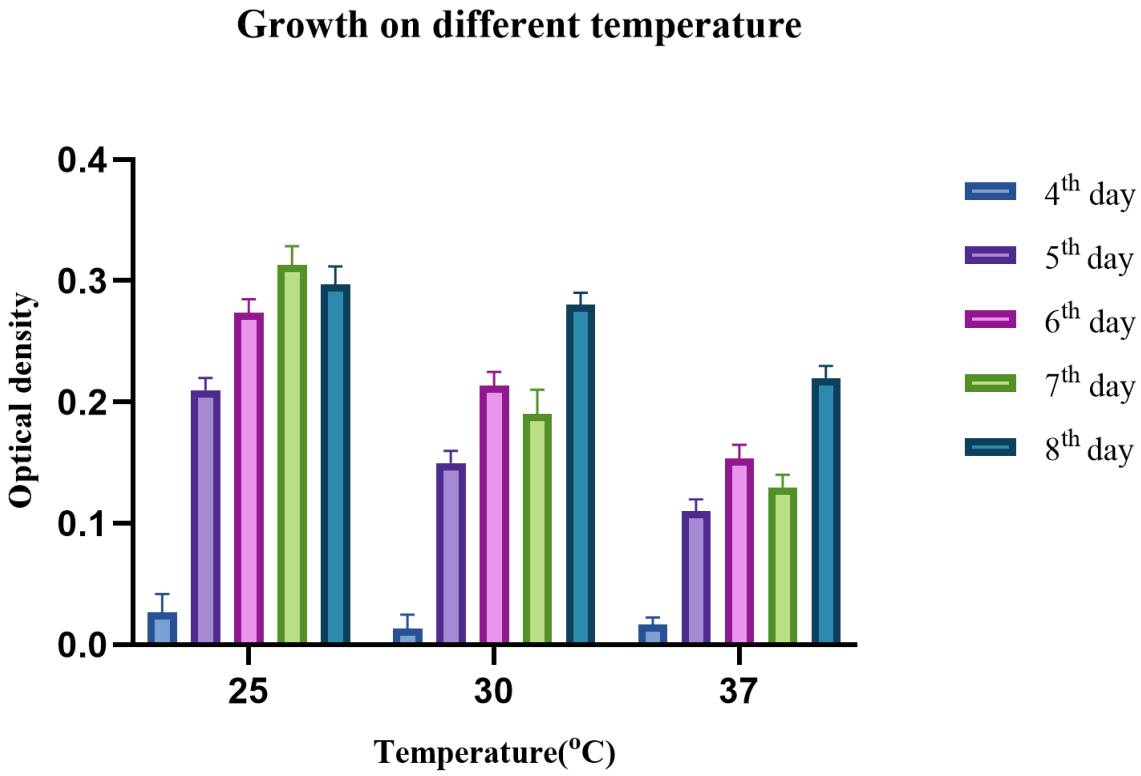
CTAGGTGTGGGTTTCCTTCCACGGGATCCGTGCCGTAGCCAACGCATTAAGCGCC
CCGCCTGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGAAGAACCTTACCTG
GGTTTGACATGTACCGGACGACTGCAGAGATGTGGTTTCCCTTGTGGCCGGTAGA
CAGGTGGTGCATGGCGTCGTCAGCTCGTGTCGGAGATGTTGGGTTAAGTCCCGCA
ACGAGCGCAACCCTTGTCCTGTGTTGCCAGCACGTGATGGTGGGGACTCGCAGG
AGACTGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCC
CCTTATGTCCAGGGCTTCACACATGCTACAATGGTCGGTACAGAGGGCTGCATAC
CGTGAGTGGAGCGAATCCCTTAAAGCCGGTCTCAGTTCGGATCGGGGTCTGCAA
CTCGACCCCGTGAAGTCGGAGTC

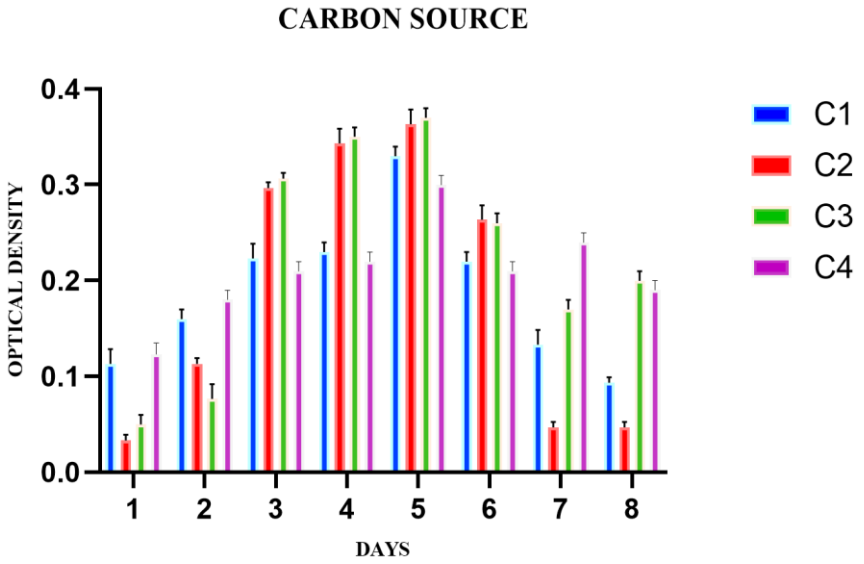
Rhodococcus pyridinivorans

(Figure: 2) Blast sequence of the Rhodococcus strain

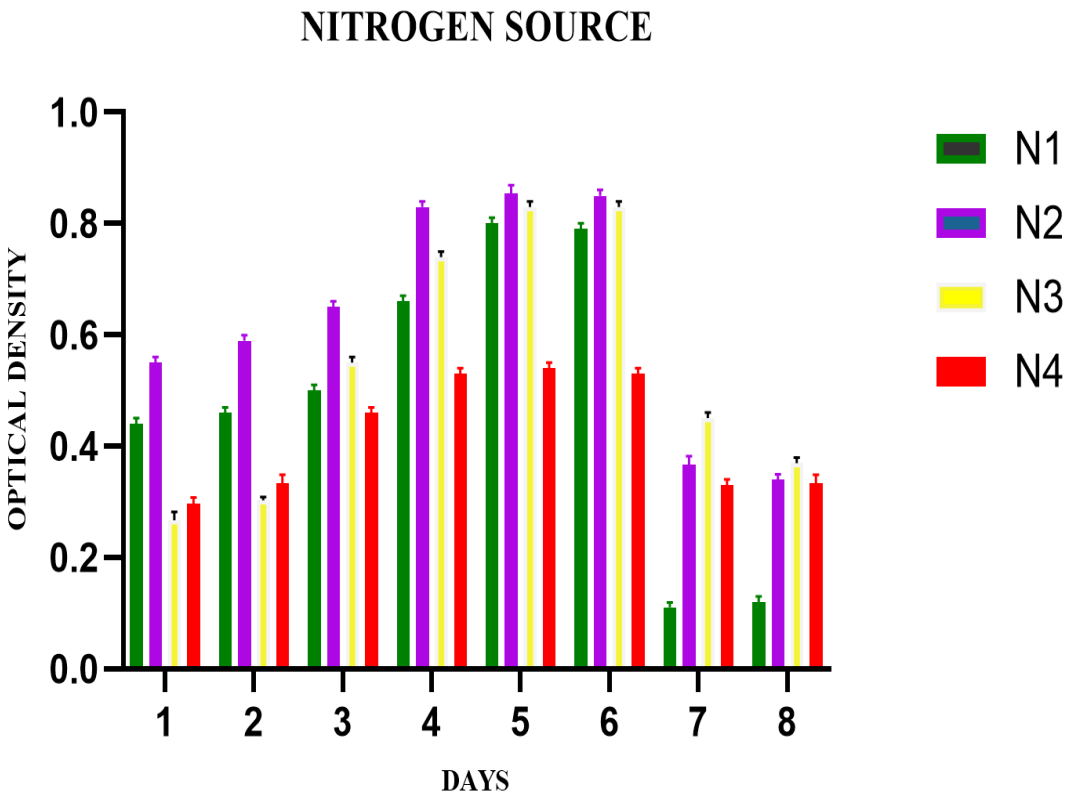
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Rhodococcus sp. (in: high G+C Gram-positive bacteria) strain OPS-004 16S ribosomal RNA gene, partial sequence	2281	2281	100%	0.0	98.91%	MT328061.1
Rhodococcus rhodochrous strain ATCC BAA870 chromosome, complete genome	2281	9075	100%	0.0	98.91%	CP032675.1
Rhodococcus pyridinivorans strain ISJ30 16S ribosomal RNA gene, partial sequence	2281	2281	100%	0.0	98.91%	MK990024.1
Rhodococcus sp. 2G, complete genome	2281	9071	100%	0.0	98.91%	CP018063.1
Rhodococcus pyridinivorans strain P23 chromosome, complete genome	2281	9126	100%	0.0	98.91%	CP113798.1
Rhodococcus sp. FF2 16S ribosomal RNA gene, partial sequence	2281	2281	100%	0.0	98.91%	KT894768.1
Rhodococcus pyridinivorans SB3094, complete genome	2281	9128	100%	0.0	98.91%	CP006996.1
Rhodococcus pyridinivorans gene for 16S ribosomal RNA, partial sequence, strain: PD7-2	2281	2281	100%	0.0	98.91%	AB506120.1
Rhodococcus rhodochrous strain BX2 chromosome, complete genome	2281	9126	100%	0.0	98.91%	CP027557.1
Rhodococcus sp. BX2 16S ribosomal RNA gene, partial sequence	2281	2281	100%	0.0	98.91%	FJ617562.1
Rhodococcus sp. gf-6 16S ribosomal RNA gene, partial	2281	2281	100%	0.0	98.91%	EU287449.1

(Figure: 3) Growth of organisms on pH 7 and different temperatures.

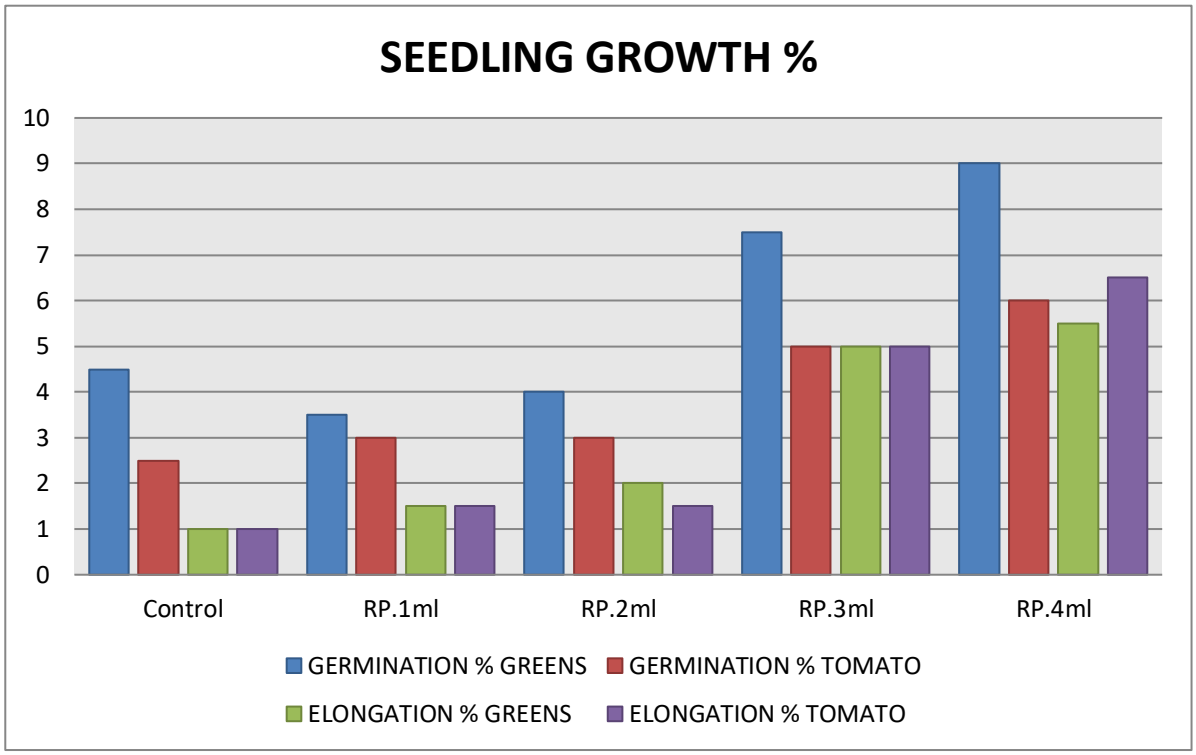




(Figure:4) Effect of carbon source on microbial growth. The bars represent the standard deviation of the triplets. (C1- glucose, C2- Sucrose, C3-Fructose, C4- sodium citrate). A significant difference at level p>0.05 between the different carbon sources.



(Figure: 5) Effect of Nitrogen source on microbial growth and the bars represent the standard deviation of the triplets. (N1- Peptone+KNO3, N2- Tryptone+NH4CL, N3- Urea+NH4H2PO4, N4- Boron+KNO3). A significant difference at level p>0.05 between the different nitrogen sources.



(Figure- 6) Seedling growth percentage by a different proportion of R.pyridinivorans

Greens : Amaranthus viridis
Tomato : Solanum lycopersicum
RP : Rhodococcus pyridinivoran

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

***Ethics approval – Not applicable**
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*** Authors' contributions**

Mr. J.Ramprasath : Investigation, Original draft and Project
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