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Screening, Characterization and Antimicrobial Activity of Soil Bacteria *Paenibacillus elgi*

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Abstract: ***A novel gram positive, spore forming bacteria was isolated from the soil. Existing methods of screening together with some novel procedures were done for checking the antimicrobial effect of the isolated bacterial strain. Modified agar streak method and culture filtrate assays were used in primary screening for bacterial antibiosis. Dual culture bioassay was used to assess the antagonistic activity against seven pathogenic fungi in vitro. It was found that the isolate JBL-1 was found to inhibit the pathogens to higher degrees. These results highlight the potential for industrially important antifungal secondary metabolites from the soil. This potent isolate was tentatively identified using standard morphological and biochemical tests. Genomic DNA was extracted and their 16s rDNA genes were amplified and sequenced for homology analysis using the GenBank database. NCBI nucleotide BLAST homology search of the 16s rRNA gene sequence revealed high homology with the genus Paenibacillus showing 99.75% sequence similarity with strain of P.elgii.***

Keywords: *Paenibacillus elgii*, Antifungal activity, Secondary metabolites, Dual culture technique, antimicrobial compounds

1.Introduction

Soil microorganisms, particularly spore-forming bacteria, represent a vast reservoir of unexplored biodiversity with significant potential for antimicrobial compound discovery (Grady *et al*., 2016). Among these, the genus *Paenibacillus* has emerged as a promising source of bioactive secondary metabolites with diverse antimicrobial properties and biocontrol applications. *Paenibacillus* species are gram-positive, spore-forming bacteria that have been isolated from a wide range of environmental sources, including soil, plant rhizospheres, and extreme environments (Grady *et al.,* 2016). These bacteria are particularly noteworthy for their ability to produce an extensive array of antimicrobial compounds, including antibiotics, antifungal agents, and other bioactive metabolites that can effectively suppress pathogenic microorganisms (Eastman *et al*., 2014).

This broad-spectrum antimicrobial activity is attributed to the production of various antimicrobials and secondary metabolites that can effectively inhibit the growth of pathogenic fungi and bacteria (Luo *et al.,* 2015). *Paenibacillus elgii*, a relatively less-studied species within the genus, has shown a promise source of antimicrobial compounds. Previous studies have demonstrated that various *Paenibacillus* species, including *P. validus* and *P. chibensis*, exhibit significant biocontrol potential with broad-spectrum antimicrobial activity (Berić *et al.,* 2007). The identification of soil-derived *Paenibacillus* isolates with broad-spectrum antimicrobial activity represents a significant step toward developing sustainable alternatives to synthetic antimicrobial agents (Khan *et al.,* 2018).

This study aimed to isolate, characterize, and evaluate the antimicrobial potential of a novel *Paenibacillus* strain from soil, with particular emphasis on its antifungal properties. Through a combination of microbiological techniques and molecular identification methods, the biocontrol potential of soil-derived *Paenibacillus* species and their capacity to produce industrially relevant antimicrobial compounds was studied.

2.Materials and Methods

Sample Collection and Isolation

Soil samples were collected from different localities of Kondotty region, Kerala. Nutrient agar plates were incubated at 37°C for 12-24 hours to screen for antagonistic bacteria. Colonies showing clear zones of inhibition were isolated and purified. Pathogenic fungi were isolated from spoiled fruits and vegetables following the method of Dashwood *et al.* (1977). Sections were plated on potato dextrose agar (PDA) containing chloramphenicol (30 mg/ml) and incubated at 25°C for 3 days (Barnett & Hunter, 1998).

Antimicrobial Activity Assays

Antibacterial Activity

Agar streak plate method: Isolates were streaked as straight lines on Mueller-Hinton agar and incubated at 27°C for 4 days. Test bacterial strains (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella* spp*., and Proteus* spp.) were then streaked perpendicular to the original streak without touching it, followed by incubation at 37°C for 24 hours.

Culture Filtrate Assay: Bacterial isolates were grown in nutrient broth for 72 hours, centrifuged at 6000 rpm for 10 minutes, and supernatants were collected (Dennis & Webster, 1971). 100 µl of supernatant was added to wells in plates inoculated with test organisms, and inhibition zones were measured.

Antifungal Activity

Dual Culture Technique: A 1cm² fungal plug was placed at the centre of PDA plates, with test bacteria streaked 2.5 cm away on either side. Plates were incubated at 28°C for 72 hours with observations every 24 hours. Percent inhibition was calculated using the formula: I = (C-T/C) × 100, where C = colony diameter in control and T = colony diameter in test.

Culture Filtrate Assay: Cell-free culture filtrates were incorporated into molten PDA at concentrations of 5%, 10%, and 20% (v/v) following Dennis and Webster (1971). Fresh pathogen mycelial plugs were inoculated, and plates were incubated at 28±1°C for 5 days. Inhibitory effects were categorized as strong, moderate, or weak.

Characterization of Antagonistic Isolates

Morphological and Biochemical Characterization

Colony morphology was recorded including colour, shape, size, and pigmentation. Gram staining, motility testing using hanging drop method, and endospore staining using Schaeffer-Fulton method was done. Biochemical tests included indole production, methyl red, Voges-Proskauer, citrate utilization, and catalase tests include were carried out following standard protocols.

Enzymatic Activity Screening

The following enzymatic activities were tested: amylase using starch-iodine test (Hankin & Anagnostakis, 1975), cellulase on carboxymethyl cellulose medium with Congo red (Teather & Wood, 1982), gelatinase on gelatin agar with mercuric chloride (Frazier, 1926), protease on skim milk agar (Agrawal & Kotasthane, 2012), phosphate solubilization on Pikovskaya's medium (Pikovskaya, 1948), and lipase on tributyrin agar (Sierra, 1957).

Molecular Characterization

DNA Extraction: Genomic DNA was isolated using NucleoSpin® Tissue Kit following manufacturer's protocol.

PCR Amplification: 16S rRNA genes were amplified using primers 16S-RS-F (5'-CAGGCCTAACACATGCAAGTC-3') and 16S-RS-R (5'-GGGCGGWGTGTACAAGGC-3') (Weisburg *et al.,* 1991). PCR conditions included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 60°C for 40 seconds, and 72°C for 60 seconds, with final extension at 72°C for 7 minutes.

Sequencing and Analysis: PCR products were treated with Exo SAP-IT and sequenced using Big Dye Terminator v3.1 on an ABI 3500 DNA Analyzer (Applied Biosystems). Sequences were analyzed using Sequence Scanner Software v1 and aligned using Geneious Pro v5.1 (Drummond *et al*., 2010). NCBI BLAST was performed for homology analysis.

Fungal Identification

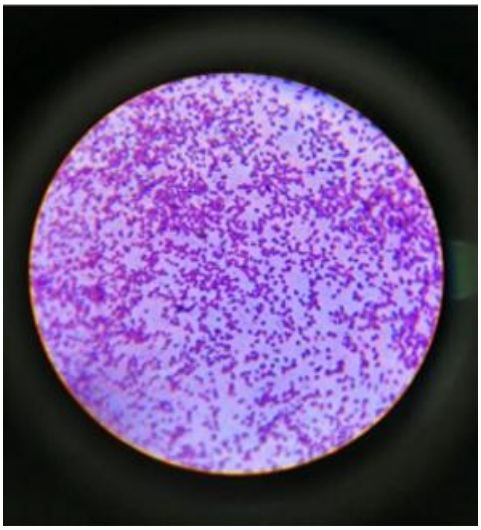
Fungal isolates were identified using cultural and morphological features including colony growth patterns, conidial morphology, and pigmentation. Cotton blue in lactophenol staining was used for microscopic examination at 10× and 40× magnifications.

3.Results

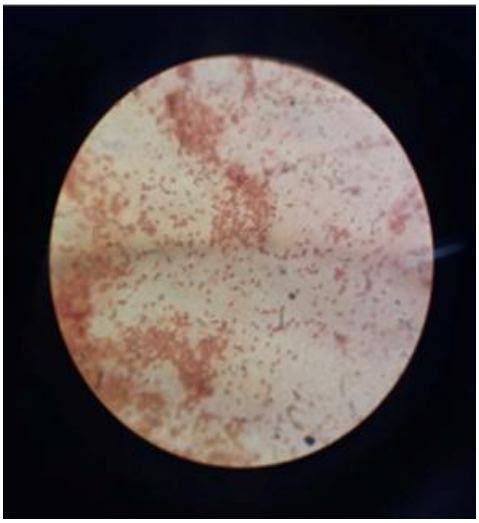
Isolation of bacteria from soil:

Out of 5 bacterial isolates, one bacterial colony giving more inhibition in the reservoir plate was selected for further studies. Plates showing inhibition zone in the primary screening fig:1.1and the selected isolate showing gram staining in fig.1.2 and endospore staining in fig. 1.3.

Fig: 1.1 Master plate



1.2 Gram positive



1.3 Endospore staining

Preliminary screening for antibiotic production:

By performing both primary and secondary screening, no antibacterial activity was observed for all the 5 bacterial strains.

Six fungal strains were identified against the potent bacterial isolate of the reservoir plate. (Table 1). The dual culture plates showed in fig.2.1-2.6, none of the fungal pathogen could over whelm the bacterial colony and the inhibition was persistent.

Table 1: Percentage inhibition against Fungus

S. No	Fungus	Colony diameter in control plate (cm)	Colony diameter in treated plate (cm)	Zone of Inhibition (%)
1	Microsporum spp.	7	3	57.15
2	Trichoderma spp.	7.5	3.5	53.33
3	Scopulariosis spp	6	3	50
4	Lichtheimia spp.	7	3	57.15
5	Fusarium spp.	5	2.5	50
6	Curvularia spp.	7.5	3	60

Fig:2.1 *Microsporum spp*.

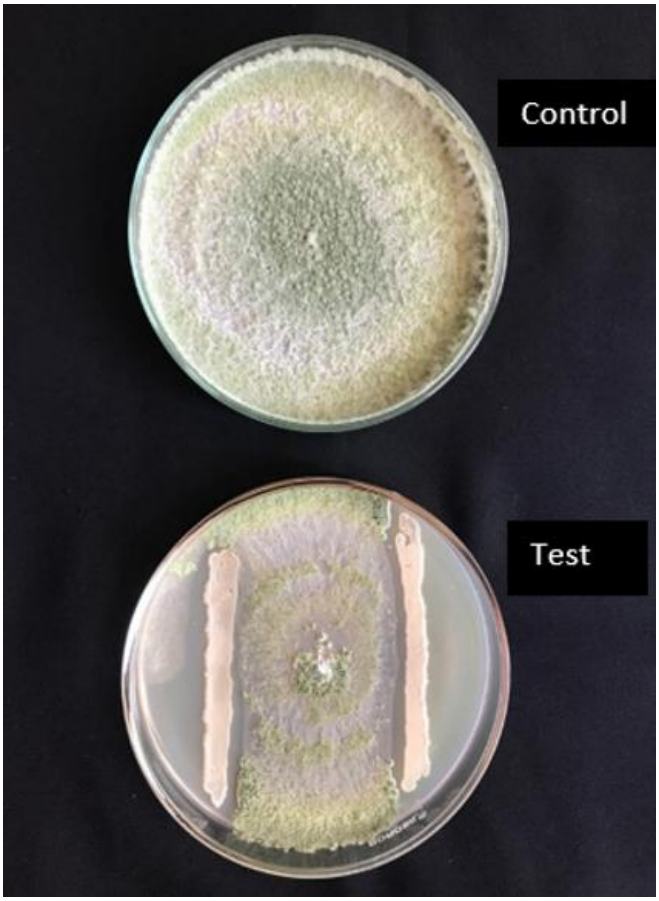


Fig: 2.2 *Trichoderma spp.*

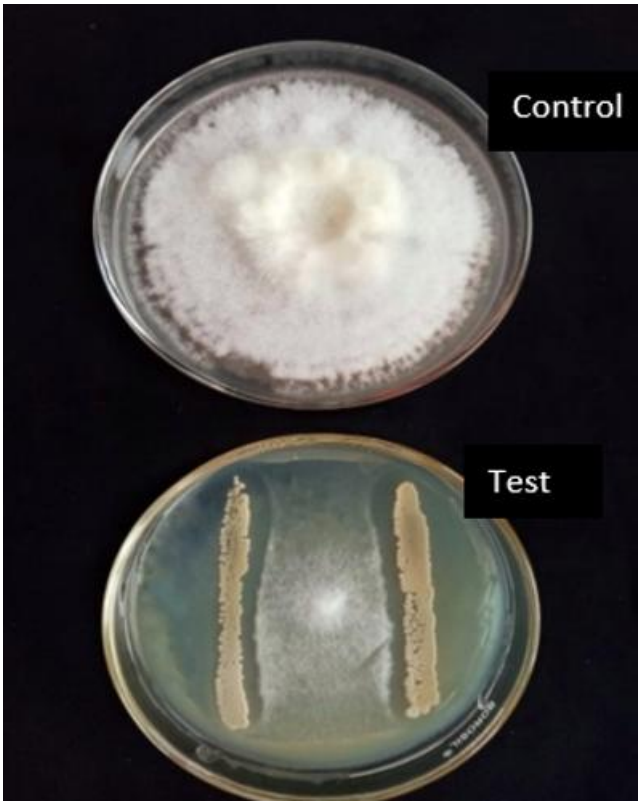


Fig:2.4 *Lichtheimia spp.*

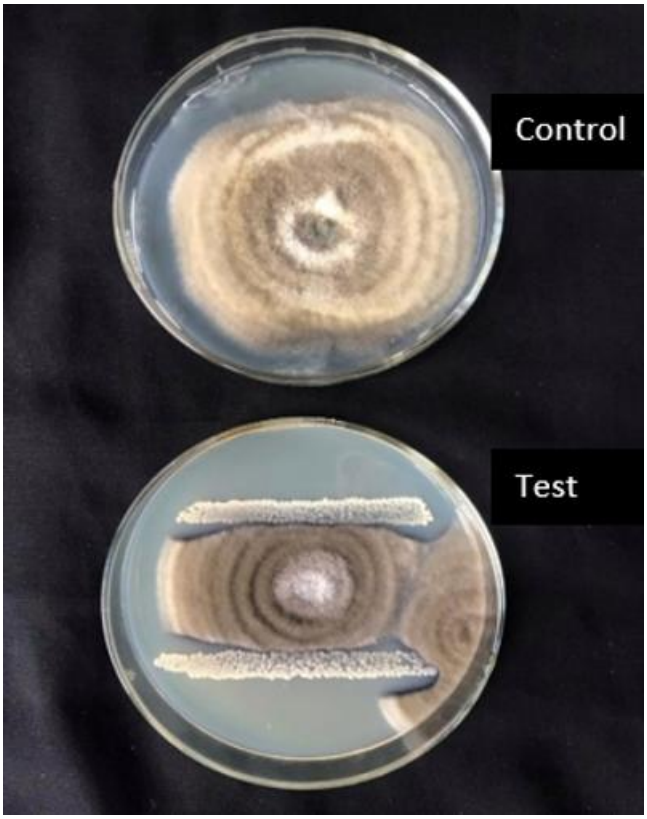


Fig:2.3 *Scopulariosis spp.*

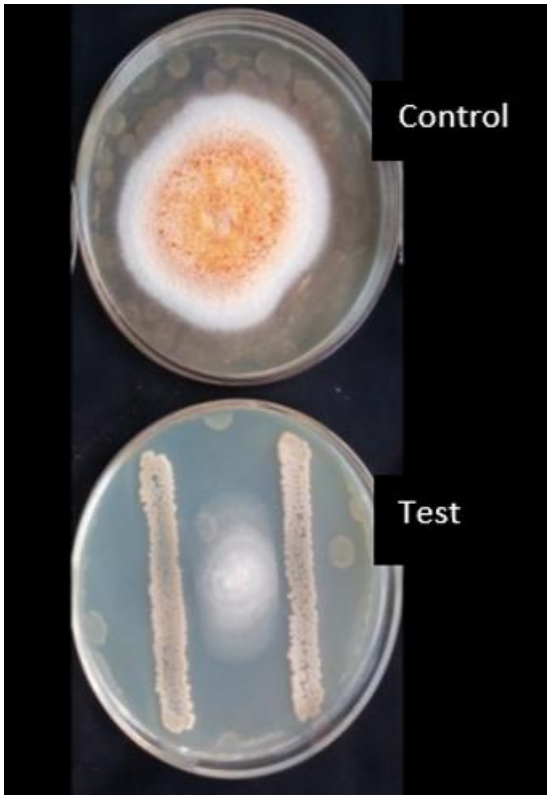


Fig: 2.5 *Fusarium spp.*

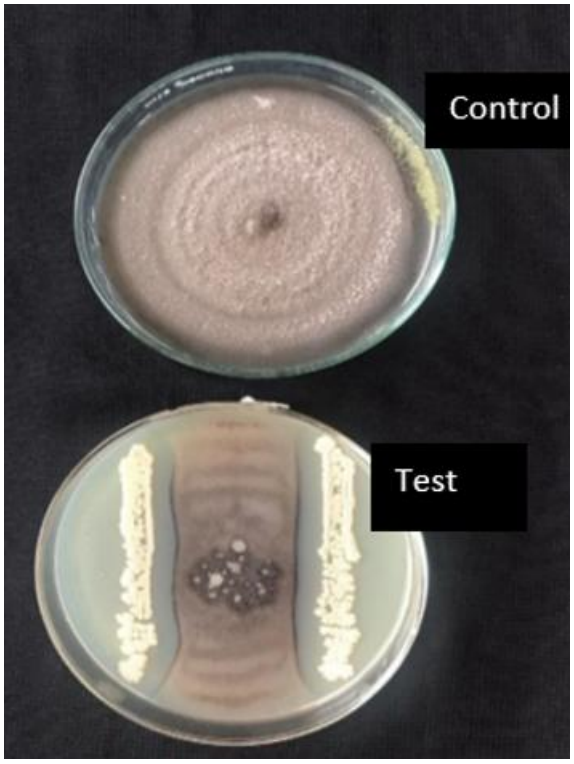


Fig: 2.6 *Curvularia spp.*

In the culture filtrate assay of the isolate against fungi, the result was positive. In the assay *Trichoderma spp*., *Scopulariopsis spp*., and *Fusarium spp*, showed a weak inhibition whereas *Microsporum spp*., and *Lichtheimia spp*., showed a moderate inhibition. But *Curvularia spp*., showed a strong inhibition in the assay.

Biochemical tests were performed to understand the physiological activity of the bacteria. The IMViC test results show that Methyl Red and Citrate are positive, while indole and Voges Proskauer are negative. Enzyme analysis revealed that the strain was positive for catalase, gelatin hydrolysis, starch hydrolysis, cellulase, and lipase (fig 3.1-3.4) negative for protease and phosphate solubilisation.


Fig: 3.1 Cellulase

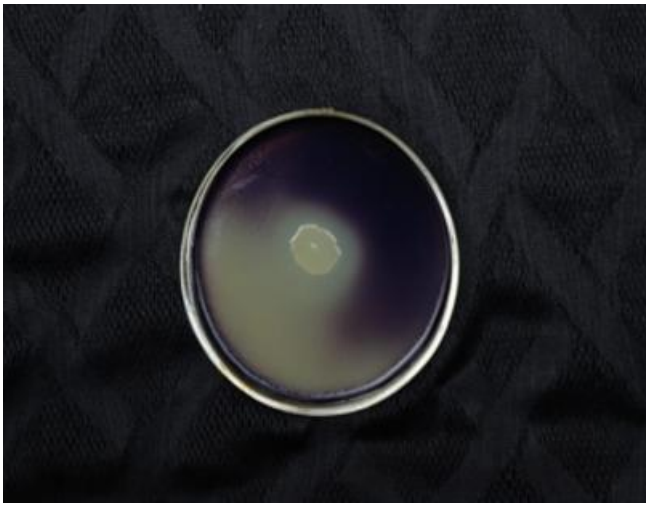


Fig: 3.2 Amylase

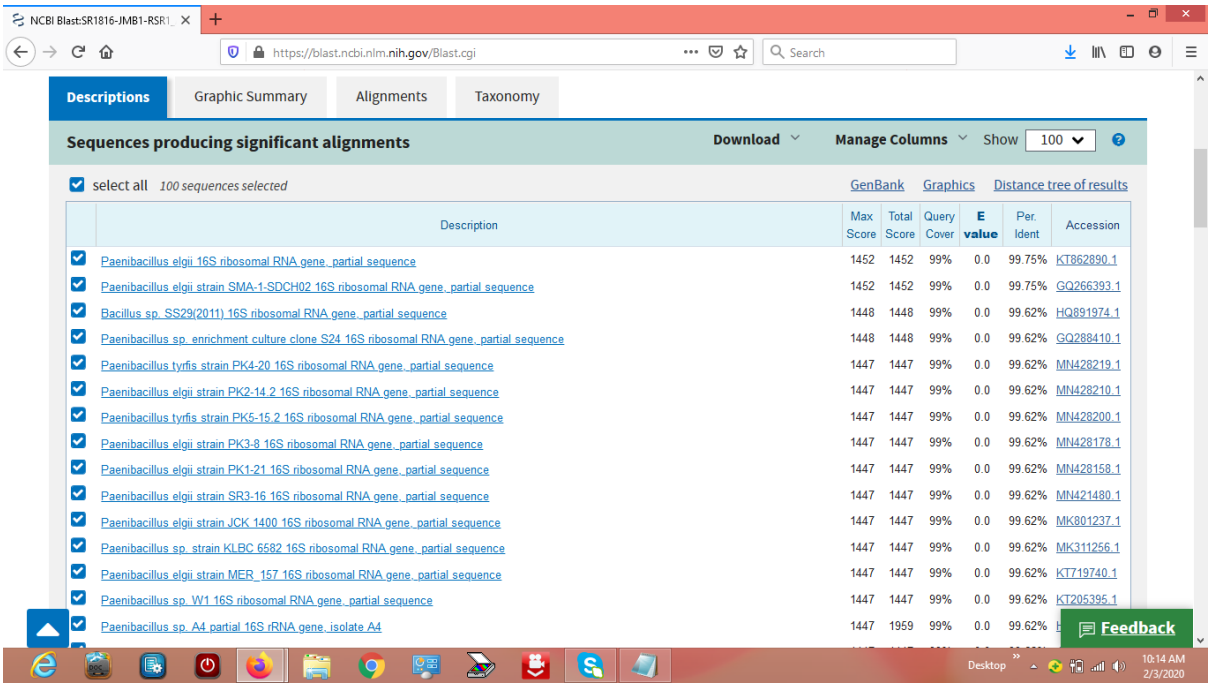

Fig: 3.3 Lipase


Fig: 3.4 Gelatin hydrolysis

After the extraction of the total genome and amplification of the 16S rRNA gene of the bacterial isolate, agarose gel electrophoresis showed that the amplified product was approximately 1,500 base pairs in size.

Prior to sequencing, the purity and concentration of the DNA sample was measured. The isolate's sequence was submitted to the NCBI Nucleotide BLAST tool, and a list of the 100 sequences most similar to the query sequence was obtained. All bacteria listed in the table were belonging to the genus Paenibacillus, confirming that the isolate also belongs to this genus. Table 2: represents the query sequence (that is rRNA gene sequence of the isolate)

Table 2: Top 10 similar sequences compared to the 16S rRNA sequence of the isolate acquired from NCBI nucleotide BLAST



The NCBI Nucleotide BLAST analysis of the 16S rDNA sequence revealed high homology with members of the genus Paenibacillus, displaying 99.75% sequence similarity with strain of P.elgii.

4.Discussion

Biological control is recognized as a sustainable alternative to chemical pesticides, particularly due to its minimal environmental impact and compatibility with integrated pest management strategies (Berg et al., 2020. This study explored the antifungal potential of a soil-isolated bacterial strain, identified as Paenibacillus elgii, known for its production of diverse antimicrobial metabolites (Grady et al., 2016). Although this strain did not exhibit antibacterial effects, it demonstrated strong antifungal activity through diffusible compounds, supporting findings that Paenibacillus species inhibit fungal pathogens via the production of antibiotics and enzymes (Raza et al., 2008).

The isolate showed notable antagonism on agar-based media but reduced efficacy in liquid extracts. Enzymatic assays confirmed the production of hydrolytic enzymes such as amylase, lipase, catalase, cellulase, and gelatinase, which contribute to the degradation of fungal cell walls. (Miller et al., 2018). The evolutionary distinction of Paenibacillus from Bacillus based on 16S rRNA gene analysis further add the phylogenetic uniqueness of this genus.

Beyond plant disease control, P. elgii and related species offer applications in bioremediation and pharmaceutical development due to their production of antimicrobial peptides and extracellular polysaccharides (Luo et al., 2015). However, environmental conditions still limit their consistent field performance, necessitating further research into their ecological adaptability.

5.Conclusion

The present study successfully identified and characterized a soil-derived bacterial isolate, Paenibacillus elgii, with notable antifungal potential. Although the isolate exhibited minimal antibacterial activity, its strong inhibitory effects against multiple fungal pathogens including Curvularia spp., Fusarium spp., and Trichoderma spp. in dual culture assays proving its promise as an effective biocontrol agent. Biochemical analyses confirmed the production of key extracellular enzymes such as amylase, cellulase, catalase, and lipase, which likely contribute to its antifungal mechanism through degradation of fungal cell wall components. Molecular identification via 16S rRNA sequencing further validated the isolate as P. elgii with 99.75% similarity. Due to its broad-spectrum antifungal activity and enzymatic versatility, P. elgii holds significant potential for applications in sustainable agriculture and integrated disease management practices. Overall, P. elgii emerges as a promising microbial resource with considerable utility in environmentally friendly pest and pathogen control strategies.

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