



The Molecular Identification of Isolated Fluoride-resistant Plant Growth-promoting Fungi

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ABSTRACT

Background: This study is about finding plant growth-promoting fungi (PGPF) that can resist fluoride, which is important for sustainable farming, especially in areas with fluoride contamination. Fluoride is commonly found in water and soil and it can harm plant health and agricultural productivity. Some fungi have developed ways to grow in high-fluoride conditions, helping plants grow and aiding in cleaning up the environment. We focused on identifying useful PGPF from the soil around plant roots, which is known for having many diverse microbes.

Method: We tested the successful fungi isolates for their PGPF capabilities by using PCR to amplify the Internal Transcribed Spacer (ITS) region, a commonly used technique for identifying different fungal species. After amplifying the DNA, we sequenced the PCR products and analyzed the sequences using the BLAST database to identify the species.

Result: Our research identified important fluoride-tolerant PGPF species, including *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium chrysogenum*. These fungi showed abilities such as breaking down phosphate, producing growth hormones and stopping pathogens in high fluoride conditions. Adding these PGPF to farming practices has the potential to create strong fungal inoculants that can boost crop growth and productivity in areas affected by fluoride. Overall, this study lays the groundwork for more research and field tests to confirm how these fungi can be used practically, which may help improve sustainable farming and food security.

Key words: Fluoride resistance, ITS Region, Molecular identification, Plant growth-promoting fungi, Sustainable agriculture.

INTRODUCTION

Fluoride is a naturally occurring element found in various environmental sources such as minerals, magmatic gases and industrial processes. It disperses through the atmosphere and water, leading to widespread exposure. (Prabhu *et al.*, 2023; Singh *et al.*, 2023). Fluoride can enter plants through water, soil, or leaves, leading to various biochemical, physiological and molecular changes. (Kumar *et al.*, 2021). Continuous exposure to fluoride can result in stunted growth, chlorosis and necrosis of leaves, ultimately affecting the overall health and productivity of crops. (Chahine *et al.*, 2023b). Fluoride levels in soil can negatively affect crop productivity and pose dangers to environmental health (Rizzu *et al.*, 2021). Elevated levels of fluoride, which are prevalent in industries, artificial fertilizers (Type NPK) and or irrigation systems that utilize contaminated water, can affect soil microbiological activities and nutrients, thereby impairing soil fertility (Malik *et al.*, 2024; Singh *et al.*, 2023). The accumulation of fluoride in plants inhibits fundamental physiological mechanisms, including photosynthesis, respiration and enzyme activity, which restricts crop growth, leads to leaf chlorosis and reduces yield (Kamruzzaman *et al.*, 2025). Long-term fluoride residuals can bioaccumulate into edible plant portions and cause transfer through food systems, which may be detrimental to human and animal health. To combat this issue, the implementation of sustainable agricultural practices and pollution controls is warranted. Researchers have been examining whether the molecular identification

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of fluoride resistant plant growth promoting fungi (PGPF) may present new opportunities for sustainable agricultural practices. The fungi contribute to plant growth via a variety of pathways and demonstrate resistance to environmental threats such as fluoride contamination which is a risk to agricultural soil quality (Boorboori *et al.*, 2022). Fluoride from varying sources (soil, water) can affect plant health

which limits agricultural productivity. The capacity of some fungi to survive and thrive in high fluoride levels marks them as potentially valuable organisms, capable of being used for bioremediation as well as in eco-friendly agricultural systems. The reported benefits of the fungi include enhancing nutrient uptake while improving plant health by suppressing plant disease (Whipps, 2001).

The accurate molecular identification is needed for Fungi, that resist fluoride, using ITS region sequencing of rRNA genes because this method is common due to its high species variability according to Schoch *et al.* (2012). Fungi identification relies on the ITS region, which functions like a genetic barcode for precise classification (Nilsson *et al.*, 2019; Nizamani *et al.*, 2020).

Researchers have recently identified PGPF species in fluoride-contaminated areas which demonstrate both survival capabilities and the ability to boost plant growth under these adverse conditions (Kanthiya *et al.*, 2025). The ability to perform two functions simultaneously is essential for creating biotechnological advancements that improve crop production in polluted locations. For instance, various species related to the genera *Trichoderma*, *Penicillium* and *Aspergillus* have shown potential for PGP and fluoride resistance. The molecular characterization of these fungi through Sanger sequencing and bioinformatic analysis helps in understanding their mechanisms of action and potential applications (Raja *et al.*, 2017; Schoch *et al.*, 2014). This knowledge allows the development of fungal inoculants that can be used in fluoride-affected soils, thereby promoting sustainable agriculture and food security. In the present study, we molecularly identified fungi that demonstrated positive results in various assays, including siderophore production, phosphate solubilization, ammonia production, hydrogen cyanide (HCN) production and biocontrol activity against pathogenic fungi in high-fluoride soil environments. We employed molecular techniques to accurately determine the fungal species of the most promising Plant Growth-Promoting Fungi (PGPF) isolate.

MATERIALS AND METHODS

In the present study, we have isolated the fungus species from evaluated were isolated from the rhizosphere of plants in fluoride-impacted areas of Sikar District of Rajasthan and Bhopal District of Madhya Pradesh and the work performed at Department of Bioscience at Mody University of Science and Technology, Lakshmangarh, Sikar (Rajasthan) and CMBR Biotech Pvt Ltd, Bhopal, MP during 2023 to 2024.

The present study involved the molecular identification of fungi that showed positive results in various assays, such as siderophore production, phosphate solubilization, ammonia production, hydrogen cyanide (HCN) production

and biocontrol activity against pathogenic fungi in high-fluoride soil environments. The following methods we used for the molecular validation of the most promising Plant Growth-Promoting Fungi (PGPF) isolates:

Pure culture preparation

The pure fungal cultures were prepared by inoculating fungal isolates in Potato Dextrose (PD) broth, a nutrient-rich medium commonly used for cultivating fungi. This medium encourages fast fungal growth, which is needed for the development of sufficient biomass for subsequent genomic analyses. The cultures were incubated under controlled environmental conditions, including appropriate temperature and humidity levels, to facilitate robust fungal growth and minimize contamination risks. Care was taken to monitor the growth phase of the fungi, ensuring maximum yield before proceeding to DNA extraction (Kanthiya *et al.*, 2025).

Genomic DNA extraction

The extraction of genomic DNA from the fungal biomass was accomplished using the CTAB (cetyltrimethylammonium bromide) method (Prakash *et al.*, 2019; Choudhary *et al.*, 2024), a reliable and widely used technique for isolating DNA from fungi and plants. This process began with the lysis of fungal cell walls and membranes using a combination of chemical reagents and physical disruption. The CTAB solution effectively bound contaminants such as polysaccharides, proteins and other cellular debris, allowing for the separation and purification of DNA. The final product was high-quality DNA that was free from impurities and suitable for downstream applications like amplification and sequencing.

PCR amplification

The polymerase chain reaction (PCR) was employed to amplify the Internal Transcribed Spacer (ITS) region of the fungal rRNA gene a region widely recognized for its utility in fungal identification and phylogenetic studies (Jadhav *et al.*, 2021). Universal fungal primers (listed in Table 1) were utilized to target this conserved region. The PCR process included three main stages: denaturation of the DNA double helix at high temperatures to produce single-stranded templates, annealing of primers to complementary sequences and extension of primers by DNA polymerase to synthesize new DNA strands. This resulted in specific and reproducible amplification of the ITS region, which serves as a genetic marker for fungal identification.

Sequencing and analysis

The amplified PCR products were then purified to remove residual primers, dNTPs and other reaction components.

Table 1: Universal primer sequence used in present study are given below.

Primer	Sequence	PCR product	Reference
ITS1 Fw	TCCGTAGGTGAACCTGCGG	≈ 300 bp	White <i>et al.</i> , 1990
ITS4 Rv	GCTGCGTTCTTCATCGATGC		

The purified samples were sequenced to determine the nucleotide composition of the ITS region (Nuratika *et al.*, 2020). This sequencing data was subsequently analyzed using the Basic Local Alignment Search Tool (BLAST) database—a powerful resource for comparing genetic sequences. The fungal ITS sequences obtained in the study were matched against known sequences in the database, facilitating accurate identification of the fungal species. This step provided valuable insights into the genetic diversity and phylogenetic relationships of the fungi under investigation.

RESULTS AND DISCUSSION

The molecular analysis conducted in this study validated the species identification of fluoride-resistant fungal organisms that may contribute significantly to sustainable agriculture in fluoride-affected areas. In the present study, we molecularly characterized *Trichoderma harzianum*, which proved to be the most effective biocontrol agent due to its ability to enhance plant resistance against fungal pathogens through the production of secondary metabolites and enzymes with antipathogenic properties (Haque *et al.*, 2025).

Other key representatives were also validated, including *Cladosporium cladosporioides* and *Penicillium chrysogenum*, both recognized for their capabilities in phosphate solubilization and production of auxin-type plant growth hormones, particularly indole acetic acid (IAA) (Seibold *et al.*, 2024). These molecular identifications confirm the taxonomic classification of these fungal species and provide essential information regarding their functional roles in improving plant growth under fluoride stress conditions.

All of these fungi demonstrated robust growth in high fluoride environments, indicating their effective stress management capabilities. Moreover, these fungal taxa function as competitive inhibitors and produce antifungal metabolites. Specifically, *Cladosporium cladosporioides* could be classified as an antifungal pathogen suppressor, while *Penicillium chrysogenum* and *Aspergillus niger* emerged as significant isolates that solubilized phosphate and generated bioactive compounds supporting plant flexibility and productivity (Hasan *et al.*, 2025).

The findings for all isolated fungi were successfully validated through molecular techniques that amplified the Internal Transcribed Spacer (ITS) region of fungal DNA. This sequencing confirmed both the identification and taxonomic classification of these fungal species, legitimizing their traits and contributing to our understanding of their functional characteristics as crucial contributors to plant growth, particularly in stressful conditions (Fig 1-3).

Collectively, these results demonstrated the functional diversity associated with the identified PGPF and provide a foundation for applying these fungi to enhance crop productivity, supporting sustainable and resilient agricultural practices in fluoride-contaminated conditions.

Molecular techniques, particularly ITS region sequencing, have revolutionised fungal species identification, providing crucial insights into their functional roles and agricultural applications. Fungal species can be identified with high precision using ITS region sequencing, which remains the “gold standard” for fungal identification (Wu *et al.*, 2023). Recent advancements in metabarcoding

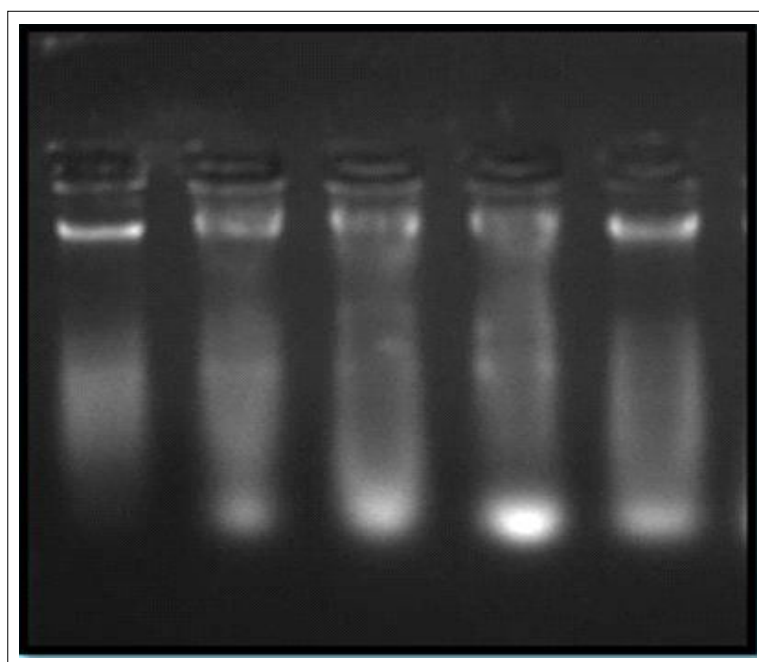


Fig 1: Present figure showing the results of genome extraction from fungi.

approaches have further enhanced the resolution and throughput of this method (Abarenkov *et al.*, 2022). This technique has proven especially valuable in differentiating cryptic fungal species that traditional culturing methods struggle to identify (Raja *et al.*, 2022). Characterized plant growth-promoting fungi (PGPF) demonstrate multiple functional traits including phosphatase production, phosphate solubilization, enhanced iron availability through siderophore production and biosynthesis of ammonia and hydrogen cyanide (HCN), while also suppressing pathogenic fungi. These capabilities are particularly valuable in fluoride-contaminated environments where soil fertility and plant growth are severely compromised. For instance, recent studies have demonstrated that

Trichoderma harzianum not only exhibits strong biocontrol properties against soil-borne pathogens but also significantly enhances nutrient acquisition pathways, effectively improving overall plant health and stress tolerance (Sharma *et al.*, 2023). Similarly, the phosphate solubilization capabilities of *Aspergillus flavus* and *Aspergillus niger* have been shown to mitigate nutrient deficiencies commonly associated with fluoride-contaminated soils. These fungi play a critical role in converting insoluble phosphate forms into bioavailable forms that plants can readily utilize (Verma *et al.*, 2024). Recent metabolomic analyses have revealed the specific organic acids responsible for this solubilization process, providing new insights into optimization strategies (Liu *et al.*,

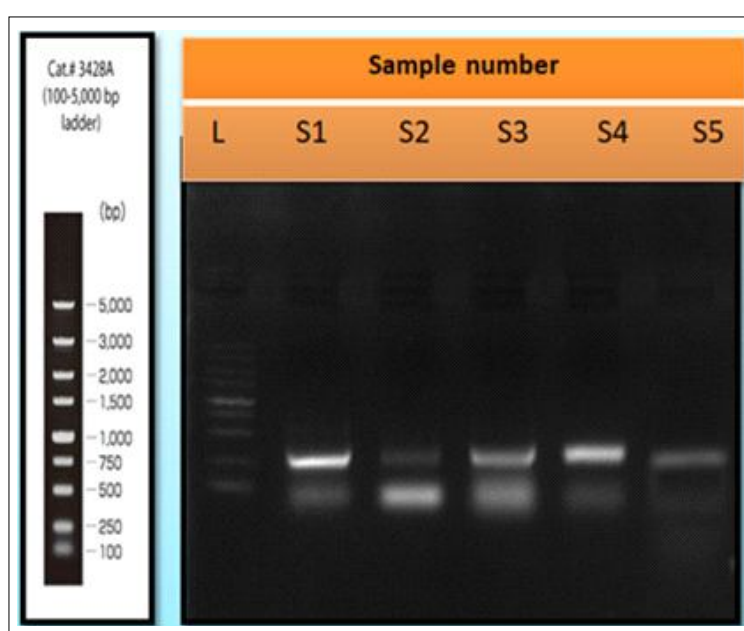


Fig 2: Present figure showing the results of molecular analysis as PCR of ITS region.

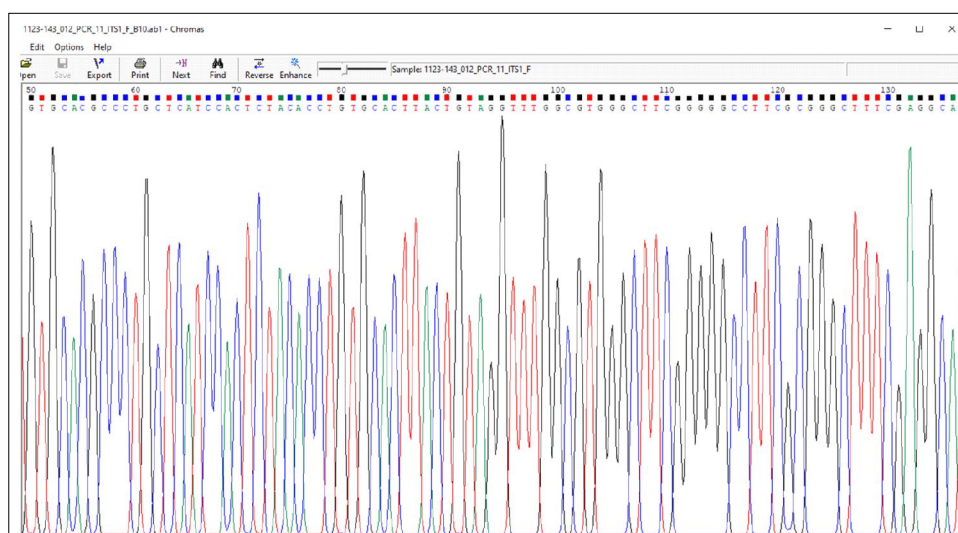


Fig 3: Representative image displaying a high-quality electropherogram during the present study.

2023). The antifungal activity of *Cladosporium cladosporioides* and the bioactive compounds produced by *Penicillium chrysogenum* further complement these functions by suppressing detrimental pathogens and enhancing plant stress tolerance (González-Rodríguez *et al.*, 2024). Meta-analysis of field trials has demonstrated that these antifungal properties remain effective across diverse soil conditions, including those with moderate fluoride contamination (Kumar *et al.*, 2023). Integration of these fungi into agricultural systems presents a viable approach for rehabilitating soil and addressing nutrient deficiencies resulting from fluoride contamination. Implementation of PGPF inoculants can potentially reduce dependence on chemical fertilizers and pesticides, thereby promoting alternative and sustainable agricultural practices with reduced synthetic inputs. While laboratory studies consistently demonstrate the effectiveness of these fungi, comprehensive field evaluations are essential to ensure that fungicide formulations and bioactive compounds can be practically implemented and their efficacy optimized under real-world conditions. Addressing the challenges associated with fluoride contamination, recent research emphasizes the significant potential of molecularly identified PGPF in contributing to sustainable agricultural practices. A 2024 meta-analysis by Patel and colleagues demonstrated that PGPF applications resulted in average yield increases of 18-27% in crops grown in moderately contaminated soils (Patel *et al.*, 2024). The deployment of PGPF in agricultural systems creates opportunities to enhance soil health while simultaneously improving crop yields, ultimately supporting environmental sustainability, promoting food security and building resilience against pollution challenges. Future research should focus on understanding the genetic basis of fluoride tolerance in these fungi and exploring the potential for developing improved strains through traditional selection or genetic approaches. Additionally, field trials investigating the performance of these molecularly characterized fungi under various fluoride contamination levels would provide practical validation of their effectiveness in real agricultural settings.

CONCLUSION

This molecular study successfully validates several key fluoride-resistant plant growth-promoting fungi (PGPF), including *Trichoderma harzianum*, *Aspergillus flavus*, *Aspergillus niger*, *Cladosporium cladosporioides* and *Penicillium chrysogenum*. These species demonstrate significant potential for enhancing sustainable agricultural practices in fluoride-stressed environments through their roles in nutrient cycling, pathogen suppression and growth promotion.

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Conflict of interest and ethical statement

The authors state that there are no conflicts of interest concerning the publication of this article. Additionally, no funding or sponsorship affected the study's design, data collection, analysis, publication decision, or manuscript preparation. Additionally, no ethical approval was required for this work, as the research did not involve human or animal subjects and did not entail any procedures subject to ethical review.

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