



Exploring Plant Growth-promoting Fungi from Rhizosphere for Improving Aromatic Rice Growth

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ABSTRACT

Background: The prolonged and unregulated application of inorganic fertilizers and pesticides declines soil productivity and fertility. This scenario has prompted the exploration of environmentally sustainable and economically reasonable alternatives for enhancing crop production.

Methods: Therefore, the present investigation focused on the plant-growth-promoting (PGP) activities of fungi isolated from the rhizospheric region of rice plants (*Oryza sativa* L.) through serial dilution techniques to be utilized as potential biofertilizers. Then they were screened for several plant growth promoting abilities. The most potent one was selected for *in vitro* growth promotion of aromatic rice.

Result: Five fungal strains (NF1, NF2, NF3, SP1 and SP2) with distinct morphological features were isolated and screened for PGP properties. NF3 (*Paradendryphiella arenariae*) exhibited positive results for most PGP traits and was selected for further investigations. Seed bio-priming of two local aromatic rice cultivars (Gobinda Bhog or GB and Badshah Bhog or BB) with the selected fungal strain enhanced seed germination percentage (1.2-fold), root-length (1.25 fold) and shoot length (1.40-fold) and dry weight (1.22-fold and 1.3-fold), along with an increase in tillers per plant (1.6-fold) and elevated pigment production (1.18-fold). The findings provided vital insights for the development of a biofertilizer for aromatic rice. Consequently, further research will be carried out to assess the efficacy of this isolate under field conditions, focusing on the growth, tillering, yield and aroma content of aromatic rice.

Key words: Badshah bhog, Biofertilizer, Gobinda bhog, *Paradendryphiella arenariae*.

INTRODUCTION

The rhizomicrobial community surrounding plant roots, known as the microbiome, plays a pivotal role in crop quality by offering essential services such as organic matter decomposition, nutrient acquisition, water absorption and biocontrol (Hossain and Farjana, 2020). Among these communities, plant growth-promoting microbes (PGPM) include beneficial fungi (PGPF) that establish non-pathogenic relationships with plants, enhancing their growth and health (Berg *et al.*, 2020). However, excessive use of chemical fertilizers and pesticides has disrupted the rhizospheric microflora, posing threats to human health, the environment and crop quality (Adedayo and Babalola, 2023 and Dey *et al.*, 2024a). Farmers often overuse fertilizers in rice fields to meet high-yield demands, negatively impacting the aroma and quality of aromatic rice varieties, which are prized for their unique flavor and fragrance (Verma *et al.*, 2020). With increasing consumer demand for fragrant rice, preserving its quality while maintaining sustainable farming practices is essential. Modern agricultural practices and the heavy reliance on inorganic fertilizers have led to a progressive decline in the aroma quality of aromatic rice (Deshmukh *et al.*, 2016). Biofertilizers, particularly fungal biofertilizers, have emerged as eco-friendly alternatives to enhance crop production, plant health and soil fertility (Ranipa *et al.*, 2023). Recent studies underscore their potential for sustainable agriculture, reducing dependence on harmful chemicals.

Paradendryphiella arenariae, a marine fungus known for its unique secondary metabolites (Pan *et al.*, 2008), has been isolated from the rice rhizosphere in the Purba

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Bardhaman district. Despite limited research on its role as a plant growth-promoting fungus, our study investigates its potential as a biofertilizer for two local aromatic rice varieties, GB and BB. This study aims to explore the *in vitro* effects of *P. arenariae* on aromatic rice growth and yield, promoting environmentally sustainable and economically viable agricultural practices. By incorporating fungal biofertilizers, we seek to enhance rice production while mitigating the adverse impacts of inorganic fertilizers on human health and the ecosystem.

MATERIALS AND METHODS

Soil collection was carried out in West Bengal, Purba Bardhaman district, from the rhizosphere region of rice

plants. Potato Dextrose Agar (PDA) was utilized as a minimal medium for fungus isolation. The soil suspension (1 g collected soil in 9 mL sterile double distilled water) was serially diluted (10^{-1} to 10^{-5}) and appropriate dilutions were spread plated in PDA medium (supplemented with Chloramphenicol 0.5%). The culture plates were incubated for 7 days at $28 \pm 2^\circ\text{C}$ in an incubator. The fungal isolates were distinguished based on morphological (colour, growth and texture) and microscopic characteristics (mycelium, conidia/spore structure) using a light microscope (Leica D750, Germany) from 7 days old plates (Debbarma *et al.*, 2021). The cultural features of fungal isolates were identified using the Royal Horticultural Society Colour Charts Edition V, London. The unidentified dominant species was sent to the National Council for Microbial Resources (NCMR), Pune, for molecular identification. The phosphate solubilization potential of isolates was assessed using NBRIP medium, calculating the Phosphate Solubilizing Index (PSI) as per Gupta *et al.* (2022). Indole-3-acetic acid (IAA) production was determined following Kumar *et al.* (2017) by incubating fungal discs in Czapek Dox broth with L-tryptophan, followed by centrifugation and colorimetric analysis with Salkowsky reagent at 533 nm. Ammonia production was evaluated in peptone broth using Nessler's reagent, with absorbance measured at 450 nm (Dey *et al.*, 2024b). Exo-polysaccharide production was quantified using the phenol-sulfuric acid method at 490 nm. Organic acid production was qualitatively tested via a plate assay with bromocresol green, assessing yellow zone formation and pH reduction (Narisetty *et al.*, 2023). Hydrolytic enzyme activity (amylase, protease, cellulase and pectinase) was detected using specific reagents: Lugol's iodine for amylase, mercuric chloride for protease, Congo red for cellulase and CTAB for pectinase (Yasmin *et al.*, 2020). Based on plant growth-promoting criteria especially phytohormone production and phosphate solubilization abilities of the five isolated strains (NF1, NF2, NF3, SP1, SP2), a single best-performing strain (NF3) was selected. The identification of the selected NF3 isolate was done by the 18S rDNA sequencing method analysis at the National Centre for Microbial Resources- National Centre for Cell Science (NCMR-NCCS), Pune, India. The isolate (Accession No. MCC9730) was deposited at NCMR, Pune, India. The sequence data was analyzed phylogenetically to decipher the evolutionary relationship among the selected gene of interest by using MEGA (Molecular Evolutionary Genetics Analysis version 7.0.18) software. The evolutionary distances were computed using the Maximum Composite Likelihood method (Kumar *et al.*, 2016).

Paddy seeds of two aromatic rice varieties GB and BB were treated with conidial suspension (1×10^8 conidium/L) and kept in an incubator shaker (100 ± 5 rpm at $28 \pm 2^\circ\text{C}$) for 3 h and 6 h, respectively, to facilitate the penetration of the inducer. The seeds treated with sterile distilled water served as control. The PGPF-treated seeds were aseptically air-dried and placed equidistantly on moistened sterile blotter

discs (3 layers) in Petri dishes to evaluate percent germination. Finally, treated and control sets of seeds (6 h) were sown in earthen pots filled with pre-sterilized potting medium (soil, farmyard manure and sand in the ratio of 2:1:1) and were maintained under greenhouse conditions [$28 \pm 2^\circ\text{C}$ at 80% relative humidity (RH)] (Naziya *et al.*, 2019). Rice seedlings were harvested 30 days and 60 days after fungal treatment. Shoots and roots length and dry weight of root-shoot were measured (Singh *et al.*, 2020). The germination percentage of seeds and tiller numbers per plant were counted in the treated and untreated sets. The total chlorophyll and carotenoids contents were estimated following the method of Singh *et al.* (2020) and Kumari *et al.* (2020) with some modifications. The concentration of the total chlorophyll and carotenoids content was calculated as per the standard formula.

The qualitative and quantitative analyses of plant growth-promoting properties were carried out in triplicate. The fungal inoculation and growth promotion studies consisted of three replicates of 10 seedlings observed per treatment. All experiments were carried out in the Applied and Molecular Mycology and Plant Pathology Laboratory, Department of Botany, Burdwan University, in 2024 (June to December). The experimental data of the current study was subjected to Analysis of Variance (ANOVA) using SPSS v. 20.0 (SPSS Inc., Chicago, IL, USA). Significant effects of treatments were determined from the magnitude of the P-value ($p \leq 0.05$) (Naziya *et al.*, 2019).

RESULTS AND DISCUSSION

Isolation of plant growth promoting fungi

The present study resulted in five fungal isolates namely, NF1, NF2, NF3, SP1 and SP2, from the rhizospheric soil of the two aromatic rice varieties taken from the Purba Bardhaman district of West Bengal, India. All of them appeared with distinct colony morphological features.

Morphological characterization and identification of the colonies

In the current study, the isolates showed morphological character variations like colony colour, growth, texture and margin (Table 1). The microscopic study revealed a variation in the mycelial and conidial structures. Identification revealed NF1 as *Penicillium* sp., NF2 as *Aspergillus* sp. and primarily unidentified NF3 was further sent to NCMR, Pune for molecular identification and was identified as *Paradendryphiella arenariae*, SP1 as *Saccharomyces* sp. and SP2 as *Alternaria* sp. In the studies of David *et al.* (2023), *Aspergillus* and *Penicillium* are the dominant fungal isolates found to colonize rice rhizosphere, whereas Ikram *et al.* (2023) have found *Alternaria* species in the soils of rice farmland.

Screening of isolated fungi for PGP properties

The plant growth promoting properties like P-solubilization and IAA production, were checked both qualitatively and quantitatively for all the isolates. All were found positive for

P-solubilization except SP2 (*Alternaria* sp.) (Table 2). The phosphate solubilization index, determined by measuring the diameter of the halo zone, resulted in a maximum value in NF3 (1.571) followed by NF1 (1.546) (Table 3). The quantitative estimation of dissolved phosphate content revealed a corresponding result where NF3 was found to be the most efficient phosphate solubilizer (0.804 $\mu\text{M/mL}$) (Table 3). Each of the isolated fungi was able to produce IAA and NF3 (9.413 $\mu\text{M/mL}$) was the best among all (Table 2; Table 3). Ammonia, organic acid and exopolysaccharide production are the indirect method of plant growth promotion and isolates were found positive in these traits too. Among all, SP2 (*Alternaria* sp.) and NF3 (*Paradendryphiella arenariae*) were the best EPS releasers (Table 2; Table 3). Here in this study, the two isolates NF3 (*Paradendryphiella arenariae*) and SP2 (*Saccharomyces* sp.) gave positive results for both amylase and protease enzyme test whereas NF2 (*Aspergillus* sp.) was positive for cellulase production and SP2 was positive for only protease enzyme and all of them was unable to produce pectinase enzyme (Table 2). Several researchers (Syamsia *et al.*, 2021, Numponsak

et al., 2018, Passos *et al.*, 2018, Wang *et al.*, 2020, Mukherjee *et al.*, 2023) also support our study and reported several plant-growth promoting traits of the fungi like *Alternaria*, *Aspergillus*, *Penicillium* and *Saccharomyces* etc.

Selection of single strain

Based on plant growth promoting abilities, especially phosphate solubilization, IAA production and EPS production, the strain NF3 (*Paradendryphiella arenariae*) was chosen as an ideal isolate for plant inoculation.

Growth pattern of the selected isolate

The growth curve of the selected fungi was recorded by measuring the dry weight of mycelia cultured over different periods (1 to 7 days). The growth curve depicted (Fig 1A) that after 5 days of inoculation the fungus reached its maximum biomass. An accentuated fall in the mycelial mass after 5th day was observed, possibly because the nutrient of the medium had been exhausted, leading to the death phase. There have been a few experiments regarding the growth of *Paradendryphiella arenariae*, but the studies of Landeta-Salgado *et al.* (2021) have reported that

Table 1: Colony morphological and microscopic features of the isolated fungi.

Isolates	Cultural characteristics					Microscopic observation	
	Colony colour		Growth	Texture	Margin	Mycelium	Conidia/spore
	Front	Back					
NF1	Deep green	Dull white	M	CF	R	Hyphae is hyaline	Globose conidia in chain
NF2	Black	Pale white	SL	P	I	Mycelium is septate and hyaline	Spores round with irregular projections on wall
NF3	Black	Yellowish black	F	FV	R	Mycelium is septate	Oval shaped multicellular conidia
SP1	Creamy white	Pale white	F	Flat	C	Mycelium is septate, branched and hyaline.	Round shaped conidia separated or in chain
SP2	Yellowish black	Olive green	M	P	C	Septate and branched mycelium	Multicellular conidia

SL: Slow; F: Fast; M: Medium; C: Circular; I: Irregular; R: Regular; CO: Cottony; P: Powdery; FV: Flat and velvety; CF: Cottony and fluffy.

Table 2: Various plant growth promoting traits of the isolated fungi.

Isolates	NF1	NF2	NF3	SP1	SP2
Phosphate solubilization	+	+	+	+	-
IAA production	+	+	+	+	+
Ammonia production	+	+	+	-	+
Exo-polysaccharide production	+	+	+	+	+
Organic acid production	+	+	+	+	+
Amylase production	-	-	+	+	-
Protease production	-	-	+	+	+
Cellulase production	-	+	-	-	-
Pectinase production	-	-	-	-	-

+ indicates positive; - indicates negative.

Paradendryphiella salina shows optimum growth at 4 days of incubation.

Molecular identification and phylogeny analysis of the selected isolates

The selected strain NF3 was further identified through molecular approach from NCMR, Pune, India. The 18S rDNA sequence of the strain was 1567 bp in length and comparing the sequence with nucleotide data bank EzBioCloud (www.ezbiocloud.net) and to sequences in the NCBI database by BLAST search analysis (<http://www.ncbi.nlm.nih.gov/>) showed *Paradendryphiella arenariae* CBS 181.58 as the closest neighbour of NF3 isolate having 99.73% sequence similarity. The strain was also deposited for public access in microbial culture centre (MCC), Pune, India with the accession number MCC9730. It has been well documented in the studies of Yoiprommarat *et al.* (2015) that *Paradendryphiella arenariae* is a marine fungus, no information is available on the occurrence of species in soil. We have isolated it from the rhizospheric soil and first time it will be documented as a potent plant growth promoting fungi. Subsequently, we performed a phylogenetic analysis using MEGA 7 software by the neighbor-joining method (Fig 1B). NF3 clustered with representatives of taxa that exhibited the highest level of

sequence identity and formed a clad that clustered with *Paradendryphiella arenariae* (NG 062992.1) with a bootstrap value of 99%. Based on molecular and phylogenetic similarities, isolate NF3 was therefore categorized as a member of the genus *Paradendryphiella* that is similar to *Paradendryphiella arenariae*.

Greenhouse experiment on aromatic rice

Plant growth parameters

Aromatic rice seedlings of GB and BB grown in pots with fungal (NF3) inoculum showed association with rice plants and significantly enhanced growth parameters. Root and shoot length and root and shoot dry weights are much higher in comparison with the control (Distilled water) in 30 days and 60 days old plants. A greater impact of inoculation in the GB variety was seen as compared to BB in terms of root (1.25-fold in 60 days) and shoot growth (1.40-fold in 60 days). Similar results were found in the case of root (1.22-fold in 60 days) and shoot (1.3-fold in 60 days) dry weight. After seed bioprimering, the germination percentage was also greatly enhanced (1.1-fold in GB and 1.2-fold in BB) in treated sets as compared to untreated. There was a significant difference ($p < 0.05$) in tillers per plant in the fungal inoculated plants compared to the control (Table 4). In contrast to previous results, tiller per plant

Table 3: Quantification of PGP traits by the isolated fungi.

Quantitative analysis ($\mu\text{M/mL}$)	NF1	NF2	NF3	SP1	SP2
Amount of IAA produced	6.548 ^a	5.879 ^{ab}	9.413 ^b	4.804 ^c	5.731 ^{ab}
Amounts of EPS produced	0.335 ^a	0.382 ^a	0.698 ^b	0.426 ^c	0.711 ^d
Amount of P-solubilized	0.765 ^a	0.704 ^a	0.804 ^a	0.512 ^b	0.065 ^c
P-solubilization Index	1.546 $\pm$ 0.09 ^a	1.525 $\pm$ 0.01 ^a	1.571 $\pm$ 0.03 ^a	1.428 $\pm$ 0.17 ^a	0
p-value	0.144	0.008	0.001	0.001	0.020

Different letters indicate statistically significant differences between groups (mean $\pm$ SD, $n = 3$, $p < 0.05$).

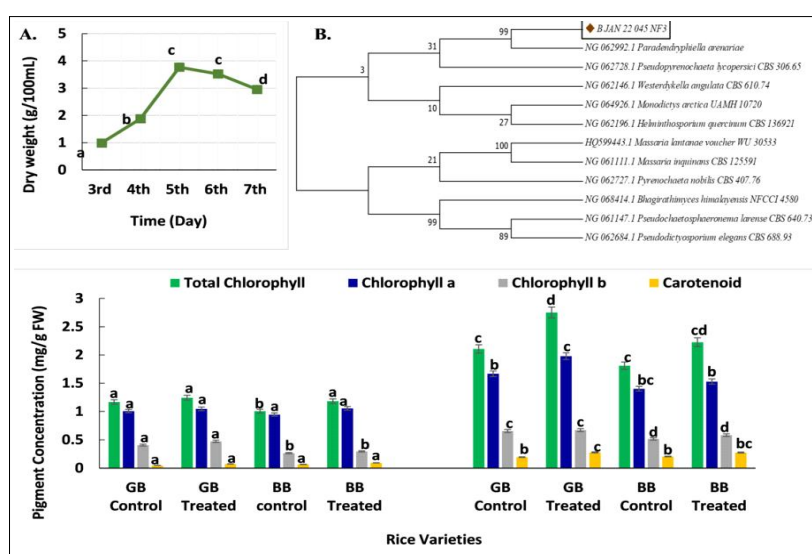


Fig 1: (A) Growth curve of NF3, (B) Phylogenetic tree of NF3 using MEGA7 software, (C) Estimation of total chlorophyll and carotenoid content in aromatic rice plants after inoculation.

Table 4: Treatment of aromatic rice cultivars with the selected fungal isolate and its effect on growth parameters under greenhouse condition.

Rice varieties	Sets	Germination percentage (%)	Root length (cm)			Shoot length (cm)			Root dry weight (g)			Shoot dry weight (g)			Tiller No. per plant (90 days)
			30days			60days			30days			60days			
			30days	60days	90days	30days	60days	90days	30days	60days	90days	30days	60days	90days	
Gobinda bhog	Control	80.15±0.64 ^c	12.54±3.01 ^{ab}	21.84±1.03 ^a	36.12±2.07 ^a	42.49±0.04 ^{ab}	3.81±0.04 ^a	8.13±1.07 ^a	9.9±0.05 ^b	18.01±0.4 ^b	5.8±0.01 ^d	9.9±0.05 ^b	18.01±0.4 ^b	5.8±0.01 ^d	
	Treated	88.76±2.88 ^a	16.90±2.62 ^b	27.41±1.79 ^a	47.08±3.11 ^b	59.60±1.05 ^c	5.1±0.02 ^b	9.92±2.01 ^b	15.54±0.01 ^{ab}	23.49±0.7 ^b	7.0±0.1 ^c	15.54±0.01 ^{ab}	23.49±0.7 ^b	7.0±0.1 ^c	
Badshah bhog	Control	77.97±1.08 ^b	10.65±0.84 ^a	20.60±2.04 ^a	31.39±0.89 ^a	56.49±2.17 ^b	3.05±0.02 ^a	8.76±0.07 ^a	9.48±0.01 ^b	17.05±0.2 ^a	6.5±0.05 ^b	9.48±0.01 ^b	17.05±0.2 ^a	6.5±0.05 ^b	
	Treated	95.41±1.46 ^a	11.82±1.50 ^a	25.17±2.74 ^b	38.85±1.73 ^a	61.04±2.11 ^a	4.7±0.11 ^a	9.89±0.12 ^a	12.19±0.01 ^a	21.68±0.25 ^a	10.5±0.09 ^a	12.19±0.01 ^a	21.68±0.25 ^a	10.5±0.09 ^a	
	p-value	0.001	0.031	0.028	0.026	0.033	0.040	0.033	0.021	0.008	0.002	0.021	0.008	0.002	

Different letters indicate statistically significant differences between groups (mean±SD, n = 3, p<0.05).

was more improved in BB treated variety (1.66-fold) compared to GB treated plants (1.4-fold). Similar results were reported by David *et al.* (2023) where rice plants treated with *Aspergillus niger* and *Penicillium crysogenum* responded with better shoot and root growth and dry biomass. Investigations by Singh *et al.*–(2023) have revealed that Pusa Basmati rice when treated with the plant growth promoting fungi *Trichoderma harzianum*, improved growth as well as enhanced drought stress tolerance.

Total chlorophyll and total carotenoids content

Both the rice varieties inoculated with fungal strain possessed significance variance ($p<0.05$) in terms of photosynthetic pigment status compared to control plants. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids contents were highest in 60 days old GB treated plants (1.98 mg/g FW, 0.673 mg/g FW, 2.752 mg/g FW and 0.278 mg/g FW respectively) (Fig 1C). Plant growth promoting fungi, with their phosphate solubilizing efficiency, indirectly affects pigment production in plants. In the current study, the selected isolate (NF3) was also proven to be an excellent P- solubilizer, which is reflected in the chlorophyll and carotenoids content of the two rice varieties. Similarly, Singh *et al.* (2023) have reported that Pusa Basmati rice variety inoculated with *Trichoderma harzianum* have increased pigment content in greenhouse conditions.

CONCLUSION

In conclusion, this study identifies five rhizospheric fungal isolates with plant growth-promoting potential, notably *Paradendryphiella arenariae*, a marine fungus first reported in rhizospheric soil. Molecular identification and *in vitro* experiments on aromatic rice varieties (GB and BB) confirm its growth-enhancing capabilities. These findings underscore the potential of leveraging beneficial fungi for sustainable agriculture. By elucidating plant-fungal interactions, this research offers eco-friendly strategies to improve crop yield and quality, addressing global food security challenges. The study highlights the importance of microbial resources in developing innovative, sustainable crop management practices.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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