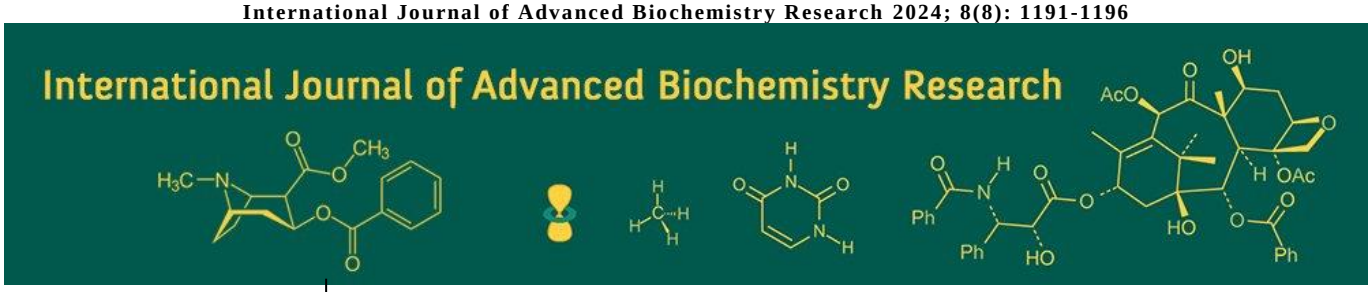

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Sukanya Das
Microbiology and Molecular Systematics Laboratory, Department of Botany Gauhati University, Guwahati, Assam, India

Jintu Rabha
Microbiology and Molecular Systematics Laboratory, Department of Botany Gauhati University, Guwahati, Assam, India

Diganta Narzary
Microbiology and Molecular Systematics Laboratory, Department of Botany Gauhati University, Guwahati, Assam, India

Corresponding Author:
Diganta Narzary
Microbiology and Molecular Systematics Laboratory, Department of Botany Gauhati University, Guwahati, Assam, India

Soil yeast diversity and their plant growth promoting activities in some forest soils of Assam, North-East India

Sukanya Das, Jintu Rabha and Diganta Narzary

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Abstract
Soil yeasts are important components of soil ecosystems and play crucial roles in maintaining soil health by participating in various soil processes. Soil yeasts are also gaining importance for their plant growth-promoting activities. However, yeasts are less explored in the soils of North-East India, including Assam. This study investigates the diversity of soil yeasts from two forest soils in Assam. A total of 44 yeast isolates were obtained from six different soil samples using two different techniques in four different culture media. Screening of all the isolates revealed their potential plant growth-promoting (PGP) activity, of which five isolates showing multiple PGP activities were considered for molecular identification. These yeasts with multiple PGP activities belong to the genera *Meyerozyma*, *Candida*, and *Debaryomyces*, respectively, which indicate the presence of diverse yeast species in the natural soils of Assam. The use of various media for yeast isolation might have helped in isolating different yeast species. These insights can channel sustainable agricultural practices involving soil yeasts and effective soil management strategies in the region.

Keywords: Forest soil, PGP activity, soil health, yeast diversity

Introduction
Natural soils harbor a vast array of microorganisms, and yeasts are one of them. Microorganisms play crucial roles in biogeochemical cycling and in maintaining soil quality. Fungi are known to play essential functions like nutrient recycling, organic matter decomposition, and plant growth promotion (Botha, 2011; Das *et al*., 2023; Nimsi *et al*., 2023) [2, 5, 15]. Unlike filamentous fungi, yeasts are primarily unicellular and not yet known for causing any major diseases in plants, except some *Cryptococcus* spp., which are reported to interact parasitically on plant surfaces to complete their sexual cycle (Xue *et al*., 2007) [22]. Yeasts produce extracellular polysaccharides that enhance soil aggregation and contribute to soil stability (Botha, 2011; Yurkov, 2018) [2, 24]. Despite their significant ecological roles, soil yeasts are less studied compared to other soil microorganisms, resulting in a limited understanding of their functions and contributions (Yurkov, 2017) [23].
The north-eastern region of India, including Assam, is a biodiversity hotspot with diverse flora and fauna (Chatterjee *et al*., 2006) [4]. The region's varied climate and soil types form unique environments for soil microorganisms, making it an ideal location for studying yeast diversity (Giri *et al*., 2022) [8]. However, there are limited studies focusing on the isolation and characterization of soil yeasts from Assam (Sood and Lal, 2009) [18].
Soil yeasts have been documented from several types of forest soils (Jaiboon *et al*., 2016; Mašínová *et al*., 2018; Mestre and Fontenla, 2021) [10, 11, 13]. Yeasts isolated from various forest soils have been shown to promote plant growth through several mechanisms, including the production of phytohormones (Jaiboon *et al*., 2016) [10]. Furthermore, Yurkov (2018) [24] has highlighted the role of yeasts isolated from forest soil. In the present study, yeasts have been isolated from two forest soils in Assam, using four different culture media to explore their diversity and ecological roles comprehensively.

Materials and Methods
Sample collection
Soil samples were collected from Garbhanga and Pobitora Wildlife Sanctuaries. Collection

of soil was done randomly from a depth of 0-10 cm after removing surface litter, such as dry twigs and fallen leaves. The collected samples were then placed in sterile polypropylene bags and stored at 4 °C until processing. The geographical locations, along with other descriptions of the sampling sites, are presented in Table 1.

Analysis of soil physico-chemical characteristics

The pH of soil samples was measured using an electronic digital pH meter (Eutech Cyberscan 510) in 1:2 (w/v) soil:water suspensions (https://anlab.ucdavis.edu/analysis/soils/207). Moisture content was determined as per the Methods Manual, Soil Testing in India, Department of Agriculture and Cooperation, Govt. of India, New Delhi (2011). Organic carbon content was assessed using the Walkley and Black (1934) [21] method. Available nitrogen was measured by following Subbiah and Asija (1956) [19]. Available phosphorus was determined using the Bray and Kurtz (1945) [3] method, while potassium content was determined according to Toth and Prince (1949) [20].

Yeast isolation

Yeast isolation was carried out using the method of Ferraz *et al*. (2016) [6], which employ a serial dilution and plating technique. For samples where yeasts isolation was unsuccessful through this method, an enrichment technique described by Hui *et al*. (2012) [9] was followed. For serial dilution and plating, 10 g of soil was dissolved in a 0.85% saline solution containing 0.85% NaCl and 0.05% Tween 80. This mixture was then agitated in an orbital shaking incubator (REMI) at 28±2 °C for 30 minutes at 250 rpm. Subsequently, one milliliter of the soil suspension was serially diluted. From each dilution, 1 ml was plated in triplicate on four media: yeast extract peptone dextrose agar (YPDA: 1% yeast extract, 2% peptone, 2% glucose, 2% agar; pH 6.5±0.2), yeast malt agar (YMA: 0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose, 2% agar; pH 6.2±0.2), potato dextrose agar (PDA, HiMedia), and sabouraud dextrose agar (SDA, HiMedia) supplemented with chloramphenicol (200 mg/L) and sodium propionate (0.25 g/L). All the plates were incubated in a Biochemical Oxygen Demand (BOD) incubator at 28±2 °C for 5-7 days.

Plant growth promoting (PGP) activity screening

All the isolates were screened for the presence of different PGP activities *viz*. production of indole-3-acetic acid (IAA), solubilization of phosphate, zinc as well as production of ammonia. The IAA production was evaluated by following the method of Nutaratat *et al*. (2014) [16] in the presence and absence of L-Tryptophan. The qualitative solubilization of phosphate was evaluated according to Nutaratat *et al*. (2014) [16] while the quantitative estimation was done by following Das *et al*. (2023) [5]. The solubilization of zinc was done according to the method given by Millan *et al*. (2020) [14]. The ammonia production ability of yeasts was evaluated by following the method of Fu *et al*. (2016) [7].

Genomic DNA isolation, nrDNA-ITS PCR and sequencing

For molecular characterization, genomic DNA was isolated following. The Internal Transcribed Spacer region of the nuclear rDNA (nrDNA-ITS) was amplified using the universal primer pairs ITS1 and ITS4. The amplified PCR product was purified with the HiMedia PCR purification kit and sent for Sanger sequencing to the service providers. The forward and reverse sequences were used to form contigs, which were then subjected to a BLAST homology search in the NCBI database to identify the fungal species.

Results

Analysis of soil physico-chemical characteristics

The pH of the collected soil samples ranged from 4.70±0.06 to 7.07±0.02 (Fig. 1A). Soil moisture content (Fig. 1B) was lowest in sample FS-A GWS (10.23±0.21) and highest in sample FS-B GWS (21.64±0.88). The highest percentage of organic carbon (Fig. 1C) was observed in sample FS-D GWS (2.67±0.05) and the lowest in sample FS-F PWS (1.14±0.06). The available nitrogen content (Fig. 1D) was highest in sample FS-D GWS (394.09±0.62 kg/ha) and lowest in sample FS-C GWS (180.64±1.76 kg/ha). Available phosphorus content (Fig. 1E) peaked in samples FS-A GWS (8.75±0.03 kg/ha) and FS-C GWS (8.75±0.06 kg/ha), while sample FS-D GWS (2.25±0.10 kg/ha) had the lowest. Similarly, available potassium content (Fig. 1F) was highest in sample FS-D GWS (277.78±4.69 kg/ha) and lowest in sample FS-F PWS (88.27±4.60 kg/ha).

Yeast isolates and CFU counts

From the six collected soil samples (A, B, C, D, E, and F), a total of 44 isolates were obtained (Table 2). Of these 20 isolates were obtained using the serial dilution and plating technique, and 24 isolates were obtained using the enrichment culture technique. In addition, as indicated in Table 2, yeasts were isolated from the soil samples B, E, and F by serial dilution and plating technique. Therefore, CFU counts of yeasts from these samples in the four media used for yeast isolation are presented in Table 3. Among all the media tested, YPDA medium supplemented with chloramphenicol and sodium propionate was found to be most suitable for isolating yeasts.

Plant growth promoting soil yeasts

The plant growth-promoting activities of soil yeasts are summarized in Table 4. Remarkably, all isolates demonstrated the ability to produce IAA when L-Tryptophan was present, and 38 out of 44 isolates were also capable of producing IAA without the addition of tryptophan. The yeasts exhibited effective solubilization of inorganic phosphate, as confirmed by both qualitative and quantitative assessments detailed in Table 4. In contrast, only 8 isolates showed the ability to solubilize zinc oxide. Additionally, 28 isolates tested positive for ammonia production, further highlighting their potential role in promoting plant growth (Table 4).

Molecular identification of yeast isolates with high PGP activities

For molecular identification, five representative isolates that produced relatively high levels of IAA and exhibited positive results for other plant growth-promoting (PGP) activities were selected. The resulting nrDNA-ITS sequences for these isolates were submitted to the NCBI GenBank, and the corresponding GenBank accession numbers are listed in Table 5. The yeast species identified in this study include *Meyerozyma guilliermondii*, *Candida* spp., *Candida tropicalis*, and *Debaryomyces castellii*.

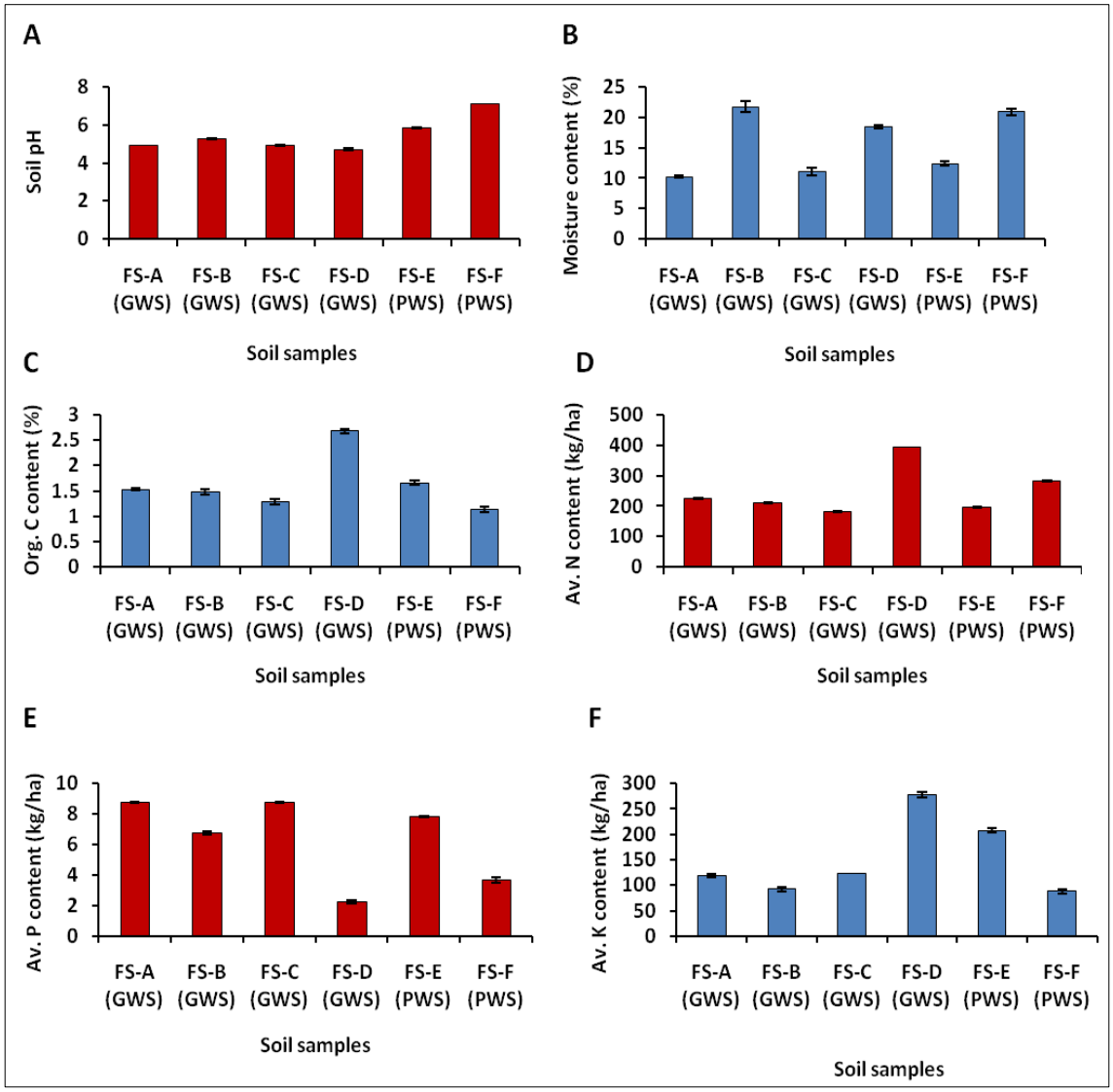

Fig 1: Soil physico-chemical characteristics of collected samples. A, Soil pH; B, Moisture content; C, Organic carbon content; D, Available nitrogen; E, Available phosphorus; F, Available potassium. Each column represents the mean of three replicates±standard error as bars above them. Av., available; C, carbon; N, nitrogen; P, phosphorus; K, potassium.

Discussion

Soil physico-chemical characteristics

The pH values of the soil samples ranged from acidic (4.70±0.06) to near-neutral (7.07±0.02). The high organic carbon content observed in all samples indicates substantial organic matter, which is essential for microbial activity and soil health. The variability in moisture content, from 10.23±0.21% in sample FS-A GWS to 21.64±0.88% in sample FS-B GWS, reflects differences in soil texture, structure, and water retention capacities across the sampled sites.

Yeast isolation and diversity

The present study reveals significant diversity among soil yeasts isolated from forest soils in Assam. A total of 44 yeast isolates were obtained from the six soil samples collected from the two sanctuaries. The isolation of yeasts from forest soils aligns with previous studies suggesting that undisturbed natural habitats tend to harbor a higher diversity of microorganisms due to their stable and heterogeneous environments (Jaiboon *et al*., 2016; Mašínová *et al*., 2017) [10, 11].

The use of two different isolation techniques: serial dilution and plating, as well as enrichment culture proved effective in observing the diverse yeast populations present in the soils. Notably, the enrichment technique yielded a higher number of isolates (24) compared to the serial dilution method (20), indicating its efficacy in recovering yeasts that might be present in lower numbers within the soil matrix (Hui *et al*., 2012) [9]. This finding underscores the importance of employing multiple isolation methods to obtain a comprehensive understanding of microbial diversity in soil ecosystems. Additionally, use of different media for isolation is recommended as it reveals the metabolic capabilities and physiological properties of the isolated microorganisms. This is crucial for their characterization and potential applications in various sectors.

PGP activities of yeasts in soil

The yeast species isolated from forest soils are likely to play significant roles in their ecosystems. Previously, Nimsi *et al*. (2023) [15] have reviewed the potential role of yeasts in plant growth promotion and development. Species such as *Meyerozyma guilliermondii* and *Candida tropicalis* have been shown to enhance plant growth through mechanisms like phytohormone production and inorganic phosphate solubilization (Amprayn *et al*., 2012; Sarabia *et al*., 2018). Furthermore, Jaiboon *et al*. (2016) [1, 17, 10] reported that

yeasts isolated from forest soils are capable of producing IAA. Zinc, an essential micronutrient for plant growth and development, can be solubilized from its insoluble forms, such as zinc oxide, by yeasts, as previously demonstrated by Millan *et al*. (2020) [14]. Additionally, yeasts that produce ammonia can support plant growth by supplying nitrogen, an essential macronutrient (Fu *et al*., 2016) [7]. These attributes indicate that soil yeasts contribute to soil fertility, making them promising candidates for biofertilizer development. Moreover, yeasts isolated from forest soils are recognized for their role in nutrient cycling and soil stability (Yurkov, 2018) [24]. The presence of these yeasts in forest soils also supports the idea that they contribute to the resilience of natural ecosystems (Botha, 2011) [2]. Therefore, understanding the ecological functions of soil yeasts can aid in the development of sustainable agricultural practices and effective soil management strategies in this region.

The potential parasitic interactions of some yeast species with plants, as reported by Xue *et al*. (2007) [22], require additional investigation to determine their impact on plant health. However, the overall benefits of yeasts in increasing soil stability and nutrient availability highlight their importance in both natural and managed ecosystems. Further research is needed to determine the specific functions of various yeast species in soil ecosystems, particularly in terms of plant-microbe interactions. Molecular approaches such as metagenomics and transcriptomics may provide more information about the functional diversity and metabolic capacities of soil yeasts. Additionally, studying the impact of environmental factors such as climate change and land-use practices on yeast diversity and function will be critical.

Table 1: Study area and soil sampling sites. FS, forest soil; GWS, Garbhanga Wildlife Sanctuary; PWS, Pobitora Wildlife Sanctuary.

S. No.	Study area	Sampling site	GPS location	Soil ID
1.	Garbhanga Wildlife Sanctuary (GWS), Kamrup, Assam, India	Site-1	26°00'02.1" N 91°30'03.4" E	FS-A (GWS)
		Site-2	25°59'16.0" N 91°31'44.0" E	FS-B (GWS)
		Site-3	26°00'59.7" N 91°30'16.2" E	FS-C (GWS)
		Site-4	25°58'52.3" N 91°32'05.1" E	FS-D (GWS)
2.	Pobitora Wildlife Sanctuary (PWS), Morigaon, Assam, India	Site-5	26°14'59.0" N 92°01'10.8" E	FS-E (PWS)
		Site-6	26°14'09.1" N 92°02'23.4" E	FS-F (PWS)

Table 2: Total number of yeast isolates obtained from the collected soil samples. FS, forest soil; GWS, Garbhanga Wildlife Sanctuary; PWS, Pobitora Wildlife Sanctuary.

Soil ID	Isolates obtained		Sub-total
	Serial dilution and plating technique	Enrichment technique	
FS-A(GWS)	-	10	10
FS-B (GWS)	9	-	9
FS-C (GWS)	-	10	10
FS-D (GWS)	-	4	4
FS-E (PWS)	6	-	6
FS-F (PWS)	5	-	5
Total	20	24	44

Table 3: Colony forming unit (CFU) counts of yeasts obtained by serial dilution and plating technique in different culture media. PDA, potato dextrose agar; SDA, Sabouraud dextrose agar; YMA, yeast malt agar; YPDA, yeast extract peptone dextrose agar. Isolates obtained from enrichment technique were not considered for CFU count.

Soil ID	Culture medium	CFU (per g soil)
FS-B (GWS)	YMA	3.0×10^2
	YPDA	8.25×10^2
	PDA	3.25×10^2
	SDA	2.75×10^2
FS-E (PWS)	YMA	3.0×10^2
	YPDA	5.5×10^2
	PDA	3.0×10^2
	SDA	2.75×10^2
FS-F (PWS)	YMA	5.5×10^2
	YPDA	5.75×10^2
	PDA	5.5×10^2
	SDA	Nil

Table 5: Yeast species name and GenBank accession number of the representative isolates. FS, forest soil; GWS, Garbhanga Wildlife Sanctuary; PWS, Pobitora Wildlife Sanctuary.

Isolate no.	Soil ID	Yeast species	Gen Bank accession no.
S-15	FS-A (GWS)	*Meyerozyma guilliermondii*	PQ083848
S-19	FS-A (GWS)	*Candida* sp.	PQ093787
S-21	FS-A (GWS)	*Candida tropicalis*	PQ156033
S-25	FS-E (PWS)	*Candida tropicalis*	OQ947271
S-34	FS-C (GWS)	*Debaryomyces castellii*	PQ084084

Table 4: Plant growth promoting (PGP) characteristics of yeast isolates recovered from two forest and soils of Assam. Values are expressed as the mean of three replicates±standard error, except for IAA where five replicates were considered. FS, forest soil; GWS, Garbhanga Wildlife Sanctuary; PWS, Pobitora Wildlife Sanctuary; IAA, indole-3-acetic acid; YPD, yeast extract peptone dextrose broth; w/o, without; TCP, tri-calcium phosphate; SE, solubilization efficiency in mm; ZnO, zinc oxide; NH_3, ammonia; '++' strong positive result; '+' weak positive result; '-' not detected.

Sl. No.	Soil ID	Isolate no.	IAA production (µg/ml)		TCP solubilization		ZnO solubilization (SE)	NH_3 production
			YPD with L-tryptophan	YPD w/o L-tryptophan	Qualitative (SE)	Quantitative (µg/ml)		
1	FS-E (PWS)	S-14	2.03±0.12	-	1.67±0.85	139.40±6.61	-	++
2	FS-A (GWS)	S-15	22.37±3.95	0.97±0.10	3.08±1.73	176.30±7.47	-	++
3	FS-A (GWS)	S-16	3.45±0.12	1.75±0.10	0.80±0.4	74.57±11.09	-	++
4	FS-E (PWS)	S-17	8.60±0.13	5.05±0.10	2.00±1.04	136.07±6.83	-	++
5	FS-A (GWS)	S-18	1.97±0.13	1.50±0.11	1.60±0.80	96.01±7.06	-	++
6	FS-A (GWS)	S-19	18.67±0.12	10.69±1.16	2.25±1.13	183.20±7.96	0.25±0.03	++
7	FS-E (PWS)	S-20	3.64±0.11	2.19±0.11	1.88±0.97	103.20±5.23	-	++
8	FS-A (GWS)	S-21	17.24±0.12	1.84±0.10	2.61±1.35	178.43±3.17	-	++
9	FS-E (PWS)	S-22	2.21±0.13	1.32±0.13	1.42±0.72	80.67±3.44	-	-
10	FS-E (PWS)	S-23	3.07±0.13	0.50±0.10	1.87±0.93	117.62±5.67	0.12±0.09	++
11	FS-E (PWS)	S-24	10.34±3.73	2.31±0.12	2.38±0.06	143.71±8.92	-	++
12	FS-F (PWS)	S-25	19.27±0.45	2.68±0.12	1.50±0.75	184.17±0.34	-	++
13	FS-F (PWS)	S-26	0.70±0.09	0.57±0.11	2.81±0.10	196.01±4.14	-	++
14	FS-A (GWS)	S-27	7.83±0.10	1.24±0.12	1.77±0.89	41.93±1.67	-	-
15	FS-A (GWS)	S-28	1.49±0.11	0.45±0.13	2.38±1.31	108.66±5.54	-	-
16	FS-A (GWS)	S-29	2.43±0.13	1.61±0.13	1.56±0.79	70.15±5.48	0.48±0.07	-
17	FS-A (GWS)	S-30	16.34±0.11	2.86±0.12	3.38±0.33	187.62±1.60	-	++
18	FS-A (GWS)	S-31	8.59±3.59	3.25±0.12	0.77±0.38	-	0.99±0.04	+
19	FS-B (GWS)	S-32	3.17±0.14	-	1.38±0.81	84.52±2.63	-	-
20	FS-C (GWS)	S-33	5.77±0.27	0.65±0.10	0.79±0.40	-	-	-
21	FS-C (GWS)	S-34	20.23±0.70	1.42±0.10	1.76±0.04	146.01±5.83	-	++
22	FS-C (GWS)	S-35	1.45±0.11	-	-	-	-	+
23	FS-C (GWS)	S-49	2.42±0.11	0.97±0.11	-	-	-	++
24	FS-C (GWS)	S-50	1.31±0.11	0.42±0.10	-	-	-	++
25	FS-C (GWS)	S-51	1.69±0.09	0.88±0.10	-	-	-	-
26	FS-C (GWS)	S-52	5.67±0.20	0.78±0.11	-	-	-	++
27	FS-C (GWS)	S-53	6.60±0.45	1.80±0.10	-	-	-	-
28	FS-B (GWS)	S-60	2.70±0.12	-	1.43±0.72	97.16±1.15	-	++
29	FS-B (GWS)	S-61	2.12±0.12	1.18±0.11	1.33±0.69	100.90±1.79	-	++
30	FS-F (PWS)	S-62	4.03±0.11	1.96±0.11	1.31±0.71	50.32±3.74	-	++
31	FS-F (PWS)	S-65	3.45±0.11	0.95±0.11	-	-	-	++
32	FS-C (GWS)	S-66	0.83±0.10	0.65±0.11	-	-	-	-
33	FS-C (GWS)	S-68	4.48±0.20	-	1.33±0.67	63.83±1.00	-	+
34	FS-D (GWS)	S-69	3.11±0.12	0.40±0.09	-	-	-	++
35	FS-D (GWS)	S-70	1.31±0.10	0.51±0.09	-	-	-	++
36	FS-D (GWS)	S-71	3.07±0.16	0.91±0.10	-	-	-	+
37	FS-D (GWS)	S-72	2.35±0.11	2.06±0.10	1.32±0.68	82.28±1.21	-	-
38	FS-B (GWS)	S-73	4.02±0.17	-	-	57.39±3.60	0.53±0.03	-
39	FS-B (GWS)	S-74	5.90±0.88	0.49±0.10	-	-	-	-
40	FS-B (GWS)	S-75	1.22±0.10	0.95±0.10	-	-	-	++
41	FS-F (PWS)	S-76	1.11±0.09	0.44±0.10	-	-	-	-
42	FS-B (GWS)	S-78	4.75±0.46	2.26±0.12	-	-	0.70±0.20	-
43	FS-B (GWS)	S-79	0.55±0.12	0.37±0.12	-	-	1.62±0.04	-
44	FS-B (GWS)	S-80	2.71±0.13	0.45±0.10	-	-	1.10±0.10	-

Conclusion

This study presented a comprehensive assessment of soil yeast diversity in two forest soils of Assam, emphasizing its ecological significance and potential uses in soil health management. The isolates obtained also showed multiple PGP activities which indicates their potential role in plant growth enhancement. These findings advance our understanding of soil microbial diversity and pave the way for future research into the benefits of soil yeasts for sustainable agriculture and ecosystem management.

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Declaration

The author(s) declare no competing interests.

Author(s) contribution

Sukanya Das: formal analysis, data curation, execution of research and writing of original draft Jintu Rabha: investigation, supervision and evaluation of the manuscript Diganta Narzary: project administration, supervision, review and editing of the manuscript. All the authors have agreed to the final draft of the manuscript.

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