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Bioprospecting of fungi from ecological niches of Assam for industrial biomolecules

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ABSTRACT

Assam is situated in the northeast region of India and is unexplored for its fungal diversity. Fungi are capable of utilizing waste materials as their substrates. In order to do so, their metabolism is designed so that they generate several biomolecules of great value to humans. The climate and biomass availability in Assam provides optimal nutrient and environment for the growth of a wide variety of fungus. In the present study, soil and decaying leaf samples were collected from tea gardens, vegetable and rice fields located in the Cachar District of Assam. These samples were screened for fungal isolates on potato dextrose agar medium. A total of thirty-six (36) fungal isolates were obtained. The morphological and phenotypic characterization of these isolated fungal strains were performed and the majority of fungal isolates were identified from genus *Aspergillus, Penicillium, Rhizopus, Schizophyllum, Trichoderma,* etc. The isolated strains were screened for their antagonistic properties and four (4) isolate showed antagonist properties against *Aspergillus* suggesting its antifungal potential. Also, the isolated strains were subjected to qualitative plate assays for their enzyme production abilities critical for biorefinery industries such as xylanase, cellulase, and amylase. The isolates LC1 and COC showed high xylanase and cellulase activity, respectively. These high cellulases and xylanase-producing isolates were identified as *Aspergillus oryzae* (LC1) and *Schizophyllum commune* (COC) through molecular identification. These isolates were subjected to enhanced enzyme production and subjecting them to several biorefinery applications for the production of biofuel and value-added compounds (oligosaccharides). Similarly, the inherited properties of other isolates can be used for different industrial applications to solve the problem of mankind.

Keywords: Fungi, Bioprospecting, Biomolecules, Enzymes, Industrial applications, Assam

INTRODUCTION

Assam is one of seven northeastern states of India and boasts one of the prominent ecological hotspots with rich flora and fauna diversity (MoEF, 2011). However, the fungal diversity of these regions is still unexplored. Although the fungus is ubiquitous and can exist in the diverse ecological niche, the climatic condition around Assam is suitable for the existence of the fungal community (Redman *et al.,* 2002; Frey-Klett *et al.,* 2011; Hernandez and Martinez, 2018). The fungus is a group of eukaryotic microorganisms and exists as unicellular to multicellular forms. The fungi are capable of surviving under harsh conditions due to their spore-forming ability or via the ability to overcome stress by the generation of bioactive compounds (enzymes, metabolites, antioxidants, etc.,) that help in overcoming stress situations themselves (Hyde *et al.,* 2019; Fadiji and Babalola, 2020). This is the reason that isolation of fungal isolates from different unexplored ecological niches interests microbiologists. The bioactive molecules from the fungal source have a widespread application in agriculture, medical, and industry sectors (Kumaresan and Suryanarayanan, 2001; Sun *et al.,* 2011; Yadav, 2018). The ability of the fungus to produce a wide range of biomolecules have helped in replacing earlier existing harsh chemical based methods (Agrawal *et al.,* 2018; Bhardwaj *et al.,* 2019; Anwar *et al.,* 2020). The attempt to screen new fungal strain and their associated biomolecules can be critical to the development of a greener environment (Purchase, 2016; Franco-Duarte *et al.,* 2019). The role of fungus in biodegradation during the food chain system is well known and thus the interrelation between fungi and the environment is central to many natural processes that occur in the biosphere (Hyde *et al.,* 2019). The present study attempted to understand the existence of different fungi in the ecological niches of Assam to uncover their industrial potential. In the present study screening for fungal isolates was carried out from the soil and decaying tea leaves samples that were collected from tea gardens, rice, and vegetable fields located around the Cachar district of Assam. The isolated fungal strains were characterized based on morphological and phenotypic characterization. Different industrially important properties such as hydrolyzing enzyme production potential, the antagonistic, antibiotic, and dye decolorization properties of some of the fungal isolates were also performed to establish the fact that bioprospecting fungal diversity of Assam can be the bio-logic solution for various industrial applications based on their inherited biotechnologically relevant properties.

MATERIAL AND METHODS

Isolation of fungal isolates

An extensive screening was carried out from the soil and decaying samples of various agro-wastes such as tea leaves wastes/litter, and decomposed leaf samples of *Lantana camara* collected from forest regions in Cachar district of Assam, India. The selected locations were various tea gardens and rice and vegetable fields located around the Cachar district. The potato dextrose media was prepared and autoclaved. After cooling, the medium was transferred aseptically to pre-sterilized Petri plates. Plates were allowed to stand for solidification. Serial dilution of samples was performed using dilution series from 10^{-1} - 10^{-6}. Then, 0.1mL of 10^{-3}, 10^{-5}, and 10^{-6} dilutions were spread on Petri plates. The Petri plates were then incubated for 7 days at 25±1°C in a BOD incubator. The phenotype and colony character of each fungus was recorded. Also, each fungal mycelium was stained with lactophenol cotton blue and their hyphae

appearances were observed under a compound microscope. Based on their morphological and phenotypic characteristics, the isolates were classified and the data are mentioned below:

Primary screening of fungal isolates for enzyme production

Xylanase: Screening for all the fungal isolates was performed to determine their ability to produce xylanase when growing in Czapek's Dox media containing xylan as a carbon source. Medium composition (g/L): 0.1% birchwood xylan (HiMedia), 5.0 ammonium tartrate, 0.1 yeast extract, 1.0 KH_2PO_4, 0.5 $MgSO_4.7H_2O$, 20.0 agar. Plates were incubated for 48 hrs at 28 ± 2°C and stained with 0.25% iodine solution (Varghese *et al.,* 2017). The isolates with a clear zone were considered positive to produce xylanase enzyme.

Cellulase: Screening of all fungal isolates was performed for their ability to produce cellulase in a basal salt medium containing CMC as the main carbon source. Medium composition (g/L): 1.0 $NH_4H_2PO_4$; 0.2 KCl; 1 $MgSO_4.7H_2O$; 1.0 yeast extract; 1% CMC; 20 agar. Plates were incubated for 48 hrs at 28 ± 2°C. Stained with 0.1% Congo red dye and flooded with 1M NaCl solution. The fungus with orange hollow with a red background was considered as positive to produce cellulase enzyme.

Amylase: Screening for all fungal isolates was performed for their ability to produce amylase in basal salt medium containing soluble starch as the main carbon source. Medium composition (g/L): 5.0 soluble starch; 2.0 yeast extract; 0.5 $MgSO_4.7H_2O$; 1.0 KH_2PO_4; 20 agar plugs. Plates were incubated for 48 hrs at 28 ± 2°C. The plates were stained with iodine solution containing 1:2 ratios of potassium iodide and iodine. The isolates with a clear zone are considered positive to produce amylase.

Enzyme production in submerged fermentation

Two isolate (2) 17 and 20 were selected among all 36 isolates for the production of xylanase and cellulase respectively in submerged fermentation. Quantitative analysis of enzyme activity was carried out in 100mL of Erlenmeyer flasks containing 1% xylan and 1% CMC as a carbon source in previously selected Mendel's and Sternberg's (MS) BS medium (M4-composition as mentioned above) at pH5 after sterilization in 120°C for 20 min and incubated in a rotary incubator shaker at 100rpm in 28 ± 2°C.

Quantitative enzymatic assay

Cellulase and xylanase activity was assayed by DNS method using 1% Carboxy Methyl Cellulose and Birch Wood Xylan (SIGMA) prepared in acetate buffer (50mM, pH 5.0) and 50mg FPA as substrates. The reaction mixture consisted of 1 mL of respective substrates (1% Xylan, 1% CMC), 50 mg of Whatman filter paper No.1 in case of filter paper assay, 0.5 mL of crude cell-free culture fluid, and 0.5 mL of acetate buffer was incubated for 60 min in the water bath at 50°C. The reaction was asserted by adding 1mL of DNS reagent and boiling at 100˚C. Absorbance was measured at 540nm in a spectrophotometer. Quantification of the amount of released reducing sugar was performed using xylose and glucose as standard. The activity was expressed as the number of µmoles of reducing sugars produced per minute of hydrolysis per mL of enzyme used, i.e., the number of international units per mL. (Miller, 1959)

$$\textbf{Enzyme activity} = A_{540} \times DF \times (1/X) \times (1/Y) \times (1/t) \times (1/slope)$$

Where A_{540} is the absorbance at 540nm of the total reducing sugar produce; X is the volume of enzyme used; Y is the volume of enzymatic digest (hydrolysate) used for assay of reducing sugars; t is the time of hydrolysis (60 min); 1/slope is determined from the standard curve of A_{540} versus xylose and glucose concentration. (µmoles/mL) and DF is the dilution factor (either for enzyme or hydrolysate dilution). Enzyme activity is expressed as the number of µmoles of reducing sugars produced per minute of hydrolysis per mL of enzyme used, i.e., the number of international units per mL.

Molecular identification of xylanase and cellulase producing isolates

The fungal isolates after the primary screening were identified based on their morphological characteristics and internal transcribed spacer (ITS) sequences (Buchan *et al.,* 2002). The genomic DNA (gDNA) was extracted using the Prabha *et al.* (2013) method. PCR amplification and DNA sequencing were performed on the partial rRNA region, as described by (Schoch *et al.,* 2012). The PCR amplification and sequence identification was performed using outsourcing at Xcelris Genomics Labs (Ahmedabad, Gujarat, India). The strain was identified with ITS sequence analysis using BLASTn search tools (http://www.ncbi.nlm.nih.gov).

Antagonistic property assay

These isolates were tested for the antagonistic property against *Aspergillus* sp. to understand various biomolecules secreted by isolated fungi. Agar plug (5mm) was taken from 5 days old plates of *Aspergillus* and other fungal isolates, inoculated on PDA plates for a week. Also, *Aspergillus* was grown in a basal salt medium and their extract was prepared at different.

Antibacterial assays

The antibacterial activity was performed on the Muller Hinton agar plate. In this assay, the filter paper disc was kept overnight in the fungal broth. 100µl of *E. coli* and *Enterococcus* sp. bacterial suspension was spread plate on the Muller Hinton Agar plate and saturated disc in the fungal broth was kept on the bacterial plate to check the antibacterial activity of the fungus. The plate was kept overnight at 37±1°C. The zone of inhibition was measured in cm.

Dye decolorization assay

The dye degradation plate assay was performed to check the potentiality of fungus to degrade or decolorize azo dyes. The plate assay was performed on PDA with a 100 mg/L concentration of three different dyes named Acid Blue 9, Sudan I, and Sudan Black. The fungus was inoculated and kept at 28°C for 4-6 days. The plates were checked every day for color decolorization against the control.

RESULTS

Isolation of fungal isolates

Various fungal isolates from a different source from Assam were obtained on PDA plates**,** after isolation of a single colony subjected to morphological and phenotypic characterization. The phenotypic characterization highlights the morphological character of the fungal colonies such as form (circular, irregular, rhizoids), elevation (flat, convex, raised, umbonate), and margins (filiform, undulate, septate, branched) along with the microscopic observation such as hyphae and spore color are used for the identification of the fungal isolates. Based on morphological identification major fungal genera isolated from different samples (air, water, soil, degraded leaf) of the Assam region are *Aspergillus*, *Trichoderma, Trichophyton, Penicillium, Rhizomucor, Schizophyllum,* and *Fusarium* (**Table 1**).

Table 1: Screening of morphological characteristics of fungal isolates

Sample No.	Sample Source & Location	Form	Elevation	Hyphae	Spore	Margin	Identified fungal spp.
1	Soil (Cachar Assam)	Circular	Umbonate	White	Light brown	Filliform	*Paecilomyces*
2	Soil (Cachar,Assam)	Irregular	Flat	White	Yellow	Filliform	*Microsporum*
3	Soil (Cachar Assam)	Irregular	Flat	White	Creamy white	Undulate	*Hortea*
4	Soil (Cachar Assam)	Circular	Flat	White	Dark green	Undulate	*Aspergillus*
5	Soil (Cachar Assam)	Circular	Flat	White	Green	Entire	*Trichoderma*
6	Leaf (Cachar Assam)	Irregular	Convex	Light yellow	Yellow	Undulate	*Blastomyces*
7	Leaf (Cachar Assam)	Irregular	Flat	White	Creamy white	Entire	*Pycnoporus*
8	Leaf (Cachar Assam)	Irregular	Convex	White	Green	Lobate	*Alternaria*
9	Leaf (Cachar Assam)	Circular	Flat	White	White	Entire	*Arthrographis*
10	Leaf (Cachar Assam)	Irregular	Convex	White	Light yellow	Entire	*Rhizopus*
11	Leaf (Cachar Assam)	Irregular	Raised	White	Green	Undulate	*Botrytis*
12	Leaf (Cachar Assam)	Rhizoid	Raised	White	Dark brown	Undulate	*Neosartorya*
13	Water (Cachar Assam)	Rhizoid	Convex	White	White	Filliform	*Trichophyton*
14	Water (Cachar Assam)	Irregular	Raised	White	Black	Undulate	*Trichophyton*
15	Soil (Cachar Assam)	Circular	Raised	Light yellow	Green	Filliform	*Aspergillus*
16	Soil (Cachar Assam)	Irregular	Raised	White	White	Filliform	*Trichophyton*
17	Leaf (Cachar Assam)	Filamentous	Raised	Light green	Pale yellow	Entire	*Aspergillus*
18	Leaf (Cachar Assam)	Circular	Raised	Light green	White	Filliform	*Penicillium*
19	Leaf (Cachar Assam)	Irregular	Umbonate	Green	White	Undulate	*Rhizomucor*
20	Leaf (Cachar Assam)	Irregular	Convex	Pale yellow	White	Lobate	*Schizophyllum*
21	Leaf (Cachar Assam)	Irregular	Umbonate	Green	Green	Undulate	*Mucor*
22	Leaf (Cachar Assam)	Irregular	Convex	White	Pink	Lobate	*Aspergillus*
23	Leaf (Cachar Assam)	Circular	Flat	White color	Pink/violet	Branched	*Fusarium*
24	Litter (Cachar Assam)	Circular	Flat	Brown	Black	Septate	*Bipolaris*
25	Leaf (Cachar Assam)	Circular	Flat	Black/Brown	White	Septate	*Idriella*
26	Leaf (Cachar Assam)	Circular	Flat	Red	Red	Septate	*Fusarium*
27	Litter (Cachar Assam)	Circular	Raised	Light green	Green	Septate	*Penicillium*
28	Litter (Cachar Assam)	Circular	Flat/raised	Yellow	Green	Septate	*Gliocladium* or *Cladosporium*
29	Litter (Cachar Assam)	Rhizoid	Flat	White	Light yellow	Septate	Not identified
30	Litter (Cachar Assam)	Circular	Raised	White/green	Gray	Septate	*Gliocladium*
31	Pond (Cachar Assam)	Circular	Flat	Light green	Yellow	-	*Penicillium*
32	Pond (Cachar Assam)	Concentric	Raised	Golden yellow	-	Branched	*Aspergillus*
33	Leaf (Cachar Assam)	Rhizoid	Flat	Black/brown	Brown	Septate	Not identified
34	Leaf (Cachar Assam)	Irregular	Raised	White	Black	Septate	*Rhizopus*
35	Soil (Cachar Assam)	Radial	Raised	White	Green	Septate	*Trichoderma*
36	Soil (Cachar Assam)	Radial	Raised	White	Green	Septate	*Trichoderma*

Note: All samples **(**soil, leaf, litter, and pond**)** were collected from the forest region and tea gardens of Chachar district of Assam.

Enzyme production from fungal isolates

Primary screening of fungal isolates for enzyme production

The isolated fungal strains from Assam regions have been subjected to qualitative enzyme assay for estimating the industrially important carbohydrate hydrolyzing i.e., cellulase, xylanase, and amylase producing potential. As most of the sample was isolated from the samples such as soil, degraded leaf, and litters rich in ligno-cellulosic components, thus showing high hydrolyzing enzyme production potential.

Table 2. Qualitative enzyme production potential of isolated strains

Isolates	1	2	3	4	5	6	7	8	9	10	11	12
Xylanase	+	+	+	+	+	+	+	+	+	+	-	+
Amylase	+	+	+	+	+	+	-	+	+	+	+	+
Cellulase	+	+	+	+	+	+	-	+	+	+	+	+
Isolates	13	14	15	16	17	18	19	20	21	22	23	24
Xylanase	+	+	-	+	++	+	+	+	+	+	+	+
Amylase	+	-	+	-	+	+	+	+	+	+	-	-
Cellulase	+	-	+	-	+	+	+	++	+	+	-	-
Isolates	25	26	27	28	29	30	31	32	33	34	35	36
Xylanase	+	+	+	+	+	+	-	+	+	+	+	+
Amylase	-	+	-	-	-	+	-	-	-	-	-	-
Cellulase	-	+	-	-	-	+	-	-	-	-	-	-

Table 2 consists of lists of all isolates (36) and their hydrolyzing enzyme production potentials as confirmed by zone clearance assay using CMC, xylan, and starch as a carbon source in the growth medium followed by staining with congo red (cellulase) and iodine (xylanase, amylase). The basic principle behind this qualitative assay is the binding of CMC, xylan, and starch to dyes used for staining. The unbound dye is washed out during washing with salt water (Varghese *et al.,* 2017). In the present study, most of the isolated strains (except isolates 11, 15, 31) could utilize xylan as the carbon source in absence of glucose in the medium and thus forming a clear zone around the fungal colony and observed after staining and washing with salt (**Table 2**) (Javed *et al.,* 2017). The maximum zone of clearance was observed for strain 17 suggesting high xylanase producing potential. More than 50% of the isolated strains i.e., 1-6, 8-13, 15, 17-20, 21-22, 26, 30 showed cellulase and amylase production ability (zone clearance) with maximum cellulase production potential for strain 20. The two isolates 17 and 20 showing high zone clearance via xylan and CMC utilization thus minimizing binding of dyes in the particular region where the substrate is utilized. Most of the isolates showing cellulase enzyme activity has shown similar pattern of amylase utilizing potential. Thus, the isolated strains can be utilized for cellulase, xylanase production utilizing cellulose and xylan rich agro-residues and wood-based waste substrates.

Xylanase and cellulase production using selected isolates in submerged medium

Mandel's and Sternberg's (MS) basal medium with xylan and CMC as carbon sources has been used for the production of xylanase and cellulase respectively in isolates 17 and 20. Isolate 17 (LC1) showed high xylanase production of 133 ± 2.6 IU/mL with comparatively low cellulase yield. The strain 20 (COC) showed high cellulase production with 36.8 IU/mL obtained in MS medium with low xylanase yield.

Molecular identification of xylanase (17) and cellulase (20) producing isolates

The molecular identification suggested that strain 17 and strain 20 were *Aspergillus oryzae* and *Schizophyllum commune* respectively based on ITS sequence-based phylogenetic tree of various fungal strains consensus ML dendrogram with bootstrap values was based on multiple sequence alignment using MUSCLE program (**Table 3**). The gene sequences were submitted to GenBank and the culture was deposited at a different culture collection center in India (**Table 3**).

Table 3: Molecular identification of all selected isolates by using sequencing methodology

Strain	Isolates	Identified strain	GenBank Number	Culture Accession Number
17	**LC1**	*Aspergillus oryzae*	MG923342	ITCC-8571
20	**COC**	*Schizophyllum commune*	MG923341.1.	NAIMCC-F-03379

Antagonistic property assay

All the isolated strains showed good cellulo-xylanolytic enzyme production potential. Additionally, these isolates were tested for the antagonistic property against *Aspergillus* sp. to understand various biomolecules secreted by isolated fungi. A total of fourteen isolates (23-36) were checked against the *Aspergillus* and found that 6 isolates i.e., 23 (*Fusarium*), 24 (*Bipolaris*), 26 (*Fusarium*), 28 (*Gliocladium* or *Cladosporium*), 29 (not Identified), and 35 (*Rhizopus*) showed inhibition (**Fig. 1**).

Also, to check the anti-fungal properties of the isolated strains *Aspergillus* extract was prepared at different concentrations to check the inhibition. The preliminary analysis showed a broad spectrum of antifungal activity-producing isolates. These isolates can be further explored against phyto-pathogenic fungi.

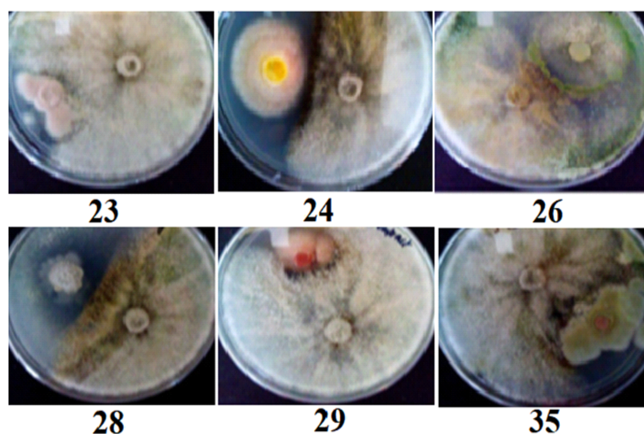

Fig. 1: Antagonistic property assay of isolates 23 (*Fusarium*), 24 (*Bipolaris*), 26 (*Fusarium*), 28 (*Gliocladium* or *Cladosporium*), 29 (not Identified), and 35 (*Rhizopus*) against *Aspergillus*

Antibacterial assays

The antibacterial activity was performed on the Muller Hinton agar plate using *E. coli* and *Enterococcus* sp. as model bacteria. The saturated disc in the fungal broth was kept on the bacterial plate to check the antibacterial activity of the fungus. Strain 25 showed a positive test against *Enterococcus* (E8) and *E. coli*. The zone of inhibition was 1.1 cm and 0.4 cm respectively after two days of incubation. Thus, evaluation of antagonistic and antibacterial activity suggested that some of the isolates have efficient antibiotic (25) activity utilizing antifungal fungal (23,24,26, 28, 29, and 35) ability.

Dye decolorization assay

The ability of isolated fungal strain for degradation of the azo dyes such as acid blue, Sudan I, and Sudan black was identified. A dye degradation plate assay was performed to check the potentiality of fungus to degrade or decolorize azo dyes. The stains 16, 19, 25, 28, 29, 32, and 35 showed the potential to decolorize at least one of the azo dyes. Isolates 16, 19, 25, 28, 29, and 32 showed decolorization of acid blue dye, and isolate19, 29, and 32 showed decolorization of Sudan I dye. Further, only strain 25 showed the ability to decolorize Sudan black (**Table 4**).

Table 4: Fungal isolates showing potential for dye decolorization

Strain	Acid Blue	Sudan I	Sudan Black
16	+	-	-
19	+	+	-
25	+	-	+
28	+	-	-
29	+	+	-
32	+	-	-
35	-	+	-

DISCUSSION

A total of 36 different fungal isolates were isolated from the different samples collected from the Assam regions with prominent genera being *Aspergillus, Trichoderma, Trichophyton, Penicillium, Rhizomucor, Schizophyllum,* and *Fusarium.* Thiyam and Sharma (2013) performed isolation fungal isolates from local fruits of Barak valley, Assam, and observed that *Aspergillus, Trichoderma, Penicillium,* and *Rhizopus* were prominent genera. Similarly, Ray *et al.* (2014) also observed *Mucor, Alternaria, Trichoderma, Penicillium,* and *Aspergillus*. Thus, suggesting the major genus of the fungal isolates found in Assam regions are *Aspergillus, Trichoderma,* and *Penicillium*. Garaga *et al.* (2019) studied the fungal diversity in the air in the largest city of Assam (Guwahati), and suggested that fungal spores of fungi namely *Aspergillus, Penicillium, Cladosporium, Fusarium, Curvularia,* and *Rhizopus*. Therefore, suggests that the Assam region is rich in filamentous fungus diversity with a major contribution from some of the industrially important genera such as *Aspergillus, Penicillium,* and *Trichoderma.* The strain *Aspergillus, Trichoderma,* and *Penicillium* has shown great potential in cellulolytic and xylanolytic enzyme production (Bhardwaj *et al.,* 2019; Siqueira *et al.,* 2020). In the present study, isolates 17 and 20 showed high xylanolytic and cellulolytic enzyme production respectively as confirmed by a large clear zone in plate assay and through quantitative enzyme production using MS media substituted with appropriated substrates. Strain 17 and 20 were suggested to be from *Aspergillus* and *Schizophyllum* genus based on morphological identification which was confirmed by the molecular identification confirming them as *Aspergillus oryzae* and *Schizophyllum commune*. Several studies have confirmed that *Aspergillus oryzae* and *Schizophyllum commune* has high cellulase and xylanase-producing potential. The xylanase and cellulase enzyme production in the isolated strains (17 and 20) has been improved by the medium and physical condition modification in the study by Bhardwaj *et al.* (2017) and Kumar *et al.* (2018) with the maximum enzyme activity of 935 IU/mL (xylanase) and 169.98 IU/mL (cellulase) respectively. Bhardwaj *et al.* (2020) also performed the over-expression of the xylanase gene from *Aspergillus oryzae* in *Escherichia coli* BL21(DE3) with a high titer of the specific activity of 1037.3 U/mg. Thus, suggesting that enzyme production can be improved using different biotechnological approaches.

Several isolated fungal isolates from Assam regions have shown ability antagonistic properties against *Aspergillus* sp thus suggesting a broad spectrum of antifungal activity by 6 different isolates isolated in the present study. The fungal isolates showing the ability to produce antifungal compounds are not much explored. Antifungal and antibiotics ability are most studied in medicinal plant extracts (Salhi *et al.,* 2017; Costa *et al.,* 2019; Liu *et al.,* 2019). Thus, the isolated strains showing good antifungal potential can be explored. Strain 25

also showed antibiotic production potential as confirmed by zone clearance plate assay against *Enterococcus* and *E. coli*. Kumar *et al.* (2010) demonstrated the antibiotic-producing potential of fungal isolates from the soil sample of Kaziranga National Park in Assam. The most potent antibiotic-producing fungal strain belongs to *Aspergillus* genus. Fungal isolates have shown great potential in azo dye decolorization and similarly in the present study 07 isolates showed dye decolorization potential against azo dyes i.e. acid blue, Sudan I, Sudan black. Several fungal isolates have shown great potential in azo dye decolorization. Based on morphological identification fungal isolates showing the dye decolorization belonged to different genera such *Trichophyton, Rhizomucor, Idriella, Cladosporium, Aspergillus,* and *Trichoderma*. Several studies suggested that the ligninolytic enzyme-producing genes from *Aspergillus* sp. could degrade the azo dyes (Liu *et al.,* 2020). Further, He *et al.* (2018) suggested that the fungus *Trichoderma tomentosum* can efficiently degrade azo dyes (acid red) and also suggested a high level of manganese peroxidases during dye decolorization by the fungus. Thus, it can be suggested that the isolated strains in the present study are capable of degrading azo dyes due to ligninolytic enzyme production ability. The ligninolytic enzymes are capable of decomposing the complex phenolic lignin from biomass helping the fungus to utilize them for fulfilling their growth need and serving in the purpose of bioremediation and degradation of recalcitrance lignin and dyes.

CONCLUSION

The present study helped to understand the unexplored fungal diversity of the different ecological niches of Assam. The major fungal genus obtained from different samples, i.e degraded leaves, soil, and water were *Aspergillus, Trichoderma, Schizophyllum, Rhizopus, Penicillium,* etc. More than 50 percent of the isolates showed xylanase and amylase activity and one-third showed cellulase activity. The isolates were mostly isolated from soil and degraded leaf sample rich in decomposed lignocellulosic structures thus showing high xylanase and cellulase activity. The two isolates i.e. isolate 17 and 20 showed maximum xylanase and cellulase activity respectively confirmed both by plate assay and quantitative assay. These two isolates were identified as *Aspergillus oryzae* and *Schizophyllum commune* and were explored further for enzyme production using several biotechnological approaches. The isolates showed the ability to antifungal and antibiotic properties suggesting great potential from fungal strains isolated from Assam regions due to its ecological unexplored terrains. The isolated fungal isolates can also help in bioremediation and biowaste remediation as suggested by the dye degradation potential of the isolates. With the help of a literature survey, it can be established that isolates were also capable of producing ligninolytic enzymes thus assisting in the dye degradation potential of isolates. Thus, these numerous applications of the isolates suggested that the Assam can act as reservoirs for the fungal diversity which can be a biological solution for various industrial applications based on their inherited properties.

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