Journal of Scientific & Industrial Research
Vol. 81, November 2022, pp. 1173-1180
DOI: 10.56042/jsir.v81i11.60811

Isolation and Screening of Potent Cellulolytic Soil Fungi from Raipur City of Chhattisgarh State, India

Ajay James[1], Srishti Pandya[1], Priya Sutaoney[1], Veenu Joshi[1] & Prabir Ghosh[2]*

[1]Center for Basic Science, Pt Ravishankar Shukla University, Raipur 492 010, Chhattisgarh, India
[2]Department of Chemical Engineering, NIT Raipur, Raipur 492 010, Chhattisgarh, India

Received 24 February 2022; revised 10 October 2022; accepted 10 October 2022

The current research focuses on the diversity of fungal isolates obtained from the soil and their ability to produce cellulolytic enzymes. Out of 76 colonies obtained, 11 fungal colonies were isolated viz. *Aspergillus*, *Humicola* and *Rhizopus* being the major genera of the isolates. From the 11 isolates, the identification of potent cellulase producing cultures was done using qualitative carboxy methyl cellulase screening test. Following the preliminary screening, five cultures viz., *Aspergillus sydowii*, *Humicola* sp-1, *Aspergillus niger*, *Aspergillus ustus*, *Aspergillus flavus* were identified to be potent with enzymatic indices ranging from 1.25 to 2.29. These were further selected for quantitative enzyme analysis. *Aspergillus sydowii* was found to have highest enzymatic activity and *Aspergillus flavus* was found to have the least enzymatic activity. Our findings revealed that *Aspergillus sydowii* appeared to be a promising candidate for cellulase production and can be utilized for waste recycling and other biotechnological applications.

Keywords: *Aspergillus*, Carboxy methyl cellulase, Endoglucanase, *Humicola*, Lignocellulose

Introduction

Population explosion has resulted in increased industrialization, which has escalated annual waste production. In the current economic scenario, the production of energy is also becoming an issue. Hence, there is a need to find new sources of raw materials for the generation of energy. One of the most promising forms available is a lignocellulosic waste. Due to their wide existence in nature, they have been exploited for the production of biofuels. Lignocellulosic biomass is the most abundant biological material derived from the biosphere. The major contribution to the cellulose pool is made by plants through photosynthesis.[1,2] Even though cellulose has numerous applications in fields such as paper manufacturing, nanomaterials, and bioenergy production, it is estimated that 1.5×10^{12} tonnes of cellulose gets wasted each year.[3] The main source of cellulosic waste is from industries such as forestry, agriculture, and municipal waste. However, 35–45% of cellulosic wastes are present in these residues.[4] Since, the tropical and sub-tropical region hosts the majority of agriculture, the zone inputs a large quantity of cellulosic waste in form of agricultural residues, which is either burned or removed from the site. This leads to the downfall in productivity, soil quality, and organic sources and indirectly causes some health issues in humans, loss of biodiversity and climate change.[5] Scientifically, rapid accumulation of this residual waste makes its management challenging. Hence, to cope up with upcoming environmental challenges, bioconversion of lignocellulosic waste into valuable biobased products can be achieved by a significant class of enzymes termed cellulases.[6,7] Cellulases are complexes of hydrolytic enzymes capable of hydrolyzing cellulosic residues into smaller sugar units like glucose. This complex shows high breakdown action on crude natural cellulosic residues and is found most commonly in fungi. Cellulase is a complex enzyme system that contains endo-1,4-β-D-glucanase (endoglucanase), exo-1,4-β-D-glucanase (exoglucanase) and β-D-glucosidase (β-D-glucoside glucanhydrolase).[8–10] The endoglucanase attacks randomly in the internal glycosidic bonds, producing glucan chains of different lengths, while the exoglucanase breaks down cellulose from the end of the chain. Endoglucanase also acts on cellodextrins and converts them into cellobiose and glucose. The completion of the hydrolysis process is mediated by β-glucosidase which cleaves cellobiose and removes glucose from the non-reducing end of the oligosaccharide.[11] The combination of cellulases is also used with other enzymes for the breakdown of biomass into simple sugars, which are further fermented to

*Author for Correspondence
E-mail: prabirg.che@nitrr.ac.in

bio-ethanol.[12,13] The rapid rise in energy demand caused by population growth, resulted in the depletion of fossil fuels and an increase in global warming. This highlights the need for the exploration and development of a cost-effective, inexhaustible, and sustainable source. In this regard, cellulase has the potential to solve the fuel crisis while also reducing the energy crisis and environmental pollution.[14,15] Unlike bacterial cellulases, which are known to synthesize solitary enzymes in aerobic bacteria or form cellulosomes in anaerobic bacteria, fungal cellulases are less complicated and also do not aggregate. *Trichoderma reesi* has attracted much interest as a promising cellulase producer, although it has very little β-glucosidase activity.[16] Numerous attempts have been made to explore and characterize cellulases from different microbial taxa, including bacteria, fungi, and actinomycetes.[17] *Acinetobacter junii*, *Anoxybacillus* sp., *Bacillus amyloliquefaciens*, *Cellulomonas biazotea* and *Streptomyces drozdowiczii* are potent cellulolytic bacteria. Some prominent fungi capable of synthesising extracellular cellulolytic enzymes include *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp.[18] The synthesis, formulation, characterization, and application of cellulase enzymes have been the preliminary aim of several research groups over the past three decades. Cellulase enzyme sales account for 8% of all annual enzyme sales, and it is anticipated that they will eventually surpass those of proteases. Since the cost of cellulase production is a major obstacle in biomass hydrolysis and industrialization, several research groups are concentrating on the synthesis of cellulase for the conversion of biomass and bio-fuel synthesis. In this study, an attempt has been made for isolation, identification, and screening of soil fungal communities having cellulolytic capability. Authors in the present investigation have reported *Aspergillus sydowii* as potent cellulolytic fungi from Raipur, Chhattisgarh. Even though, few reports with *A. sydowii* possessing cellulolytic potential are available, there has been no survey till date showing it as most promising when compared with *A. niger* or other *Aspergillus* sp. The current study indicates that it is a rare isolate documented till now from this region and can be further exploited in industries as well as in waste management procedures leading to the decreased environmental pollution.

Materials and Methods

Sampling Site

The soil sampling for the study was done in the rainy season from the campus of Pt. Ravishankar Shukla University, Raipur, city of Indian state Chhattisgarh (21.2406°N, 81.6014°E) (Fig. 1). The sample collection was done in August 2021. The climatic condition of Chhattisgarh is tropical with

Fig. 1 — (a) Outline Map of Chhattisgarh State (India) denoting the location of sample collection; (b) Site of sample collection

distinct seasons. The soil sample was collected in a sterile petri plate and was stored at 40°C for 7 days in hot air oven to remove any moisture, till the isolation procedures were initiated.

Isolation and Identification of Fungi from the Soil

The fungal isolation was performed by serial dilution agar plating method.[19] The soil samples with different concentrations obtained after serial dilution were further inoculated on agar plates and incubated for 5 days at 28°C. The media used for isolation was potato dextrose agar (PDA). Once the growth of fungi was visible, the fungal sub cultures were prepared on PDA plates and were further purified on PDA slants and stored at 4°C. Morphological characterization was done using the slide culture method and was stained using lactophenol cotton blue. Identification of fungal species was done following Barnett and Hunter[20] and Klich.[21]

Screening

Preliminary Screening

The qualitative screening of fungal cultures having the potential to produce cellulase enzyme was done by a plate assay incorporating carboxymethyl cellulose as a substrate in the agar medium. After the growth was observed, the presence of cellulase enzyme production was confirmed using Gram's iodine.[22] The CMC agar was composed of (*g/l*) sucrose 30 g; $NaNO_3$ 2 g; KH_2PO_4 1g; KCl 0.5 g; $MgSO_4$ 0.5g; $FeSO_4$ 0.001g; carboxy methyl cellulose sodium salt 5 g; peptone 2 g; agar- agar 15 g. The pH of the medium was maintained at 5.0. After inoculation, the plates were incubated at 28°C for 48 hours. Following incubation, the diameter of the fungal colony was measured and a 5 ml aliquot of Gram's Iodine was added to each plate. A bluish-black complex was formed with cellulose and the presence of cellulase was confirmed by the formation of pale halos around the fungal colony due to the hydrolysis of cellulose. The diameter of the halos was measured for the calculation of enzymatic index (EI):

$$Enzymatic\ Index(EI) = \frac{Diameter\ of\ hydrolysed\ area}{Diameter\ of\ fungal\ colony}$$

Potent cellulase producing fungal colonies with an EI greater than 1.25 were considered potent and were subjected to quantitative screening.[23]

Quantitative Screening

For the production of cellulase enzyme for quantitative analysis, 150 ml Erlenmeyer flask was used. Each Erlenmeyer flask was filled with modified Czapek Dox medium with 1% carboxymethyl cellulose sodium salt (CMC), maintaining a pH of 5.0, and was sterilized for 15 minutes at 121°C. The medium was cooled, inoculated with a 10 mm mycelial disc from five days old culture and incubated for 7 days at 28°C under controlled environment. The filtrate from the culture was preceded for the quantitative analysis of the enzymatic activity.

Carboxy Methyl Cellulase (CMCase) Assay

The determination of the enzyme activity of the potent isolates were carried out according to Wood and Bhat.[24] The dinitrosalicyclic acid method[25] was used for the estimation of reducing sugar. The reaction mixture, 0.5ml of 1% CMC (w/v) was prepared, and 0.5ml of undiluted enzyme filtrate was added. The mixture was incubated at 50°C for 30 minutes, and the further reaction was ceased by the addition of 3 ml of DNS reagent to the mixture. The mixture was boiled for 15 minutes until colour change followed by addition of 1 ml Rochelle salt before the cooling of the reaction mixture to stabilize the colour change whereas, 1 ml citrate buffer and 0.5 ml of undiluted enzyme extract were used as controls. The absorbance at 540 nm was noted.

Statistical Analysis

The means of data and standard error were calculated using the data analysis tool in Microsoft Excel.

Results and Discussion

Fungi, being an important agent in biomass decomposition, play a major role in the carbon cycle of an ecosystem. Soil is the sink of all lignocellulosic waste from the environment in the form of detritus as well as industrial and agricultural wastes. In this study, the isolation of fungi from the soil was done and the exploration of mycoflora associated with the degradation of cellulosic waste in the soil was assessed. The process of isolation was performed during the rainy season on PDA medium. The isolation was performed by the serial dilution agar plating method. Incubation period for all dilution factor was kept as 5 days. The colony forming units were calculated at different concentrations (Table 1, Fig. 2). Isolation of 76 fungal cultures was done from

Table 1 — Colony Forming Unit (CFU) determination of the fungal colonies isolated from soil

Dilution	Dilution factor	No. of colonies per plate	Average no. Of colonies per dilution	Organisms per gram of soil
10^{-2} (A)	10^2	11	10.6	1.06×10^3
10^{-2} (B)	10^2	—		
10^{-2} (C)	10^2	21		
10^{-3} (A)	10^3	2	3.66	0.366×10^4
10^{-3} (B)	10^3	4		
10^{-3} (C)	10^3	5		
10^{-4} (A)	10^4	3	7.66	0.766×10^5
10^{-4} (B)	10^4	5		
10^{-4} (C)	10^4	14		
10^{-5} (A)	10^5	4	3.66	0.36×10^6
10^{-5} (B)	10^5	5		
10^{-5} (C)	10^5	2		
Total Colonies:		**76**		

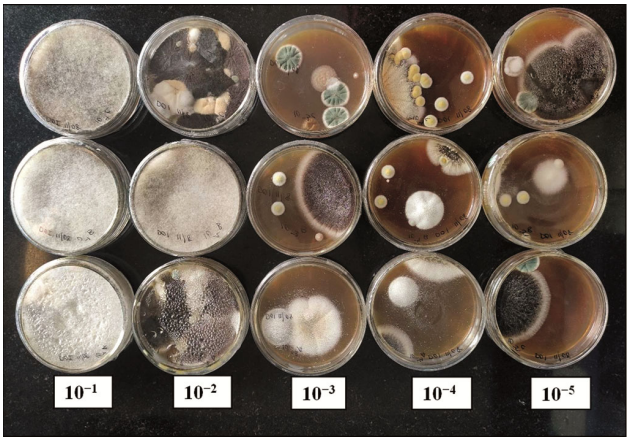


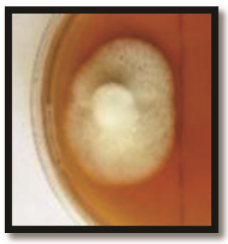
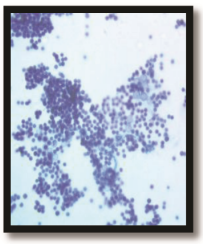
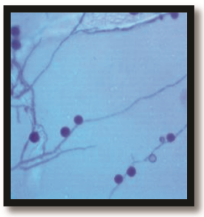
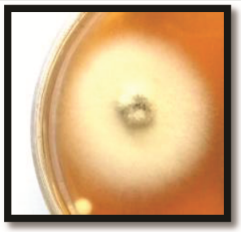
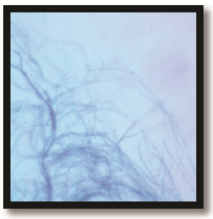
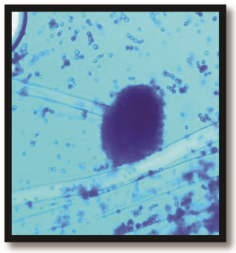
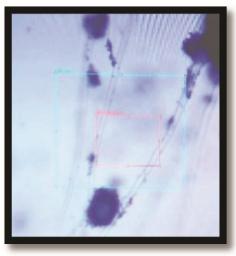
Fig. 2 — Soil fungi isolated via serial dilution agar plating method

the soil sample, out of which 11 fungal colonies were purified. These 11 fungal isolates include members of mainly 3 genera, viz., *Aspergillus*, *Humicola*, and *Rhizopus*. An isolate with sterile mycelia was also obtained. The overall fungal load was maximal at a concentration of 10^{-5} (0.36×10^6). The colonies were isolated by the spread plate method and purified on PDA slants. The microscopic analysis of 11 fungal colonies was done and identification to species level was also accomplished. The photographs representing colonial and morphological characteristics of fungal isolates have been presented in Table 2. It is in accordance with many studies showing soil as a source of cellulolytic fungi. Similar findings were also reported by Sari *et al.*[26], who isolated cellulase-producing fungi from the leaf litter. Twelve fungal isolates were obtained, out of which eight species were found to possess potent cellulolytic ability and were identified as *Aspergillus*, *Penicillium*, *Paecilomyces* and *Theilaviopsis*.

The results of cellulase screening of 11 fungal isolates based on the clear zone of hydrolysis on the CMC media show 5 isolates, viz., F1, F2, F4, F7, F11 as potential cellulose degraders, ranging in enzymatic index from 1.25 to 2.29 (Table 3). The digestion of carboxymethyl cellulose was visible by the appearance of pale halos around the fungal colony, when treated with Gram's iodine (Fig. 3). The isolates were further identified as *Aspergillus sydowii, Humicola* sp.1*,* sterile mycelium*, A. ustus*, and *A. flavus*, respectively. Various reports suggest *Aspergillus* and *Humicola* as potent producers of cellulase in the fermentation industry process. The maximum cellulolytic index was given by *Aspergillus sydowii*, followed by *Aspergillus flavus*, sterile mycelium, followed by *Humicola* sp.1, and least by *Aspergillus ustus*. These results were similar to those of Khokhar *et al.*[27], who isolated cellulolytic fungi from different soil sources and industrial effluents. The results were also in agreement with Kale and Zanwar[28], who isolated fungi from sawmills and paper mills. Out of 12 fungal isolates obtained, only four were found to have positive cellulolytic activity. The maximal zone of hydrolysis after plate assay was shown by *Trichoderma* sp. along with *A. niger* and *A. flavus*.

All five fungal isolates were further subjected to enzymatic production by a submerged fermentation technique using carboxymethyl cellulose as a substrate for seven days of incubation (Fig. 4). The highest cellulase activity was exhibited by *Aspergillus sydowii* (F1) with an enzymatic activity of 0.210 IU/ml followed by *Humicola* sp. 1 (F2) with an enzymatic activity of 0.157 IU/ml. These findings were in agreement with Jahangeer *et al.*[29], who reported the highest enzymatic activity by *A. niger* (0.193 IU/ ml) and comparatively low cellulolytic activity by *A. flavus* (0.060 IU/ ml). Delabona *et al.*[30] also reported comparable cellulase production from *A. fumigatus* (43.65 IU g^{-1}) and *A. niger* (39.15 IU g^{-1}). Additionally, the outcomes were identical to those described by Kachlishvili *et al.*[31] whose documents consisted of findings from three potential cellulolytic white-rot basidiomycetes. The highest CMCase activity was reported by *Lenzites betulina* (2.9 U/ml) followed by *Stereum hirsutum* (2.1 U/ml) and lowest by *Irpex lacteus* (0.9 U/ml). Similar results were

Table 2 — Colonial and morphological characterization of fungal isolates

Fungal Isolates Macroscopic characterstics	**Image**	**Microscopic characteristics**	**Image**
F1 *Aspergillus sydowii* • Greenish velvety and constricted colony with smooth margins and irregular form. • Crateriform elevation • Honey brown colour pigment present. • Exudates absent		• Hyphae septate. • Conidia globose. • Rough walled. • Presence of echines. • Colourless, smooth walled phialoconidia present.	
F2 *Humicola* sp.1 • Colony effuse and cottony. • Initially white becoming grey later. • Elevation is raised. • Irregular form with entire margin. • Constricted colony at reverse. • Pigments present. • Exudates absent.		• Hyphae septate and branched. • Conidiophores smooth. • Hemispherical vesicles possessing two series of phialides. • Conidia arranged in chains. • Conidial wall spinulose. • Sexual spores absent	
F3 *Humicola* sp.2 • Colonies growing rapidly. • Initially white turning greyish black later.		• Hyphae septate. • Presence of chlamydospores. • Single chlamydospores arrangement. • Chlamydospores thick walled.	
F4 Sterile mycelia • Cottony white in colour. • Elevation flat. • Entire smooth margin. • Pigments absent. • Exudates absent.		• Spores absent. • Mycelia sterile.	
F5 *Aspergillus niger* • Colonies having moderate growth with carbon black in colour. • Powdery colonies. • Flat elevation. • Circular form. • Smooth, entire margin. • Pigments absent. • Minute drops of exudates present.		• Conidiophores smooth and aseptate. • Vesicles globose. • Biseriate phialides, both brownish in colour. • Conidia globose possessing echines. • Conidial head is split in defined columns.	
F6 *Aspergillus nidulans* • Greenish Velvety colony • Ambonate elevation • Irregular form • Undulate margin. • Pigments present. • Exudates absent.		• Hyphae septate. • Presence of cleistothecia and hülle cells. • Conidial heads slightly radiate, short columnar. • Conidiophores light brown in colour. • Hemispherical vesicles. • Biseriate phialides. • Conidia globose.	

contd.

Table 2 — Colonial and morphological identification of fungal isolates

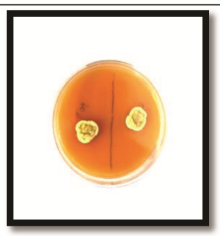
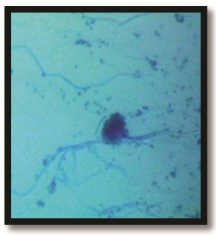
F7 *Aspergillus ustus* • White floccose colonies at first turning olive grey later. • Colonies circular with crateriform elevation. • Undulate margin. • Yellowish brown pigments present. • Exudates present.		• Conidiophores are light brown and sparsely septate with hemispherical vesicles present with biseriate phialides. • Conidia globose and echinulate, brownish in colour.	
F8 *Aspergillus* sp.1 • Pale yellowish floccose and cottony colony. • Crateriform elevation. • Irregular, undulate margin. • Pigments present. • Exudates absent.		• Hyphae septate. • Biseriate phialides. • Conidia globose, smooth walled. • Rough walled conidiophores. • Hemispherical vesicle present.	
F9 *Aspergillus* sp.2 • Rapidly spreading colony. • Powdery colony with dark brown colour. • Dull brown colour at reverse. • Flat elevation. • Entire, smooth margin present. • Circular or rhizoid form. • Exudates absent.		• Hyphae septate. • Biseriate phialides. • Conidia in chains.	
F10 *Rhizopus* sp.1 • Rapidly growing colony. • Pale greyish in colour. • Aerial hyphae present.		• Aseptate hyphae. • Rhizhoid arises from the nodes of the implantation. • Nodal region with two or more unbranched sporangiophore. • Oval, brown, smooth sporangiospores present. • Flattened columella present.	
F11 *Aspergillus flavus* • Yellowish green irregular colony with flat elevation. • Undulated margin. • Conidial heads radiate with poorly splited columns. • Yellowish pigment present. • Presence of sclerotia. • Exudates absent.		• Hyphae septate. • Rough walled conidiophores with sub globose vesicle, predominantly uniseriate. • Conidia yellowish green in colour. • Conspicuously echinulate.	

Table 3 — Enzymatic indices of fungal isolates

Colony Name	Colony diameter	Zone diameter	Enzymatic Index
F1 (*Aspergillus sydowii*)	1.7 ± 0.02	3.9 ± 0.06	2.29
F2 (*Humicola* sp. 1)	1.85 ± 0.05	2.8 ± 0.05	1.51
F3 (*Humicola* sp. 2)	3.75 ± 0.25	4.4 ± 0.15	1.17
F4 (Sterile mycelia)	2.05 ± 0.05	3.3 ± 0.05	1.60
F5 (*Aspergillus niger*)	2.6 ± 0.1	NA	NA
F6 (*Aspergillus nidulans*)	2.5 ± 0.01	NA	NA
F7 (*Aspergillus ustus*)	1.55 ± 0.05	1.95 ± 0.1	1.25
F8 (*Aspergillus* sp. 1)	1.2 ± 0.06	NA	NA
F9 (*Aspergillus* sp. 2)	3.6 ± 0.02	NA	NA
F10 (*Rhizopus* sp.)	9.8 ± 0.01	NA	NA
F11 (*Aspergillus flavus*)	3 ± 0.01	5.7 ± 0.05	1.9

Note: NA = not applicable

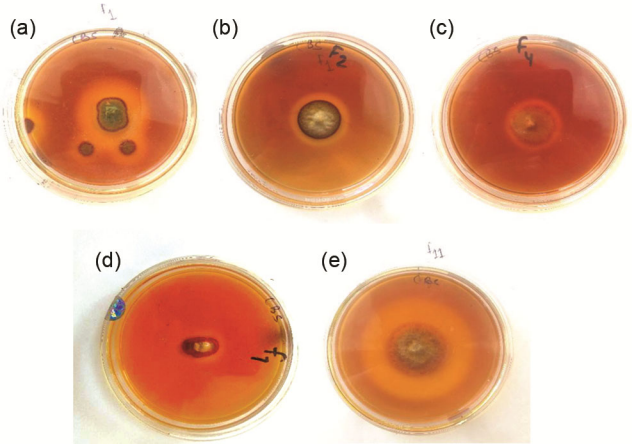


Fig. 3 — Cellulolytic activity of isolated soil fungi viz.: (a) F1 (*Aspergillus sydowii*), (b) F2 (*Humicola* sp. 1), (c) F4 (Sterile mycelia), (d) F7 (*Aspergillus ustus*), (e) F11 (*Aspergillus flavus*), Pale halos around the fungi indicates positive cellulase activity

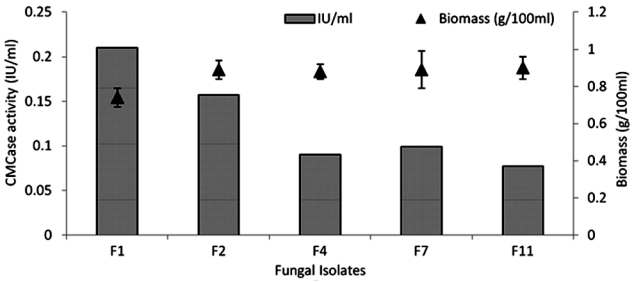


Fig. 4 — Quantitative estimation of carboxy methyl cellulase (CMCase) activity: F1 (*Aspergillus sydowii*); F2 (*Humicola* sp.1); F4 (Sterile mycelia); F7 (*Aspergillus ustus*); F11 (*Aspergillus flavus*)

obtained by the studies of Santos *et al.*[32] and Bhatia *et al.*[33], in which the enzyme activity of marine derived strain of *A. sydowii* was found to be 0.02 FPU ml^{-1} and *Humicola* species from paddy field samples ranged from 0.79–1.90 IU g^{-1}, respectively, when provided with similar climatic conditions. *A. sydowii's* cellulolytic potential and its capability for hydrophyte breakdown were reported by Zhang *et al.*[34] The foregoing finding is supported by the fact that *Canna indica* medium showed maximum cellulase activity (0.31 U/ml). Saryono *et al.*[35], who isolated a thermophilic *Aspergillus flavus* from an Indonesian hot spring called Sungai Pinang, also reported similar findings.

Conclusion

In the present investigation, the fungal diversity and cellulolytic potential of soil fungi were studied. A total of 76 fungal cultures were isolated, out of which *Aspergillus* was found to be the most prominent fungi, followed by *Humicola* and *Rhizopus*. The potent cellulolytic fungus was found to be *Aspergillus sydowii*. This study signifies that the soil is a sink of lignocellulosic waste as well as a habitat for a lot of cellulolytic fungi. However, it is necessary to conduct additional research on the optimization of culture conditions for enhanced enzyme production and their catalytic characteristics. Additionally, an approach of co-cultivating various fungal strains could also result in increased cellulase production. The current research findings on fungi may augment the demand for cellulolytic enzymes in wide range of industries and can also be applied in reprocessing of waste and formation of value added products.

Acknowledgment

An extension of gratitude is due to the Head, Center for Basic Sciences, Pt. Ravishankar Shukla University for providing the research facility. The authors are grateful towards Microbiology Research Laboratory, School of Studies in Life Science, Pt. Ravishankar Shukla University for providing chemicals.

References

1 Saxena R C, Adhikari D K & Goyal H B, Biomass-based energy fuel through biochemical routes: A review, *Renew Sustain Energy Rev*, **13** (2009) 167–178, https://doi.org/10.1016/j.rser.2007.07.011.

2 Nidhi C, Sharma B & Singh P K, Energy value in biomass and plastic components of municipal solid waste, *MATTER: Int J Sci*, **3** (2017) 80–92, https://doi.org/10.20319/mijst.2017.32.8092.

3 Al-Kharousi M M, Sivakumar N & Elshafie A, Characterization of cellulase enzyme produced by *Chaetomium* sp. isolated from books and archives, *Eur Asian J Bio Sci*, **9** (2015) 52–60.

4 Narayana T, Municipal solid waste management in India: From waste disposal to recovery of resources? *Waste Manag*, **29** (2009) 1163–1166, https://doi.org/10.1016/j.wasman.2008.06.038.

5 Sahu A, Manna M C, Bhattacharjya S, Thakur S, Mandal A, Rahman M M, Singh U B, Bhargav V K, Srivastava S, Patra A K, Chaudhari S K & Khanna S S, Thermophilic ligno-cellulolytic fungi: The future of efficient and rapid bio-waste management, *J Environ Manage*, **244** (2019) 144–153, https://doi.org/10.1016/j.jenvman.2019.04.015.

6 Mathew G M, Sukumaran R K, Singhania R R & Pandey A, Progress in research on fungal cellulases for lignocellulose degradation, *J Sci Ind Res*, **67** (2008) 898–907.

7 Manavalan T, Manavalan A & Heese K, Characterization of lignocellulolytic enzymes from white-rot fungi, *Curr Microbiol*, **70** (2015) 485–498, https://doi.org/10.1007/s00284-014-0743-0

8 Sukumaran R K, Singhania R R & Pandey A, Microbial cellulases-production, applications and challenges, *J Sci Ind Res*, 64 (2005) 832–844.

9 Wilson D B, Microbial diversity of cellulose hydrolysis, *Curr Opinion Microbiol*, **14** (2011) 259–263, http://doi.org/10.1016/j.mib.2011.04.004.

10 Singh A, Adsul M, Vaishnav N, Mathur A & Singhania R R, Improved cellulase production by *Penicillium janthinellum* mutant, **55** (2017) 436–440.

11 Da Silva Almeida L E, Fernandes P & de Assis S A, Immobilization of fungal cellulases highlighting β-Glucosidase: Techniques, supports, chemical, and physical changes, *Protein J*, **41** (2022) 274–292.

12 Ranganathan S, Mahesh S, Suresh S, Nagarajan A Z, Sen T M & Yennamalli R, Experimental and computational studies of cellulases as bioethanol enzymes, *Bioengineered*, **13** (2022) 14028–14046.

13 Singh S, Kumar A, Sivakumar N & Verma J P, Deconstruction of lignocellulosic biomass for bioethanol production: Recent advances and future prospects, *Fuel*, **327** (2022), 125109.

14 Pointing S B, Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi, *Fungal Divers*, **2** (1999) 17–33.

15 Debnath G, Das P & Saha A K, Screening and characterization of extracellular cellulase enzyme produced by wild edible mushroom *Pleurotus giganteus*, *Indian J Nat Prod Resourc*, **10** (2020) 195–199.

16 Peterson R, Nevalainen H, *Trichoderma reesei* RUT-C30–thirty years of strain improvement, *Microbiology*, **158** (2012) 58–68.

17 Bhat M K, Cellulases and related enzymes in biotechnology, *Biotechnol Adv*, **18** (2000) 355–383.

18 Kuhad R C, Gupta R & Singh A, Microbial cellulases and their industrial applications, *Enzyme Res*, **2011** (2011) 1–11, https://doi.org/10.4061/2011/280696.

19 Waksman S A, A method for counting the number of fungi in the soil, *J Bacteriol*, **7** (1922) 339–341, https://doi.org/10.1128/jb.7.3.339-341.1922.

20 Barnett H L & Hunter B B, *Illustrated genera of imperfect fungi*, **3rd edn**, (Burgess Pub Co) 1972, 930–932.

21 Klich M A, *Identification of common Aspergillus species*, (Centraal bureau voor Schimmel cultures, Utrecht: The Netherlands) 2002.

22 Kasana R C, Salwan R, Dhar H, Dutt S & Gulati A, A rapid and easy method for the detection of microbial cellulases on agar plates using gram's iodine, *Curr Microbiol*, **57** (2008) 503–507, https://doi.org/10.1007/s00284-008-9276-8.

23 Florencio C, Couri S & Farinas C S, Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by *Trichoderma* strains, *Enzyme Res*, **2012** (2012) 1–7, https://doi.org/ 10.1155/2012/793708.

24 Wood T M & Bhat K M, Methods for measuring cellulase activities, in *Methods in Enzymology* (Elsevier) 1988, 87–112.

25 Miller G L, Use of DNS reagent for the measurement of reducing sugar, *Anal Chem*, **31** (1959) 426–428.

26 Sari S, Setyaningsih R & Wibowo N, Isolation and screening of cellulolytic fungi from *Salacca zalacca* leaf litter, *Biodiversitas*, **18** (2017) 1282–1288, https://doi.org/10.13057/biodiv/d180355.

27 Khokhar I, Haider M S, Mushtaq S & Mukhtar I, Isolation and screening of highly cellulolytic filamentous fungi, *J Appl Sci Environ Manag*, **16** (2012) 223–226.

28 Kale R A & Zanwar P H, Isolation and screening of cellulolytic fungi, *IOSR J Biotechnol Biochem*, **2** (2016) 57–61.

29 Jahangeer S, Khan N, Jahangeer S, Sohail M, Shahzad S, Ahmad A & Khan S A, Screening and characterization of fungal cellulases isolated from the native environmental source, *Pak J Botany*, **37** (2005) 739–748.

30 Silva Delabona D, Pirota P, Codima R, Tremacoldi C R, Rodriguez A & Farinas S A, Using Amazon forest fungi and agricultural residues as a strategy to produce cellulolytic enzymes, *Biomass Bioenerg*, **37** (2012) 243–250, https://doi.org/10.1016/j.biombioe.2011.12.006

31 Kachlishvili E, Khardziani T, Metreveli E, Kobakhidze A & Elisashvili V, Screening of novel basidiomycetes for the production of lignocellulolytic enzymes during fermentation of food wastes, *J Waste Convers Bioprod Biotechnol*, **1** (2012) 9–15.

32 Santos D A, Oliveira M M, Curvelo A A S, Fonseca L P & Porto A L, Hydrolysis of cellulose from sugarcane bagasse by cellulases from marine-derived fungi strains, *Int Biodeterior Biodegr*, **121** (2017) 66–78, https://doi.org/10.1016/j.ibiod.2017.03.014

33 Bhatia S, Kaur B & Phutela U, Optimization of process parameters for cellulase production from *Humicola fuscoatra* MTCC 1409 using response surface methodology, *Agricul Res J*, **55** (2018) 122–128, https://doi.org/10.5958/2395-146X.2018.00020.0.

34 Zhang H P, Li, Q Z, Zhou Q H & Wu Z B, Endoglucanase activity of novel *Aspergillus sydowii* isolate WIU-01 and application for hydrophyte degradation, *Glob Nest J*, **23** (2021) 280–287.

35 Saryono S, Novianty R, Suraya, N, Piska F, Devi S, Pratiwi N W & Ardhi A, Molecular identification of cellulase-producing thermophilic fungi isolated from Sungai Pinang hot spring, Riau Province, Indonesia, *Biodiversitas*, **23** (2022) 1457–1465.