Annals of Plant and Soil Research 24(3):347-354 (2022)
https://doi.org/10.47815/apsr.2022.10174

Isolation of lignocelluloses degrading microbes from soil and their screening based on qualitative analysis and enzymatic assays

RESHMA SHINDE*[1], DHIRENDRA KUMAR SHAHI[2], PRABHAKAR MAHAPATRA[2], SUSHANTA KUMAR NAIK[1], CHANDRA SHEKHAR SINGH[2], SHIKHA VERMA[2] AND AURN KUMAR SINGH[1]

[1]ICAR- Research Complex for Eastern Region, Farming system Research Centre for Hill and Plateau Region, Ranchi- 834010, Jharkhand, India

Received: April, 2022; Revised accepted: May, 2022

ABSTRACT

Crop residues (CRs) composed of structurally complex lignocellulosic material which is resistant to degradation due to various biologically stable linkages present in it. Enhancing the process of its biological degradation using microbial strains capable of decomposing lignocellulose would not only solve the problem of CR disposal but also recycle the organic carbon, NPK and other plant nutrients back into the soil. This study was aimed at isolating and screening of the lignocellulose decomposing microbes from various sources such as crop residue incorporated soil, forest soil, soil near decaying wood, compost pit and dump yard. Total of 15 fungi and 6 bacteria were isolated in the form of pure colonies and screened for cellulose and lignin decomposing ability using carboxymethylcellulose (CMC) and tannic acid (TA) agar media on the basis of appearance of clear zone. Five fungal and two bacterial cultures identified as lignocellulose decomposers were further screened quantitatively for CMCase and filter paperase enzyme assay by standard protocols. Two fungal strains, identified as Trichoderma species and Aspergillus species showed significant enzymatic activity and can be utilized as a potential lignocellulose decomposer.

Keywords: crop residue, fungi, bacteria, enzyme, degradation

INTRODUCTION

Crop residues (CRs) refer to the left over of the crops in the fields after the grains or economic part is harvested. The major components of CR are cellulose, hemicellulose and lignin (Andlar *et al*., 2018) which make a compact structure bound together. Crystalline structure of cellulose, water resistance of lignin and protective coating of lignin-hemicellulose matrix on to the cellulose fibres, collectively make lignocellulosic biomass a resistant material to degrade by the microbial enzymes(Isikgor and Becer, 2015).Cellulose fibrils are cross-linked with hemicellulose and the voids between these two are occupied with a polyphenolic-aromatic biopolymer, lignin, which by forming cross-linked network, protects the cellulose-hemicellulose core(Anwar *et al.*, 2014; Wang *et al.*, 2021).

Being voluminous by-product of the agriculture, collection of these residues from field and its management is a costly and time-consuming process which ultimately delays the next sowing in the crop intensive agriculture (Shinde *et al.*, 2022). To avoid this, in many countries, farmers burn this residue on site, which seems good short cut for CR disposal but in the long term it is very harmful to the sustainability of the agriculture and the entire ecosystem. CR burning also releases substantial amount of GHGs in air, responsible for global warming. Moreover, CRs are the major reservoirs of organic carbon, NPK and other plant nutrients, which if not recycled back into the soil cause huge nutrient depletion of soil every year drastically reducing its fertility and quality (Porichha*et al.*, 2021). Retention of this CR on the field is a sustainable method by which the carbon and nutrients can be recycled back in to the soil. For this, accelerating degradation of CR by use of lignocellulose degrading microbes is an eco-friendly management alternative to avoid environmental pollution (Garg, 2017). Several microbes present in soil and other substrates, especially fungi and bacteria, have the ability to decompose lignocellulose materials under aerobic or anaerobic environment (Harindintwali*et al.*, 2020). The complex lignocellulose is broken down through different mechanisms such as oxidative attacks and depolymerization of lignocellulose by specific enzymes (Cragg *et al.*, 2015).Fungi with two kinds of enzyme systems, including hydrolases enzymes (decomposing cellulosic materials) and

*Corresponding Author: reshma.icar@gmail.com
[2]Birsa Agriculture University, Kanke, Ranchi- 834006, Jharkhand, India

lignolytic system (degrading lignin by opening aromatic rings) (Cragg *et al.*, 2015), have better CR decomposing potential as compared to bacteria. They also have added advantage to supply nutrients from one point to other through their long mycelium which helps their entry to even the nutrient poor substrate. Such fungi mostly include *Trichoderma, Aspergillus, Fusarium, Neurospora, Pleurotus*, *Coniophora, Phanerochaete, etc. (Ghazali et al., 2019; Keerthana et al., 2019).* Whereas, well known lignocellulose degrading bacteria are from the genera *Trichonympha, Clostridium, Bacteroides, Ruminococcus, Methanobrevibacter* (Islam, 2019). With the hypothesis that the pure culture of lignocellulosic microbes obtained from decaying wood, compost, residue rich soil, etc. would accelerate the decomposition rate of the CR, this study was conducted. The major focus of this work was on isolation of the lignocellulolytic fungi and bacteria from various substrates and their screening by qualitative and quantitative methods, which can be used as potential crop residue decomposition accelerators in the soil.

MATERIALS AND METHODS

Microbes having lignocellulolytic potential were isolated from different sources such as, crop residue incorporated soil, forest soil, soil near decaying wood, compost pit and dump yard.

Isolation of fungi: For isolation, one gram of soil sample was thoroughly mixed in 99 ml of sterilized distilled water with the help of vortex shaker. Clear supernatant thus obtained after allowing heavy particles to settle down was used for serial dilution. For fungi, 1 ml of the diluted sample was poured on potato dextrose agar (PDA) medium, having chloramphenicol (50 mg/l). The plates were incubated for 7 days at 28 ± 1°C and observed for fungal growth. Morphologically different colonies of fungi were then isolated and purified through repeated streaking on PDA media (Gomashe*et al*., 2013; Choudhary *et al.*, 2016). The purified cultures were maintained at 4°C and sub-cultured as per requirements.

Isolation of bacteria: For isolation of bacteria, clear supernatant obtained from vertexin-gcollected sample with distilled water was used for serial dilution. The sample so obtained was inoculated on nutrient agar medium to allow bacterial growth. The plates were incubated for 48 hours at 30˚C. Bacterial colonies were purified by repetitive streaking. The pure colonies were maintained at 4°C for further identification and screening for lignocellulose degradation (Kameshwar and Qin, 2018).

Screening for lignocellulosic activity: The microbial cultures selected were multiplied in laboratory of ICAR-Research Complex for Eastern Region, FSRCHPR, Ranchi. Purified fungal and bacterial isolates were evaluated by qualitative analysis on the basis of cellulolytic and lignolytic activity using carboxy-methyl-cellulose (CMC) and tannic acid (TA) agar plates, respectively (Choudhary *et al.*, 2016).

Fungi: Purified fungal inoculum in the form of circular disc of approximately 5mm size, was inoculated on the CMC and TA containing agar medium and incubated for a week at 28 ± 1°C. After 7 days, 1% Congo red dye solution was poured in the CMC containing media (10 ml in each petri dish) for 15-20 minutesand then poured off. The coloration was terminated by flooding the plates with 10 ml of 5% NaCl solution, gently shaken and emptied after another 20 minutes. The unstained zone from where CMC is actually consumed by the microbes was measured to note the cellulolytic activity of the microbes (Gomashe *et al.*, 2013).Similarly, lignin degrading ability of fungi was screened by using TA agar media confirming their polyphenol oxidase activity. Appearance of dark brown coloration on the TA agar media was observed and noted which is an indicator of the poly-phenol oxidase (ppo) activity (Choudhary *et al.*, 2016).

Bacteria: Isolated bacterial cultures were inoculated on the CMC agar and TA agar plates and incubated for 48 hours at 30˚C. Similar to fungi, the bacterial plates were also flooded with dilute Congo red (1%) dye solution for 15 minutes and then drained off. Coloration was terminated by gentle shaking with 5% NaCl solution for 20 minutes and the unstained zone was recorded for measuring cellulolytic activity (Andro *et al.,* 1984). For lignolytic activity plates were observed for growth of the brown pigments zone as a measure for PPO activity (Choudhary *et al.,* 2016).

Enzymatic activity: Carboxy methyl cellulase (CMCase) or Endo-b-1,4-glucanase and Filter paperase (FPase) were measured as per the process described by Ghose (1987) Ghose and Bisaria (1987)using CMC and Whatman No.1 filter paper, respectively.

CMCase *Assay*: CMCaseor Endo-b-1,4-glucanase activityof the selected strains was estimatedas per the methoddescribed by Ghose (1987). The reaction mixture with0.5ml of 1% CMC in 0.05M Na-acetate buffer, pH 5.0, and0.5ml of appropriately diluted crude enzyme was used(Sethi *et al.,* 2013).IMThe reaction was terminated by adding 3,5-dinitrosalicylic-acid (DNS) and the readings were taken on UV Vis spectrophotometer at 540 nm (Singh, 2017). The quantity of enzyme liberating 1μmole of reducing sugars from CMC is counted as 1 unit of endoglucanase activityunder the assay conditions.

FPase Assay: The cellulase activity was estimated for FPase usingstrips of Whatman No.1 filter papers of 1 × 6 cm equivalent to 50mg of substrate as perstandard protocol (Ghose, 1987). Minimum two dilutions were prepared, one with the release of just less than 2mg and the other with more than 2mg. The reaction mixture containing 1ml of 0.05M sodium citrate, pH 5.0, filter paper strip, and 0.5ml of crude enzyme diluted accordingly was incubated at50°C for one hour. The reducing sugar released by the mixture was assessedby adding 3,5-dinitrosalicylic-acid (DNS) with glucoseas a standard(Miller, 1959). The absorbance was tested at 540nm with UV-Vis spectrophotometer. All the experiments including controls were carried out in triplicate. Filterpaperase activity is referred to the quantity of enzyme required to release 2 mg of glucose from approx. 50mg filter paper strip within one hour time, divided by 0.37.The best performing cellulose degrading fungi were identified on the basis of morphological and cultural characters. Effective bacterial strains were identified as per the Bergey's Manual of Systematic Bacteriology (Whitman, 2009).

RESULTS AND DISCUSSION

From the different soil samples collected colonies of fungi and bacteria are obtained by serial dilution plating on PDA and nutrient agar medium, respectively. A total of 15 fungi and 6 bacteria were isolated in the form of pure colonies through repeated sub-culturing, from various soil samples collected from of Ranchi region. Details of the various isolates, their source and types are given in the Table 1.

Qualitative analysis by zone of clearance formation

The cellulosic activity of these cultures was evaluated by inoculating them on the CMC containing agar media, where spot inoculation for bacteria and 5mm disc inoculation for fungi was done (Choudhary *et al.,* 2015; Choudhary *et al.,* 2016). Out of the different isolated microbial cultures (IMC) tested, 5 IMCs of fungi and 2 cultures of bacteria showed formation of clear zones around the inoculated site, which can be seen clearly after staining with Congo red dye (Fig 1). The diameter of the zone formation was found to be in the range of 1.1 to 2.9 cm for the seven active microbial cultures (Table 1).

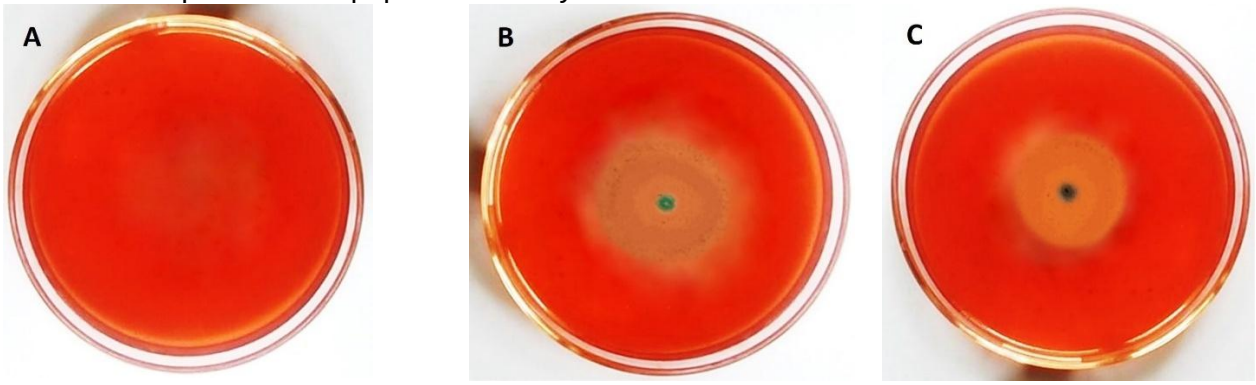

Fig 1: Cellulosic activity and clear zone formation in CMC agar stained by Congo red dye. A. Control- without inoculum, B. IMC 18, C. IMC 4

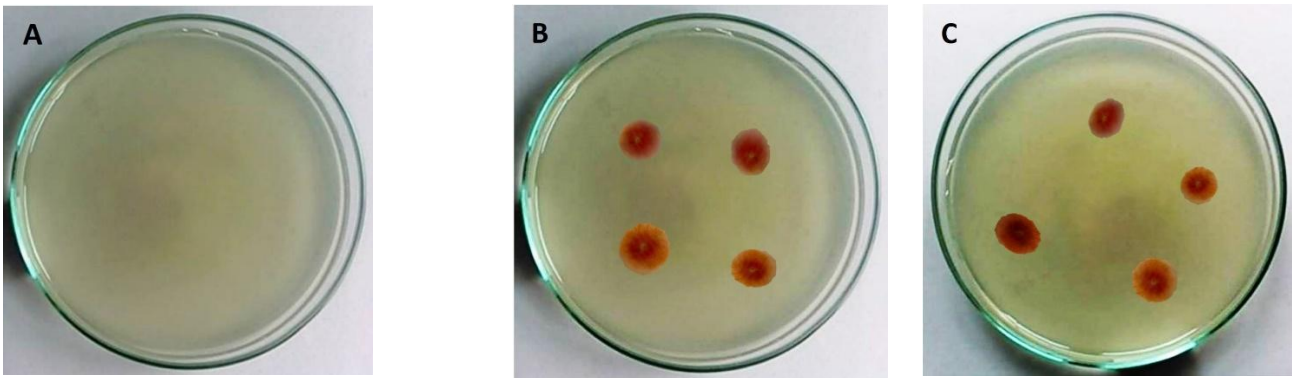

Fig 2. Lignolytic activity and brown zone formation in TA agar. A. Control- without inoculum, B. IMC 18, C. IMC 4

The maximum zone of 2.9 cm was observed for the sample IMC 18, followed by 2.1 cm for the IMC 4. IMC 11 revealed moderate clear zone of 1.9 cm and zone diameter of 1.2 cm for IMC 6 &17 and 1.1 cm for IMC 1 and 20 was observed. For remaining 14 cultures no obvious clear zone was observed.

Interestingly, the cultures showing cellulosic activity have also showed lignolytic activity tested on the tannic acid containing agar media (Fig 2). The range of diameter of the brown growth developed was in the range of 0.3 to 1.8 cm, with maximum 1.8cm for IMC 18, followed by 1.2 cm for IMC 4 (table 1). Minimum activity of 0.3 cm was observed in the case of IMC 1 and 6. The graphical representation of the zone of clearance by different IMCs for CMC agar and TA agar is depicted (Fig 3). All the experiments were conducted in triplicates and mean values are presented in the tables.

Table 1: Source, type and lignocellulosic activity of the various IMCs

Sl. No.	Microbial strain	Type	Source	Ligno-cellulosic Activity	Zone of clearance (cm)	
					CMC agar- Congo red staining	Tannic acid agar
1	IMC-1	Fungus	CRIS	√	1.1	0.3
2	IMC-2	Fungus	CRIS	-	-	-
3	IMC-3	Bacteria	CRIS	-	-	-
4	IMC-4	Fungus	CRIS	√	2.1	1.2
5	IMC-5	Fungus	SNDW	-	-	-
6	IMC-6	Fungus	SNDW	√	1.2	0.3
7	IMC-7	Fungus	SNDW	-	-	-
8	IMC-8	Fungus	FS	-	-	-
9	IMC-9	Fungus	FS	-	-	-
10	IMC-10	Bacteria	FS	-	-	-
11	IMC-11	Fungus	DY	√	1.9	0.4
12	IMC-12	Fungus	SNDW	-	-	-
13	IMC-13	Bacteria	DY	-	-	-
14	IMC-14	Fungus	CP	-	-	-
15	IMC-15	Fungus	FS	-	-	-
16	IMC-16	Fungus	DY	-	-	-
17	IMC-17	Bacteria	DY	√	1.2	0.4
18	IMC-18	Fungus	CP	√	2.9	1.8
19	IMC-19	Fungus	CP	-	-	-
20	IMC-20	Bacteria	CP	√	1.1	0.5
21	IMC-21	Bacteria	DY	-	-	-

IMC: Isolated microbial culture, CRIS: Crop residue incorporated soil,
SNDW: Soil near decomposing wood, FS: Forest soil, DY: Dump yard, CP: Compost pit

The cellulolytic microbes produce cellulase enzyme, which is able to break downcelluloseinto its monomeric or dimeric form which are soluble in water and then it is utilized as a carbon or food source for the growth of colony (Romsaiyud *et al.,* 2009). The purpose of

Congo red staining was to form visible reddish complex with the intact CMC in the plate. The spots where CMC is utilized by the microbes did not form any color complexes and gave clear zone formation. Similar results were obtained by several researchers (Choudhary *et al.,* 2016; Lakshmi, 2016; Ghazali *et al.,* 2019; Keerthana *et al.,* 2019).

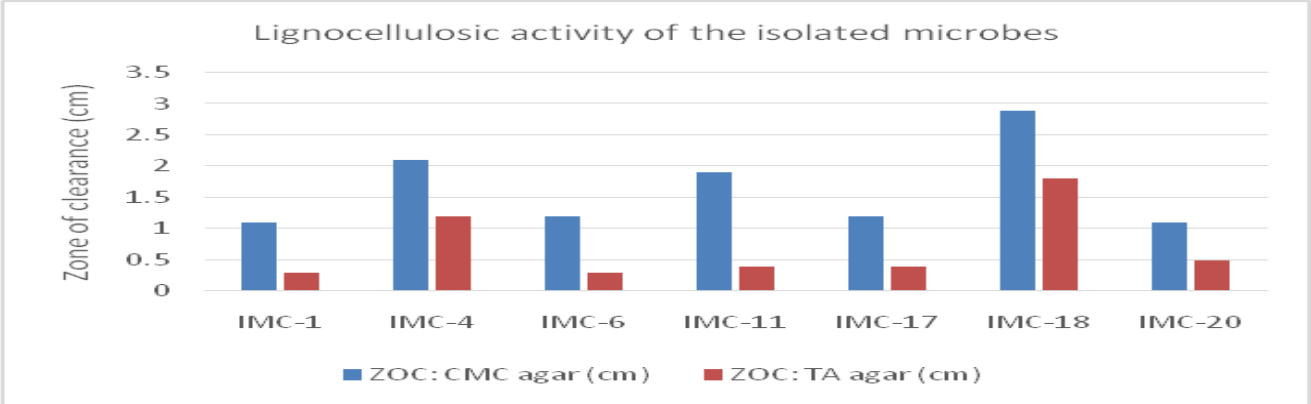

Fig 3: The zone of clearance by different IMCs for CMC agar and TA agar

Quantitative analysis by enzymatic activity assay

Previously screened 5 fungi and 2 bacteria were further evaluated using enzymatic assay to test their CMCase and FPase activity. The reaction mixture was inoculated with the selected microbe and allowed to produce reducing sugars by incubating for 30 minutes at 50°C. Out of five active fungi, two fungal isolates were found far better, showing maximum enzyme activity as compared to other selected fungi and two bacterial cultures (Table 2). In the case of CMCase activity, IMC 18 showed maximum activity 0.26IU/ml followed by IMC 4 (0.21) and IMC 11 (0.16), as depicted in Fig 4. Minimum activity was recorded for IMC1 (0.05) and other cultures including IMC 6 (0.07), IMC 17 (0.09) and IMC 20 with 0.08 were found to have intermediateCMCase activity. For FPase activity quantified by using Whatman no. 1 filter paper, again IMC 18 exhibited highest activity of 0.14 IU/ml and IMC 4 was second highest with 0.11 MU/ml. IMC 6 showed minimum FPase activity (0.008), whereas IMC 1, 11, 17 & 20 were in the middle range. Overall FPase activity was in the range of 0.02 to 0.14 for all seven colonies screened.

Table2: CMCase and FPase activity of the IMCs through enzyme assay

Sl. No.	Microbial strain	Type	Enzymatic activity (IU/ml)	
			CMCase activity	FPase activity
1	IMC-1	Fungus	0.05	0.02
2	IMC-4	Fungus	0.21	0.11
3	IMC-6	Fungus	0.07	0.008
4	IMC-11	Fungus	0.16	0.09
5	IMC-17	Bacteria	0.09	0.04
6	IMC-18	Fungus	0.26	0.14
7	IMC-20	Bacteria	0.08	0.06

Hence, based on the results of clear zone formation and quantitative enzymatic assay IMC 18 was found to most efficient lignocellulose decomposer followed by IMC 4. Both the IMC 18 and IMC 4 were fungi and interestingly they have shown dual ability for decomposing cellulose as well as lignin in the form of tannic acid. The top two performing fungi, IMC 18 and IMC 4 were identified as *Trichoderma species and Aspergillus species*, respectively, based on their morphological and cultural characteristics. The efficiency of the *Trichoderma sp.*(Pachauri *et al.,* 2017; Li *et al.,* 2019)and *Aspergillus species*(Lakshmi, 2016; Lin *et al.,* 2017; Hasanin *et al.,* 2019) revealed in this work is also in the agreement with the earlier studies. The *Trichoderma sp.* was isolated form the compost pit whereas, the *Aspergillus sp.* was purified

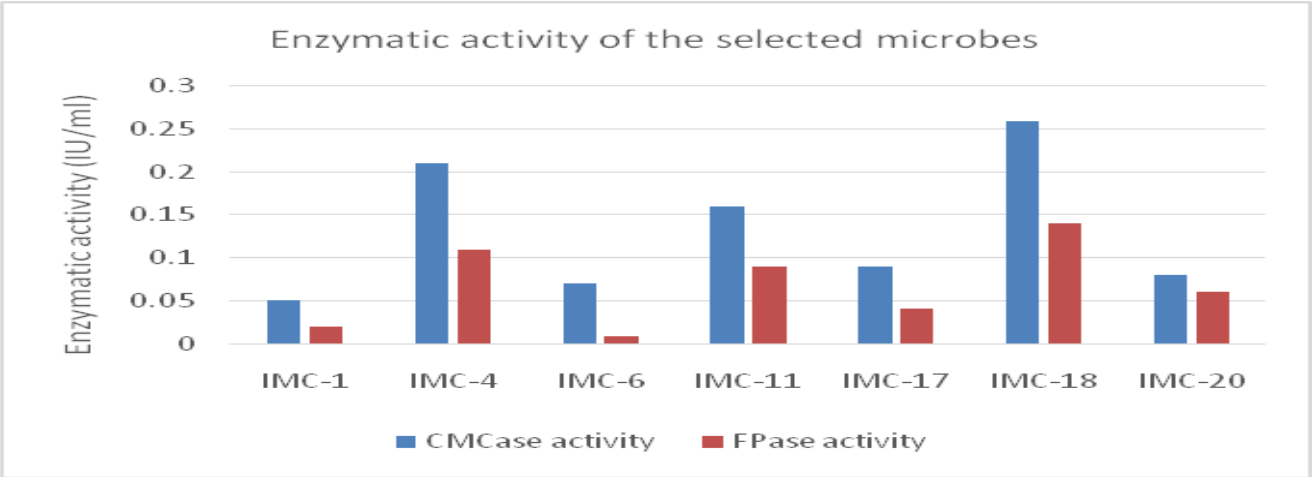

Fig 4: Identification of best performing isolated microbial cultures

from the crop residue incorporated soil. Two bacteria primarily screened from the clear zone formation test, performed fairly for cellulose and lignin decomposition but the extent of their activity was lesser than that of the fungi on an average.

Total of 15 fungi and 6 bacteria were isolated in the form of pure colonies from various sources such as crop residue incorporated soil, forest soil, soil near decaying wood, compost pit and dump yard. Seven isolated microbial cultures (IMC) including five fungal (IMC1, IMC4, IMC6, IMC11 and IMC18) and two bacterial cultures (IMC17 and IMC20) were identified as lignocellulose decomposers by appearance of clear zone in the CMC and TA agar media. Quantitative analysis of these selected cultures through CMCase and filter paperase enzyme assay. IMC 18 and IMC 4 emerged with highest zone of clearance and interestingly both these fungi have shown dual ability for decomposing cellulose as well as lignin in the form of tannic acid. These two fungal strains were identified as *Trichoderma species* and *Aspergillus species* also showed significant enzymatic activity in the CMCase and FPase assay. The efficiency of these strains can be tested through on-field trials to establish them as a potential lignocellulose decomposer.

ACKNOWLEDGEMENT

The authors earnestly express their immense gratitude to the Director, ICAR Research Complex for Eastern Region, Patna, India and the Vice Chancellor, Birsa Agricultural University, Ranchi, India for their continuous encouragement and support. They also thankfully acknowledge the financial assistance fully from Indian Council of Agricultural Research, New Delhi, India.

REFERENCES

Andlar, M., Rezić, T., Marđetko, N., Kracher, D., Ludwig, R., Šantek, B. (2018) Lignocellulose degradation: An overview of fungi and fungal enzymes involved in lignocellulose degradation.*Engineering in Life Sciences* **18**(11): 768–778.

Andro, T., Chambost, J.P., Kotoujansky, A., Cattaneo, J., Bertheau, Y., Barras, F., Van Gijsegem, F., Coleno, A. (1984) Mutants of *Erwinia chrysanthemi* defective in secretion of pectinase and cellulase. *Journal of Bacteriology* **160**(3): 1199–1203.

Anwar, Z., Gulfraz, M., Irshad, M. (2014) Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review.*Journal of Radiation Research and Applied Sciences* **7**(2): 163–173.

Choudhary, M., Sharma, P.C., Jat, H.S., Nehra, V., McDonald, A.J., Garg, N. (2016) Crop residue degradation by fungi isolated from conservation agriculture fields under rice–wheat system of North-West India. *International Journal of Recycling of Organic Waste in Agriculture* **5**(4): 349–360.

Choudhary, M., Sharma, P., Garg, N. (2015) Crop residue degradation by autochthonous fungi isolated from cropping system management scenarios. *Bio Resources* **10**(3): 5809–5819.

Cragg, S.M., Beckham, G.T., Bruce, N.C., Bugg, T.D.H., Distel, D.L., Dupree, P., Etxabe, A.G., Goodell, B.S., Jellison, J., McGeehan, J.E., McQueen-Mason, S.J., Schnorr, K., Walton, P.H., Watts, J.E.M., Zimmer, M. (2015) Lignocellulose degradation mechanisms across the tree of life.*Current Opinion in Chemical Biology* **29**: 108–119.

Garg, S. (2017) Bioremediation of agricultural, municipal, and industrial wastes, in Handbook of research on inventive bioremediation techniques. IGI Global.

Ghazali, M., Zainudin, N., Nor, A., Mustafa, M. (2019) Screening of lignocellulolytic fungi for hydrolyzation of lignocellulosic materials in paddy straw for bioethanol production. *Malaysian Journal of Microbiology* **15**(5): 379–386.

Ghose, T.K. (1987) Measurement of cellulase activities.*Pure and Applied Chemistry* **59**(2): 257–268.

Ghose, T.K. and Bisaria, V.S. (1987) Measurement of hemicellulase activities part 1: Xylanases.*Pure and Applied Chemistry* **59**(12): 1739–1751.

Gomashe, A., Gulhane, P. and Bezalwar, P. (2013) Isolation and screening of cellulose degrading microbes from Nagpur region soil.*International Journal of Life Sciences* **1**(4): 291–293.

Harindintwali, J.D., Zhou, J. and Yu, X. (2020) Lignocellulosic crop residue composting by cellulolytic nitrogen-fixing bacteria: A novel tool for environmental sustainability, *Science of the Total Environment* **715**: 136912.

Hasanin, M.S., Darwesh, O.M., Matter, I.A., El-Saied, H. (2019) Isolation and characterization of non-cellulolytic Aspergillus flavus EGYPTA5 exhibiting selective ligninolytic potential.*Biocatalysis and Agricultural Biotechnology* **17**: 160–167.

Isikgor, F. and Becer, C. (2015) Lignocellulosic biomass: A sustainable platform for the production of bio-based chemicals and polymers. *Polymer Chemistry* **6** (25): 4497–4559.

Islam, F. (2019) Isolation and characterization of cellulase-producing bacteria from sugar industry waste.*American Journal of Bio Science* **7**(1): 16.

Kameshwar, A.S. and Qin, W. (2018) Isolation and screening of cellulose-degrading microorganisms from different ecological niches, In Lubeck, M. (ed.) Cellulases: methods and protocols, methods in molecular biology. Springer Nature: 115–122.

Keerthana, S., Kalaiselvi, P., Maheshwari, M., Kalaiselvi, T. (2019) Isolation and screening of lignin and cellulose degrading proficient microbial strains from diverse biotic substrates based on qualitative traits.*International Journal of Current Microbiology and Applied Sciences* **8**(07): 475–483.

Lakshmi, C. (2016) Cellulase from isolates of banana fields in Nanjangud and Bannur villages of Mysore district.*International Journal of Current Research* **8**(4): 29039–29043.

Li, J.X., Zhang, F., Li, J., Zhang, Z., Bai, F.W., Chen, J., Zhao, X.Q. (2019) Rapid production of lignocellulolytic enzymes by *Trichoderma harzianum* LZ117 isolated from Tibet for biomass degradation. *Bioresource Technology* **292**: 122063.

Lin, C., Shen, Z. and Qin, W. (2017) Characterization of xylanase and cellulase produced by a newly isolated *Aspergillus fumigatus* N2 and its efficient saccharification of barley straw.*Applied Biochemistry and Biotechnology* **182**(2): 559–569.

Miller, G.L. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar.*Analytical Chemistry* **31**(3): 426–428.

Pachauri, P., Aranganathan, V., More, S., Sullia, S.B., Deshmukh, S. (2017) Purification and characterization of cellulase from a novel isolate of *Trichoderma longibrachiatum. Biofuels* **11**(1): 85–91.

Porichha, G.K., Hu, Y., Rao, K.T.V., Xu, C.C. (2021) Crop residue management in India: Stubble burning vs. other utilizations including bioenergy.*Energies*, **14**(14): 1–17.

Romsaiyud, A., Songkasiri, W., Nopharatana, A.,

Chaiprasert, P. (2009) Combination effect of pH and acetate on enzymatic cellulose hydrolysis.*Journal of Environmental Sciences* **21**(7): 965–970.

Sethi, S., Datta, A., Gupta, B.L., Gupta, S. (2013) Optimization of cellulase production from bacteria isolated from soil.*ISRN Biotechnology* **2013**: 1–7.

Shinde, R., Shahi, D.K., Mahapatra, P., Singh, C.S., Naik, S.K., Thombare, N., Singh, A.K. (2022) Management of crop residues with special reference to the on-farm utilization methods: A review.*Industrial Crops and Products* **181**: 114772.

Singh, G. (2017) Assessment of microbial consortia for paddy straw degradation. MSc Thesis. Punjab Agricultural University.

Wang, F., Ouyang, D., Zhou, Z., Page, S.J., Liu, D., & Zhao, X. (2021) Lignocellulosic biomass as sustainable feedstock and materials for power generation and energy storage. *Journal of Energy Chemistry* **57**: 247–280.

Whitman, W.B. (ed.) (2009) Bergey's Manual of Systematic Bacteriology. London: Bergey's Manual Trust.