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Qualitative analyses of lignocellulolytic enzymes produced by *Emericella* sp strain HST9 under diverse culture conditions

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Abstract

Aim : Optimization of cultural conditions for improved lignocellulolytic enzyme production by an ascomycete HST9 isolated from leaf and litter waste.

Methodology : The fungus HST9 was isolated from leaf and litter waste collected from Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. Culture experiments were conducted at different temperature, pH, incubation periods and aeration conditions. Effects of addition of different concentrations of various metal ions, different carbon complexes and nitrogen salts on enzyme production were also studied under submerged culture condition. Enzyme activities were measured by standard protocols using spectrophotometer.

Results : HST9 grew well and produced optimum enzymes at 30°C on 7th day of incubation at stationary conditions and pH- 6. Overall enzyme activities decreased after addition of metal salts. Carboxymethyl cellulose (300 mg l⁻¹) and alkali lignin (200 mg l⁻¹) were observed to be the best carbon complexes for cellulolytic and ligninolytic activities. Ammonium sulfate was found to be a better nitrogen source compared to others. Under optimum conditions, different enzyme activities observed were 0.011 IU m l⁻¹ FPase, 0.015 IU m l⁻¹ CMCase, 6.5 IU m l⁻¹ Lac, 57.5 IU m l⁻¹ LiP and 4 IU m l⁻¹ MnP. Molecular phylogenetic analysis of the strain confirmed that strain HST9 showed closeness with genus *Emericella*.

Interpretation : Lignocellulolytic enzyme activity of *Emericella* isolate HST9 enhanced at optimum culture conditions, signifying that it can be used as a biological agent to degrade lignocellulosic waste.

Key words: Ascomycetes, Compost, *Emericella* sp, Endoglucanases, Lignocellulose

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Introduction

The most copious biomass on earth is the plant biomass which is considered as an attractive source of bioenergy and bio-based chemicals. Agro-wastes generated from plant biomass also provide an excellent alternative for chemical fertilizers in the form of compost. In India, the most prevailing problem these days is that generated agro-wastes are abundantly destroyed by burning. Burning of paddy straw by farmers in north-western India causes environmental pollution and degradation of soil health (Kaur and Mahal, 2017). It is responsible for the loss of many essential components of top, fertile layer of soil including nitrogen, carbon, phosphorus, sulphur and potassium and also reduces the number and diversity of soil microbiota, thereby reducing the fertility and viability of agricultural land in the long run. Efforts have been made to increase the avenues for the alternate usage of crop residues. They can be used as fuel in biomass based power plants for generating of bio-fuels (biogas and bioethanol) for automobiles, raw material in paper, cardboard and textile industries and also as organic fertilizers.

Compost can be prepared from agro wastes using natural biological phenomena. These agro wastes are mainly composed of three types of polymers: Cellulose, hemicellulose and lignin, with varying ratio between the three fractions depending on plant species and from the plant part biomass is collected (Lohre *et al.*, 2017). They are strongly intermeshed and chemically bonded by non-covalent forces and covalent cross-linkages. Plant cellulose is efficiently hydrolyzed to glucose by the synergistic action of three types of cellulases, i.e., endoglucanases also known as 1,4- β -D glucan 4-glucosidase, β -glucosidases (β -D-glycoside glucosidase) and exoglucanases (β -1,4-D-glucan cellobiohydrolase) (Padaria *et al.*, 2014). However, the main hurdle in this process is lignin, which blocks artificial cellulases to reach cellulose polymer. Due to its complex aromatic heteropolymer and high molecular weight, lignin is extremely recalcitrant to biodegradation (Zhao *et al.*, 2012).

Many microorganisms that produce lignocellulolytic enzymes have been studied from several decades. Some fungal and bacterial species produce either ligninase or cellulase or both isoforms (Kubicek *et al.*, 2008), however, fungi are considered to be better degraders of lignocellulose because they comprise all the lignocellulolytic activities. Besides, fungal enzymes are extra-cellular, adaptive and usually secreted in large quantities during growth. Lignocellulose in compost is degraded and transformed into fungal biomass and accounts 85% of fruiting body and 45% of mycelial dry weights (Zhou *et al.*, 2018). However, the number of microbes identified for degradation of lignocellulosic biomass, till date, are still unable to fulfill the industrial demands (Shary *et al.*, 2007; Afrida *et al.*, 2009; Daur, 2016). Thus, identification of new microorganisms and development of methods to utilize them completely for lignin degradation is important (Tanvi *et al.*, 2016).

Enzyme production by fungi and their function totally depends on the appropriate cultural and physico chemical

conditions and media composition. The objectives of current study were to isolate and identify fungal species that can effectively produce lignocellulolytic enzymes and to optimize its biochemical and physical conditions to obtain optimal conditions for mycelial growth rate and activities of produced enzymes.

Materials and Methods

Isolation and selection of lignocellulolytic fungi: Samples of leaf and litter remains in soil were collected from Chaudhary Charan Singh Haryana Agricultural University campus (29.1504° N, 75.7057° E), Hisar, India. Enrichment culture technique was performed on liquid media consisting of malt extract and Mary Mandel broth for isolation of ligninolytic and cellulolytic fungi and then the flasks were kept on rotary shaker at 140 rpm, at $28 \pm 2^\circ\text{C}$ for incubation. After 7 days, 1 ml culture was withdrawn and a sequential dilution of enriched sample was prepared (Tanvi *et al.*, 2018). This process was repeated two times within a gap of 14 days and, subsequently, microorganisms from enriched cultures were isolated on carboxy methyl cellulose (CMC) agar (Hankin and Anagnostakis, 1977) and malt extract agar media plate (Erden *et al.*, 2009). Cellulolytic activity of the fungus was screened on CMC agar plates. Screening procedure was based on degradation of CMC by the fungal enzymes resulting in a transparent zone around the fungal colony which was observed after inundation of culture plates with 0.1% Congo red dye solution for 15-20 min, followed by detaining the plate for 15-20 min with 1 M NaCl. Index of Relative Enzyme Activity (Ircmc) of the fungus was estimated as per Bradner *et al.* (1999) and Peculyte (2007). For ligninolytic activity, zone of clearance (ratio of clear zone diameter to colony diameter) on aniline blue (0.04%) supplemented malt extract agar medium was calculated.

Enzyme assay: Cellulytic enzymes like CMCase and FPase were anticipated by the method of Ghose (1987) and the reducing sugars produced were determined by DNS method of Miller (1959). Ligninolytic enzymes like laccase, lignin peroxidase and manganese peroxidase were estimated by the respective methods of Shandilya and Munjal (1983), Tien and Kirk (1988) and Paszczynski *et al.* (1988). The quantity of enzyme required to release 1 μmol of reducing sugars in a minute is designated as single International Unit (IU) of enzyme.

Culture conditions characterization: Different culture conditions like temperature, pH, aeration, incubation period, addition of metal ions, carbon and nitrogen sources were studied. Equal amounts of mycelial biomass were grown at 25, 30 and 35°C at stationary and shaking conditions and pH 4, 5, 6 and 7. To check the relationship between enzyme activity and incubation period, samples from broth were withdrawn daily for 10 days. All enzyme activities were estimated by standard procedures stated above. Different concentrations of MnSO_4 (0.003 g l⁻¹, 0.006 g l⁻¹ and 0.009 g l⁻¹), FeSO_4 (0.0025 g l⁻¹, 0.005 g l⁻¹ and 0.0075 g l⁻¹) and ZnSO_4 (0.0015 g l⁻¹, 0.003 g l⁻¹ and 0.0045 g l⁻¹) were supplemented to the broth for estimating cellulase activity. For determining ligninase activity, 0.25 g l⁻¹, 0.5 g l⁻¹ and 1.0 g l⁻¹ of

three metallic salts were amended in broth. Varying concentrations (50 g l^{-1} , 100 g l^{-1} , 200 g l^{-1} and 300 g l^{-1}) of different carbon sources like alkali lignin, cellulose powder and carboxymethyl cellulose powder were added to the respective broths to check their effect on enzyme activities. Four different ammonium salts (nitrogen source) viz., ammonium nitrate, ammonium chloride, ammonium sulfate and ammonium oxalate (0.5 g l^{-1}) were added to the broth and their effect on lignocellulolytic enzymes was observed. Standard culture of *Phanerochaete chrysosporium* MTCC-787 was obtained from MTCC, Institute of Microbial Technology (IMTECH), Chandigarh and *Trichoderma viride* QM- 9414 from Department of Microbiology, CCS HAU, Hisar for comparison. All the experiments were performed in triplicate and the results obtained were expressed as mean $\pm$ SD.

Morphological characterization: Morphological characteristics like colony color and size, surface, margin and pigmentation, front and reverse morphology, septation and branching in hyphae, reproductive structures, sporulation and arrangement of spores were studied in (Toledo et al., 2013).

Molecular characterization: Fungal isolate was outsourced for molecular characterization to CHROMOUS BIOTECH Pvt. Ltd., Bangalore, where the sample was subjected to PCR using 600-650 bp internal transcribed spacer (ITS) specific primers for 18S rRNA. The gel purified sample was sequenced bidirectionally. Sequence obtained from forward and reverse primers was subjected to BLAST tool of NCBI for comparison. The sequence along with other similar sequences was then

aligned using BioEdit software and a phylogenetic tree was constructed using MEGA (version X) software (Fig. 1) (Kumar et al., 2018). The sequence was submitted to GenBank (NCBI) with accession number MH032749.

Results and Discussion

Fungal isolate HST 9 showed lignocellulolytic activity after primary screening with 1.12 mm zone of clearance and 0.070 lcmc on carboxymethyl cellulose agar plates. Similarly, Kale and Zanwar (2016) isolated, screened and compared cellulolytic activity of four fungal species belonging to *Trichoderma* and *Aspergillus*. They also screened the fungi using assay described by International Union of Pure and Applied Chemistry (IUPAC), i.e., by Congo Red staining and NaCl washing. The clear zone diameter around the fungi indicated the presence of cellulolytic enzyme. However, Selvam et al. (2012) used poly R-478 and remazol brilliant blue dyes for screening of ligninolytic fungi. Dye removal in 57 samples ranged 11.5 to 38.4% in poly- R dye and 11.1 to 72.0 % in remazol brilliant blue.

In this study, at 30°C (Fig. 1a) and pH 6 (Fig. 1b), the lignocellulolytic activity of HST9 was highest. Although the enzyme activities improved with rise in the pH from 4 to 7; but, no significant change was observed in the enzyme activities at pH 6 and 7 (Fig. 1b). Also, at stationary conditions, enzyme activities were better as compared to shaking conditions with $0.011 \text{ IU m l}^{-1}$ FPase, $0.015 \text{ IU m l}^{-1}$ CMCcase, 7.5 IU m l^{-1} Lac, 53.5 IU m l^{-1} LiP and 4 IU m l^{-1} MnP activity (Fig. 1c). Some other researchers have also reported better lignocellulose utilization under static conditions

Fig. 1 : Evolutionary history of fungal strain HST9 and related fungal species constructed by neighbor-joining method. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. This analysis involved 11 nucleotide sequences. Evolutionary analyses were conducted by the MEGA X program.

Table 1 : Lignocellulolytic activities of strain HST9 at different incubation periods

Incubation periods (Days)	Cellulolytic activities (IU ml ⁻¹)			Ligninolytic activities (IU ml ⁻¹)	
	FPase	CMCase	Lac	LiP	MnP
Day 0	0.000	0.000	0	0	0
Day 1	0.000	0.000	0	0	0
Day 2	0.000	0.000	0	0	0
Day 3	0.002 ± 0.001	0.000	3 ± 0.020	4 ± 0.119	1 ± 0.109
Day 4	0.004 ± 0.001	0.002 ± 0.001	4.5 ± 0.117	6.5 ± 0.045	1 ± 0.025
Day 5	0.005 ± 0.001	0.007 ± 0.002	6 ± 0.021	47.2 ± 1.265	1 ± 0.213
Day 6	0.010 ± 0.003	0.008 ± 0.002	7.5 ± 0.136	49 ± 1.376	3.5 ± 0.564
Day 7	0.012 ± 0.003	0.016 ± 0.008	7.5 ± 0.025	58.3 ± 3.412	3.5 ± 0.358
Day 8	0.010 ± 0.002	0.016 ± 0.005	6.6 ± 0.267	6 ± 0.750	3 ± 0.257
Day 9	0.010 ± 0.003	0.015 ± 0.008	6 ± 0.870	2 ± 0.025	2 ± 0.063
Day 10	0.010 ± 0.007	0.015 ± 0.004	6 ± 0.875	2.5 ± 1.450	2 ± 0.764

Values are mean of three replicates ± SD for all enzymes

Table 2 : Enzyme activities of strain HST9 in different concentrations of metallic salts

Cellulolytic (IU ml ⁻¹)			Ligninolytic (IU ml ⁻¹)			
MnSO ₄ (g l ⁻¹)	FPase	CMCase	MnSO ₄ (g l ⁻¹)	Lac	LiP	MnP
0.003	0.002 ± 0.001	0.001 ± 0.001	0.25	11 ± 2.598	5 ± 0.368	1 ± 0.048
0.006	0.011 ± 0.002	0.015 ± 0.866	0.50	21 ± 1.478	10 ± 0.075	2 ± 0.065
0.009	0.014 ± 0.005	0.015 ± 0.003	1.00	44 ± 1.476	13 ± 0.489	2 ± 0.153
FeSO ₄ (g l ⁻¹)	FPase	CMCase	FeSO ₄ (g l ⁻¹)	Lac	LiP	MnP
0.0025	0.007 ± 0.002	0.006 ± 0.003	0.25	0	0	2 ± 0.866
0.005	0.010 ± 0.001	0.010 ± 0.002	0.50	10 ± 0.500	14 ± 0.288	1 ± 0.576
0.0075	0.007 ± 0.002	0.011 ± 0.002	1.00	1 ± 0.024	9 ± 0.5	2 ± 0.763
ZnSO ₄ (g l ⁻¹)	FPase	CMCase	ZnSO ₄ (g l ⁻¹)	Lac	LiP	MnP
0.0015	0.005 ± 0.001	0.003 ± 0.001	0.25	5 ± 0.478	0	2 ± 0.068
0.003	0.011 ± 0.004	0.015 ± 0.006	0.50	20 ± 1.076	5 ± 0.474	0
0.0045	0.010 ± 0.003	0.013 ± 0.005	1.00	5 ± 0.367	9 ± 0.056	2 ± 0.110

Values are mean of three replicates ± SD

as compared to that of shaking conditions (Balaji *et al.*, 2012; Muthukumar *et al.*, 2014); however there are also reports stating that shaking conditions are helpful in lignocellulose degradation. Abdel-Fatah *et al.* (2012) observed that for *Aspergillus terreus* strain DSM 826, favorable conditions for the production of CMCase were 30°C incubation temperature, 150 rpm shaking, pH 4.5-5 with rice straw and sugar cane bagasse as lone carbon source and ammonium chloride as nitrogen source. On third day of incubation, enzyme yield was maximum.

While observing enzyme activity at successive days, an increase in activities was seen up to 7 days of incubation, reaching the highest value of 0.012 IU ml⁻¹ FPase and 0.016 IU ml⁻¹ CMCase. After 7th day, no significant changes were observed. Laccase, lignin peroxidase and manganese peroxidase activities also showed similar trend with maximum values of 7.5 IU ml⁻¹, 58.30 IU ml⁻¹ and 3.5 IU ml⁻¹ respectively, on 7th day. Therefore, for further experiments, estimation of enzyme activities on 7th day of

incubation was standardized (Table 1). Addition of different concentrations of MnSO₄, FeSO₄ and ZnSO₄ to the growth medium surprisingly decreased all enzyme activities (Table 2).

This concluded that these metal ions were inhibiting the fungal activity. In a similar study, Guillén *et al.* (2011) studied the effect of copper and cadmium ions on the lignocellulolytic activities of 71 fungal isolates and concluded that maximum fungal strains showed tolerance to 3mM copper in solid medium, but were inhibited in the presence of 1mM cadmium. From Table 3, it is clear that, lignocellulolytic activities increased with increase in substrate concentration up to 200 mg l⁻¹. Several many other substrates like pre-treated groundnut shell (Vyas *et al.*, 2005), oil palm empty fruit bunch fiber (Shahriarnour *et al.*, 2011), coir waste (Mrudula and Murugammal, 2011), rice straw and sugarcane bagasse (Abdel-Fatah *et al.*, 2012; Kumar and Parikh, 2015) have also been reported for producing cellulases by fungi. In a similar study conducted by Kausar *et al.* (2010), the lignocellulolytic

Fig. 2 : Dynamics of different (a) temperature, (b) pH, (c) aeration conditions and (d) ammonium ions on lignocellulolytic enzyme activities. Error bar indicates standard error (SE) of mean values of three replicates. Different numbers on graph bars show the actual enzyme activities. *Ammonium ions were added in the form of ammonium salts.

Table 3 : Enzyme activities of strain HST9 at different concentrations of carbon sources

Conc.* (mg l ⁻¹)	Cellulolytic activities (IU ml ⁻¹)				Ligninolytic activities (IU ml ⁻¹)		
	Cellulose powder		CMC powder		Alkali lignin		
	FPase	CMCase	FPase	CMCase	Lac	LiP	MnP
50	0.009 ± 0.002	0.011 ± 0.002	0.010 ± 0.002	0.011 ± 0.001	6 ± 0.256	50.5 ± 2.647	1 ± 0.856
100	0.010 ± 0.001	0.013 ± 0.003	0.012 ± 0.003	0.011 ± 0.001	7 ± 1.547	54.5 ± 3.764	0.5 ± 0.025
200	0.013 ± 0.005	0.015 ± 0.002	0.019 ± 0.008	0.017 ± 0.004	11 ± 0.149	55.5 ± 1.488	4.5 ± 1.043
300	0.010 ± 0.002	0.015 ± 0.005	0.017 ± 0.009	0.015 ± 0.005	7 ± 0.236	55 ± 3.767	4 ± 0.727

Values are mean of three replicates ± SD

Table 4 : Comparison of different enzyme activities of strain HST9 with standard cultures

Fungal isolate	Cellulolytic activities (IU ml ⁻¹)			Ligninolytic activities (IU ml ⁻¹)		
	FPase	CMCase	Lac	LiP	MnP	
Strain HST9	0.011 ± 0.002	0.015 ± 0.002	6.5 ± 0.736	57.5 ± 2.547	4 ± 0.763	
<i>T. viride</i> -QM 9414	0.121 ± 0.017	0.106 ± 0.023	14.5 ± 1.765	52.69 ± 2.877	3.5 ± 0.043	
MTCC 787	0.080 ± 0.008	0.029 ± 0.005	0.50 ± 0.053	93.500 ± 3.856	6.210 ± 0.975	

Values are mean of three replicates ± SD

activity of ten lignocellulolytic isolates were tested and an increase in enzyme activity was observed with increase in the concentration of substrate i.e., powdered rice straw in the media showing their ability to utilize complex lignocellulosic materials by producing oxidative and hydrolytic enzymes. Similarly, Neethu *et al.* (2012) supplemented basal salt medium with synthetic (CMC, glucose, sucrose, dextrose, lactose or maltose) and natural (flours of banana, banana peel, jack seed, potato or tapioca) carbon sources and observed improved cellulase activity of 173 U ml⁻¹ at 1.25% CMC concentration. Also, 1.0 and 0.5 % CMC was able to induce CMCase effectively in sugar cane bagasse and rice straw, (Abdel-Fatah *et al.*, 2012). Likewise, Naseeb *et al.* (2015) studied the effect of different substrates like xylan CMC and salicin on enzyme activities and observed that these substrates were responsible for inducing the production of hydrolytic enzymes like xylanase endoglucanases and β-glucosidase respectively.

Nitrogen in the form of ammonium ions is easily accessible and acts as a nutrient for fungal growth as it is essential for the regeneration of NADH (Subramanian *et al.*, 2014). Amongst four different ammonium salts added, ammonium sulfate proved to be a better nitrogen source giving 0.015 IU ml⁻¹ FPase activity, 0.007 IU ml⁻¹ CMCase, 4 IU ml⁻¹ Lac, 15.50 IU ml⁻¹ LiP and 0.50 IU ml⁻¹ MnP activity (Fig. 1d). Similarly, Membrillo *et al.* (2008) studied the effect of ammonium sulfate on enzyme production and reported that among the two strains of *Pleurotus ostreatus* under consideration, i.e., *Pleurotus ostreatus* IE-8 and *Pleurotus ostreatus* CP-50, strain IE-8 produced highest levels of all enzymes with and without the addition of AS but

strain CP-50 showed better enzyme production i.e. 48% on substrate particle size of 1.68 mm after adding of ammonium sulfate as nitrogen source when compared to media with no additional nitrogen source.

The enzyme activity of HST9 was compared with enzyme activities of *Phanerochaete chrysosporium* (MTCC- 787) and *Trichoderma viride* QM- 9414 under optimized conditions (Table 4). HST9 showed less FPase and CMCase activities in comparison to MTCC- 787 and *T. viride* QM- 9414. The isolate was showing 6.5 IU ml⁻¹ Lac activities which was better than the Lac activity of MTCC- 787, however, less than *T. viride* QM- 9414. LiP activity was more in the standard culture MTCC- 787 (93.5 IU ml⁻¹) than the selected isolate (57.5 IU ml⁻¹). MnP activity of HST9 was equivalent to the standard culture *T. viride* QM- 9414 and slightly less than MTCC-787 (Table 4). Mishra and Pandey (2007) also studied the lignocellulolytic activities of two *Phanerochaete chrysosporium* strains (NCIM- 1073 and MTCC- 787). They observed that MTCC- 787 possessed higher CMCase (0.36 – 0.42 IU ml⁻¹) and FPU (0.55 – 0.88 IU ml⁻¹) activities after 15 days of submerged fermentation and lignin peroxidase activity of 0.857 IU ml⁻¹ and 1.477 IU ml⁻¹ after 7 and 15 days, respectively. However, laccase activity of 0.116 IU ml⁻¹ was recorded after 15 days of incubation.

HST9 grew very fast, covering full plate after 7 days of incubation. Hyphae were white, cottony and septate with separate conidia. These morphological characteristics revealed that fungal isolate belongs to phylum *Ascomycota* and it might be of genus *Aspergillus*. After sequencing and molecular analysis, it was confirmed by BLAST that the fungal strain HST9 was 99.48%

similar to *Emericella rugulosus* strain APBDSF 104 and *Emericella rugulosa* isolate 14. Production of lignocellulolytic enzymes by fungal isolate HST9 (*Emericella dentate*) could be enhanced by growing the fungus under optimum culture conditions. This fungus in association with other fungi could be used as a consortium to degrade lignocellulosic waste. The above findings are important not only for farmers to convert the residual lignocellulose into compost but also for different lignocellulose based industries.

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References

- Abdel-Fatah, O.M., M.M. Hassan, A.M. Elshafei, B.M. Haroun, H.M. Atta and A.M. Othman: Physiological studies on carboxymethyl cellulase formation by *Aspergillus terreus* DSM 826. *Braz. J. Microbiol.*, **43**, 1-11 (2012).
- Afrida, S., Y. Tamai, T. Watanabe and M. Osaki: Screening of white rot fungi for bio-bleaching of *Acacia* oxygen-delignified kraft pulp. *World J. Microbiol. Biotechnol.*, **25**, 639-647 (2009).
- Balaji, V., D. Vinayagamoorthi, A. Palanisamy and S. Anbalagan: Degradation of reactive red HE7B and yellow FN2R dyes by fungal isolates. *J. Acad. Ind. Res.*, **1**, 132-136 (2012).
- Bradner, J.R., M. Gillings and K.M.H. Nevalainen: Qualitative assessment of hydrolytic activities in Antarctic micro fungi grown at different temperatures on solid media. *World J. Microbiol. Biotechnol.*, **15**, 131-132 (1999).
- Daur, I.: Study of commercial effective microorganism on composting and dynamics of plant essential metal micronutrients. *J. Environ. Biol.*, **37**, 937-941 (2016).
- Erden, E., C. Ucar, T. Gezer and N. Pazarlioglu: Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of remazole marine blue. *Braz. J. Microbiol.*, **40**, 346-353 (2009).
- Ghose, T.K.: Measurement of cellulase activities. *Pure Appl. Chem.*, **59**, 257-268 (1987).
- Guillén, Y., G. Palfner and A. Machuca: Screening for lignocellulolytic enzymes and metal tolerance in isolates of wood-rot fungi from Chile. *Interciencia*, **36**, 860-868 (2011).
- Hankin, L. and S.L. Anagnostakis: Solid media containing carboxymethylcellulose to detect Cx cellulase activity of microorganisms. *J. Gen. Microbiol.*, **98**, 109-115 (1977).
- Kale, R.A. and P.H. Zanwar: Isolation and screening of cellulolytic fungi. *IOSR J. Biotechnol. Biochem.*, **2**, 57-61 (2016).
- Kaur, J. and S.S. Mahal: Influence of paddy straw mulch on crop productivity and economics of bed and flat sown wheat (*Triticum aestivum*) under different irrigation schedules. *J. Environ. Biol.*, **38**, 243-250 (2017).
- Kausar, H., M. Sariah, H.M. Saud, M.Z. Alam and M.R. Ismail: Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. *Int. Biodegrad. Biodegrad.*, **64**, 594-600 (2010).
- Kubicek, C.P., M. Komon-Zelazowska and I.S. Druzhinina: Fungal genus *Hypocrea trichoderma*: From barcodes to biodiversity. *J. Zhejiang Univ. Sci. B.*, **9**, 753-763 (2008).
- Kumar, A.K. and B.S. Parikh: Cellulose-degrading enzymes from *Aspergillus terreus* D34 and enzymatic saccharification of mild-alkali and dilute-acid pretreated lignocellulosic biomass residues. *Bioresour. Bioprocess.*, **2**, 7 (2015) doi:10.1186/S40643-015-0038-8
- Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura: MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.*, **35**, 1547-1549 (2018).
- Löhre, C., H.V. Halleraker and T. Barth: Composition of lignin-to-liquid solvolysis oils from lignin extracted in a semi-continuous organosolv process. *Int. J. Mol. Sci.*, **18**, 225 (2017) doi: 10.3390/ijms18010225
- Membrillo, I., C. Sa'nchez, M. Meneses, E. Favela and O. Loera: Effect of substrate particle size and additional nitrogen source on production of lignocellulolytic enzymes by *Pleurotus ostreatus* strains. *Bioresour. Technol.*, **99**, 7842-7847 (2008).
- Miller, G.L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, **31**, 426-428 (1959).
- Mishra, B.K. and A.K. Pandey Lata: Lignocellulolytic enzyme production from submerged fermentation of paddy straw. *Indian J. Microbiol.*, **47**, 176-179 (2007).
- Mrudula, S. and R. Murugammal: Production of cellulose by *Aspergillus niger* under submerged and solid state fermentation using coir waste as a substrate. *Braz. J. Microbiol.*, **42**, 1119-1127 (2011).
- Muthukumar, P., M. Priya, R. Divya, E. Indhumathi and C. Keerthika: Biodecolorization of reactive dyes by using *Pleurotus ostreatus*. *J. Biotechnol. Biosaf.*, **2**, 94-101 (2014).
- Naseeb, S., M. Sohail, A. Ahmad and K.S.A. Ahmed: Production of xylanases and cellulases by *Aspergillus fumigatus* MS16 using crude lignocellulosic substrates. *Pak. J. Bot.*, **47**, 779-784 (2015).
- Neethu, K., M. Rubeena, S. Sajith, S. Sreedevi, P. Priji, K.N. Unni, M.K. Sarath Josh, V.N. Jisha, S. Pradeep and S. Benjamin: A novel strain of *Trichoderma viride* shows complete lignocellulolytic activities. *Adv. Biosci. Biotechnol.*, **3**, 1160-1166 (2012).
- Padaria, J.C., K. Sarkar, S.A. Lone and S. Srivastava: Molecular characterization of cellulose-degrading *Bacillus pumilus* from the soil of tea garden, Darjeeling hills, India. *J. Environ. Biol.*, **35**, 555-561 (2014).
- Paszczynski, A., B. Huynhv and R. Crawford: Enzymatic activities of an extracellular, manganese-dependent peroxidase from *Phanerochaete chrysosporium*. *FEMS Microbiol. Lett.*, **2**, 37-41 (1988).
- Patel, K., H. Patel and G. Shah: Production and optimization of cellulase enzymes from newly isolated fungi. *J. Pure Appl. Microbio.*, **8**, 4163-4169 (2014).
- Peculyte, D.: Isolation of cellulolytic fungi from waste paper gradual recycling materials. *Ecology*, **53**, 11-18 (2007).
- Selvam, K., M. Shanmuga Priya, C. Sivaraj and K. Arungandhi: Identification and screening of wood rot fungi from Western Ghats area of South India. *Int. J. Chem. Tech. Res.*, **4**, 379-388 (2012).
- Shahriarinnour, M., M.N.A. Wahab, S. Mustafa, R. Mohamad and A.B. Ariff: Effect of various pretreatments of soil palm empty fruit bunch fibres for subsequent use as substrate on the performance of cellulase production by *Aspergillus terreus*. *Bioresour.*, **6**, 291-307 (2011).
- Shandilya, J.R. and R.L. Munjal: Some experiments with casing layer. *Tai-wan-Yang-ku: Taiw Mush*, **7**, 32-38 (1983).
- Shary, S., S.A. Ralph and K.E. Hammel: New insights into the ligninolytic capability of a wood decay ascomycete. *Appl. Environ. Microbiol.*, **20**, 6691-6694 (2007).
- Subramanian, T., T. Ramesh and M. Kalaiselvam: Degradation of triphenylmethane dye: Malachite green by *Aspergillus flavus*. *World J. Pharm. Sci.*, **3**, 44-50 (2014).
- Tanvi, B., P. Kumari and S. Goyal: Ligninolytic activity of a promising

- fungal isolate from tropical areas of Haryana. *Remarking*, **2**, 27-30 (2016).
- Tanvi, B., S. Chaudhary, R. Dhanker, S. Devi and S. Goyal: Optimization of physical and nutritional factors for enhanced production of lignocellulolytic enzymes by *Aspergillus terreus* FJAT-31011 under submerged conditions. *Int. J. Curr. Microbiol. App. Sci.*, **7**, 150-162 (2018).
- Tien, M. and T.K. Kirk: Lignin peroxidase of *Phanerochaete chrysosporium*. *Met. Enzymol.*, **161**, 238-249 (1988).
- Toledo, A.V., M.E. Simurro and P.A. Balatti: Morphological and Molecular characterization of a fungus, *Hirsutella* sp., isolated from planthoppers and psocids in Argentina. *J. Insect Sci.*, **13**, 1-11 (2013).
- Vyas, A., D. Vyas and K.M. Vyas: Production and optimization of cellulases on pretreated groundnut shell by *Aspergillus terreus* AV 49. *J. Sci. Ind. Res.*, **64**, 281-286 (2005).
- Zhao, X., L. Zhang and D. Liu: Biomass recalcitrance. Part I: The chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuel. Bioprod. Biorefin.*, **6**, 465-482 (2012).
- Zhou, S., J. Zhang, F. Ma, C. Tang, Q. Tang and X. Zhang: Investigation of lignocellulolytic enzymes during different growth phases of *Ganoderma lucidum* strain G0119 using genomic, transcriptomic and secretomic analyses. *PLoS ONE*, **13**, e0198404 (2018).

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