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Isolation, identification and screening of xylanase and glucanase-producing microfungi from degrading wood in Nigeria

Adesina F. C.[1,2]* and Onilude A. A.[1]

[1]Department of Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.
[2]Department of Microbiology, Lead City University, Ibadan, Oyo State, Nigeria.

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The objective of this present study was to isolate, identify and screen for potential fungal isolates from local degrading wood chips with respect to glucanase and xylanase production. The fungal strains were isolated from degrading wood chips over a period of six months. Five hundred and twenty six isolates were obtained from the different degrading wood chips with different frequencies of occurrence. Using cultural and morphological observations, the isolates were identified and grouped into thirteen fungal species. All the fungal strains were screened for their ability to produce both glucanase and xylanase by plate screening method using Congo red as indicator. Twenty isolates were further selected among them and screened for the production of the enzymes in liquid medium. Among the twenty isolates, *Trichoderma reesei* TRAR had the highest glucanase activity of 10.18 U/ml while *Fusarium compactum* FCGA had the highest xylanase activity of 9.33 U/ml.

Key words: Xylanase, glucanase, microfungi, degrading wood, screening.

INTRODUCTION

Hemicellulose and cellulose are the major components of lignocellulosic materials of which hardwood is part (Malherbe and Cloette, 2003). Hardwood is comprised of 40 to 45% and 20 to 35% of cellulose and hemicellulose respectively (Capoe et al., 2000) with the basic structural components of its hemicelluloses being xylan and about 20 to 35% of the total dry weight in tropical plant biomass (Levasseur et al., 2005). Xylan is branched heteropolysaccharide consisting of β-1,4-xylopyranosyl units substituted with arabinosyl, glucuronyl acetyl residues (Shallom and Shoham, 2003). Wood can be subjected to degradation either by using alkaline (Arantes and Saddler, 2011), acid (Nguyen, 1993) or microbial hydrolysis by microbial enzymes.

Microbial enzymes for hydrolysis are preferred to Others since they are specific biocatalysts which can operate under much milder reaction conditions and do not produce undesirable products and are environmentally friendly. Among the sources of enzymes microbial sources are preferred industrially because of the short generation times of the microbes and so large volumes of enzymes can be obtained within a short time Filamentous fungi are particularly interesting as source of cellulases and hemicellulases since they secrete these enzymes into the medium and have higher activities in contrast to yeasts and bacteria (Krisana et al., 2005). This feature makes fungal enzymes more attractive for various industrial processes. Fungi are the best known microorganisms capable of degrading lignin, cellulose and hemicelluloses. Because the substrates are insoluble, fungal degradation occurs exocellularly, either in association with the outer cell envelope layer or

*Corresponding author. E-mail: adesinafelicia@yahoo.com. Tel: +2348123329944, 07034945799.

extracellularly (Asha, 2006). Fungi that degrade cellulose and hemicelluloses, but very little lignin, are termed soft rot fungi (Irbe et al., 2001). Soft rot is characterized by soft decayed surface of wood in contact with excessive moisture however, soft rots can also occur in dry environments (Blanchette et al., 1998).

Generally, hydrolytic enzymes like cellulases and xylanases are produced by fungi for hydrolysis of complex substrates (such as lignocelluloses) as carbon souce for their growth. Fungi are highly diverse in nature; they have been recognized as a target for screening to find out the appropriate source of enzymes with constructive and novel characteristics (Bakri et al., 2008). *Trichoderma* spp. and *Aspergillus* spp. have most widely been used for production of these enzymes ((Mansfield et al., 1999). Cellulose degrading fungi produce a battery of cellulases which act synergistically to degrade cellulose (Lynd et al., 2002). The hydrolysis of xylan backbone of wood is accomplished by endoxylanases and β-xylosidases along with a variety of debranching enzymes like a-arabinofuranosidases, α - glucuronidases and acetyl esterases (Bhat, 2000). Cellulases and hemi-cellulases are the focus of several studies for their use in the bioconversion of agricultural wastes and to replace pumice in the manufacture of stone washed denim in the textile industry (Garcia et al., 2002). In the paper and pulp industry, xylanases are used for biobleaching of kraft pulp while the enzyme in addition to cellulases is used to improve the manufacture of recycled papers (Ghatora et al., 2011). For instance, in pulp and paper industry, the xylanases are exploited in the pre bleaching process to decrease the utilization of the toxic chlorine chemicals (Herpoel et al., 2002). Besides that, xylanase treatment serves to increase the brightness of the pulp, which is very essential in developing chlorine free bleaching processes (Yadov et al., 2010). Sequential treatment of pulp by xylanase/laccase system was found to be promising to remove 60% and up to 70% of the residual lignin from softwood Kraft pulp and wheat straw chemical pulp respectively (Hoerpel et al., 2002). Since degrading wood could serve as substrate for cellulose and hemicellulose degrading fungi this study was carried out to isolate, identify and screen fungal isolates from degrading common wood samples in Nigeria. From this study, isolates with high hemicellulase and cellulase activities could be used for further studies.

MATERIALS AND METHODS

Sample collection

Chip samples of different wood types in Nigeria were collected from sawmills and conditioned to degrade for six months. The wood types used were T*erminalia superba*, *Anogeisus leiocarpus*, *Chlorophora excelsa*, *Albizia sativum*, *Holoptera grandis*, *Gmelina arborea*, *Albizia zygia, Mansonia altissima* and *Sweetenia macrophylla*. Sampling for culturing was carried out on the degrading wood fortnightly for duration of six months.

Sterilization of glass ware, other apparatus and media

All the chemicals used in every aspect of this work were of analytical grade and were sterilized by autoclaving at a temperature of 121°C, 15 pounds per square inch for 15 min. Glassware was fully sterilized using hot air oven (Gallenkampus, Model NYC –101) at 180°C for two hours; inoculating needles were sterilized by flaming.

Isolation procedure

This was carried out using the method of Onilude (1996). A sterile 0.5 mm cock borer was used to remove tissue disk from area near the edge of symptomatic colonization and attack to obtain actively growing mycelia. Such disk was then inoculated aseptically at the centre of sterile Potato dextrose agar (PDA) plate. This procedure was carried out aseptically in a lamina flow chamber. The inoculated plates were then incubated at 28±2^{o}C. Each plate was examined for growth after 72 h during which mixed culture of fungal mycelia were observed.

Identification of pure cultures of isolates

The fungal isolates obtained were subcultured on sterile culture plates containing PDA and Czapek medium until pure culture of each isolate was obtained. For observation of microscopic characteristics of each isolate, sterile forceps was used to aseptically pick a strand of mycelium with the fruit body and it was carefully placed on a clean microscopic slide, stained with lactophenol blue and was subsequently covered with a clean cover slip. The mycelium was then observed under the x40 objective of the light microscope. Isolates were subjected to both cultural and morphological characterization and compared with characteristics in the Fungal Compendium (Domsch et al., 1980). Kiffer and Morellet (2000) for proper identification. *Aspergillus* spp., *Penicillium* sp., *Emericella nidulans* and *Fusarium* spp. identities was further established using Singh et al. (1991). Each isolate was code labeled and subcultured regularly to maintain viability.

Screening of fungal isolates for glucanase and xylanase activities

Primary screening of isolates

The fungal isolates were subjected to screening for their cellulolytic and xylanolytic properties using the modified agar diffusion test method (Downie et al., 1994; Whitaker et al., 2002). Ten microlitres (10 µl) of spore suspension of each isolate was dropped onto 6 mm diameter of sterile paper disc cut out of Whatman No. 1 filter paper. The inoculated paper discs were dried at room temperature in a lamina flow chamber and put onto the center of special medium plates in which xylan or carboxylmethyl cellulose had been incorporated. Xylan agar medium consisting of 0.8% Birch-wood xylan (Sigma) (Cordeiro et al., 2002) was used to evaluate xylanase activity of the isolates. Cellulase activity was evaluated by using a cellulose-agar medium containing 1% Carboxyl methyl cellulose (Sigma). Each medium also contained the following constituents (g/L): 0.05 g $MgSO_4$. $7H_20$, 0.005 g $CaCl_2$, 0.005 g $NaNo_3$,0.009 g $FeS0_4.7H_20$,0.002 g $ZnSO_4$, 0.012 g $MnSO_4$, 0.23 g KCl, 0.23 g KH_2PO_4, 2 g peptone, 19 g Agar (LAB M). After seventy two hours (72 h) of incubating the plates at 28±2°C, each was flooded with 0.4% Congo red for 10 min and then destained with 1 M NaCl. The hydrolysis zones on the plate media were measured and the relative enzyme activity of each isolate was determined using the following formula:

$$\text{Relative enzyme activity} = \frac{\text{Diameter of clearing (mm)}}{\text{Diameter of growth (mm)}}$$

Isolates with wide clear zones (high relative enzyme activities) were selected for further work. Pure cultures of the selected fungal isolates were regularly sub-cultured onto fresh sterile Potato Dextrose Agar (PDA), (LABM) slants every 2 to 3 weeks to maintain viability and kept in refrigerator at 4°C.

Secondary screening of isolates

Selected fungal isolates were subjected to secondary screening using birch wood xylan and carboxymethyl cellulose as main carbon sources; for xylanase (0.8% birch wood xylan) and cellulase (1.0% carboxymethyl cellulose) production respectively. Other components of each of the secondary screening medium were (g/L): 0.05 g $MgSO_4$. $7H_20$, 0.005 g $CaCl_2$, 0.005 g $NaNO_3$,0.009 g $FeS0_4$.7H20,0.002 g $ZnSO_4$, 0.012 g $MnSO_4$, 0.23 g KCl, 0.23 g KH_2PO_4, 7 g peptone. Erlenmeyer flasks (250 ml) containing 90 ml of the medium were each inoculated with 72 h culture of each selected fungus using a sterile 5 mm cork borer which is flame sterilized for each inoculation. Each flask was inoculated with three 5 mm fungal culture and incubation was done at 28±2°C in an incubator with shaker at 120 rpm for 6 days. Samples were taken after 72 h, the cell free supernatant was recovered by centrifuging samples at 12,000 rpm at 4°C for 15 min in a cold centrifuge (Model: IEC B-20A). Dry weight of mycelia, total soluble protein (Lowry et al., 1951) and glucanase and xylanase activities of the isolates were calculated from the amount of reducing sugar present in the fermentation medium which was estimated using the method of Miller (1959).

Glucanase assay

Carboxymethyl cellulose (CMCase) or Endo-β-1, 4- glucanase activity was determined according to the method of Mandels and Webber (1969). 0.5 ml of 1% Carboxymethyl cellulose (CMC) (Sigma) in 0.1 M citrate buffer pH 5.6 was placed in a test tube and 1.0 ml of culture filtrate added. The test tube was incubated at 40°C in a water bath with shaker (Uniscope SM 101) for 30 min (Baldrian and Gabriel, 2003). The reaction was terminated by adding 2.0 ml of 3,5- dinitrosalicylic acid (DNS) reagent to the reaction mixture, boiled for 5 min (Miller, 1959). The absorbance of the appropriately diluted reaction mixture was read at 540 nm using a spectrophotometer (752W, UV-VIS Grating Spectrophotometer). One unit of glucanase was defined as the amount of enzyme that released I µmol reducing sugar as glucose equivalent per minute in the reaction mixture under the specified assay conditions. All enzyme assays were performed in triplicates.

Xylanase assay

Xylanase activity was determined according to the method of Bailey et al. (1992). 0.5 ml of 0.8%w/v of birch wood xylan (Sigma) (0.8 g in 100 ml of 0.1 M citrate buffer at pH 5.6) was placed in a MacCartney bottle and 1 ml of enzyme filtrate added. The reaction mixture was incubated in a water bath with shaker (Uniscope SM 101), at 50°C for 30 min and the reaction terminated by adding 2.0 ml of 3,5-dinitrosalicylic acid (DNSA) reagent to the reaction mixture, heated for 5 min at 80°C in a water bath. Absorbance was read at 540 nm using spectrophotometer (Model 752W, UV-VIS Grating Spectrophotometer) to determine the concentration of sugar released by the enzyme. One unit (U) of Xylanase was defined as the amount of enzyme that released 1.0 µmol reducing sugar as xylose equivalent per minute in the reaction mixture under the specified assay conditions. All enzyme assays were performed in triplicates

RESULTS

All the wood samples were colonized by different fungi however, while some wood types supported heavy colonization of the fungi, others only supported few fungal types as shown on Table 1. A total of five hundred and twenty six fungal isolates were obtained from the nine wood samples allowed to degrade for six months. Fungal isolates were identified as *Aspergillus flavus, A. niger, Emericella nidulans, Laesodiploida theobromae, Fusarium chlamydosporium, F. compactum, F. oxysporium, Penicillium purpurogenum, Rhizopus stolonifer, Trichoderma harzianum* and *T. reesei.* Among all the isolates *A. niger, T. harzianum, T. reesei* and *R. stolonifer* consistently have high frequency of occurrence on all the wood samples. *L. theobromae* and *E. nidulans* occurred least on most of the degrading wood chip samples (Table 1). *T. superba, H. grandis* and *S. macrophylia* sample supported relatively high number of fungal colonization while *Chlorophora excelsa* supported the least number of fungi as indicated on Table 1.

Table 2 shows the relative enzyme activity of each isolate on plate screening test. The maximum relative cellulase activity of 1.59 was obtained for *T. harzianum* from *Mansonia altissima* wood chips, followed by the same isolate from *Gmelina arborea* chips. The cellulase activity recorded for *F. chlamydosporium* from *Holoptera grandis* wood sample was least (1.10) among the isolates tested. Generally *Trichoderma* spp. from all the wood samples used in this research work had high relative cellulase activity as compared to other isolates obtained during the course of this work.

In screening the individual isolates for their relative hemicellulase activities, Table 2 shows that *A. niger* from *M. altissima* and *G. arborea* had the highest hemicellulase activity of 1.55 each. *R.stolonifer* from *A. adianthifolia* and *C. excelsa* had the least hemicellulase activity of 1.10 each and it was observed that generally, *R. stolonifer* from all the wood sample types was low as compared to that of other isolates. Some of the isolates were also observed not to have either cellulase or hemicellulase activity or both as shown on Table 2.

This result however only gave a more or less qualitative result of the hydrolytic enzyme producers with the clear zones only able to distinguish producers from nonproducers among the isolates. There was therefore a need for a more quantitative result to be able to distinguish the high producers of hydrolytic enzymes among the isolates so a quantitative screening was done. Results of the quantitative screening of selected isolates were presented on Table 3.

Table 1. Frequency of occurrence (F.O.) and the % F.O. of isolates on each degrading wood chip samples.

Isolates	*T. superba*		*A. leiocarpus*		*A. sativum*		*H. grandis*		*A. zygia*		*C. excelsa*		*S. macrophylia*		*M. altissima*		*G. arborea*	
	F.O	%*	F.O	%*	F.O	%*	F.O	%*	F.O	%*	F.O	%*	F.O.	%*	F.O.	%*	F.O	%*
E. nidulans	5	5.6	4	3.3	3	7.1	2	2.3	0	0	2	6.5	0	0	2	4	3	5.3
A. flavus	4	4.4	0	0	0	0	4	5.5	2	3.9	0	0	1	1.4	0	0	2	3.5
A.niger	12	15.6	12	31.7	11	26.2	12	20.5	12	33.3	4	6.5	12	25	12	12	12	26.3
L. theobromae	4	2.2	0	0	1	1.24	0	0	0	0	0	0	2	2.8	0	0	5	8.8
F. chlamydosporium	8	8.9	0	0	0	0	5	4.2	5	5.9	3	9.7	12	18.1	0	0	7	12.3
F. compactum	8	0	6	3.3	4	9.5	7	9.6	5	5.9	0	0	9	2.8	0	0	5	8.8
F. oxysporium	6	6.7	6	10	4	7.1	6	8.2	0	0	0	0	4	8.3	3	6	0	0
P. purpurogenum	8	5.6	0	0	3	11.9	6	8.2	3	5.9	1	3.2	0	0	5	10	0	0
R. stolonifer	11	12.2	11	18.3	12	28.6	7	8.2	6	11.8	6	19.4	8	11.1	9	18	10	17.5
T. harzianium	12	17.8	9	10	0	0	12	16.4	9	11.8	6	19.4	12	18	12	24	4	7
T. reesei	12	21.1	12	20	3	7.1	12	16.4	11	21.6	9	29	12	12.5	7	14	9	10.5

F.O. represents frequencies of occurrence. %* means percentage frequency of occurrence of isolates.

Table 2. Relative enzyme activities of fungal isolates on plate screening.

Isolate codes	Isolates identities	Average relative glucanase activities	Average relative xylanase activities	Isolate codes	Isolates identities	Average relative glucanase activities	Average relative xylanase activities
AFAA	*Aspergillus flavus*	1.22±0.05	1.20±0.00	FOGA		1.24±0.00	0.00
AFAL		1.32±0.01	0.00	FOHG			1.24±0.01
AFAR		1.32±0.01	1.23±0.01	FOMA		0.00	0.00
AFCE		0.00	0.00	FOSM		0.00	1.27±0.00
AFGA		0.00	1.25±0.01	LTAA	*Laesodiploida theobromae*	1.26±0.01	0.00
AFHG		1.23+0.02	0.00	LTAL		0.00	0.00
AFMA		1.26±0.02	0.00	LTAR		1.31±0.01	0.00
AFSM		0.00	1.26±0.01	LTCE		1.28±0.04	0.00
ANAA	*A. niger*	1.36±0.01	1.42±0.00	LTGA		0.00	1.16±0.01
ANAL		1.41±0.00	1.44±0.03	LTHG		1.27±0.02	1.14±0.01
ANAR		1.36±0.01	1.42±0.01	LTMA		1.33±0.02	0.00
ANCE		1.36±0.01	1.40±0.01	LTSM		0.00	1.14±0.00
ANGA		1.46±0.02	1.55±0.01	PPAA	*Penicillium purpurogenum*	1..26±0.01	1.19±0.01
ANHG		0.00	1.42±0.05	PPAL		0.00	0.00
ANMA		1.44±0.01	1.55±0.02	PPAR		1.31±0.01	1.23±0.01
ANSM		1.44±0.01	1.49±0.01	PPCE		1.28±0.041	0.00
ENAA	*Emericella nidulans*	0.00	1.19±0.01	PPGA		0.00	0.00

Table 2. Contd.

ENAL		1.32±0.02	1.20±0.01	PPHG		1.27±0.02	1.22±0.01
ENAR		1.32±0.01	1.24±0.04	PPMA		1.33±0.02	1.24±0.00
ENCE		1.28±0.03	1.19±0.01	PPSM		0.00	1.20±0.00
ENGA		1.29±0.02	1.25±0.01	RAA	*Rhizopus sp.*	1.23±0.02	1.10±0.00
ENHG		1.23±0.02	1.22±0.01	RAL		1.22±0.01	1.14±0.02
ENMA		0.00	1.26±0.00	RAR		1.22±0.02	1.13±0.01
ENSM		0.00	0.00	RCE		1.23±0.02	1.10±0.00
FHAA	*Fusarium chlamydosporium*	1.34±0.00	1.23±0.01	RGA		1.24±0.01	0.00
FHAL		0.00	0.00	RHG		1.22±0.02	1.14±0.02
FHAR		1.27±0.01	1.25±0.01	RMA		1.25±0.02	1.13±0.01
FHCE		1.32±0.01	1.22±0.00	RSM		1.23±0.01	1.14±0.02
FHGA		1.35±0.02	1.29±0.01	THAA	*Trichoderma harzianum*	1.52±0.02	1.36±0.00
FHHG		1.19±0.01	0.00	THAL		1.45±0.01	1.39±0.01
FHMA		0.00	0.00	THAR		1.50±0.03	1.42±0.00
FHSM		1.31±0.00	1.28±0.01	THCE		1.42±0.01	1.39±0.01
FCAA	*F. compactum*	0.00	1.19±0.01	THGA		1.52±0.02	1.40±0.00
FCAL		1.27±0.00	1.19±0.01	THHG		1.29±0.02	0.00
FCAR		1.30±0.01	1.22±0.01	THMA		1.59±0.01	1.45±0.00
FCCE		0.00	1.22±0.01	THSM		1.51±0.01	1.43±0.00
FCGA		1.24±0.01	1.47±0.01	TRAA	*T. reesei*	1.43±0.04	1.44±0.01
FCHG		0.00	1.20±0.01	TRAL		1.43±0.01	1.33±0.01
FCMA		0.00	0.00	TRAR		1.43±0.00	1.45±0.01
FCSM		1.25±0.01	1.23±0.01	TRCE		1.45±0.00	1.39±0.02
FOAA	*F. oxysporium*	0.00	0.00	TRGA		1.50±0.01	1.51±0.01
FOAL		0.00	1.24±0.02	TRHG		1.34±0.01	1.34±0.01
FOAR		0.00	1.25±0.01	TRMA		1.45±0.00	1.48±0.01
FOCE		0.00	1.22±0.01	TRSM		1.53±0.00	1.49±0.01

Values represent means of replicates ± standard error.

Cultural and microscopic characteristics of identified isolates

E. nidulans

Colonies on potato dextrose agar at 25°C were initially white, quickly becoming brown with conidial production.

A. flavus

Rapid growing yellowish green mycelia becoming green with age. Reverse was yellow to red brown. Microscopically, the conidial head was radial, stipe was long and varicose, dome shaped vesicle, small ampliforms Phialides with globose conidia.

A. niger

The upper part of colony had blackish brown with yellowish submerged mycelia and creamish reverse. Under the microscope, the head appeared globose with long stipe and thick walled globose vesicle. Phialides appeared short.

Table 3. Average Biomass profile, Total protein, Cellulase and Hemicellulase activities of selected fungal isolates in quantitative screening.

Isolates	No of strains tested and codes	Average total protein (mg/ml)	Average biomass (mg/ml)	Glucanase activity (U/ml)	Average biomass (mg/ml)	Xylanase activity (U/ml)
	ANGA	2.461	39.21	7.45	35.13	6.16
A. niger	ANSM	2.006	39.15	5.35	35.09	4.10
	ANMA	1.891	38.98	5.41	34.95	4.29
F. compactum	FCGA	2.331	75.08	2.49	80.44	9.33
F. oxysporium		2.004	65.09	3.47	31.31	3.09
L. theobromae	LTMA	1.774	28.18	1.92	21.10	1.03
P. purpurogenium	PPMA	1.366	47.12	3.47	28.11	1.61
R. stolonifer	RMA	1.662	38.12	3.42	20.18	1.29
	THMA	3.771	88.24	7.04	61.64	7.34
	THAR	2.743	87.19	6.01	60.68	4.15
T. harzianum	THAA	2.914	87.96	6.55	61.15	4.12
	THSM	3.352	88.17	6.43	60.96	5.99
	THAL	3.452	88.09	6.89	61.56	6.12
	TRAR	3.524	67.46	10.18	70.04	3.37
	TRGA	2.915	65.16	8.15	70.54	4.32
T. reesei	TRSM	3.165	68.13	8.66	70.12	3.31
	TRMA	3.359	67.32	7.94	69.89	3.46
	TRGE	3.415	67.14	7.96	69.54	3.21

Each value is a mean of triplicate determination.

L. theobromae

Colonies on potato dextrose agar at 25°C was initially creamy in colour with wooly mycelia but became dark on the observe and the reverse with longer period of incubation.

F. chlamydosporium

Colonies appeared floccose with creamish yellow observe and deep burgundy reverses on PSA. Microconidia produced rabbit ear shaped septate conidiophores. Macroconidia was not many and are sickle shaped while chlamydospores were many and appeared in chains.

F. compactum

Whitish cream floccose observes with reverse that started with red colour and turned to burgundy with age. Microconidia is absent while Macroconidia were borne on structures that look like Phialides. Chlamydospores appear in chains.

F. oxysporium

Observe colony appeared creamy blue while the reverse gave violet like colour. Microconidia appeared like beads, macroconidia have pointed apical cells and each had three septa and they were borne on monophialides.

P. purpurogenum

Dark green observe with deep red reverse. Stipe of conidiophores was small and conidia appeared ellipsoidal under the light microscope.

Rhizopus sp.

Colonies on potato dextrose agar at 25°C temperature first appeared wooly or cottony and white initially and gradually turned to grey with black dots that represented the mature sporangia with time. Growth was very rapid filling the culture plates within 2 to 3 days.

T. harzianum

Mycelia was initially white and downy, became light green and later deep green with compact tufts and rings with green coloured spores and fast growing, the reverse was brownish. Microscopically, the conidia were repeatedly branched with flask shaped phillides.

T. reesei

Mycelia were initially scanty and white, became light green and later very deep green with scanty tufts and rings having hallows in between with deep green coloured spores. There was yellowish secretion into the agar. Microscopically, the conidia were repeatedly branched with oval shaped phillides.

DISCUSSION

Components of each wood differ in availability, complexity and polymerization which may be the reason different wood chip samples support fungal colonization differently. The presence of long units of coumaroyl and feruloyl groups in wood affects the ease at which fungi and other microbes colonise wood and attack its components (Chesson and Forsberg, 1988). Isolates were identified as *Aspergillus flavus*, *A. niger*, *E. nidulans*, *F. compactum*, *F. chlamydosporium*, *F. oxysporium*, *L. theobromae, P. purpurogenum, T. harzianum* and *T. reesei*. These fungal isolates have been previously isolated from lignocellulosic materials of which wood chips is one (Immanuel et al., 2007; Picart et al., 2007). *Trichoderma* spp. was reported to be associated with wood degradation and *A. niger* is an ubiquitous fungi found in decaying woods.

Plate screening method has previously been used for screening hydrolytic enzymes producing fungi and was reported to be suitable (Bhallal and Joshi, 1993; Abdel-Sater et al., 2000). A clear zone of hydrolysis was noticed when the culture filtrate was dropped in wells on carboxyl methyl cellulose (CMC) and birch wood xylan plates stained with Congo red. Tables 2 and 3 revealed that even isolates in the same specie differ in their xylanase and glucanase activities. This further affirms that the environment of a microbe dictates its metabolic capability. *T. reesei* in an environment where the substrate that induces synthesis of certain enzyme is available in limited amount will secrete less of such enzyme as compared to when it is found in an environment with abundant supply of the inducing substrate thus the enzyme activity of each strain will differ. Observing the area of clear zone produced differentiated the potent xylanase and cellulase producers. However hallows around the isolates on solid agar medium may sometimes be due to the presence of membrane bound hydrolases which cause formation of the clearing when the substrates are being hydrolysed. This can also be due to the regional presence of enzyme secreted near the growing colony in the agar plates. This however will not occur in liquid medium, so isolates with relatively high enzyme activities were selected for liquid fermentation using birch wood xylan and carboxyl methyl cellulose followed by xylanase and cellulase assays respectively to get a clear picture of hydrolytic enzyme produced as well as a quantitative estimation of the enzymes. The selected fungal isolates are dominant heterologous enzyme producing microorganism (Sathiyavathi and Parvatham, 2011), which are very much attracted by industries for a varying reasons, including their high growth rates leading to short fermentation cycle times, their capacity to secrete extracellular proteins. The biochemistry, physiology and generics of some of these isolates are well studied, facilitating further development and greater exploitation for industrial purposes (Alexopoulus and Mims, 1952). Low enzyme activity of the isolates on plate agar as compared to that obtained on liquid medium might be due to the adsorption of the enzymes on the surface of insoluble carboxylmethyl cellulose and xylan particles present in the solid culture medium (Irwin et al., 1994). The fact that the enzyme activity of some of the isolates were higher in liquid medium is an indication that the clearing found on plate agar plate of most of the isolates is due to the regional presence of glucanase and xylanase and or membrane bound glucanase and xylanase that has led to good clearing zone on solid agar plate (Kazuhisa, 1997).

Conclusion

Of all the isolates from the degrading wood chips, only four were selected because of their relatively high potential for production of glucanase and/ xylanase. These fungi are dominant hydrolytic enzyme producing microorganisms on lignocellulosic substrates (Kazuhisa, 1997; Leghlimi et al., 2013).These isolates can further be used for production of these enzymes in solid state fermentation of these wood shavings that litter our environments and have proven difficult to dispose off thus becoming environmental nuisance and pollutants.

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