Isolation, screening and identification of potential cellulolytic and xylanolytic mold from oil palm waste

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Abstract. The objective of this research was to isolate, screen, and identify the cellulase and xylanase-producing mold from oil palm waste. There are thirty-two isolates from oil palm waste which are able to degrade and grow on media containing cellulose or xylan as a sole carbon source. Screening to determine cellulolytic and xylanolytic activity was performed by paper disc diffusion method using Congo Red as an indicator. All the thirty-two mold isolates showed cellulolytic and xylanolytic activity with relative enzyme activity (ratio of hydrolysed zone diameter and diameter of colony growth) ranging from 1.04 to 1.62. Based on the macro- and micromorphology characteristics, these isolates were identified as genus *Trichoderma*, *Aspergillus*, *Rhizopus*, *Talaromyces* and *Penicillium*, with the number isolates in each genus was 22, 4, 3, 2, and 1 respectively. The highest cellulolytic and xylanolytic activity was achieved from the isolate namely OPT1(4) which was identified as *Talaromyces pinophilus* using morphological and molecular analysis. Under the optimum condition (30°C, 72 hours) in liquid medium containing carboxymethyl cellulose (CMC) and birch wood xylan as the main carbon source, *Talaromyces pinophilus* OPT1(4) presented glucanase and xylanase activity of 15.17 U/mL and 13.35 U/mL, respectively.

1. Introduction

Lignocellulose biomass is one of the biggest renewable resources, derived from waste from agriculture and forestry industry. In recent years, there is an increasing interest in exploiting lignocellulose biomass because of the potential to be converted into fuel and chemicals [1]. The conversion of lignocellulose waste into chemicals of added value is increasingly attracting attention due to the depletion of fossil fuels and the increasing demand for energy, food, chemicals, and sustainable development.

One of the biggest sources of lignocellulosic compounds in Indonesia is oil palm waste in the form of oil palm empty fruit bunches, oil palm frond, and oil palm trunk. The waste of the oil palm contains large amounts of cellulose and hemicellulose, which can be hydrolysed to produce hexose and pentose sugars and can be further fermented into value-added products. Cellulose, the main component of lignocellulose, is a polymer consisting of glucose units that are connected by β-1-4 glycosidic bonds [2]. Hemicellulose is the most polysaccharide after cellulose found in plants, binds strongly covalently and non-covalently with lignin and cellulose. Hemicellulose is a sugar polymer which is composed of various types of sugar. Xylan is the main component of hemicellulose, which has the main xylopyranose chain which contains β-D-xylopyranose units that bind to each other with β-1,4-glycoside bonds. Xylan...
has O-acetyl, arabinose, and 4O methyl-D-glucuronic acid residues, which are attached to the main chain [3].

One of the pathways for utilizing lignocellulose waste is to convert the cellulose and hemicellulose contained in it into sugar, which undergoes further changes to fuel and other chemicals. The conversion of cellulose and hemicellulose into sugar is mostly done using the appropriate enzymes. For the degradation of cellulose and xylan, complete synergistic activity of several types of enzymes is needed [4, 5]. Cellulase and xylanase production usually starts when there is a cellulose or xylan substrate in the environment around the enzyme-producing microbes. Many cellulase and xylanase-producing strains, therefore, are found in environments rich in lignocellulose waste such as oil palm waste. The aim of this research was to isolate, screen, and identify the cellulase and xylanase-producing mold from oil palm waste.

2. Materials and Method

2.1. Sample collection, isolation and purification of the fungal strains

Oil palm waste samples were collected from three oil palm plantations, namely Sampurna-Lampung, PTPN VI Lampung, and Kayangan (PT. Salim Ivomas Pratama)-Riau. Oil palm waste samples were air dried and ground. Mold isolates that able to degrade oil palm wastes were isolated by direct plate method on plates containing approximately 15 mL of cellulose and xylan agar medium which consists of (L): 10 g cellulose powder (Sodium Carboxymethylcellulose-Sigma) or 8 g xylan (Birchwood Xylan-Sigma); 0.5 g (NH4)2SO4; 1 g K2HPO4; 0.5 g KCl; 0.2 g MgSO4.7H2O; 0.1 g CaCl2; 0.5 g yeast extract; 0.01 g FeSO4.7H2O and 20 g agar. Chloramphenicol was added as a bacteriostatic agent with concentration at 0.015% (w/v). The plates were incubated at 30°C for 72 h, and the resulting colonies were purified on potato dextrose agar medium (PDA). The stock cultures of the isolated mold were maintained on PDA slants. The cultures were transferred from the stock onto a freshly prepared agar plate, and fresh cultures were used for experimental work [6].

2.2. Screening of cellulolytic and xylanolytic activity

Thirty-two mold isolates that can degrade and grow in a medium containing cellulose or xylan as the sole carbon source were screened for their cellulolytic and xylanolytic activity using the agar paper disc diffusion method and Congo Red solution as indicator. 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 put onto the center of medium plates in which xylan or carboxymethyl cellulose had been incorporated. The xylanolytic activity was evaluated by using a medium consisting of 0.8% Birch-wood xylan (Sigma). Cellulose-agar medium containing 1% Carboxyl methylcellulose (Sigma) was used to evaluate the cellulolytic activity of the isolates. Each medium also contained the following constituents (L): 0.05 g MgSO4.7H2O, 0.005 g CaCl2, 0.005 g NaNO3, 0.009 g FeSO4.7H2O, 0.002 g ZnSO4, 0.012 g MnSO4, 0.23 g KCl, 0.23 g KH2PO4, 2 g peptone, 19 g Agar (Merck). After five days of incubating the plates at 30°C, each plate was added with 0.4% Congo red for 10 min and then destained with 1 M NaCl [7]. The hydrolysis zones on the plate media were measured, and the relative enzyme activity of each isolate was determined using the following formula:

\[
\frac{\text{Relative enzyme activity}}{= \frac{\text{hydrolysed zone diameter (mm)}}{\text{colony growth diameter (mm)}}}
\]

(1)

2.3. Identification of mold isolates based on macro- and micromorphological characteristic

Two different media viz. Malt Extract Agar (MEA-Merck) and Potato Dextrose Agar (PDA-Merck) were used for the macromorphological characterization of fungi. The isolates grown on two different media at 30°C were observed daily to identify the morphological characteristics of each culture. Important macromorphology characters used for describing mold isolates include colony texture, the colour of conidia, the abundance, texture, and colour of mycelia, the presence of soluble pigments and exudates, colony reverse colours and the presence or absence of radial sulcation [6].
Slide cultures were prepared for observation of micromorphological characteristics. Incubation was done at 30°C for 48-72 hours. After incubation, a drop of lactophenol mounting fluid was placed on a clean microscope slide. Carefully, the cover glass was lifted from the slide culture, and the block of agar was discarded. A drop of 95% ethanol was added to the mold hyphae on the cover glass. After the alcohol has evaporated, the cover glass was placed, mold side down, on the drop of lactophenol mounting fluid on the slide. All isolates were examined using oil immersion with microscopes with up to 400 × magnification. Identification of each fungal isolate at the genus level was obtained by comparing morphological characteristics with the literature [8].

2.4. Molecular identification
Molecular identification was carried out by 28S rDNA sequence analysis [9] and amplification using NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTCAGACGG-3′) primers. The separation of amplified gene products was performed using agarose gel electrophoresis and purified using PCR purification and gel extraction kits. The amplified products were sequenced. DNA sequences were obtained in the Clustalx program and analysed by the Basic Local Alignment Search Tool (BLAST) database (http://www.ncbi.nlm.nih.gov/).

2.5. Endoglucanase and xylanase production and assay
The isolate with the highest relative enzyme activities was subjected to secondary screening using birch wood xylan and carboxymethyl cellulose (CMC) as sole carbon sources; for xylanase (0.8% birch wood xylan) and cellulase (1.0% CMC) production respectively. Other components of each of the secondary screening medium were (L): 0.05 g MgSO₄, 7H₂O, 0.005 g CaCl₂, 0.005 g NaNO₃, 0.009 g FeSO₄.7H₂O, 0.002 g ZnSO₄, 0.012 g MnSO₄, 0.23 g KCl, 0.23 g KH₂PO₄, 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 sterilised for each inoculation. Each flask was inoculated with three 5 mm fungal culture and incubation was done at 30°C in an incubator with shaker at 120 rpm for 6 days. Samples were taken after 72h, the cell free supernatant was recovered by centrifuging samples at 6,000 rpm for 30 min [7].

Endoglucanase (carboxymethyl cellulase activity, CMCase), and xylanase activity were assayed and estimated based on standard procedure recommended by the Commission on Biotechnology, IUPAC [10, 11]. One unit of glucanase was defined as the amount of enzyme that released 1 μmol reducing sugar as glucose equivalent per minute in the reaction mixture under the specified assay conditions. 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. The amount of reducing sugar present in the fermentation medium which was estimated using dinitrosalicylic acid (DNS) reagent [12]. All enzyme assays were performed in separately triplicates.

3. Results and Discussion
A total of thirty-two mold isolates, which were obtained from the oil palm waste in the form of a chip of decayed oil palm trunk, oil palm empty fruit bunch and oil palm frond, were able to degrade and grow on media containing xylan or cellulose as the sole carbon source. The isolates also showed relative glucanase (CMC ase) and xylanase activity in the screening with paper disc diffusion method using Congo Red as an indicator as presented in Table 1. The maximum relative cellulase activity of 1.63 was obtained for isolates that is identified as the genus of *Tallaromycetes* with code OPT1(4), followed by the isolate for the genus of *Penicillium* with code OPT8C. The cellulase activity recorded for isolate that is identified as *Rhizopus* spp. from was the smallest (1.043) among the isolates tested.

Based on morphology characteristics, the mold isolates were identified as the genus of *Trichoderma*, *Aspergillus*, *Rhizopus*, *Talaromycetes*, and *Penicillium* as shown in Table 3. The ability of filamentous fungi including the genus of *Trichoderma*, *Aspergillus*, *Rhizopus*, *Talaromycetes*, and *Penicillium* to produce cellulase and xylanase has been extensively studied and reviewed [13-15]. The production of cellulase and xylanase is inducible therefore enzyme production usually occurs if suitable substrates are
available. Lignocellulosic biomass waste which is rich in cellulose and hemicellulose such as oil palm waste can be a source of potential cellulase and xylanase-producing mold. Cellulolytic and xylanolytic activity of various mold however very diverse and suitable for certain different substrates.

| Table 1. Relative enzyme activity of mold isolates |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Isolate identities               | Isolates Code   | Relative glucanase activities | Relative xylanase activities | Isolate identities | Isolates Code   | Relative glucanase activities | Relative xylanase activities |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Trichoderma                       | R5(4), SL7(2), R5(2), R3, SL7(5), R6, R2, SL9(2), R5(3), R1(3), R2(2), SL7(3), SL7(4), R1(4), R4, SL1(1) | 1.056 | 1.086 | Trichoderma | SL4, OPT5B, | 1.147 | 1.079 |
| Aspergillus                       | OPT5A, | 1.091 | 1.094 |
| Rhizopus                          | OPT6B, | 1.137 | 1.093 |
| Talaromyces                       | OPF3C, | 1.095 | 1.088 |
| Penicillium                       | OPT3A | 1.089 | 1.092 |
|                                   | OPT6A | 1.057 | 1.160 |
|                                   | EFB3C | 1.164 | 1.339 |
|                                   | R2(1) | 1.057 | 1.052 |
|                                   | R3(1) | 1.156 | 1.151 |
|                                   | EFB3B | 1.204 | 1.190 |
|                                   | OPT8A | 1.051 | 1.043 |
|                                   | SL2 | 1.043 | 1.036 |
|                                   | OPT1(1) | 1.045 | 1.014 |
|                                   | OPT1(4) | 1.378 | 1.456 |
|                                   | OPT8C | 1.623 | 1.488 |
|                                   | Penicillium | 1.617 | 1.086 |

Even isolates in the same species differ in their glucanase and xylanase activities, due to the environment of a microbe influence its metabolic capability. Cellulase and xylanase production by mold is a complex process; strains induced by different substrates may produce different cellulase and xylanase which are more suitable for degrading certain substrates. This is the reason, although many cellulase and xylanase-producing strains available commercially, it is still necessary to obtain new isolates that are more efficient on a specific substrate.

For molecular identification of isolate OPT1(4) that has the highest cellulolytic and xylanolytic activity, the PCR amplicon of the D2 region of LSU of 28S rDNA was sequenced. Based on nucleotide homology, the isolated mold strain was identified as Talaromyces pinophilus (Table 2). Talaromyces species are generally isolated from soil, indoor environment, and food products. Their ability to produce enzymes and soluble pigments make Talaromyces an important genus for biotechnological purposes.

| Table 2. Isolates Similarity based on 28Sr-RNA sequences |
|---------------------------------------------------------|
| Mold isolate | Identical Mold | % Similarity |
|-------------|----------------|--------------|
| OPT1(4)     | Talaromyces pinophilus strain EMM | 99 |

Some of the important enzyme producers of Talaromyces include T. pinophilus (endoglucanase, cellulase; [16]), T. funiculosus (cellulase; [17]) and Acremonium cellulolyticus (= T. pinophilus), T. leycettanus (xylanase; [18], T. thermophilus (glucosidase, xylanase; [19, 20]), T. stipitatus (cellulase, xylanase [21]), which is reported to produce cellulase for biomass degradation. The enzymes derived from various mold species belonging to the genus Talaromyces often show performance superior to those from Hypocrea jecorina, which is the best-known cellulase producer in lignocellulosic saccharification [22, 23].
Table 3. Macro- and micromorphology characteristics of mold isolates

| Isolates Identities | Macromorphology on Different Media | Micromorphology |
|---------------------|------------------------------------|-----------------|
|                     | MEA                                | PDA             |
| **Trichoderma**     | Colonies size ranging from 70-80 mm in 5 days, hairy texture; mycelia initially white and downy, became light green and later deep green with compact tufts and rings with green spores; colour from reverse was light tan but turn dark, with radial sulcation. | Colonies size ranging from 70-80 mm in 5 days, hairy texture; mycelia initially white and downy, became green and later deep green with compact tufts and rings with green spores; colour from the reverse was light tan to pale orange, with radial sulcation. | Hyphae is septate and hyaline; conidiophores are short branching at wide angle (approaching 90°) giving pyramidal appearance; phialides are flask shaped; conidia are globose to ellipsoidal with smooth walled; conidia often accumulated at the tips of phialides in slimy balls. |
| **Aspergillus**     | Colonies size ranging from 50-60 mm in 5 days; texture initially hairy which turn dry powdery after 5 days; colour from top is white covered with dark black spores with heavy sporulation within a week; colour from reverse is creamish brown, with radial sulcation. | Colonies size ranging from 50-60 mm in 5 days, texture initially hairy which turn powdery after 5 days; colour from top is dark black due to heavy sporulation within a week; colour from reverse is creamish yellow but turns dark, with radial sulcation. | The head globose with long stipe and thick wall globose vesicle; phialides short; conidiophores smooth, hyaline or faintly brownish near the apex; conidia typically spherical at maturity, very dark or spiny and very dark in colour or with conspicuous longitudinal striations |
| **Rhizopus**        | Colonies.size ranging from 80-90 mm in 3 days, texture woolly or cottony, colour from top is initially white and turned to grey with black dots that represented the mature sporangia. Colour from reverse is cream. | Colonies size ranging from 80-90 mm in 3 days, texture woolly or cottony; colour from top is white initially and gradually turned to grey with black dots that represented the mature sporangia. Colour from reverse is cream. | Sporangio-phores singly or in small groups from nodes on the stolons, brown, non-septate; sporangia spherical, black with numerous spores; columellae light brown, sub-spherical; stolons are hyaline; rhizoid short and brown. |
| **Tallaromyces**    | Colonies sizes ranging from 30-40 mm in 7 days, texture appeared hairy; colour from top initially white, turn greyish, to deep green; colour from reverse is orange in centre but white in periphery. | Colonies sizes ranging from 30-40 mm in 7 days; mycelia white, yellow and red; texture floccose with radially sulcate; exudates present as clear droplets; colour from top deep green presented the con-idia; colour from reverse is reddish brown. | Conidiophores is biverticillate, branch only once and then bears a fruiting structure consisting of the metulae and phialides; phialides 3-5 per stipe; stipes smooth; conidia are smooth, spherical to ellipsoidal; ascormata not observe |
| **Penicillium**     | Colonies size ranging from 30-40 mm in 7 days; surface texture is velutinous (velvety surface) with radial sulcation; colour from top grey with weak greenish overtones; | Colonies size ranging from 30-40 mm in 7 days; surface texture is velutinous with radial sulcation; colour from top has a central greyish turquoise with white periphery; colour from reverse is pale yellow to light yellowish brown | Hyphae is septate and hyaline; smooth walled conidiophores and biverticillate; metulae found in whors of 3 – 5 differ structures; flask-shaped phialides; conidia are globose to sub-globose, smooth or finely roughened surface, form rather long chains. |
Glucanase and xylanase activities of *Talaromyces pinophilus* OPT1 (4) isolated from palm oil waste compared to other species on the same substrate and conditions are shown in Table 4.

### Table 4. Glucanase and xylanase production from different mold under submerge fermentation

| Organism                  | Substrat               | Glucanase (U/mL) | Xylanase (U/mL) | Reference |
|---------------------------|------------------------|------------------|-----------------|-----------|
| *Talaromyces pinophilus* OPT1(4) | CMC / birchwood xylan | 15.17            | 13.35           | This work |
| *Aspergillus niger* ANGA   | CMC / birchwood xylan | 7.45             | 6.16            | [7]       |
| *Trichoderma reesei* TRAR  | CMC / birchwood xylan | 10.18            | 3.37            | [7]       |
| *Aspergillus fumigatus* MS16 | CMC / birchwood xylan | 0.311            | 0.391           | [24]      |
| *Aspergillus niger* MS80   | - / birchwood xylan    | -                | 1.24            | [24]      |
| *Aspergillus fumigatus* ABK9 | CMC/-                 | 1.56             | -               | [24]      |

Table 4 shows that the glucanolytic and xylanolytic activity of *Talaromyces pinophilus* OPT1 (4) was higher than that of *Aspergillus niger*, *Aspergillus tereus* and *Trichoderma reesei* isolate as reported in several studies (7, 24), but the glucolytic activity was lower than *Aspergillus fumigatus* ABK9 isolate reported in another study (9). Table 3 also shows that enzyme activity is strain dependent so that the same species can produce different catalytic activities. This reason led to the need for research to characterize the enzymatic activity of the newly isolated strains.

## 4. Conclusions

Thirty-two cellulase and xylanase-producing mold were isolated from oil palm waste. Among these isolates OPT1 (4) had the highest relative cellulase and xylanase activity and was identified as *Talaromyces pinophilus* OPT1 (4). In liquid media containing CMC and xylan as the sole carbon source, *Talaromyces pinophilus* OPT1 (4) produces glucanase and xylanase in the amount of 15.7 and 13.35 U/mL, respectively. Cellulase and xylanase producing mold obtained from this study have the potential to be applied in the degradation of lignocellulosic biomass waste to produce sugars that are further converted into chemicals and fuels.

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## References

1. Chundawat S P, Beckham G T, Himmel M E, Dale B E 2011 Deconstruction of lignocellulosic biomass to fuels and chemicals *Annu. Rev. Chem. Biomol. Eng.* 2 121-145.
2. Keegstra K 2010 Plant cell walls *Plant Physiol.* 154 2 483-486.
3. Girio F M, Fonseca C, Carvalheiro F, Duarte L C, Marques S, Lukasik R M 2010 Hemicelluloses for fuel ethanol: a review *Biores. Technol.* 101 13 4775-4800.
4. Bayer E A, Belaich J P, Shoham Y, Lamed R 2004 The cellulases: multi-enzyme machines for degradation of plant cell wall polysaccharides *Annu. Rev. Microbiol.* 58 521-554.
5. Fontes C M, Gilbert H J 2010 Cellulases: highly efficient nanomachines designed to deconstruct plant cell wall complex carbohydrates *Annu. Rev. Biochem.* 79 655-681.
6. El-Naggara N E, Harounb S A, Owaisb E A, Sherief A A 2015 Identification of newly isolated *Talaromyces pinophilus* and statistical optimization of β-glucosidase production under solid state fermentation *Prep. Biochem. Biotechnol.* 45 7 712-729.
7. Adesina F C, Onilude A A 2013 Isolation, identification and screening of xylanase and glucanase-producing microfungi from degrading wood in Nigeria *African J. Agric. Res.* 8 34 4414-4421.
[8] Rahayu E S, Sardjono, Samson R A 2014 Jamur benang (mold) pada bahan pangan (Mold in food) Kanisius. [In Indonesian]

[9] Das A, Paul T, Halder S K, Maity C, Mohapatra P K D, Pati B R, Mondal K C 2013 Study on regulation of growth and biosynthesis of cellulolytic enzymes from newly isolated *Aspergillus fumigatus* ABK9 *Polish J. Microbiol.* 62 1 31-43.

[10] Ghose T 1987 Measurement of cellulase activities *Pure Appl. Chem.* 59 257-268.

[11] Ghose T, Bisaria V S 1987 Measurement of hemicellulase activities. Part 1: Xylanases *Pure Appl. Chem.* 59 1739-1752.

[12] Miller G L 1959 Use of dinitrosalicylic reagent for the determination of reducing sugars *Anal. Chem.* 31 426-428.

[13] Hansena G H, Lübeckb M, Frisvada J C, Lübeckb P S, Andersena B 2015 Review-Production of cellulolytic enzymes from ascomycetes: comparison of solid state and submerged fermentation *Process Biochem.* 50 1327–1341.

[14] Champreda V, Mhuantong W, Lekakarn H, Bunterngsook B, Kanokratana P, Zhao X-Q, Zhang F, Inoue H, Fujii T, Eurwilaichitr L 2019 Designing cellulolytic enzyme systems for biorefinery: from nature to application *J. Biosci. Bioeng.* 126 6 637-654.

[15] Liu G, Qu Y 2019 Research review paper - engineering of filamentous fungi for efficient conversion of lignocellulose: tools, recent advances and prospect *Biotechnol. Advances.* 37 4 519-529.

[16] Pol D, Laxman R S, Rao M 2012 Purification and biochemical characterization of endoglucanase from *Penicillium pinophilum* MS20 *Indian J. Biochem. Biophys.* 49 189-194.

[17] Maeda R N, Barcelos C A, Anna L M M S 2013 Cellulase production by *Penicillium funiculosum* and its application in the hydrolysis of sugar cane bagasse for second generation ethanol production by fed batch operation *J. Biotech.* 163 38-44.

[18] Wang X, Huang H, Xie X, Ma R, Bai Y, Zheng F, You S, Zhang B, Xie H, Yao B, Luo H 2016 Improvement of the catalytic performance of a hyperthermostable GH10 xylanase from *Talaromyces leycettanus* JCM12802 *Bioresour. Technol.* 222 277-284.

[19] Mallek-Fakhfakha H, Fakhfakh J, Wallha K, Hassairi H, Gargouri A, Belghith H 2017 Enzymatic hydrolysis of pretreated Alfa fibers (*Stipa tenacissima*) using *d*-glucosidase and xylanase of *Talaromyces thermophilus* from solid-state fermentation *J. Int. Biol. Macromol.* 103 543–553.

[20] Li Q, Suna B, Xiong K, Teng C, Xub Y, Li L, Xiuting  L 2017 Improving special hydrolysis characterization into *Talaromyces thermophilus* F1208 xylanase by engineering of N-terminal extension and site-directed mutagenesis in C-terminal *J. Int. Biol. Macromol.* 96 451–458.

[21] Bharti A K, Kumar A, Kumar A, Dutt D 2018 Exploitation of *Parthenium hysterophorous* biomass as low-cost substrate for cellulase and xylanase production under solid-state fermentation using *Talaromyces stipitatus* MTCC 12687 *J. Rad. Res. Appl. Sci.* 11 271-280.

[22] Fujii T, Fang X, Inoue H, Murakami K, Sawayama S 2009 Enzymatic hydrolyzing performance of *Acremonium cellulolyticus* and *Trichoderma reesei* against three lignocellulosic materials *Bioresour. Biofuels* 2 24 1-8.

[23] Gusakov AV. 2011 Alternatives to *Trichoderma reesei* in biofuel production *Trends Biotechnol.* 29 419-425.

[24] Sohail M, Naseeb S, Sherwani S K, Sultana S, Aftab S, Shahzad S, Ahmad A, Khan S A 2009 Distribution of hydrolytic enzymes among native fungi: aspergillus the pre-dominant genus of hydrolase producer *Pak. J. Bot.* 41 2567-2582.