Isolation and Characterization of Cellulase- and Xylanase-Producing Microbes Isolated from Tropical Forests in Java and Sumatra

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Abstract: Efficiency of cellulases enzyme is one of determining factors in bioethanol-from-biomass production. However, it could be hampered by the presence of xylan. Employing microbes that can produce both cellulase and xylanase may help to overcome the current challenges in bioethanol production. Biodiversity of microorganisms in tropical forests offers more possibility for obtaining the potential microbes for this purpose. This research, therefore, was aimed to collect, screen, characterize and identify potential cellulase- and xylanase-producing microbes from five different tropical forest ecosystems in Java and Sumatra. Numbers of 553 isolates that have been collected from the fields were screened using selective media (i.e. CMC- and Xylan- agar) in order to determine the potency of microbes in producing cellulase and xylanase which were indicated by clear zones exhibited around the cultures. This qualitative screening showed that 304 isolates were positive producing cellulase and 323 isolates were positive producing xylanase. Eight potential isolates which showing greater cellulose- and xylano- lytic indexes were subjected to enzyme activity tests and DNA analyses for species identification. The assay for enzymatic activity of cellulase and xylanase were determined based on the release of glucose and xylose respectively that were detected using dinitrosalycylic acid (DNS). The potential isolates were identified as Rhizomucor variabilis, Fusarium sp., F. solani, Aspergillus niger, Bacillus thuringiensis, and Sphingobacterium daejeonense. Other two bacterial isolates (FORDA-CC 3128, 3171) were remains unidentified due to faint PCR results. Fusarium sp. And F. solani showed greater cellulase activity than the commercial cellulase after 19 hours of incubation. B. thuringiensis, FORDA-CC 3128 and 3171 showed greater cellulase and xylanase activities than the commercial enzymes after 15 min
incubation. Stability of cellulase and xylanase activities was still an issue to be solved in the next experiments. Optimization of pH and temperature are suggested.

**Keywords:** Characterization, cellulase-producing microbes, enzymatic activity, xylanase-producing microbes.

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1. Introduction

Bioethanol is a promising alternative energy in dealing with the depletion of fossil fuel and the increasing of greenhouse gases effects. It offers many advantages, such as lower energy content, lower price and less emission than gasoline and diesel (Chandel et al., 2007; Tomás-Pejo et al., 2008; Mutreja et al. 2011). Lignocellulolytic biomass represents the better prospect for bioethanol production compare to starch-containing sources which is compete with food consumption. In addition, the abundance of lignocellulolytic biomass that came from agricultural and forestry waste offers a renewable and in-expensive feedstock for long-term supply of fermentable sugar sources (Maki et al., 2011; Held, 2012).

The use of lignocellulosic material for ethanol production present a number of challenges related to its structures. Lignocellulose is a complex structure that contains of cellulose – a homologous polymer of glucose molecules connected by β-1,4 linkages, hemicellulose – a heterologous polymer of pentose and hexose, and lignin – a complex aromatic polymer (Maki et al., 2011). A crucial step in bioethanol production from lignocellulosic materials is to maximize the effectiveness of saccharification of celluloses and hemicellulos to fermentable sugars. Hemicellulose has been proposed as a physical barrier for cellulose hydrolysis (Yoshida et al., 2008). Xylan which is a major constituent of hemicelluloses performs a ‘blocking effect’ that limited the accessibility of cellulase enzymes to the cellulose (Hu et al., 2011). Hemicellulose-degrading enzyme activities are usually detected in most of commercial cellulase enzymes but they are insufficient to achieve significant conversion of the residual hemicelluloses (Hu et al., 2011; Qing and Wyman, 2011). It has been reported that supplementation of cellulase with hemicelluloses-degrading enzymes, such as xylanase, improves cellulase digestion by reducing xylan coating and linkages to cellulose (Kumar & Wyman, 2009; Hu et al., 2011; Qing & Wyman, 2011).

In relation with supplementation of cellulase, microbial co-cultures that could produce various enzymatic hydrolysis for cellulose and hemicelluloses, has been proposed as a highly efficient approach. In co-culture strategy, symbiotic association of microorganisms causes better substrate colonization which is each species having its own niche for growth and substrate degradation (Kaushal et al., 2012). For example, cellulose is degraded by certain strain and produce cellubiose and glucose;
at the same time, hemicelluloses can be hydrolysed into pentoses like xylobiose and xylose by other strains (Cheng and Zhu, 2012). However, prior to construct a co-culture strategy, compatibility among microorganisms should be characterized in order to ensure their synergistic effect to improve cellulose hydrolysis rate (Cheng & Zhu, 2012).

In order to contribute in the improvement of biodegradation of cellulosic biomass through microbial co-cultures, exploration, selection and characterization of potential cellulase- and xylanase producing microbes may have important roles. Humid tropical forest soils that house an immense and un-explored microbial diversity, abundance of biomass, and unique microbial communities present an attractive target for the discovery of novel enzymes which is potential for the improvement of efficiency of bioethanol production derived from cellulosic feedstock (Parton et al., 2007; Liptzin and Silver, 2009; DeAngelis et al., 2010; DeAngelis et al., 2011). The objective of this study, therefore, is to collect, screen, identify and characterize cellulase- and xylanase-producing microbes isolated from soils, plant litters, herbivores dunks, rotten woods and sporocarps that have been collected from several tropical forests in Java and Sumatra for possible use in the large scale of bioconversion of lignocellulosic materials into ethanol.

2. Materials and Methods

2.1. Sample Collection and Microbial Isolation

Samples for isolation of cellulase- and xylanase-producing microbes were obtained from particular sites in five National Parks in Java and Sumatra as listed in Table 1. Exploration for collecting potential samples was carried out by walking-through method within 60 – 90 min for each site. Samples of soils, litter, rotten woods, herbivores dunks and sporocarps were put into plastic or paper bags. Sample details were recorded, photographs were taken.

To isolate fungi and bacteria from soil and litter, 1 g of each crushed-sample was suspended in 10 ml of sterile water by vortexing for 2 min on maximum speed, following by a 10x serial dilution of the suspension that was made in 10 ml of 0.85% NaCl solution. One ml of each dilution in the series was spread onto the surface of ½ PDA medium + 150 ppm of chloramphenicol, and ½ NA media + 15 ppm of cyclohexamide using the standard spread plate technique in order to isolate fungal and bacterial isolates, respectively. All plates were incubated at 28-30°C for 2 days before sampling and then they were incubated for another additional 2-4 days to allow growth of slower growing microorganisms for further sampling. Various colonies were selected based on their morphology, size and colour appear on the agar plates. The selected colonies were then subcultured onto separate agar plates to ensure their purity.
Fungal isolations of rotten wood and sporocarps were carried out by placing small pieces of the wood samples and sporocarps that had been surface-sterilised onto plates of PDA medium + 150 ppm of Chloramphenicol. All plates were incubated at 28-30°C for 1-2 weeks with daily observation of mycelia growth. Mycelia that grown from the samples were subcultured into fresh PDA plates. Colonies were further subcultured if more purification was required.

### Table 1. Descriptions of The Exploration Sites

<table>
<thead>
<tr>
<th>Site Code</th>
<th>National Parks (NP)</th>
<th>Locality</th>
<th>Type of ecosystem</th>
<th>GPS Coordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>Berbak NP, Jambi Province</td>
<td>Sungai Air Hitam Dalam:</td>
<td>Fresh-water swamp forest</td>
<td>a) E: 104° 09.133’ S: 01° 15.624’&lt;br&gt;b) E: 104° 09.059’ S: 01° 16.173’&lt;br&gt;c) E: 104° 09.078’ S: 01° 15.421’</td>
</tr>
<tr>
<td>Site 2</td>
<td>Gunung Leuser NP, North Sumatra Province</td>
<td>Bukit Lawang:</td>
<td>Secondary forest</td>
<td>a) E: 98° 06.947’ N: 3° 32.958’&lt;br&gt;b) E: 98° 06.912’ N: 3° 33.060’</td>
</tr>
<tr>
<td>Site 3</td>
<td>Bukit Barisan Selatan NP, Lampung Province</td>
<td>a) Pemerihan (degraded area)&lt;br&gt;b) PAL-50 Rhino Camp (conserved area)</td>
<td>Tropical lower montane rainforest</td>
<td>a) E: 104° 24.255’ S: 05° 36.480’&lt;br&gt;b) E: 104° 25.263’ S: 05° 30.336’</td>
</tr>
<tr>
<td>Site 4</td>
<td>Gunung Gede Pangrango NP, West Java Province</td>
<td>a) Mandalawangi (conserved area)&lt;br&gt;b) Rawa Panjang (degraded area)</td>
<td>Tropical upper montane rainforest</td>
<td>a) E: 107° 00.128’ S: 06° 44.459’&lt;br&gt;b) E: 106° 59.909’ S: 06° 44.371’</td>
</tr>
<tr>
<td>Site 5</td>
<td>Gunung Merapi NP, Central Java Province</td>
<td>a) Dusun Turgo (less impacted by Mt. Merapi eruption)&lt;br&gt;b) Kaliurang (highly impacted by the Mt. Merapi eruption)</td>
<td>Highland tropical forest after eruption</td>
<td>a) E: 100° 31.166’ S: 14° 03.830’&lt;br&gt;b) E: 110° 26.089’ S: 07° 35.873’</td>
</tr>
</tbody>
</table>

#### 2.2. Screening for Cellulase- and Xylanase- Producing Microbes

Prior to the screening process, the isolates were overnight grown in ½ PDB (Potato Dextrose Broth) and ½ NB (Nutrient Broth), for fungi and bacteria, respectively. Due to the large number of microbes that had been isolated, screening of cellulase- and xylanase- producing microbes was carried out in two steps. Firstly, screening was conducted using a 48-pin replicator which was dipped into microbial cultures in 96-microwell plates, then spot plated on CMC- and xylan- agar plates (Kasana et al., 2008; Agustini et al., 2011) for detecting cellulase and xylanase, respectively. The ingredient of xylan agar was similar to CMC agar with replacement of C source by xylan from beechwood (SIGMA). There was 48 isolates tested in one plates; five replications were performed. Plates were
incubated at 28°C for 24 hours and then were flooded with Gram’s iodine (2.0 g KI and 1.0 g iodine in 300 ml distilled water) for 3-5 minutes (Kasana et al., 2008). Isolates that showing positive reaction, which was determined by the zone of clearance around the colony, were subject to further screening test (Fig.1).

At the second test, 5 µl of the overnight grown culture was spotted on CMC- and xylan- agar plates (one isolate per plate). After being incubated at 28°C for 48 hours, plates were flooded with Gram’s iodine as it was conducted in the first test. Diameters of the colony and the clear zone were measured. Ratio of the diameter of clear zone to the diameter of colony was determined as cellulo- (for those that grown in CMC agar) and xylano- lytic indexes (for those that grown in xylan agar). Eight isolates that shown greater either cellulolytic- or xylanolytic- index or both were selected for characterization of the cellulase and xylanase activities.

Figure 1. The first step of screening: Cellulase and xylanase production on CMC- and xylan- agar plates

2.3. Characterization of Cellulase and Xylanase Activities through Reducing Sugar Measurement

Cellulase and xylanase activities of the selected isolates were measured using the 3,5-dinitrosalicylic acid (DNS) method (Ghose, 1987) for determination of the amount of reducing sugars released during certain times of reaction mixture. Prior to cellulase and xylanase assay, the fungal isolates were prepared in 100 ml of Malt Extract Broth (MEB) and being incubated on a shaker at 110 rpm for 4 days; and bacterial isolates were in 100 ml of Nutrient Broth (NB) and being overnight incubated on a shaker at 110 rpm. Culture supernatants obtained after centrifugation at 3600 g for 30 minutes were used to determine the cellulase and xylanase activities. Three tenths (0.3) ml of the
supernatants were suspended into 2.7 ml of 1% CMC solution in 50 mM citrate buffer (pH 5.0) for measuring total cellulase activity or into 2.7 ml of 1% xylan solution in 50 mM citrate buffer (pH 5.0) for measuring xylanase activity. The mixture cultures were incubated in room temperature for 1, 2, 3, 5, 19 and 24 hours for fungal cultures; and 15, 30, 60, 90, and 120 minutes for bacterial cultures. Enzymatic reactions were terminated by adding 3ml of DNS reagent and then being incubated at 80°C in a waterbath for 5 minutes. After reaching room temperature, 1ml of 40% potassium sodium tartrate (KNaC\textsubscript{4}H\textsubscript{4}O\textsubscript{6}.4H\textsubscript{2}O) was added. Absorbance of the final solution was read at 540 nm. Glucose and xylose standards (ranging from 0 – 5 mg/ml) were prepared in the same manner like the samples. The amount of reducing sugar present in the samples was calculated refers to the standard curves that have been prepared before. The cellulase and xylanase activities of the selected microbes were compared to the commercial cellulase and xylanase produced by SIGMA-Aldrich Inc.

2.4. Molecular Identification of the Potential Microbes

Bacterial cultures were grown on NA (Nutrient Agar) media for one day before their DNA was extracted. A loop of the bacterial colony was then diluted in 100 µL of sterile miliQ. The bacterial suspension was heated using a thermocycler at 95°C for 3 min, 4°C for 3 min and then left at 15°C before it used for PCR. PCR amplification of the 16S ribosomal DNA was carried out using MyCycler, BIO-RAD with the following program: 90 sec at 95°C, followed by 30 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for 90 sec, final extension of 72°C of 5 min and terminated at 4°C. PCR reaction contained 15 µL reaction buffer of Green \textit{Go Taq Mastermix}™ (Promega Inc.USA) [400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3 mM MgCl\textsubscript{2}, \textit{Green GoTaq} reaction buffer], 10 µM primers 27F and 1492R, 28µL of sterile miliQ and 5 µL of cell suspension as DNA template that had been prepared before. Three µL of the PCR product was loaded onto 2% agarose in TAE buffer at 140V for 70 min to visualize the DNA bands. PCR products were sent to the 1st Base Inc. (Singapore) for DNA sequencing. DNA sequences were then matched against the existing database of GeneBank. Taxonomic information was derived from similarity to sequences in public DNA databases.

DNA of fungal isolates was extracted from the cultures that had been grown in PDB (Potato Dextrose Broth) medium for 4 days. About thirty (30) mg of mycelia was ground with a micro-pestle with the addition of a few drops of extraction buffer Wizard Genomic™ (Promega, USA). Process of fungal DNA extraction referred to the protocol of Wizard Genomic™. PCR amplification of the ribosomal DNA internal transcribed spacers (rDNA ITS) was carried out using MyCycler, BIO-RAD with the following program: 5 min at 94°C, followed by 24 cycles of 94°C for 1 min, 55°C for 30 1
min, 72°C for 2 min, final extension of 72°C of 7 min and terminated at 4°C. PCR reactions contained 5µL Go Taq Flexi™ buffer, 10 µM primers ITS1 and ITS4, 27.75 µL sterile milliQ, 4µL MgCl₂ 2.5mM, 1µL dNTP 10mM, and 5 µL DNA template. Three µL of the PCR product was loaded onto 2% agarose in TAE buffer at 140V for 70 min to visualize the DNA bands. PCR products were sent to the 1st Base Inc. (Singapore) for DNA sequencing. DNA sequences were edited using BLAST program. Obtained sequences were then matched against the existing database of GenBank. Taxonomic information was derived from similarity to sequences in public DNA databases.

3. Results and Discussions

3.1. Isolation and Screening of Cellulo- and Xylano-lytic Microbes

A total of 553 isolates were collected and screened based on the formation of clear zones around the microbial colonies (Table 2). Three hundred and four (304) of them showed positive reactions to qualitative test of cellulase production as indicated by the formation of clear zone (halo) in CMC agar medium. Three hundred and twenty three (323) isolates showed positive reaction to xylanase production as it indicates by clear zones in xylan agar medium. The second step of screening showed that cellulolytic indexes were ranging from 1 to 3.36; and the xylanolytic indexes were ranging from 1 to 3.68 (data not shown here).

<table>
<thead>
<tr>
<th>Exploration Site</th>
<th>Total isolates collected</th>
<th>Taxon</th>
<th>Number of isolates</th>
<th>Enzymes Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fungi</td>
<td></td>
<td>Cellulase</td>
</tr>
<tr>
<td>Site 1</td>
<td>118</td>
<td>Fungi</td>
<td>78</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>Site 2</td>
<td>70</td>
<td>Fungi</td>
<td>57</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Site 3</td>
<td>115</td>
<td>Fungi</td>
<td>73</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>42</td>
<td>21</td>
</tr>
<tr>
<td>Site 4</td>
<td>146</td>
<td>Fungi</td>
<td>87</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>59</td>
<td>24</td>
</tr>
<tr>
<td>Site 5</td>
<td>104</td>
<td>Actinomycetes</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fungi</td>
<td>74</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bacteria</td>
<td>29</td>
<td>16</td>
</tr>
<tr>
<td>TOTAL</td>
<td>553</td>
<td>Total</td>
<td>553</td>
<td>304</td>
</tr>
</tbody>
</table>
The eight isolates that have high cellulo- and xylanolytic indexes were presented in Fig. 2. It showed that isolates FORDA-CC 3128, 3123, and 3171 were the best three for cellulosolytic index, \textit{i.e.} 3.36, 3.14, and 3.00, respectively; and isolates FORDA-CC 3127, 3128, and 3154 were the best three for xylanolytic index, \textit{i.e.} 3.68, 3.28, and 2.85, respectively (Fig. 2). Isolates FORDA-CC 3123, 3127, 3128 (bacterial isolates) and 3154 (fungal isolate) presented relatively high index for both cellulase and xylanase compare to others isolates. These isolates were isolated from samples of elephant’s faeces in Bukit Barisan NP, soil in Berbak NP, litter in Gunung Merapi NP and litter in Berbak NP, respectively (Table 3). Samples of rotten wood and sporocarps resulted fungal isolates that produce either cellulase or xylanase or both, however their index of cellulo- or xylano- lytic were lower than the eight isolates (data not shown here).

In order to confirm this qualitative screening, further quantitative assay for determining enzymatic hydrolysis activity was conducted. The eight potential isolates were subject to test for their cellulase and xylanase activity in breaking down the celluloses and hemicelluloses into fermented sugars.

![Figure 2](image-url). Cellulo- and xylanolytic index of the isolates. Bars were indicated the mean standard error

\textbf{3.2. Characterization of Cellulase and Xylanase Activities}

Activities of cellulase and xylanase of all fungal isolates tested were compared to enzymes reference (Sigma-Aldrich C2605 for cellulase; H2125 for hemicellulase or xylanase) as standards (Fig.3). The reference enzymes were derived from \textit{Aspergillus} sp.
Characterization of cellulase activity showed that the standard of cellulase reached the first optimum activity after 1 hour of incubation and reached the second one after 19 hours (overnight) incubation, and yielded glucose 1.51 mg/ml. Generally, while the fungal isolates reached the optimum reactions later than the standard with lesser yield of glucose, except for isolates FORDA-CC 3154 and 3157 which produced glucose 2.75 and 2.03 mg/ml, respectively, after 19 hours incubation. This two isolates may have higher cellulase activity in a shorter time of incubation if the physical factors, such as temperature and pH were optimized. However, further experiments are required to confirm this possibility.

<table>
<thead>
<tr>
<th>Isolates code</th>
<th>Taxon</th>
<th>Type of samples</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORDA-CC 3140</td>
<td>Fungi</td>
<td>Rotten wood</td>
<td>Simpang Bayas at Air Hitam Dalam rivers of Berbak NP</td>
</tr>
<tr>
<td>FORDA-CC 3157</td>
<td>Fungi</td>
<td>Litter</td>
<td>Resort Mandalawangi, Gunung Gede-Pangrango NP</td>
</tr>
<tr>
<td>FORDA-CC 3135</td>
<td>Fungi</td>
<td>Litter</td>
<td>Rawa Panjang, Gunung Gede-Pangrango NP</td>
</tr>
<tr>
<td>FORDA-CC 3154</td>
<td>Fungi</td>
<td>Litter</td>
<td>Simpang Larang at Air Hitam Dalam rivers of Berbak NP</td>
</tr>
<tr>
<td>FORDA-CC 3123</td>
<td>Bacteria</td>
<td>Elephant's faeces</td>
<td>Resort Pemerihan, Bukit Barisan Selatan NP</td>
</tr>
<tr>
<td>FORDA-CC 3127</td>
<td>Bacteria</td>
<td>Soil</td>
<td>Simpang Larang at Air Hitam Dalam rivers of Berbak NP</td>
</tr>
<tr>
<td>FORDA-CC 3128</td>
<td>Bacteria</td>
<td>Litter</td>
<td>Dusun Turgo, Gunung Merapi NP</td>
</tr>
<tr>
<td>FORDA-CC 3171</td>
<td>Bacteria</td>
<td>Soil</td>
<td>Resort Bukit Lawang of Gunung Leuser NP</td>
</tr>
</tbody>
</table>

Even though two fungal isolates (FORDA-CC 3154 and 3140, Fig.2) showed great xylanolytic indexes, the enzymatic activity of the isolates were lesser than the enzyme reference. The standard reach an optimum hydrolytic activity after 2 hours incubation with producing 2.35 mg/ml of xylose, while the fungal isolates showed optimum reaction after 3 hours with xylose production ranging from 0.38 to 0.86 mg/ml (Fig. 3). It seems that the xylanolytic indexes are poorly correlated to the xylanase activities.

Bacteria present an attractive potencies for exploitation of cellulase and hemicellulase due to their rapid growth rate and enzyme complexity (Maki et al. 2009). Cellulase activity of FORDA-CC 3123, 3128 and 3171 were greater than the enzyme reference. The potential bacterial isolates produced 1.79, 2.29 and 1.80 mg/ml of glucose, respectively, while the standard produced 1.53 mg/ml after incubation for 15 minutes. However, cellulase activities of the bacterial isolates were less stable than
the standard which can retain its cellulose hydrolytic activity after 120 minutes of incubation (Fig. 4.A).

Figure 3. Enzymatic activities of fungal isolates in degrading cellulose into glucose (A); and hemicelluloses into xylose (B) in comparison to the commercial enzyme derived from *Aspergillus* sp. (Sigma-Aldrich).

Four bacterial isolates tested showed greater xylanase activity than the enzyme reference during the first 90 minutes of incubation. All isolates have optimum xylanase activity after 15 minutes of incubation, while the reference reached optimum reaction after being incubated for 2 hours (Fig. 4 B).

Figure 4. Enzymatic activities of bacterial isolates in degrading cellulose into glucose (A); and hemicelluloses into xylose (B) in comparison to the commercial enzyme derived from *Aspergillus* sp. (Sigma-Aldrich).

Four bacterial isolates tested showed greater xylanase activity than the enzyme reference during the first 90 minutes of incubation. All isolates have optimum xylanase activity after 15 minutes of incubation, while the reference reached optimum reaction after being incubated for 2 hours (Fig. 4 B).

Stability of enzymatic reactions of both cellulase and xylanase is still an issue for both fungal and bacterial isolates. It could be caused by unsuitable physical factors (*i.e.* pH and temperature) for
optimum reactions of cellulase and xylanase. All experiments of reducing sugars measurements using the DNS method were set at room temperature (± 30°C) and pH 5, as it was required in the DNS method (Ghose, 1987). However, it seems that the temperature and pH were not suitable for optimum reaction of cellulase and xylanase produced by the tested isolates. Perez-Avalos et al. (2008) reported that cellulase and xylanase of Cellulomonas flavigena have optimum activity at pH 6 and 9, respectively, with a general optimum temperature at 50°C. Thus, further experiments in order to determine optimum condition for hydrolytic activity and stability of the cellulase and xylanase enzymes of the tested isolates are required.

3.3. Identification of the Potential Isolates

A sequence alignment of four filamentous fungi that had been amplified using the primers ITS1 and ITS4 shown that FORDA-CC 3140 had 99% similarity to Rhizomucor variabilis (GenBank accession DQ11900.1); FORDA-CC 3157 had 99% similarity to Fusarium solani LW-1 (GenBank accession EU719658.1); FORDA-CC3135 had 100% similarity to Aspergillus niger strain 91718 (GenBank accession JN565296.1); and FORDA-CC 3154 had 99% similarity to Fusarium sp. Strain Papaya1 (GenBank accession EU707572.1).

Two of four bacterial isolates that were subjected to DNA analysis gave faint PCR products using the primers 27F and 1492R (FORDA-CC 3128 and 3171). The rDNA ITS sequences of bacterial isolate FORDA-CC 3123 collected from elephant’s feces had 98% similarity to sequence from Sphingobacterium daejeonense (GenBank accession NR041407.1), FORDA-CC 3127 had 100% similarity to Bacillus thuringiensis sequence (GenBank accession EU177581.1).

It was unfortunate that the isolates which showed high cellulase and xylanase activities (FORDA-CC 3171) and the highest cellulase activity (FORDA-CC 3128) were remain unidentified. The faint PCR products of unidentified bacterial isolates are likely to be a result of PCR inhibition. Many microbial cultures may contain substances that inhibit the polymerase enzyme. The problem can be overcome by further purification of the DNA or by attempting PCR with several dilutions of the DNA extract. However, there was insufficient time to complete this in the current project.

4. Conclusions

Out of eight isolates that have been characterized for their cellulase and xylanase activity, Fusarium sp. (FORDA-CC 3154) and F. solani (FORDA-CC3157) are potential fungal isolates due to their cellulase activities; Bacillus thuringiensis (FORDA-CC 3127) is potential bacterial isolate due to its xylanase activity; while Sphingobacterium daejeonense (FORDA-CC 3123) and other two unidentified bacteria are potential due to their high activities both for cellulase and xylanase. These
isolates are potential for further co-culture application in order to develop various strategies for the improvement of saccharification of lignocellulosic material, especially from forestry waste.

Hence isolation, screening and selection have facilitated the discovery of several cellulase complex-producing microbes from a wide variety of environments and realizing that Indonesian tropical forest ecosystems offer an abundant biodiversity of microorganisms that have not been fully explored, exploitation of cellulase-complex genes from both culturable and un-culturable microorganism in more extreme environment is suggested. It hopes that the enzymes isolated will have specific application in biorefining industry and contribute to a decrease in the current cost of bioconversion of lignocelluloses to ethanol.

Acknowledgments

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