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Evaluation of Cellulase Activity from *Trichoderma* spp. and Xylanolytic Bacteria

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ABSTRACT

Fungi and bacteria were considered as potential decomposers on various substrates. This study was aimed to evaluate the ability of *Trichoderma* spp. to degrade various cellulolytic substrates and xylanolytic bacteria to decompose lignocelluloses from rice straw. Six selected *Trichoderma* spp. isolates showed high activities of specific cellulase in given substrates. Pan23.2 isolate was shown to be superior in all cellulose substrates without glucose with the highest activity to hydrolyze cellulose crystals. Some isolates preferentially hydrolyzed crystalline (Pan23.2, Bo17, Andi2.1) while others (Kun4, Gam4.1, Pan23.1) were more dominant on amorph hydrolysis. Not all cellulases from *Trichoderma* spp. with high activity on cellulose amorph showed activity on cellulose crystals. The addition of glucose into cellulose substrates increased the cellulase productivity of Andi2.1 and Gam4.1 isolates. Combination of 1% glucose and 1% cellobiose were the best substrate for CMCase activity, while 1% glucose and 1% CMC were good for avicelase. However, xylanolytic bacteria had no ability to degrade lignocelluloses on rice straw which was indicated by low temperature and high C/N ratio during composting process. In this study, all *Trichoderma* spp. isolates demonstrated high potency as decomposers, but the xylanolytic bacteria may be used for other applications other than as decomposers.

Keywords: Bacterial xylanase, cellulose, cellulase, lignocelluloses, *Trichoderma* spp.

ABSTRAK

Kulat dan bakteri telah dianggap sebagai pengurai berpotensi pada pelbagai substrat. Kajian ini bertujuan untuk menilai keupayaan *Trichoderma* spp. dan bakteria xilanolisis untuk mengurai pelbagai substrat selulosa dan lignoselulosa pada jerami padi. Enam pencilan terpilih *Trichoderma* spp. menunjukkan aktiviti tinggi untuk menghasilkan selulase tertentu dalam substrat yang diberikan. Pencilan Pan23.2 adalah unggul dalam semua substrat selulosa tanpa gula dengan aktiviti tertinggi untuk hidrolisis kristal selulosa. Beberapa pencilan memilih untuk mengurai kristal selulosa (Pan23.2, Bo17, Andi2.1) manakala yang lain (Kun4, Gam4.1, Pan23.1) adalah lebih dominan terhadap hidrolisis amorf. Tidak semua selulase dari *Trichoderma* spp. dengan aktiviti yang tinggi pada selulosa amorf mempunyai keupayaan yang sama pada kristal selulosa. Penambahan glukosa kepada substrat selulosa telah meningkatkan produktiviti selulase pencilan Andi2.1 dan Gam4.1. Gabungan glukosa 1% dan selobiosa 1% adalah substrat yang terbaik untuk aktiviti CMC_{Case}, manakala gabungan glukosa 1% dan CMC 1% adalah baik untuk avicelase. Walau bagaimanapun, bakteria xilanolisis tidak mempunyai keupayaan untuk mengurai lignoselulosa pada jerami padi sepertimana ditunjukkan oleh suhu rendah dan nisbah C/N yang tinggi semasa proses pengkomposan. Dalam kajian ini, semua pencilan *Trichoderma* spp. menunjukkan potensi yang tinggi sebagai pengurai, tetapi bakteria xilanolisis boleh juga digunakan untuk aplikasi lain dan bukan hanya sebagai pengurai.

Kata kunci: Xilanase bakteria, selulosa, selulase, lignoselulosa, *Trichoderma* spp.

INTRODUCTION

The development of agriculture and the agricultural industry in Indonesia has led to an increase in agricultural lignocelluloses-wastes, which consist of the main components of cellulose, hemicelluloses and lignin (Nur *et al.*, 2009; Sanchez, 2009). Rice, the staple food of most of the Indonesian population, produces by-products of rice straw containing high lignocelluloses (Nur *et al.*, 2009). The rest of the rice harvest-waste could also affect environmental pollution if they are not well managed.

Lignocellulosic waste as organic material has great potency in restoring soil fertility that affects the increment of agricultural productivity. Therefore, the development of a biotechnology approach for pioneering the use of microbes in waste-bioconversion is very important. Lignocellulosic waste utilization using cellulolytic microbes has been widely studied as an environmental problem solving approach (Wilson, 2011; Gautam *et al.*, 2012). Cellulose as part of a source of lignocelluloses, is a major component of plants and non-fossil sources of

renewable carbon in nature (Wilson, 2009). Cellulases, a group of enzymes that could catalyze the degradation of the polysaccharide cellulose which is composed of glucose molecules with β -1,4 bonding unit. This enzyme group includes the endoglucanase cellulase (β -1, 4-D-glucan-4-glucanohydrolase, EC.3.1.1.4, cellulase carboxylase, EC), exoglucanase (β -1, 4-D-glucan-4-glucohydrolase, EC 3.2.1.91, cellobiohydrolase/CBH), and cellobiose (β -D-glycoside glucohydrolase, EC 3.2.1.21, β -1, 4-D-glucosidase). Endocellulase hydrolyzes internal glycoside bonds randomly, rapidly decreasing the polymer length. Exocellulase hydrolyzes cellulose chains leading to a rapid decline of the sugar but has little effect on polymer length. Endocellulase and exocellulase work synergistically on cellulose to produce glucose, cellobiose and oligosaccharides cellobiose with high molecular weight (Vlasenko *et al.*, 2010).

Materials containing cellulose could serve as a substrate for cellulase. As the biggest part of the lignocelluloses components, it might be characterized as a linear polymer of D-glucose units with high molecular weight. Several microorganisms could grow on a cellulosic substrate because they have ability to produce enzymes that can degrade cellulose derivatives or components of the crystalline cellulose or amorphous in nature extensively. Various fungal species such as *Trichoderma* spp., *Penicillium* spp., and *Aspergillus* spp. are able to produce cellulases degrading organic matter (Nur *et al.*, 2009; Gautam *et al.*, 2010, 2012). Because of its function as a decomposer of organic matter, some species of *Trichoderma* spp. could also be used as biocontrol agents to reduce pathogenic fungi causing diseases on plant roots such as *Fusarium*, *Rhizoctonia*, *Sclerotinia* and *Phytophthora* (Nederhoff, 2001; Schile, 2005).

Any materials containing high cellulose and hemicelluloses such as rice straw, provide opportunity to be used not only as substrates for cellulolytic microbes but also for xylanolytic microbes to accelerate decomposition (Nur *et al.*, 2009). Extracellular xylanase enzymes normally produced by bacteria or fungus are able to hydrolyze xylan (hemicelluloses) into xylooligosaccharides. In the paper industry, xylanase is used to remove the hemicelluloses during the bleaching process. The enzymatic process of bleaching would be cheaper and useful to replace chemical means (Ruiz-Arribas *et al.*, 1995). In addition, xylanolytic microbes are prospective sources of xylose, forage, food and beverages, as well as the possible utilization in the process of decomposition of lignocelluloses (Richana, 2002). Xylanase production by several microbes has also been known to work as a defense system against pathogens, mainly as xylanase inducer of ethylene (Hanania *et al.*, 1999). Hence, some fungal cellulolytic *Trichoderma* spp. or xylanase-producing bacteria showing characteristics of appropriate cellulolytic enzyme activity need to be applied.

The objective of this study was to obtain preliminary information on cellulase-producing *Trichoderma* spp. isolates that are indigenous to Indonesia based on the morphology, 18S rDNA amplification and its activity on different cellulosic

substrates. Some local bacterial xylanase producers were also tested for their ability to degrade lignocelluloses material.

MATERIALS AND METHODS

Microbes and Growth Conditions

The fungal and bacterial isolates used in this study were obtained from the Indonesian Centre for Agricultural Biotechnology and Genetic Resources R&D (ICABIOGRAD) culture collection consisting of six isolates of *Trichoderma* and five unknown bacterial isolates. *Trichoderma* spp. isolates tested in this study (designated Pan23.2, Pan23.1, Gam4.1, Kun4, Bo17 and Andi2.1) were grown on Potato Dextrose Agar (PDA, Difco) plates. The main media for growth of *Trichoderma* was Mandels medium treated with various sources of cellulolytic substrates. Xylanolytic bacterial isolates of ON33, ON13, AIII5, AII5 and AI5, were grown in neutral medium with oat spelt xylan as a carbon source. All trials were conducted in three replicates. Qualitative assay of bacteria for xylan hydrolysis (Pointing, 1999) was carried out using Remazol Brilliant Blue-xylan (RBB, Sigma) media.

Morphological Observation and 18S rDNA Amplification of *Trichoderma* spp. Isolates

Trichoderma spp. isolates of 3-5 day-old in PDA media were transferred onto malt extract for four days at 28 °C in the dark room, and then irradiated by UV light prior to observation. The colony culture was observed under light microscope (Wild Balplan M3).

Isolation of total DNA was carried out based on previous standard procedures (Furlong *et al.*, 2002). Approximately 300 mg of fungal mycelia were suspended by bead beating solution (containing 0.1 M NaCl, 0.5 M Tris-HCl pH 8.0 and 5% sodium dodecyl sulfate) and added with approximately 0.2 g of a mixture of glass bead. After vortexing for 10 minutes at maximum speed, mycelia mixtures were spun in a microcentrifuge (Eppendorf 5410, Germany) at $14,400 \times g$ for 10 minutes. Solution of phenol: chloroform: isoamyl alcohol (25:24:1) was added to each sample (1:1 ratio), vortexed and centrifuged for 5 min. The aqueous phase obtained was again extracted with the same volume of chloroform: isoamyl alcohol (24:1) and centrifuged for 5 minutes at $12,000 \times g$. The supernatant was added mixed with 2.5 volume isopropanol and stored at -80°C for at least 1 hour. The mixture was centrifuged at 4°C , 10 minutes, $15,600 \times g$. The resulting DNA pellet was washed 2 times with 70% ethanol, air dried, then resuspended in sterile ddH₂O and finally mixed with RNase (DNase free). DNA was electrophoresed in 1% (w/v) Ethidium bromide (EtBr)-stained agarose gel. Polymerase chain reaction (PCR) amplification was performed in a total volume of 20 μL by mixing approximately of 10-100 ng DNA with $10 \times$ PCR buffer (containing 25 mM

MgCl₂), 25 mM dNTPs, 1U KAPA *Taq* Polymerase (Biosystems) and 10 µL each of forward and reverse primers.

PCR amplification was performed in a PTC thermal cycler (MJ Research Inc., USA) using the following conditions: initial denaturation at 94 °C for 2 minutes, followed by 25 cycles of amplification (denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 1 minutes) and a final extension at 72 °C for 7 minutes. PCR products were resolved in 1% EtBr-stained agarose gel and viewed under UV transilluminator (Hoefer Scientific, USA).

Cellulase Activity of *Trichoderma* spp on Various Substrates

Trichoderma spp. isolates were grown in Mandels medium containing CMC, avicel and cellobiose substrates, respectively. Cellulase activity for CMCase, β -glycosidase, avicelase and complete cellulase activity (FPase) were determined by reducing sugars using the DNS (dinitrosalicylic acid) method. Combination treatment of cellulose sources to test avicelase and CMCase activity, included: 1) 1% glucose + 1% avicel, 2) 1% glucose + 1% CMC, 3) 1% glucose + 1% cellobiose, 4) 1% glucose + 1% Whatman filter paper (WFP) and 5) 1% WFP with the size of 1 x 6 cm². *Trichoderma* spp. mycelia of 5 day-old culture grown on PDA were removed and mixed with 10 mL of 0.85% NaCl.

A total of 1 mL of the culture was inoculated into the appropriate medium containing cellulose-treated substrates, incubated in constant shaking incubator (Stuart Scientific S150, UK) at 150 rpm under room temperature for five days. At the end of each incubation period, the culture was added with 0.2 mL of 20% NaN₃. Culture was filtered through WFP No. 42 and then centrifuged at 4,800 × *g* at 4 °C for 30 minutes. The resulting supernatant was measured for crude cellulase activity. CMCase, avicelase, β-glycosidase, F-Pase enzyme activities were calculated by mixing 0.5 mL filtrates in 1 mL of 0.05 M citrate buffer pH 4.8 and incubated for 5 minutes. Each substrate (1%) was added with 3 mL DNS solution and heated at 100 °C for 15 minutes. Absorbance was measured at a wavelength of 575 nm wavelength using a standard glucose. The β-glycosidase activity was measured using Saligenin (Sigma) standards.

Xylanolytic Test of Bacteria Degrading Lignocelluloses

Xylanolytic bacterial isolates were grown in liquid medium (Dung *et al.*, 1993) containing xylan in a shaking incubator (Stuart Scientific S150, UK) at 150 rpm under room temperature for three days. The enzyme was extracted by centrifugation at 4,800 × *g* (Eppendorf 5410, Germany) for 30 minutes in 4 °C, and then measured for cellulase activity. Bio-decomposers activities of five xylanolytic bacterial isolates against rice straw were evaluated under laboratory-scale. Biomass yields from centrifugation of 100 mL of culture were mixed in 8 mL of nutrient broth (NB) and 2 mL of sterile glycerol. Culture was inoculated into 10

g of rice straw in a fine plastic tube that kept 60-70% humidity and was incubated for four weeks. Stirring and temperature measurements were performed regularly every three days, while temperature and the C/N ratio were observed regularly until the end of incubation.

RESULTS

Morphological Characteristics and 18S rDNA Amplification of *Trichoderma* spp.

Trichoderma spp. showed asexual phase, with specific amounts of sporulation of conidia. Ellipse conidia were formed in clusters. The production of conidiophores was highly variable in branching conidiophores (Fig. 1A-B). The conidiophores' branching patterns and aggregation demonstrated in these isolates were important for species characterization. Fungal isolates observed in this study showed morphological characteristics of *Trichoderma* spp. In the present study, only three isolates of *Trichoderma* (i.e., Kun4, Gam4.1 and Pan23.1) were analyzed using 18S rDNA amplification and two of them (Kun4 and Pan23.1) showed a positive result, with amplicon size of about 800 bp while Gam4.1 did not generate any amplicon (Fig. 1C).

Evaluation of Cellulase from *Trichoderma* spp. Isolates

Complex cellulase activity (FPase, CMCCase, and β -glycosidase avicelase) of *Trichoderma* spp. isolates showed optimum activity on the particular substrate specificity (Fig. 2A-C). CMCCase activity and avicelase were mostly higher than that of cellulase activity. In general, total cellulase activities (FPase) of all *Trichoderma* spp. isolates were higher than that of avicel CMC and cellobiose substrates, with the highest activity showed by Pan23.2 isolate. Pan23.2 generated maximum cellulase activity on avicel substrate although it was still appropriate in the CMC and cellobiose substrates. Bo17 and Kun4 were more suitable on avicel, whilst Andi2.1 and Gam4.1 showed cellulase activity comparable to each other on three cellulose substrates. Pan23.2 and Bo17 isolates had more potential to hydrolyze cellulose in nature, as indicated by high activity of CMCCase and avicelase on avicel of 133.24 and 133.7 IU/mL, respectively (Fig. 2).

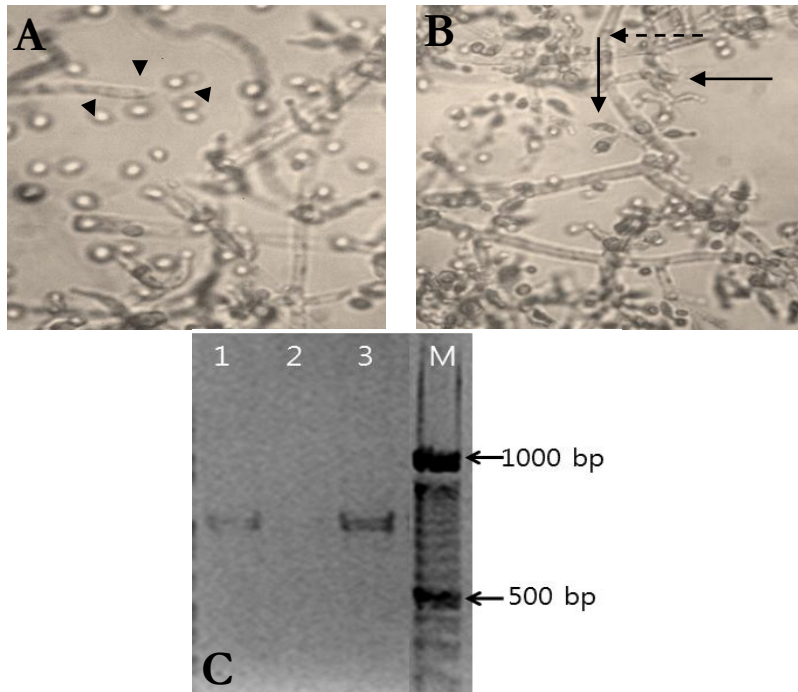


Fig. 1. Characterization of *Trichoderma* spp. A) and B) Examples of *Trichoderma* conidia (an arrow), septa (dash arrows) and branch of conidiophores (arrow) observed under Wild Balplan M3 light microscope; C) agarose gel electrophoresis of 18S rDNA amplification products from *Trichoderma* spp. (lanes 1, Kun4; 2, Gam4.1; 3, Pan23.1; and M, DNA ladder with sizes in bp indicated).

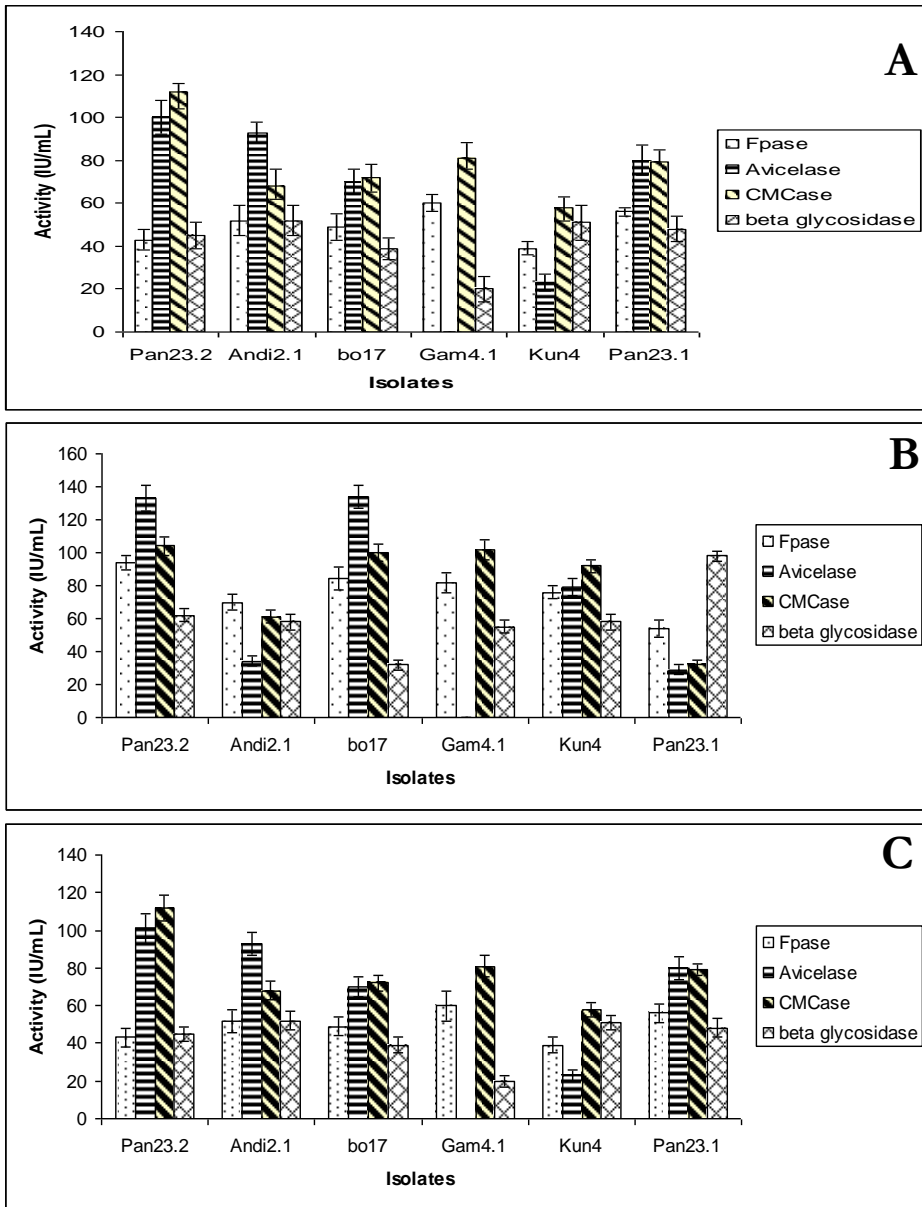


Fig. 2. Cellulase activity profiles of several superior *Trichoderma* spp. isolates on various source of cellulose substrates. A) Cellulase activity on CMC, B) cellulase activity on avicel, C) cellulase activity on cellulose.

Substrate Optimization

Optimization of the use of appropriate substrates as was conducted in this study would assist in the optimum production of cellulase to hydrolyze cellulose. Evaluation of CMCCase (Fig. 3A) and avicelase (Fig. 3B) showed that the combination of 1% glucose + 1% CMC generally generated the highest activity for all isolates.

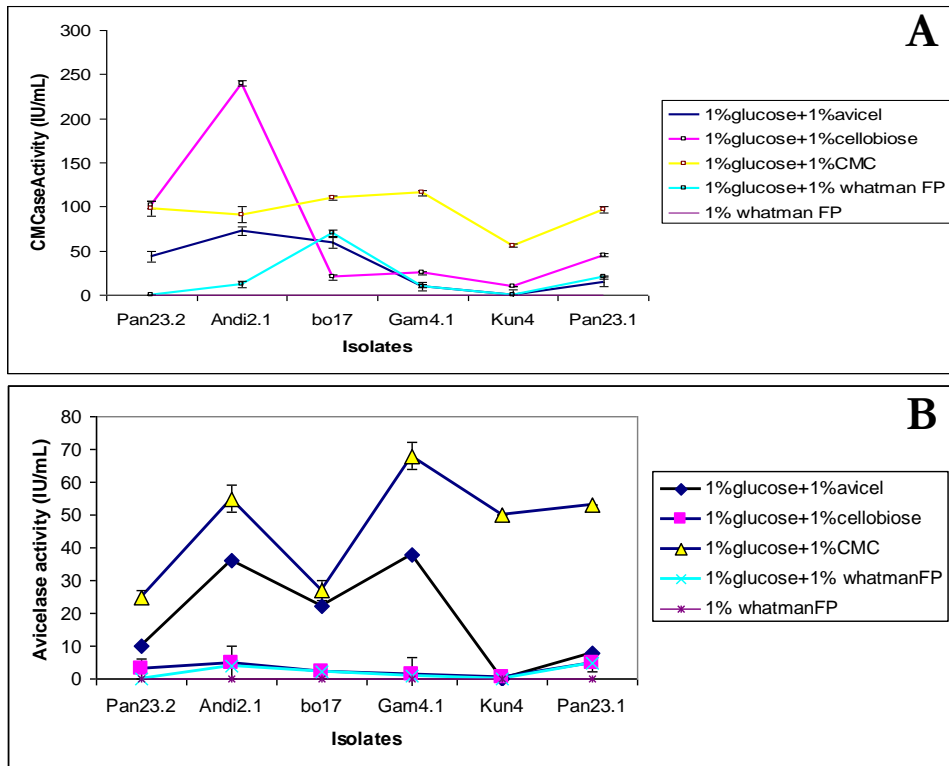


Fig. 3. Complex cellulase activity of *Trichoderma* spp. isolates with various combination of cellulose substrates. A) CMCCase activity, B) avicelase activity.

Evaluation of Xylanolytic Bacteria in Degrading Lignocellulolytic Material

On RBB-xylan medium, the bacteria proved to be able to degrade xylan. Clear zones surrounding the colonies were the result of hydrolysis due to endoxylanase activity (Fig. 4). However, the xylanolytic bacterial isolates showed cellulase activities that were lower than cellulolytic fungi, with an interval of only 1.01

IU/mL (ON13) to 11.96 IU/mL (AIII5). Further tests of the bacterial isolates to degrade lignocellulolytic materials such as rice straw, also did not show them to be as good a bioconversion agent. The C/N ratio was still high and did not differ with the controls, indicating that the degradation process for organic material was lower (Fig. 5). Composting temperature for one month was generally higher and increased in the xylanolytic bacterial treatment (26.9-30.5 °C) compared with controls of 26.5-27.1 °C (Table 1).

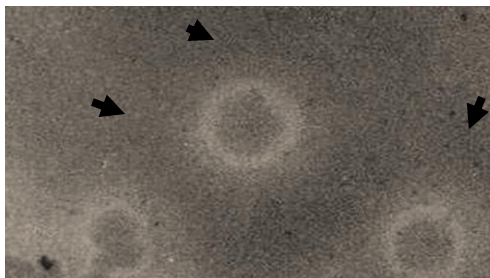


Fig. 4. Xylanolytic bacteria with clear zones (arrows) surrounding colonies on remazol brilliant blue (RBB)-xylan media.

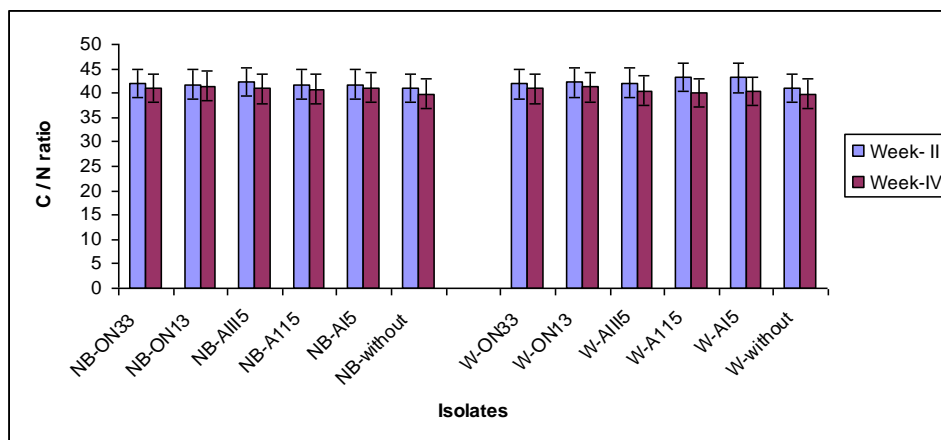


Fig. 5. C/N ratio on rice straw treated with biomass of xylanolytic cell bacteria during 2-4 weeks incubation. NB = Nutrient broth, W = water.

Table 1. Changes of rice straw temperature profiles as treated by biomass of xylanolytic bacterial isolates during one month incubation.

Isolates	Days-Temperature (°C)											
	0	3	6	9	12	15	18	21	24	27	30	
ON33	27.0 ± 0.1	27.2 ± 0.1	28.0 ± 0.2	30.0 ± 0.3	27.8 ± 0.1	30.0 ± 0.5	30.4 ± 0.4	30.6 ± 0.9	30.6 ± 0.7	30.2 ± 0.1	30.0 ± 0.5	
ON13	27.1 ± 0.1	27.3 ± 0.2	29.2 ± 0.2	30.8 ± 0.5	27.8 ± 0.1	29.6 ± 0.5	31.2 ± 0.7	30.8 ± 0.2	31.0 ± 0.7	30.8 ± 0.2	30.2 ± 0.5	
AIH5	27.0 ± 0.2	27.1 ± 0.2	27.8 ± 0.4	30.0 ± 0.4	27.4 ± 0.4	29.5 ± 0.4	31.0 ± 0.5	31.2 ± 0.4	31.2 ± 0.5	30.1 ± 0.5	30.0 ± 0.3	
AI5	26.9 ± 0.3	27.0 ± 0.2	28.1 ± 0.1	30.0 ± 0.5	27.8 ± 0.2	29.8 ± 0.7	31.4 ± 0.4	31.8 ± 0.2	31.6 ± 0.5	30.5 ± 0.5	29.5 ± 0.2	
AI5	26.9 ± 0.2	27.2 ± 0.4	28.0 ± 0.1	30.2 ± 0.1	27.9 ± 0.2	29.4 ± 0.5	30.5 ± 0.4	30.8 ± 0.2	31.0 ± 0.2	30.1 ± 0.4	30.5 ± 0.2	
Control	26.5 ± 0.5	26.8 ± 0.3	26.8 ± 0.2	26.2 ± 0.5	26.2 ± 0.3	27.0 ± 0.3	27.0 ± 0.2	27.2 ± 0.1	27.4 ± 0.2	27.0 ± 0.3	27.1 ± 0.4	

DISCUSSION

In this study, 18S rDNA amplification was utilized as a first step in identifying the *Trichoderma* spp. isolates aside from morphological characteristics. The DNA sequence of the 18S rDNA would enable us to distinguish and identify the isolates at the species level (Embong *et al.*, 2008). However, one of the isolates, Gam4.1, failed to generate any PCR-amplified product with the 18S rDNA primers. This was presumably due to the presence of PCR inhibitors or low purity of the extracted genomic DNA. Further optimization of the genomic DNA extraction protocol and PCR conditions may be needed for isolates such as Gam4.1.

Trichoderma spp. with various genetic variations produced polysaccharide degrading enzymes (Zheng and Shetty, 1998; Kullnig-Gradinger *et al.*, 2002), have been widely used as bio-decomposers (Bari *et al.*, 2007; Rahman *et al.*, 2011). Isolate Pan23.2 was found to over-secrete endoglucanase on CMC and cellobiose, indicating its preference to hydrolyze amorphous cellulose. Andi2.1 produced more cellobiohydrolase on CMC and cellobiose substrates. Other isolates (Bo17, Kun4, and Gam4.1) showed a tendency to hydrolyze amorphous cellulose. Pan23.1 produced β -glycosidase activities that were higher than other isolates. Gam4.1 was not able to hydrolyze crystalline cellulose because it did not secrete cellobiohydrolase on avicel. An absence of Gam4.1 avicelase on the cellobiose substrate showed that low β -glycosidase may affect the synergistic interaction of these three cellulase complexes. Not all of *Trichoderma* cellulases that showed high activity on amorphous cellulose were accompanied by high activity on crystalline cellulose.

Thus, the formation step of enzyme-substrate complexes and the absorption of the enzyme were essential in the enzymatic hydrolysis of cellulose. High total cellulase activity was not the sum of the components of the enzyme activity but as the result of the synergistic interaction of these three components (Klyosov, 1990). In this study, high cellobiohydrolase activity of the superior isolate Pan23.2 on avicel demonstrated its ability to hydrolyze crystalline cellulose.

The rate of degradation of the crystalline part was harder than the amorphous cellulose (Arifoglu and Ögel, 2000), which was influenced by exoglucanase (cellobiohydrolase) in removing cellobiose. Synergy of exoglucanase and endoglucanase may contribute to hydrolyze crystalline cellulose efficiently both derived from the same species or different microorganisms. Thus, *Trichoderma* spp. Pan23.2 isolate has potential as a good natural cellulose bio-decomposer.

Interestingly, Andi2.1 and Gam4.1 had low cellulase activity on the cellulose substrate without any source of glucose, indicating that glucose tends to increase cellulase activity. Gam4.1 produced CMCase (116.38 IU/mL) and the highest avicelase activity (67.71 IU/mL) at the best substrate of 1% CMC + 1% glucose. The highest CMCase activity (240.23 IU/mL) was produced by Andi2.1. Cellulase complex activity patterns between isolates showed alignment between CMCase and avicelase on the best substrate (1% CMC + 1% glucose). Whatman paper substrate was not good for *Trichoderma* spp., as shown by low cellobiohydrolase activity.

Glucose could affect the cellulose production that served as energy sources for supporting fungal growth so that it would better in hydrolyzing amorphous and crystalline cellulose. Low glucose concentrations were needed in the early stages of growth, but large quantities could inhibit the formation of cellulase. It could be seen from these trials that the use of substrates with a combination of glucose produced higher cellulase activity (> 200 IU/mL).

The best isolate (Pan23.2) in our study with a cellulose substrate without the addition of glucose was however not able to produce the optimum cellulase activity. The combination of 1% cellobiose + 1% glucose was the best substrate for CMCase activity, while 1% CMC + 1% glucose was the greatest for avicelase. Andi2.1 with the 1% cellobiose + 1% glucose substrate showed high activity in hydrolyzing amorphous cellulose, and Gam4.1 gave the highest activity on the 1% CMC + 1% glucose substrate in hydrolyzing crystalline cellulose. Whatman filter paper is known as a source of good substrates that proves by low cellobiohydrolase of microbes (Purwadaria *et al.*, 1998), hence this substrate was very important and specific to each *Trichoderma* spp. isolates, as demonstrated in this study. A high ability to hydrolyze crystalline cellulose such as *Trichoderma* Pan23.2, Andi2.1 isolates and Bo17 isolate had a greater potency to hydrolyze natural cellulose into useful products such as compost and animal feed (Abdelhamid *et al.*, 2004; Nur *et al.*, 2009). The cellulolytic enzyme activity was more efficient on cellobiohydrolase endoglucanase with the addition of β -glycosidase. The success of the hydrolysis of cellulose was determined also by the activity of β -glycosidase which produced the end product of glucose. Thus, the combination of *Trichoderma* spp. isolates in this study with the potential activity of the cellulase to three bacterial isolates made them prospective biodecomposers.

Variable C/N ratios and temperatures were often observed in the composting process (Liang *et al.*, 2003). No decrease in the C/N ratio of rice straw was detected in the mixture inoculated with xylanolytic bacterial isolates (On33, ON13, AIII5, AII5, and AI5) at four weeks. However, the temperature of the

mixture treated with five bacterial isolates reached about 30 °C which indicated there had been an internal process in the hay. These results were relevant to the results of Nur *et al.* (2009) who reported an increase in temperatures around 31-32 °C on compost treated with xylanolytic microbes after six weeks' incubation. However, the ideal temperature range for decomposition of organic matter was between 55-65 °C (Stalbrand *et al.*, 1994; Nur *et al.*, 2009), placing the microbes that carry out the processes within the generally thermophilic range (45-65 °C). The xylanolytic bacterial isolates were clearly mesophilic (23-45 °C); although the C/N ratio indicated no decomposition of organic matter, other processes may have occurred since an increase in temperature was noted.

The application of microbial decomposers was expected to shorten the composting time. When compared with the composting process using cellulolytic fungi such as *Trichoderma* spp., decrease of the C/N ratio below 20 can be reached within one month. Combination of *Trichoderma* Pan23.1, Kun4 and Is29 isolates were able to accelerate the composting rice straw to 23 days with the C/N ratio of 15.31. Inoculation with Gam4, And12.1, Pan23.1 and Pan23.2 also were able to accelerate the composting of rice straw to 27 days (C/N ratio 17.73) and 33 days (C/N ratio 13.52) (Gunarto *et al.*, 1999). Given that the *Trichoderma* spp. isolates in this study have been proven as potential biodecomposers, the use of suitable substrates to produce cellulases components would help in achieving optimum hydrolysis activity. While some microbes were capable of producing xylanolytic cellulase, or able to degrade lignin and hemicelluloses (Nur *et al.*, 2009; Dodd *et al.*, 2010), these isolates should be considered as good candidates for cellulolytic microbes. Because the xylanolytic bacterial isolates in this study did not show potential as bio-decomposer candidates, it was necessary to test their potency for other applications such as xylan hydrolysis to the sugar xylose, hydrolysis of hemicelluloses for bleaching process, xylanase as a forage mixture (Richana, 2002) or as anti-pathogens (Hanania *et al.*, 1999) so that their proper utilization will be beneficial under scale-up processes.

CONCLUSION

The results of this study indicated that the *Trichoderma* spp. isolate Pan23.2 showed the highest activity to hydrolyze cellulose crystals using all cellulosic substrates without glucose. The best substrate for CMCase activity was obtained using a combination of 1% glucose and 1% cellobiose, while 1% glucose and 1% CMC were good substrates for avicelase.

Based on these findings the xylanolytic bacteria used in this study had no ability to degrade lignocelluloses on rice straw as indicated by low temperature and high C/N ratio during the composting process. Further studies are needed to determine the specificity of these bacteria for other applications rather than as decomposers.

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