Australian Journal of Crop Science AJCS
AJCS 7(3):425-431 (2013) ISSN:1835-2707

Potential lignocellulolytic *Trichoderma* for bioconversion of oil palm empty fruit bunches

Mukhlis[1], H. Mohd Saud [*2], M. Sariah[3, 4], M. Razi Ismail[3], S. H. Habib[2] and H. Kausar[4]

[1]Research Institute for Swampland Agriculture, P.O.Box 31, Banjarbaru, South Kalimantan, Indonesia
[2]Department of Agriculture Technology, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[3]Laboratory of Food Crops, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia
[4]Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

***Corresponding author: halimi@putra.upm.edu.my**

Abstract

An experiment was conducted to evaluate the lignocellulolytic potential of *Trichoderma* spp. for bioconversion of oil palm empty fruit bunches (EFB). Thirty five *Trichoderma* isolates were tested for *in-vitro* lignocellulolytic activity by assaying their ability to develop dark brown zone and clearing zone on tannic acid, carboxymethyl cellulose (CMC) and avicel-amended media. Most of the isolates were found to synthesize polyphenol oxidase, endoglucanase and cellobiohydrolase enzymes on tannic acid, CMC and avicel-amended media, respectively. Five best selected isolates (T2, T15, T17, T20 and T35) were further tested for *in-vitro* bioconversion of oil palm EFB. All five isolates could decrease carbon content and increase nitrogen concentration at 3 to 6 weeks of incubation; thus, leading to a significant decrease in C/N ratio. The decrease in C/N ratio of the isolates, *T. harzianum* and *T. koningii* were higher than other isolates, exhibiting the potential for rapid bioconversion of lignocellulosic EFB.

Keywords: Lignocellulolytic, *Trichoderma*, bioconversion, oil palm empty fruit bunches.
Abbreviations: EFB-Empty fruit bunch, CMC- Carboxymethyl cellulose, RBA - Rose Bengal Agar, PDA - Potato Dextrose Agar, PPO- Polyphenol oxidase.

Introduction

In Malaysia, the oil palm is the largest enterprise in the agricultural sectors. The total area of oil palm plantation is about 3.875 million ha, which accounts for almost 50% of the total land under cultivation in Malaysia. Annually, 90 million tonnes of renewable biomass in the form of trunks, fronds, shells, palm press fiber, and empty fruit bunch (EFB) are accumulated, where the EFB represents about 9 % (Bari et al., 2010). The EFB is a lignocellulosic material which typically contains 25% lignin, 50% cellulose and 25% hemicellulose in their cell wall. In the past years, EFB was being used as fuel to generate steam in the palm oil industries. The burning of EFB caused serious environmental concern and the authority imposed strict rules to curb air-pollution from such activities. Nowadays, EFB is used as mulch in agricultural lands to control weeds, maintain moisture and prevent soil erosion (Thambirajah et al., 1995; Umikalsom et al., 1997; Molla et al., 2004; Alam et al., 2005; Bari et al., 2009; Alam et al., 2009; Misson et al., 2009). However, due to the increased cost of labor and transport its utilization as mulch is becoming more expensive. Therefore, a growing interest has been generated to develop an environmentally sound and cost-effective alternative to dispose the EFB in a short period of time.

Composting is the most suitable option amongst the wastes management strategies with economic and environmental profits since this process reduces the bulk volume of organic materials, eliminates the risk of spreading of pathogens, weed seeds or parasites associated with direct land application of manure and leads to final stabilized products, which can improve and sustain soil fertility. However, composting of lignocellulosic EFB takes a longer period of time which is considered as the most blocking stump of this eco-friendly disposal technique (Chen et al., 1992; Esse et al., 2001).

Lignin protects cellulose, hemicelluloses and carbohydrate in lignocellulosic materials. Lignocellulolytic micro-organisms are the key agents in depolymerizing the lignin barrier in organic materials. Therefore, the selection of effective lignocellulolytic microbe (s) is a crucial step leading to the success in accelerating composting of EFB. *Trichoderma* spp. are widely known as a lignocellulose decomposer because they are filamentous and have the ability to produce prolific spores which can invade substrates quickly (Tengerdy and Szakacs, 2003). Various studies have shown that composting of lignocellulosic materials pre-inoculated with potential *Trichoderma* spp. can reduce the time of biodegradation (Mohammad et al., 2012). *T. harzianum* was used as inoculant to enhance composting of rice straw and weeds (Cuevas et al., 1998). However, to the best of our knowledge no or a little work has been done on composting of EFB using lignocellulolytic *Trichoderma* isolated from the related ecological habitats. This work was carried out to isolate, screen and characterize the potential

lignocellulolytic *Trichoderma* isolate (s) for rapid biodegradation of EFB from the ecological habitats of oil palm.

Results

Enzymatic degradation of lignin

All the 35 tested isolates were able to grow on tannic acid-amended media where 34 isolates produced dark brown zone surrounding their colonies (Table 1). Among them, 16 isolates were found to form a dark brown zone in between 40-58 mm. Isolate T11 produced the significantly biggest dark brown zone on tannic acid-amended media with the diameter of 58 mm followed by T1 (55 mm), T9 (55mm), T15 (55mm) and T32 (54mm), respectively.

Enzymatic degradation of cellulose

All isolates grew well on both the CMC and avicel-amended media and produced a clearing zone after staining with Congo red (Table 1). Among them, 27 isolates were found to form the biggest clearing zone with the diameter of 80 mm on CMC-amended media. The least capable isolates to CMC-amended media were T6, T9 and T32 which produced clearing zones of 63, 50 and 40 mm, respectively. On the other hand, 13 isolates were found to form a clearing zone bigger than 70 mm on avicel-amended media. Isolates T17 and T28 formed the significantly biggest clearing zone with the diameter of 75 mm. Although the isolate T15 formed a colony of 68 mm in diameter but it produced a clearing zone with the diameter of 74 mm which was statistically similar with isolate T17 and T28.

The results for the 35 isolates from enzymatic degradation of cellulose and lignin tests using avicel, CMC and tannic acid media were combined in a single cluster analysis producing an UPGMA dendrogram derived from Gower's coefficient (Fig. 1). The dendrogram revealed five clusters, in which overall group dissimilarity ranged from 3.68 (cluster 1) to 7.26 (cluster 3, 4 and 5). Cluster 2 grouped five isolates (T2, T15, T17, T20 and T33) with high degree of ability to degrade avicel, CMC and tannic acid media, producing significantly bigger clearing zones and dark brown zones. Cluster 4 contained two isolates (T9 and T32) produced the highest colony growth both on CMC and avicel media but formed smaller clearing zones after staining and washing with Congo red and NaCl solutions. They also formed moderate colonies and dark brown zones on tannic acid media. On the contrary, cluster 5 consisted of only one isolate T12, produced the least colony either on CMC or avicel media and very poor growth and dark brown zone on tannic acid media.

Evaluation of potential Trichoderma isolates to in-vitro biodegradation of oil palm EFB

In the second phase of screening, five best isolates (T2, T15, T17, T20 and T33) were selected for *in-vitro* biodegradation of oil palm EFB on the basis of their *in-vitro* lignocellulolytic characteristics.

Changes in carbon, nitrogen, and the C/N ratio

The changes in organic carbon, total nitrogen, and C/N ratio after three and six weeks of incubation are presented in Tables 1 and 2, respectively. After three weeks, the decomposition of EFB resulted in a significant decrease in C content either for non-autoclaved or autoclaved treatments, where the decomposition rates were higher in non-autoclaved treatments compared to autoclaved treatments. In non-autoclaved treatments, all the isolates were statistically similar in terms of their C biodegradation potency, where the isolate T15 showed the highest potential (with 49.13 % reaming C) followed by T20 (49.3 %). In autoclaved treatments, all the isolates were also similar in terms of C biodegradation potency except isolate T2. After six weeks of incubation, in non-autoclaved treatments, the range of remaining carbon content was 44.18 to 46.55 %, whereas this range was 45.89 to 48.86% for autoclaved treatments. At the end of the biodegradation process, the significantly lowest percent remaining C was found in non-autoclaved treatments. There were significant effects of *Trichoderma* isolates on mineralization of nitrogen content during six weeks of biodegradation, where the highest efficiency was shown by T15 and T33 either on non-autoclaved or autoclaved treatments. After three weeks, the ranges of total nitrogen content of substrates inoculated with *Trichoderma* spp. were 2.20 to 2.31% (non-autoclaved) and 1.32 to 1.53 % (autoclaved), respectively. After six weeks, the total nitrogen content of the inoculated substrates increased up to 2.61 % (non-autoclaved) and 1.72 % (autoclaved), respectively.

The C/N ratio differed significantly during the six weeks of biodegradation process. After three weeks, the ranges of C/N ratio of inoculated treatments were 21.45 to 23.13 (non-autoclaved) and 32.71 to 37.35 (autoclaved), respectively. After six weeks, these were recorded as 17.51 to 20.06 (non-autoclaved) and 26.68 to 31.09 (autoclaved), respectively. The significantly lowest C/N ratio was found in non-autoclaved treatment T33 with the value of 17.51 followed by T15 (17.6).

Identification of potential Trichoderma isolates

The cultural and morphological characteristics of two selected isolates of *Trichoderma* based on mycelial growth, sporulation patterns, and pigmentation are presented in Table 4. Based on visual observation and micro-morphological characters, isolate T15 was identified as *Trichoderma harzianum and T33 as T. koningii*. The colony appearances and the color of conidia of three-day-old isolates of *Trichoderma* are shown in Fig. 2. After 5 days of incubation at room temperature (28 ± 2^0C) on PDA, the conidial color for all species was dark green.

Discussions

Empty fruit brunch (EFB) is a lignocellulosic material. In lignocellulosic materials, cellulose and hemicellulose are encrusted by lignin which protects them from biodegradation. Naturally, microorganisms produce ligninolytic enzymes to expose cellulose and hemicellulose by cleaving lignin barrier in lagnocellulosic materials. Therefore, screening of *Trichoderma* isolates, having the potential of producing ligninolytic and cellulolytic enzymes, is important in the rapid composting of EFB.

The synthesis of polyphenol oxidase is necessary for lignin depolymerization. In this study, the color change on the tannic acid medium was used as an indicator of polyphenol oxidase synthesis. Tannic acid used as an indicator for the ability of soil microorganisms to decompose phenol-like compounds. *Trichoderma* that utilized tannic acid can be considered to degrade lignin as a carbon source although lignin is structurally different from tannic acid. Nowadays,

Table 1. Ability of *Trichoderma* isolates to degrade lignin and cellulose on media, containing tannic acid, carboxymethyl cellulose and avicel.

Isolate No	Lignin degradation Tannic acid		Cellulose degradation Carboxymethyl cellulose		Avicel	
	Colony diameter (mm)	Dark brown zone (mm)	Colony diameter (mm)	Clearing zone (mm)	Colony diameter (mm)	Clearing zone (mm)
T1	55 bc	55 ab	80 a	76 b	77 a	65 ef
T2	44 f	44 d	80 a	76 b	80 a	70 cd
T3	40 g	40 e	80 a	80 a	66 c	70 cd
T4	35 hi	35 fg	80 a	80 a	80 a	65 ef
T5	32 ij	35 fg	80 a	80 a	80 a	54 i
T6	35 hi	0 j	80 a	63 c	80 a	65 ef
T7	32 ij	35 fg	80 a	80 a	80 a	70 cd
T8	50 d	52 bc	79 a	80 a	80 a	68 de
T9	60 a	55 ab	80 a	50 d	80 a	68 de
T10	40 g	40 e	80 a	80 a	80 a	50 j
T11	58ab	58 a	80 a	80 a	80 a	30 k
T12	30 jk	36 f	52 g	80 a	43 d	70cd
T13	20 l	30 h	80 a	78 ab	80 a	63 fg
T14	20 l	25 i	80 a	80 a	80 a	66 ef
T15	60 a	55 ab	69 de	80 a	68 bc	74ab
T16	30 jk	35 fg	75 bc	80 a	80 a	48 j
T17	35 hi	40 e	80 a	80 a	80 a	75 a
T18	28 k	32 gh	80 a	80 a	80 a	70 cd
T19	30 jk	36 f	70 d	75 b	71 b	65 ef
T20	50 d	52 bc	80 a	80 a	80 a	71bd
T21	32 ij	36 f	80 a	80 a	80 a	72 ac
T22	35 hi	30 h	80 a	78 ab	80 a	63 fg
T23	45 ef	45 d	80 a	80 a	80 a	60 gh
T24	40 g	45 d	78 ab	80 a	80 a	65 ef
T25	20 l	30 h	74 c	80 a	80 a	54 i
T26	48 de	50 c	66 e	80 a	80 a	68 de
T27	20 l	30 h	80 a	80 a	80 a	52 ij
T28	38 gh	38 ef	77 ac	80 a	80 a	75 a
T29	35 hi	40 e	80 a	80 a	80 a	64 f
T30	16 m	25 i	80 a	80 a	80 a	58 h
T31	28 k	32 gh	62 f	80 a	80 a	72 ac
T32	55 bc	54 b	78 ab	40 e	80 a	60 gh
T33	54 c	40 e	78 ab	80 a	80 a	70 cd
T34	35 hi	36 f	80 a	80 a	80 a	70 cd
T35	35 hi	35 fg	80 a	80 a	80 a	66 ef

Different letters in a column indicate significant difference at $P \leq 0.05$ by LSD.

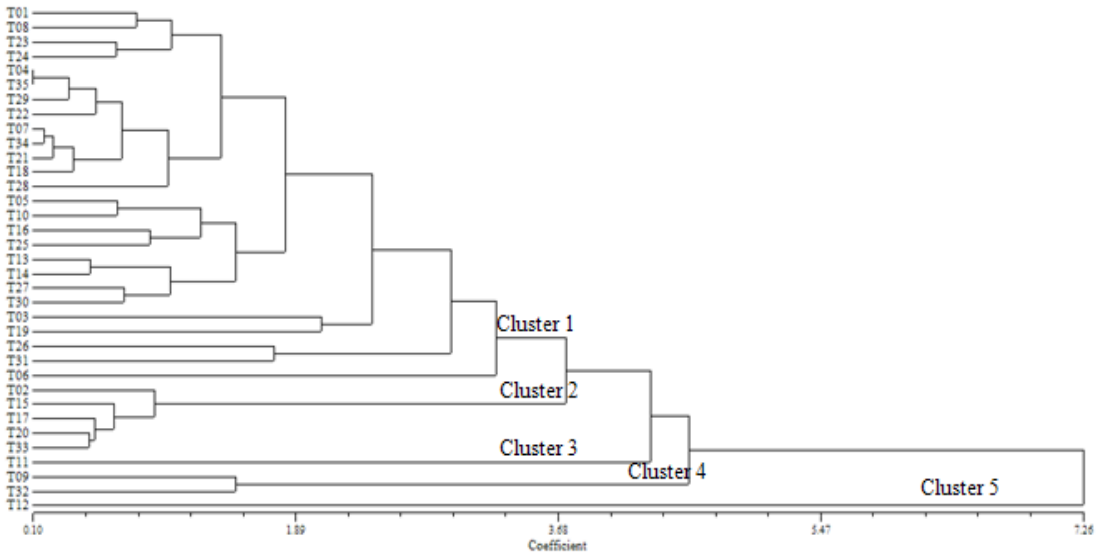

Fig 1. Dendogram of 35 *Trichoderma* isolates based on their lignocellulolytic ability prepared by using NTSYS-PC based on UPGMA.

tannic acid as an indicator of polyphenol oxidases is used to select ligninolytic microbes (Thormann et al., 2002).

The ability of all *Trichoderma* isolates to grow on CMC or avicel media showed that they had excreted cellulose degrading enzymes and were able to use these substrates as energy source. The clearing zone on CMC and avicel-amended media also confirmed that all tested isolates degraded cellulose by producing endoglucanase and cellobiohydrolase enzymes on such media. The enzymatic hydrolysis of cellulose biopolymer is initiated at internal glucosidic bonds within intact glucan chain by endoglucanase. This creates a new non-reducing chain terminus susceptible to exotype cellulase, cellobiohydrolase (Hoshino et al., 1997). *Trichoderma* spp. had the ability to produce the complete cellulases, i.e. cellobiohydrolases, endoglucanase, and ß-glucosidase enzymes (Shafique et al., 2009).

Variation in clearing zone diameter by *Trichoderma* spp could be due to the difference in cellulase activity. Salma and Gunarto (1996) found that *Trichoderma* isolates differ in cellulose activity and the highest cellulose activity was obtained from *T. harzianum*. Cluster analysis identified five clusters based on the ability of the isolates to degrade lignin and cellulose. Cluster 2 consisted of 5 isolates with high degree of ability to degrade both the carbon sources and was selected for the biodegradation of oil palm EFB.

All the *Trichoderma* isolates significantly decreased total carbon content in EFB compost than that of control throughout six weeks of biodegradation. Carbon is the building block of microorganisms. They intake sugars and different organic acids and emit CO_2 as metabolic end-product. Thus, the total carbon content of composting substrate was decreased as biodegradation proceeds. The lower reduction in total carbon content in control was indicative of lack of biodegradation activity. This result was in line with Hassan (2001) that reported greater loss of organic carbon in microbial inoculated EFB compared to control. Van Wyk and Mohulatsi, (2003) stated *Trichoderma* spp. enhanced total carbon decomposition by their lignocellulolytic enzymatic systems.

The total nitrogen accumulation in inoculated treatments throughout the biodegradation was higher than that of control. This was in agreement with the results of Inbar et al. (1993), who stated that, an increase in total nitrogen content during biodegradation was mainly due to the carbon loss and increase of nitrogen concentration. The concentration of total nitrogen usually increases during biodegradation process, when the loss of volatile solids (organic matter) exceeds the loss of NH_3 (Lee et al., 2002).

Carbon and nitrogen are the most important elements in biodegradation process as one or the other is normally a limiting factor (Richard, 2008). C/N ratio is a reliable indicator and used as an index of compost maturity (Inbar et al., 1990). The data showed that *Trichoderma* isolates (T15 and T33) significantly reduced C/N ratio during EFB biodegradation compared to control. The decrease in C/N ratio was higher in non-autoclaved treatment. This might be due to the presence of other microorganisms, besides *Trichoderma*, which also played an important role in biodegradation of EFB. *Trichoderma* spp. had the ability to mineralize the waste of high C/N ratio and enhanced composting (Stentiford and Dodds, 1992; Raimbult, 1998). The C/N ratio in microorganisms themselves is between 8 and 12 and the excess carbon in the initial mixture is required to provide the energy for synthesis and respiration.

The identification of *Trichoderma* isolates (T15 and T33) was performed based on the description of Rifai (1969). Based on colony and microscopic characteristics, the selected isolate T15 was identified as *T. harzianum* and T33 as *T. koningii*. Sariah et al. (2005) isolated and identified five species of *Trichoderma* in Malaysia, namely *T. hamatum, T. harzianum, T. koningii, T. longibrachiatum,* and *T. viride.* These spp. have been well documented as potential bio-control agents in several crops against many pathogenic organisms (Siddiqui et al., 2008; 2009). Therefore, lignocellulolytic *Trichoderma* isolates i.e., *T. harzianum* (T15) and *T. koningii* (T33) selected from this study have the potential to be used in large scale composting of lignocellulosic EFB.

Materials and methods

Isolation of lignocellulolytic Trichoderma

Trichoderma was isolated from different sources, i.e. matured EFB compost, soil and plant residues collected from oil palm plantations at Universiti Putra Malaysia. Samples were taken from five cm depth of compost heap or soil of oil palm plantation, transported in sterile plastic bags, and stored at 4^0C until use.

Isolation of lignocellulolytic isolates of *Trichoderma* was done by serial dilution technique on Rose Bengal Agar (RBA) proposed by Martin (1950) with slight modification. After autoclaving the media at 121^{o}C for 20 min, 2 ml of sterile streptomycin (50 mg ml^{-1}) was added aseptically to molten RBA and mixed homogenously. One ml of sample at selected dilution was pipetted onto each Petri dish followed by approximately 15 ml of molten RBA. The Petri dish was swirled manually for an even distribution and allowed to solidify. The plates were incubated at room temperature (28±2 ^{0}C) and examined regularly. Single colony was transferred aseptically onto Potato Dextrose Agar (PDA) plate to obtain pure culture. Pure cultures were kept on PDA slants at 4^{o}C for further studies.

In vitro screening for lignocellulolytic potential

In the first stage of screening, all the Trichoderma were tested for their potential of degrading lignin and cellulose in tannic acid and cellulose amended media.

Enzymatic degradation of lignin

Lignin degradation was tested on tannic acid medium. The Tannic acid media [5.0 g Tannic acid, 15.0g Difco malt extract agar, 20.0 g Difco agar, and 1 L distilled water] was autoclaved at 121ºC for 20 min and poured approximately 20 ml into each Petri dish. A 5.0 mm mycelia disc from 5-day-old PDA culture was placed at the centre of the plate and incubated in dark at room temperature (28±2 ºC). Formation of a dark brown pigment surrounding the point of inoculation was used as indicator of polyphenol oxidase (PPO) activity on tannic acid media (Thormann et al., 2002; Kausar et al., 2010).

Enzymatic degradation of cellulose

Carboxymethyl cellulose (CMC) and microcrystalline cellulose (avicel) were used for the determination of endoglucanase and cellobiohydrolase enzymes activity. Twenty (20) g of cellulose substrate and 15 g Difco agar

Table 2. Carbon, nitrogen, and C/N ratio on decomposition of EFB after three weeks of incubation with *Trichoderma* isolates.

Isolates	EFB Non-autoclave			EFB Autoclave		
	C (%)	N (%)	C/N	C (%)	N (%)	C/N
T2	51.60 b	2.27 ab	22.73 bcd	50.61 ab	1.53 a	33.08 c
T15	49.13 b	2.29 a	21.45 d	49.33 b	1.41 ab	34.99 bc
T17	51.58 b	2.23 ab	23.13 bc	49.30 b	1.32 bc	37.35 b
T20	49.30 b	2.20 ab	22.41 bcd	50.23 b	1.42 ab	35.37 bc
T33	49.89 b	2.31 a	21.60 cd	47.76 b	1.46 a	32.71 c
Control	54.91 a	2.07 c	26.53 a	54.06 a	1.24 c	43.60 a

Different letters in a column indicate significant difference at $P \leq 0.05$ by LSD.

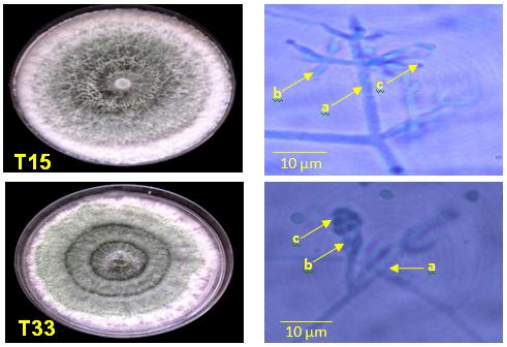

Fig 2. Cultural and morphological characteristics of *T. harzianum* (T15) and *T. koningii* (T33), (a) coniodiophore (b) phialide and (c) conidia.

Table 3. Carbon, nitrogen, and C/N ratio on decomposition of EFB after six weeks of incubation with the *Trichoderma* isolates.

Isolates	EFB Non-autoclave			EFB Autoclave		
	C (%)	N (%)	C/N	C (%)	N (%)	C/N
T2	46.55 ab	2.32 e	20.06 ab	48.86 ab	1.63 ab	29.97 bc
T15	44.18 b	2.51 bc	17.60 cd	46.89 b	1.64 a	28.59 bc
T17	45.91 ab	2.42 dc	18.97 bcd	46.52 b	1.62 abc	28.72 bc
T20	45.89 ab	2.41 de	19.04 bc	46.94 b	1.51 bc	31.09 b
T33	45.70 ab	2.61 a	17.51 d	45.89 b	1.72 a	26.68 c
Control	48.65 a	2.34 de	20.79 a	52.84 a	1.33 d	39.73 a

Different letters in a column indicate significant difference at $P \leq 0.05$ by LSD

were dissolved in 1liter distilled water. After autoclaving the media at 121^{0}C for 20 min, approximately 20 ml of these media were pipette onto each Petri dish and allowed to solidify. A 5.0 mm diameter of mycelia disc from five-day-old culture on PDA plates was placed at the center of each plate and incubated at room temperature (28±2 ^{o}C) for seven days. After day 7, the media were flooded with an aqueous solution of Congo Red (1 mg ml^{-1}) for 15 min. The plates were further treated by flooding with 1M NaCl for 15 min. Degradation of cellulose was visualized as a clearing zone around the fungal colony. The diameter of the clearing zone around colonies was used to assay the degree of endoglucanase and cellobiohydrolase activity (Teather and Wood, 1982; Kausar et al., 2010).

Evaluation of potential Trichoderma isolates to in-vitro biodegradation of oil palm EFB

Five selected lignocellulolytic *Trichoderma* isolates (T2, T15, T17, T20 and T33) based on previous *in-vitro* lignocellulolytic potency were further evaluated for *in-vitro* EFB biodegradation study.

Dried EFB was ground and sieved to 2 mm for biodegradation study. Ground EFB and chicken manure were mixed in a ratio of 2:1 (w/w). The mixture was amended with distilled water to obtain a moisture content of about 60 % (w/w). The prepared substrate was placed into individual composting jar and was sterilized by autoclaving at 121^{o}C for 20 min. The substrates were inoculated with 10% (v/w) of inoculum at a concentration of 10^6 cell ml^{-1} and incubated for 6 weeks at room temperature (28±2 ^{o}C). The substrates were turned once in a week to provide aeration. Sampling was done three and six weeks after incubation. The C/N ratio was determined by following the Loss on Ignition and Kjeldahl Method (Kausar et al., 2010; 2011).

Identification of potential Trichoderma isolates

Based on the *in-vitro* lignocellulolytic potency and EFB biodegradation study the best adapted isolates T15 and T33 were identified through macro-and micro-morphological study. Selected isolates were re-cultured from stock slant on PDA and grown for 10 days.

The mycelia growth, color and changes of media color of each isolate were observed daily. For micro-morphological studies, slide cultures were prepared and viewed in microscope and the Image Processing and Analytical System (Leica Q500 IW, Germany). The size, shape and arrangement of conidiophores, phialides and conidiospores or phialospores were observed for identification (Rifai, 1969).

Statistical analysis

All experiments were conducted in completely randomized design (CRD) with five replications. The data were subjected to analysis of variance (ANOVA) and tested for significance

Table 4. Descriptions of the cultural and morphological characteristics of *Trichoderma* on PDA medium.

Isolate	Cultural and morphological characteristics	Identification
T15	Fast growing. At first, it appears whitish and sparse mycelia. Later on, it formed ring like conidial area which green color developed in central part and gradually extended to the periphery. Conidiation predominately effuse, appearing granular or powdery. The diameter of mycelium is 3.84-6.40 μm. Long and slender conidiophores have branches forming a pyramidal structure terminated by phialides. Phialides were ampulliform and arise in 3 to 4 verticillate or in opposite direction in each point. Phialospores were glubose, subglobuse to obovoid, mostly 2.56-3.84 μm x 2.56-3.84 μm.	*T. harzianum*
T33	At 3-4 days, the mycelial were whitish to pale green and changed into whitish green to dark green color forming a concentric ring. Conidiophores were branched, long and slender and formed dendroid system. Phialides were ampulliform. They usually arise in 2-3 verticillate. The phialospores were ellipsoidal to oblong, 2.56 μm x 2.56-3.84 μm.	*T. koningii*

using Least Significant Difference (LSD) by PC-SAS software (SAS Institute Cary NC 2001). Data were subjected to cluster analysis to group of *Trichoderma* isolates, based on their ability to degrade lignin and cellulose. Pairwise similarities were computed and analyzed with NTSYS version 1.80. The dendrogram was constructed using the UPGMA algorithm (Sneath and Sokal, 1973).

Conclusions

Trichoderma isolates exhibited good growth performance to tannic acid and cellulose media, and had the high ability to utilize different carbon sources. The results implied that these isolates could play an effective role for decomposing carbon substrate. The six selected isolates could decrease carbon and increased nitrogen contents during the biodegradation of oil palm EFB and caused significant decrease in C/N ratio. The decrease in C/N ratio by T15 and T33 was higher than that of other isolates. Therefore, isolates T15 (*T. harzianum*) and T33 (*T. koningii*) had the highest ability to decompose EFB based on the C/N ratio, and exhibited the best potential for rapid composting.

References

Alam MZ, Mahamat ME, Muhammad N (2005) Production of cellulase from oil palm biomass as substrate by solid state biodegradation. Am J Appl Sci 2: 569–572

Alam MZ, Mamun AA, Qudsieh IY, Muyibi SA, Salleh HM, Omar NM (2009) Solid state biodegradation of oil palm empty fruit bunches for cellulase enzyme production using a rotary drum bioreactor. Biochem Eng J 46: 61–64

Bari MN, Alam MZ, Muyibi SA, Jamal P, Mamun AA (2010) Effect of particle size on production of citric acid from oil palm empty fruit bunches as new substrate by wild *Aspergillus niger*. J Appl Polym Sci 10(21): 2648-2652

Bari NM, Alam MZ, Muyibi SA, Jamal P, Mamun AA (2009) Improvement of production of citric acid from oil palm empty fruit bunches: Optimization of media by statistical experimental designs. Bioresour Technol 100: 3113–3120

Chen Y, Inbar Y, Hadar Y (1992) Composted residues reduce peat and pesticide use. Biocycle 33(6): 48–51

Cuevas VC, Samulde SN, Pajaro PG (1988) *Trichoderma harzianum* Rifai as activator for rapid composting of agricultural wastes. Philipp Agric 71(4):461-469

Esse PC, Buerkert A, Hiernaux P, Assa A (2001) Decomposition of and nutrient release from ruminant manure on acid sandy soils in the Sahelian zone of Niger, West Africa. Agric Ecosyst Environ 83: 55-63

Hassan HAD (2001) Solid state biodegradation of oil palm empty fruit bunches into compost by selected microbes. MSc Thesis UPM. p.151.

Hoshino E, Shiroishi M, Amano Y, Nomura M, Kanda T (1997) Synergistic Actions of Exo-Type Cellulases in the Hydrolysis of Cellulose with Different Crystallinities. J Ferment Bioeng 84 (4): 300-306

Inbar Y, Chen Y, Hadar Y, Hoitink HAJ (1990) New approaches to compost maturity. Biocycle 31(12): 64-68

Inbar Y, Hadar Y, Chen Y (1993) Recycling of cattle manure: the composting process and characterization of maturity. J Environ Qual 22: 857-863

Kausar H, Sariah M, Saud HM, Alam MZ, Ismail MR (2011) Development of compatible lignocellulolytic fungal consortium for rapid composting of rice straw. Int Biodeterior Biodegradation 64(7):594-600.

Kausar H, Sariah M, Saud HM, Alam MZ, Ismail MR (2011) Isolation and screening of potential actinobacteria for rapid composting of rice straw. Biodegradation 22(2): 367-375

Lee IB, Kim PJ, Chang KW (2002) Evaluation of stability of compost prepared with Korean food wastes. Soil Sci Plant Nutr 48(1): 1-8

Martin JP (1950) Use of acid, rose Bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. 69: 215-232

Misson M, Haron R, Kamaroddin MFA, Amin NAS (2009). Pretreatment of empty palm fruit bunch for production of chemicals viacatalytic pyrolysis. Bioresour Technol 100: 2867–2873.

Mohammad N, Alam MZ, Kabbashi NA, Ahsan A (2012) Effective composting of oil palm industrial waste by filamentous fungi: A review. Resour Conserv Recy 58: 69–78

Molla AH, Fakhrul-Razi A, Alam MZ (2004) Evaluation of solid-state biodegradation of domestic waste water sludge as promising environmental friendly technique. Water Res 38:4143–4152

Raimbult M (1998) General and microbiological aspects of solid substrate fermentation. Electron J Biotechn 1(3):1-15

Richard TL (2008) Cornell composting, municipal solid waste composting. http//www.cals.cornell.edu/dept/ compost. Accessed on July 2009.

Rifai MA (1969) A Revision of the Genus *Trichoderma*. Mycological Paper No. 116. Commonwealth Mycological Institute, Kew, Surrey, England. 56p

Shafique S, Bajwa R, Shafique S (2009) Cellulase biosynthesis by selected *Trichoderma* species. Pak J Bot 41(2): 907-916

Salma S, Gunarto L (1996) *Trichoderma* activity on cellulose degradation. Penelitian Pertanian Tanaman Pangan 15(1):43-47

Sariah M, Choo CW, Zakaria H, Norihan MS (2005) Quantification and characterization of Trichoderma spp. From different ecosystems. Mycopathologia 159(1): 113-117

Shafique S, Bajwa R., Shafique S (2009) Cellulase biosynthesis by selected *Trichoderma* species. Pak J Bot 41(2): 907-916

Siddiqui Y, Meon S, Ismail R, Rahmani M (2009) Bio-potential of compost tea from agro-waste to suppress Choanephora cucurbitarum L. the causal pathogen of wet rot of okra. Biol Control 49:38–44

Siddiqui Y, Meon S, Ismail R, Rahmani M, Ali A (2008). Bio-efficiency of compost extracts on the wet rot incidence, morphological and physiological growth of okra (Abelmoschus esculentus [(L.) Moench]). Sci Horti 117: 9–14

Sneath PHA, Sokal RR (1973) Numerical taxonomy: the principles and practice of numerical classification. W.H. Freeman and Company, San Fransisco. p.573

Stentiford EI, Dodds CM (1992) Composting. *In* Doelle, H.W. et al. (eds.) Solid Substrate Cultivation. Elsevier Science Publishers Ltd, London. pp. 211-230

Teather RM, Wood PJ (1982) Use of congo red-polysaccharide interaction in enumeration and characterization of cellulolytic bacteria from the bovin rumen. Appl Environ Microbiol 43(4):777-780

Tengerdy RP, Szakacs G (2003) Biodegradation of lignocellulose in solid substrate fermentation. Biochem Eng J 13(2-3): 169-179

Thambirajah JJ, Zulkifli MD, Hashim MA (1995) Microbiological and biochemical changes during the composting of oil palm empty fruit bunches. Effect of nitrogen supplementation on the substrate. Bioresour Technol 52: 133–144

Thormann MN, Currah RS, Bayley SE (2002) The relative ability of fungi from *Sphagnum fuscum* to decompose selected carbon substrates. Can J Microbiol 48(3): 204-211

Umikalsom MS, Arif AB, Zulkifli HS, Tong CC, Hassan MA, Karim MIA (1997) The treatment of oil palm empty fruit bunch for subsequent use as substrate for cellulase production by *Chaetomium globosum* kunze. Bioresour Technol 62:1–9

Van Wyk JPH, Mohulatsi M (2003) Biodegradation of wastepaper by cellulase from *Trichoderma viride*. Bioresour Technol 86:21-23