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Lignocellulolytic Activities among *Trichoderma* Isolates from Lahad Datu, Sabah and Deception Island, Antarctic

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Abstract

Trichoderma species have the potential for application in composting as biological control agents in controlling disease and increasing yield of production in the agricultural industry. The prevalent soil fungus of *Trichoderma* produces lignocellulolytic enzymes that assist the degradation of woody lignocellulose materials. The aim of the experimental work was to check the potential of lignocellulolytic *Trichoderma* fungi for the use of rapid composting of oil palm empty fruit bunches fibers. Fifty-two of *Trichoderma* isolates from Sabah and seven isolates from Antarctic were examined for in-vitro lignocellulolytic activity by assaying their ability to develop dark brown pigments, yellow halo zone, and clear white zone on tannic acid media (TAM) for lignin; Jensen Media (JM) for cellulose; and modified Melin–Nokrans media (MMNM) for starch. The best six Sabah *Trichoderma* isolates (5D, 10L2, 10P, 5E, 10X, and 10E2) were found to be potential lignocellulolytic agents based on their diameter of halo zone formed on amended media for further testing of in vitro bioconversion of oil palm empty fruit bunches. The diameters of halo zones were measured for the analysis of their ability in degrading lignin, cellulose, and starch. In contrast, Antarctic *Trichoderma* isolates consistently exhibited lower lignocellulolytic activities based on their smaller diameter of halo zone formed on TAM, JM, and MMNM. Most of the *Trichoderma* isolates are found to synthesize polyphenol oxidase, endoglucanases, and are able to hydrolyze starch to glucose in the three different media. Thus, the finding shows the potential of these isolates for use in large-scale composting of oil palm empty fruit bunches.

Keywords: Lignocellulolytic; Genus *Trichoderma*; Oil palm empty fruit bunches; Antarctic

Introduction

Biomass can be divided into three categories—namely, wood and agricultural products, solid waste and landfill gas, and biogas. According to Roslan et al. [1] and EU-Malaysia Biomass Sustainable Production Initiative [2], the biomass industry in Malaysia has represented renewable organic matters, including timber waste, rice husk, coconut trunk fibers, sugar cane waste, municipal solid waste, and oil palm waste. These biomasses exhibit potential in the manufacturing of value-added products such as bio-plastics, bio-composites, bio-pellets, and bio-fertilizers.

Statistics released by MPOB [3] show that Malaysia has the largest planted areas of oil palm trees in the world which occupy approximately 4.5 million ha of oil palm plantation areas. Consequently, the amounts of biomass wastes generated by this industry are abundant. In 2011, oil palm industries in Malaysia produced approximately 80 million tons of dry weight of oil palm biomass [4]. Only 10% of the oil palm tree consists of crude palm oil, and the remaining are lignocellulosic wastes which includes the trunks, fronds, palm-pressed fibers, and empty fruit bunches [5]. Hence, these products are not only under-utilized but also frequently cause pollution [6].

Composting is one of the potential ways to reduce these bio-wastes by converting them to bio-fertilizer. However, composting is always labeled as a time-consuming process owing to the wooden structure of the bio-masses. The conventional style of composting through natural degradation is slow and is not applicable to some lignocellulose materials. With the advances in biotechnology, the composting process can be accelerated. Dayana Amirah et al. [7] have shown that the soil fungus *T. virens* exhibits the best degrader of organic matter to minerals. Based on their results, the application of *Trichoderma* to the bio-waste can shorten the composting period. A consortium of effective microbes that contribute efficiently in composting process can be formulated by the inclusion *Trichoderma* spp. that has the ability to degrade lignocellulose materials effectively. Microorganisms to form the effective microbes consortium are selected and identified through a few selection processes with special focus on their ability to degrade lignocellulose materials.

A natural degrader of lignocellulosic materials, *Trichoderma* species also possesses acted as a biological control agent. It exerts strong competitive effects of space and nutrients, produces toxins against phytopathogenic species [8], and has antagonist properties toward *Ganoderma boninense* [9].

Definitions on lignocellulolytic enzymes have been provided by Mtui [10] and state that the biocatalysts are responsible for the degradation of lignocellulosic (lignin and cellulosic) materials. Lignocellulolytic fungi have a potential to degrad a range of the lignocellulosic biomass, including recalcitrant and highly toxic phenolic compounds. It possesses diverse, profitable bio-products used in industrial applications, including food, textile, pulp, paper and others [10].

Studies on cellulolytic activities are well-described by Biswas

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and Narayanasamy [11]; the inclusion of *T. virens* to the bio-waste promotes rapid degradation of cellulose and hemicelluloses, and it showed great potential by shortening the period of composting. The composting process is shortened to one month when *Aspergillus* and *Trichoderma* were added.

Sabah is the second largest state in Malaysia, and it is situated in the Island of Borneo and shares the borders with Sarawak, Brunei, and Indonesian Kalimantan. With an area spanning 72,500 square kilometers, lined with beaches surrounded by the South China Sea to the west, the Sulu Sea in the northeast, and the Celebes Sea in the East, Sabah is located in the northern portion of Borneo. Most of the landscapes in Sabah are lowland rain forests with areas of mountain rain forests. Climate throughout the state is equatorial/tropical with an estimated temperature of 32°C (90°F) in the lowlands and an average temperature of 21°C (70°F) in the highlands. Sabah is the sole producer of palm oil and cocoa in Malaysia, and Lahad Datu is the location where oil palms are extensively farmed in Sabah [12]. Lahad Datu is located in southeastern Sabah, bordered with the Kinabatangan district in the northeast; whereas Kunak, Semporna, and Tawau are located in the south. Oil palm plantations cover approximately 700,000 ha in the East Coast area and they have contributed toward almost 25% of the crude palm oil exports of the country [13].

In contrast, Antarctic is the driest, coldest, and windiest place in the world with the lowest temperature of Vostok recorded (-89.2°C) in 1983. Most of Antarctic is covered with ice that is more than 1.6 kilometers thick [14]. In short, any microorganism lives in Antarctic under the most extreme condition—between the limit of adaptability and near death, barely surviving, and rarely reproduced [15]. Nevertheless, some researchers have revealed the existence of *Trichoderma* in Antarctic that are isolated from different places; namely, *Trichoderma atroviride* by Holker et al. [16], *T. koningii* by Hughes et al. [17], *T. asperellum* by Ren et al. [18], and *T. citinoviride* by Jacklitsch [19]. The lignocellulolytic activities of tropical and Antarctic fungi are significantly different based on the studies done by some researchers [20-23]. Tropical fungi show different, unique metabolites owing to having a distinct metamorphosis and adaptability when compared with their other temperate counterparts [10]. Therefore, the aim of this study was to compare the lignocellulolytic activities between *Trichoderma* isolates from tropical areas of Sabah and those from extremely cold area in Antarctic.

Materials and Methods

Isolation of lignocellulytic fungi

Trichoderma isolates were isolated from soil samples collected from Ladang Sahabat 33 oil palm plantations, Kinabatangan, Lahad Datu, Sabah (Figure 1) and the Deception Island soil-rock (Craters Lake S62°58'56.34", W60°39'51.10" and Soto Lake S62°59'01.96" W60°39'13.21"), Antarctica. The soil samples were mixed homogeneously before 10 g was weighed out and put into a flask; after that, 100 ml of sterilized distilled water (121°C/1.05 kg/m^2 for 15 min) was added to make the stock solution following the method of Elad et al. [24]. A stock solution of 10 g soil in 100 ml of sterile distilled water solution was placed on an environmental orbital shaker that was set at 210 rpm for 20 min, after which 1 ml solution was added to 9 ml of water first for the first (10^{-1}) dilution. From preliminary trials, the serial dilutions at 10^{-3} and 10^{-5} were used for the Colony Forming Unit (CFU) estimation. One ml of soil solution was pipetted out and seeded into each Petri dish followed by pouring 9 ml of sterilized *Trichoderma* Selective Medium (TSM) [0.20 g of $MgSO_4.7H_2O$, 0.90 g of K_2HPO_4,

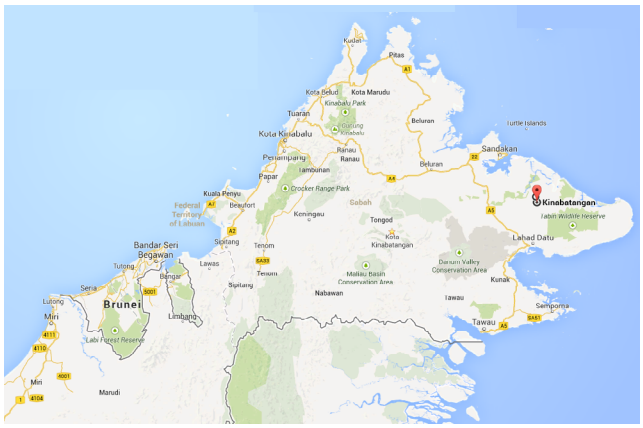

Figure 1: Sample collection site of Trichodemera species in this study.

0.15 g of KCl, 1.0 g of NH_4NO_3, 3.0 g of glucose, 0.15 g of Rose Bengal, 20.0 g of agar (Difco, USA), and 1000 ml of distilled water] [24]. The Petri dish was swirled manually before solidification and incubated at 28 ± 2°C. After a 7 day incubation period, fungal colonies could be seen as small whitish growth spots. Each single colony richly appeared on the plate and then was scored as a Colony Forming Unit (CFU). All Colony Forming Units (CFU) appeared per plate and were then checked and re-isolated onto fresh potato dextrose agar (PDA) (Oxoid, UK). Subcultures and pure cultures were maintained on PDA slants in universal bottles and maintained from 4 to 10°C at the Biotechnology Research Institute until they were required for further studies.

Enzymatic degradation on the basis of the lignocellulolytic activities between *Trichoderma* isolates was observed throughout the biochemical screening process. In this screening process, three types of enzymatic activities were tested—degradation of lignin, cellulose, and starch. Three replicate plates were done for each treatment, and the experiment was repeated twice under room temperature conditions (28 ± 2°C, 12 h daylight and 12 h darkness).

Morphological characterization

Pure cultures of *Trichoderma* were aseptically cultured from stock slants onto agar medium in the Petri dishes. All isolates were allowed to sporulate under room temperature. Mycelium was typically formed within three to four days as compact or loose tufts in shades of green, yellow, or, less frequently, white. A yellow pigment was secreted into the agar. All isolate observations were recorded especially on PDA medium. Radial growth of the *Trichoderma* colony on PDA medium was observed daily until the plate was completely covered. Pure cultures were transferred onto a new PDA medium for characterization into species aggregates.

For microscopic examination, 5 mm of mycelia was cut from the culture and transferred onto a sterile glass slide with an inoculation needle. The slides were viewed under a light biological microscope (Olympus CX31), and photographs were taken with a camera that was attached to the microscope and connected to a computer. A 40x magnification lens was used to observe conidia, conidiophores, hyphae, chlamydospores, and phialides for the selection of the *Trichoderma* species. A conidiophore was a simple/or branched fertile hypha in which conidia were produced. Phialides were observed for their appearance, which was flask like or bowling pins shaped. The cultures

were also observed for the presence of chlamydospores that were often thickened hyaline cells.

Lignocellulolytic activities of *Trichoderma* isolate

Enzymatic degradation of lignin: Enzymatic degradation of lignin was tested according to the Thormann et al. [25] method with some necessary modifications. Tannic acid media (TAM) [5.0 g tannic acid, 15.0 g malt extract agar (Difco, USA), 20.0 g agar (Difco, USA), and 1000 ml of distilled water] was autoclaved at 121°C/1.05 kg/m^2 for 15 min and poured into petri dishes at 20 ml per plate. A 5.0-mm diameter agar disc cut out from the leading edge of 5 day old pure culture of each *Trichoderma* isolate was placed at the center of each TAM plate, then incubated at 28 ± 2°C, and kept in complete darkness for 4 days. Lignin degradation was determined through the formation of dark brown pigments as an indicator of polyphenol oxidase (PPO) activity in the inoculation area.

Enzymatic degradation of cellulose: The fibrolytic ability of the fungi was tested on Jensen media [26] [20.0 g of sucrose, 1.0 g of K_2HPO_4, 0.1 g of $FeSO_4$, 0.5 g of $MgSO_4.7H_2O$, 0.5 g of NaCl, 0.005 g of $NaMoO_4$, 2.0 g of $CaCO_3$, 15.0 g of agar, and 2.0 g of Carboxymethyl Cellulose (CMC)] and autoclaved at 121°C/1.05 kg/m^2 for 15 min. After that, 20 ml of media was poured into each petri dish. Five day old pure cultures of *Trichoderma* mycelia were cut by using a 5 mm disc cork-borer, then placed at the center of each media plate, and allowed to grow for 7 days at 28 ± 2°C. Congo red (1.0 mg/ml) and 1.0 M of NaCl solutions were used to make contact with the colony for 15 min and then poured away. The yellowish halo zones surrounding the inoculation area appeared and were immediately measured for the analysis of cellulose hydrolysis according to Teather and Wood [27] protocol.

Enzymatic degradation of starch: Degradation of starch was examined through the appearance of halo zone. Modified Melin–Nokrans Media (MMNM) [1.0 g of D-glucose anhydrous, 2.0 g of malt extract agar (Difco, USA), 1.0 g of yeast extract, 10.0 g of KH_2PO_4, 5.0 g of $(NH_4)HPO_4$, 3.0 g of $MgSO_4.7H_2O$, 1.0 g of $CaCl_2$, 0.5 g of NaCl, 12.0 g of agar (Difco, USA), and 1000 ml of distilled water] along with 2.0 g of soluble starch [25,28] were autoclaved at 121°C/1.05 kg/m^2 for 15 min and poured into petri dishes at 20 ml per plate. A 5 mm diameter of pure *Trichoderma* culture on MMNM was grown for 3 days at 28 ± 2°C. After that, the cultures were flooded with iodine solution [5.0 g of KI, 1.5 g of I, and 100 ml of ddH_2O] for 5 min and decanted. A clear white zone indicated the hydrolyzed starch and exhibited amylase enzymatic activity.

Data analysis

Statistical analyses of data were performed by using the Statistical Package for the Social Science (SPSS), version 21.0. One way analysis of variance (ANOVA) was carried out to show the significance difference at $p \leq 0.05$. Tukey test was used to compare the means.

Results

Isolation of *Trichoderma* on TSM

Total number *Trichoderma* colonies seen on the agar plates were considered based on the CFU for four days during the incubation period. A total of 97 *Trichoderma* colonies were obtained from the soil samples taken from Sabah, and seven colonies were obtained from the soil samples taken from Antarctic. The soil samples from Sabah and Antarctic were found to be roughly 70% of *Trichoderma* CFU on TSM. *Penicillium* sp., *Aspergillus* sp., and unidentified fungi were also found in TSM (Table 1).

Enumeration and characterization of *Trichoderma* isolates

Trichoderma colonies on TSM were seen as white specks on agar plates; after that, they were enlarged by at least 5 to 6 mm within 6 days. By this time, the white colony turned yellowish/greenish/gray/off white on TSM (Figure 2). *Trichoderma* colonies rapidly grew and developed a green-yellow color. This feature was different from other fungi and also facilitated the identification of *Trichoderma*. A total of 97 *Trichoderma* CFU was cultured from 15 sampling points.

Subculture was done on a PDA medium; *Trichoderma* was initially white and after two days, it turned slightly green. At the moment, the wooly conidia filled up and were compact at the midpoint of a Petri dish with mostly dark green color. Usually, on the 4th and 5th days, 50-60% of the colonies appeared dark green, whereas the remaining 40-50% of the area was dull green/or off white depending on the species (Figures 3 and 4). The back of the colonies was mostly light green, dull, pale, and off white in color owing to the fluctuation in the species. Some of the *Trichoderma* remained a slightly green/yellow/white color owing to the species variations (Figure 4).

Microscopic examinations were conducted on the conidiophores that typically formed paired branches and displayed pyramidals arranged along the length of the primary axis (Figures 3 and 4). Branches toward the top and secondary branches tended to be held at 90° to the main axis formed, which they grew. Variations were seen in the phialides—typically enlarged in the middle; long, skinny shaped, bottle shaped, flask shaped, inflated at the based, and some were cylindrical. Phialides were held in the whorls that were commonly flask shaped, densely clustered on a wide main axis.

Lignocellulolytic activities of *Trichoderma* isolate

Enzymatic degradation of lignin: Most of the *Trichoderma* isolates exhibited multiple capabilities in degrading lignin. Forty three out of 52 Sabah *Trichoderma* isolates and all the Antarctic *Trichoderma* isolates were able to put out the polyphenol oxidase (Figures 5 and 6). Six Sabah isolates were found to have larger brown pigment diameter values of 4.93 cm for isolate 5D, 4.43 cm for isolate 10L2, 4.43 cm for isolate 10P, 4.27 cm for isolate 5E, 4.20 cm for isolate 10X, and 4.13 cm for isolate 10E2, respectively (Figure 7). The ligninolytic activities of the Antarctic *Trichoderma* isolates were always lower when compared with Sabah *Trichoderma* isolates (Figure 6). Most of the *Trichoderma* isolates produced a dark brown zone halo with a diameter of more than 2 cm (Figures 5 and 6).

Enzymatic degradation of cellulose: All the *Trichoderma* isolates

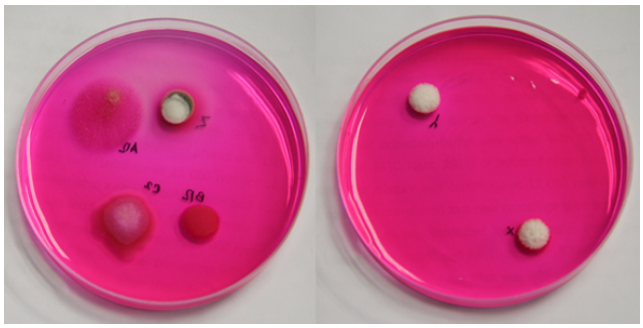

Figure 2: Five-day-old culture showing Trichoderma CFU on TSM.

Soil Samples	No. of Trichoderma CFU	Other Fungi	Percentage of Trichoderma CFU
Lahad Datu, Sabah	97	2 (Aspergillus sp.), 39 (Penicillium sp. and other fungi)	70
Graham Land, Antarctic	7	3 (Penicillium sp.)	70

Table 1: Trichoderma colonies are counted among all soil samples.

Figure 3: Cultural and morphological characteristic of Trichoderma isolate named S14 isolated from Sabah; (a) conidiophores (b) phialide (c) conidia.

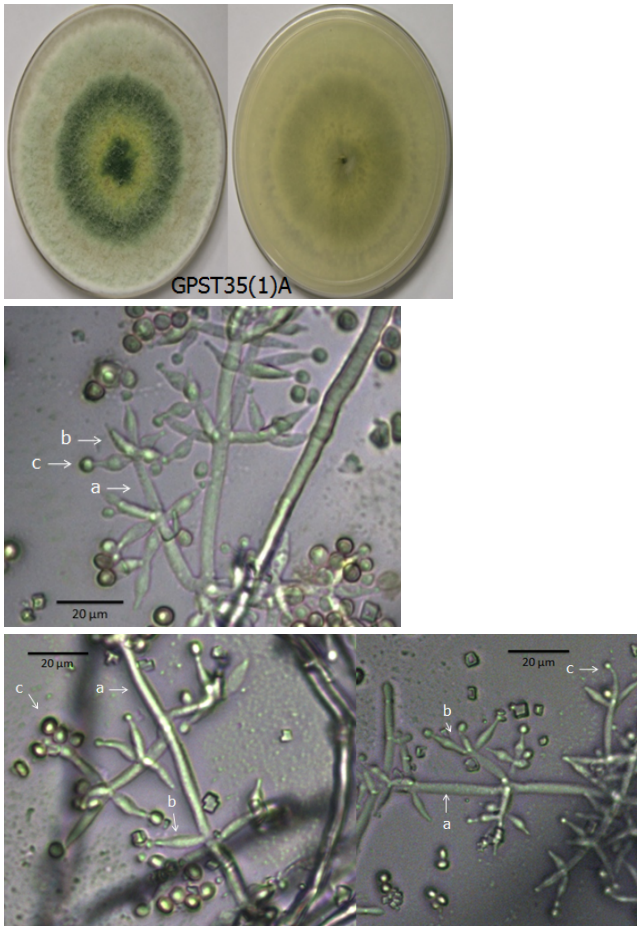

Figure 4: Cultural and morphological characteristic of Trichoderma isolate named GPST35(1)A isolated from Antarctic; (a) conidiophores (b) phialide (c) conidia.

from Sabah and Antarctic were able to degrade the cellulose (Figure 8). Cellulose degradation variations were seen in the results shown in Figures 5 and 6. All Sabah *Trichoderma* isolates were able to hydrolyze cellulose as indicated in Figure 5. Seven isolates were found to have higher cellulolytic activity values of 2.53 cm for 1 gB, 2.50 cm for 1 gE, 2.36 cm for 1gF, 2.33 cm for SIC, 2.33 cm for CI, 2.30 cm for 11A, and 2.30 cm for SIE, respectively. The isolate 1gB was found to have a significantly ($p \leq 0.05$) higher clearing zone diameter. In comparison, the cellulolytic activities of the Antarctic *Trichoderma* isolates, were always lower when compared to those from Sabah and their yellowish clearing zone ranged from 1.70 to 2.30 cm (Figures 5 and 6).

Enzymatic degradation of starch: All *Trichoderma* isolates (Sabah and Antarctic) were found to be effective in degrading starch (Figures 5, 6 and 9). Four Sabah isolates (7.47 cm for 10L2, 7.33 cm for 12L, 6.87 cm for 14V, and 6.17 cm for 14U2) showed a significantly ($p \leq 0.05$) higher halo zone diameter when flooded with iodine solution (Figure 5). However, Antarctic *Trichoderma* isolates exhibited apparently lower amylase activities with the halo zone sizes ranging from 1.53 to 3.90 cm (Figure 6).

Discussion

Among all the 33 samples collected from the plantations in Ladang Sahabat and Deception Island 70% of them harbored *Trichoderma* species. The *Trichoderma* isolates were distinguished through their morphological characteristics under the macroscope (color of conidia, sporulation pattern, and density) and microscope (structure

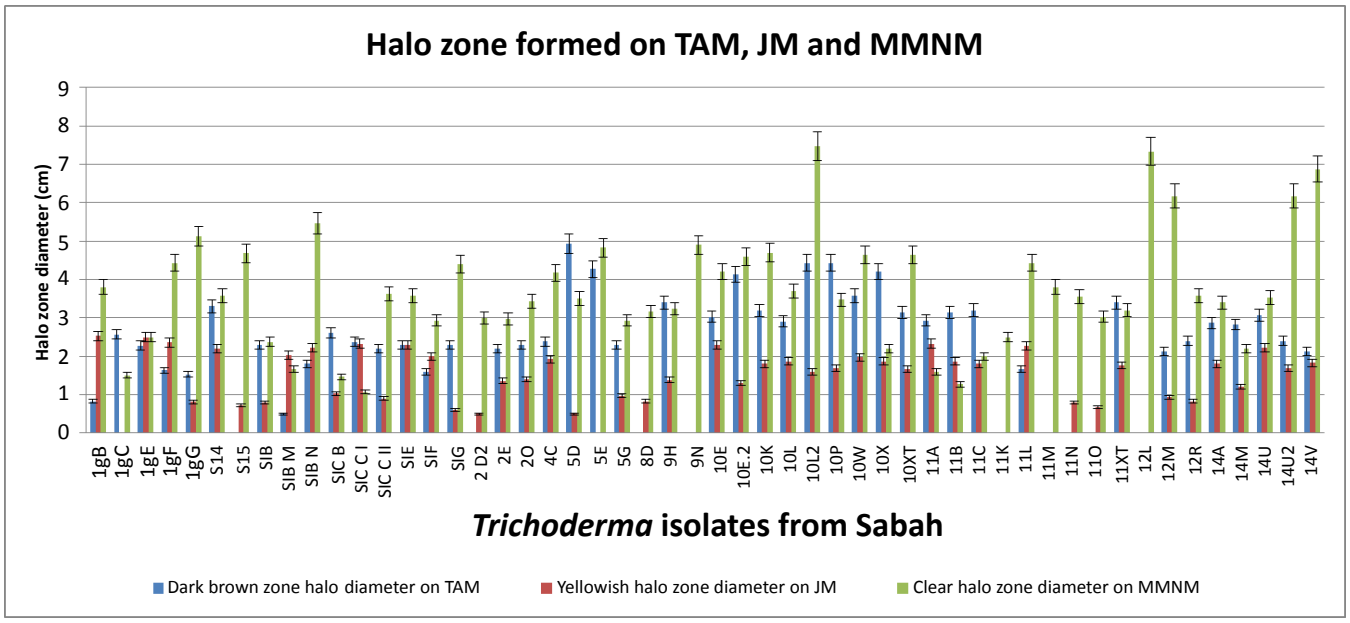

Figure 5: Comparison between Trichoderma isolates from Sabah and Antarctic on their ability to degrade lignin with a large halo zone (isolates 10X; 4.20 cm (a) and (isolates CRATERSOTOII; 2.17 cm (c)) and a small halo zone (isolates SIB M; 0.50 cm and isolates GPST351B; 1.90 cm (d)).

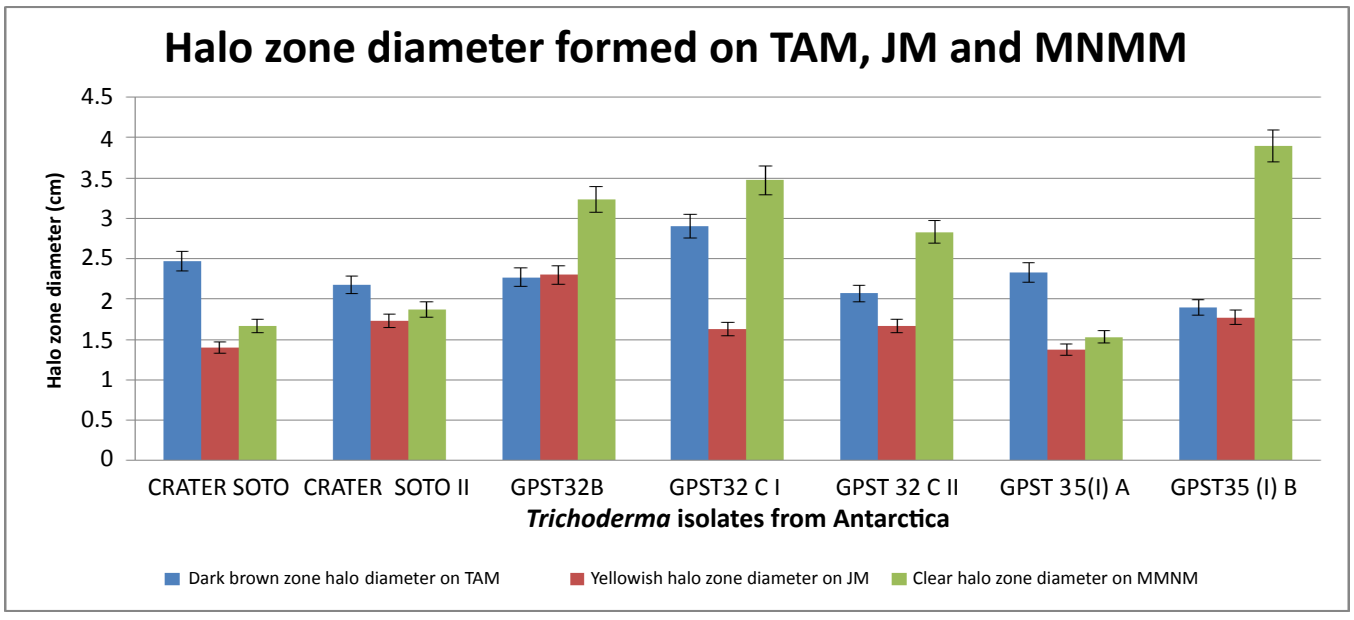

Figure 6: Comparison between Trichoderma isolates from Sabah and Antarctic on their ability to degrade cellulose with a large halo zone (isolate 1 gB; 2.53 cm (a) and isolate GPST32B; 2.30 cm (c)) and a small halo zone (isolate 2D; 0.50 cm (b) and isolate GPST351A; 1.37 cm (d)).

and arrangement of phialide, conidial size, and shape). However, morphological and cultural characteristics cannot be used to distinguish *Trichoderma* isolates up to the species level [29]. Similarly, most of the researchers are facing difficulty with regard to the identification of the *Trichoderma* species owing to the higher level of structural similarities.

The *Trichoderma* species can be found everywhere in the world, including the area with extreme climate such as the Antarctic. Some researchers [9,30,31] have analyzed the identities of *Trichoderma* spp. from tropical areas in West Malaysia. However, no in-depth studies are performed on isolates from Borneo Island in East Malaysia. To the best of our knowledge, this is the first time that the *Trichoderma* species is reported in the Island of Borneo. Isolation of fungi from the Antarctic has reported by several researchers [32-35]. However, only few researchers [17,36] have isolated fungi belonging to the *Trichoderma* genus. These results show that *Trichoderma* species may survive in in the harsh Antarctic environment. Johnson et al. [37] have also found *Trichoderma* species in the soils of Tennessee and Alaska, Canada which have cold climates. Nevertheless, the enzymatic activities of *Trichoderma* isolates from those cold climates are not characterized.

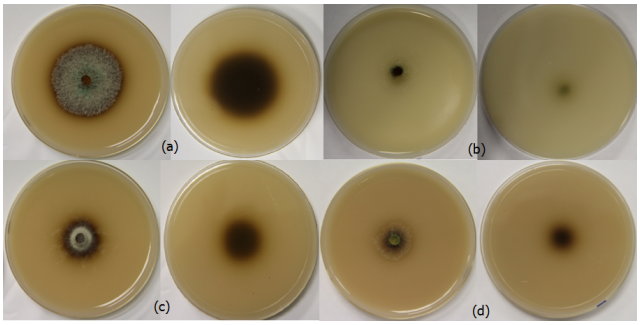

Figure 7: Comparison between Trichoderma isolates from Sabah and Antarctic on their ability to degrade starch with a large halo zone (isolates 14V; 6.87 cm (a) and isolates GPST32B; 3.23 cm (c)) and a small halo zone (isolate 5E; 4.83 cm (b) and isolates GPST32CII; 2.83 cm (d)).

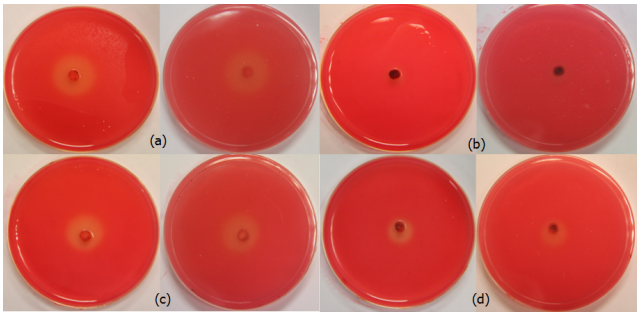

Figure 8: Halo zone diameter recorded for lignin, cellulose, and starch degradation by Trichoderma isolates isolated from Sabah on media containing tannic acid, CMC, and starch.

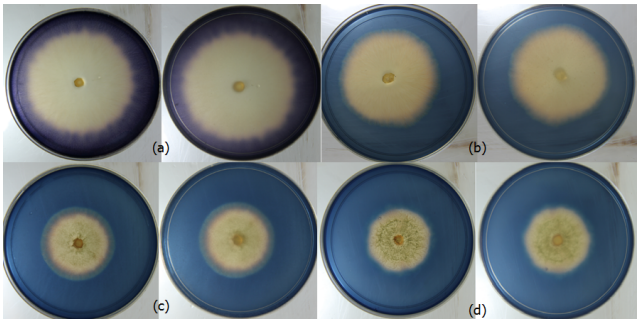

Figure 9: Halo zone diameter recorded for lignin, cellulose, and starch degradation by Trichoderma isolates isolated from Antarctic on media containing tannic acid, CMC, and starch.

All of the comparative studies on the lignocellulolytic activities between the Tropical and Antarctic *Trichoderma* isolates were conducted at 28 ± 2°C. This was possible because both the Sabah and Antarctic's isolates grew well at 28 ± 2°C. The comparisons of lignocellulolytic activities of *Trichoderma* from habitats with different extremes are new and have never been reported by any other researchers. Our results indicated that the Sabah *Trichoderma* isolated is more suited for composting in the tropical climate. Nevertheless, detection of lignocellulase activities in the *Trichoderma* isolates from the Antarctic indicating that composting probably can be conducted in temperate regions. In future the Antarctic *Trichoderma* isolates can be tested for their composting ability in cold areas. So, investigation of the lignocellulolytic activities at a lower temperature is a good idea for conducting some research in the near future.

Enzymatic degradation of lignin

Priority should be given to the degradation of lignin based on its main structure—encrusting and guarding the cellulose and hemicelluloses from being degraded. Lignin structure is unhydrolyzable, and only a few species of microorganisms are able to hydrolyze lignin [38-40]. The ability of fungi to produce lignocellulolytic enzyme activities during the biodegradation of lignocellulosic materials has been discussed by some researchers [41-44]. According to Kausar et al. [41], dark brown complexes are formed through a catalyzation reaction between polyphenol and molecular oxygen activated by the components of polyphenol oxidase: monophebol oxidase and catechol oxidase. This brown complex plays an important role in the degradation of phenol compounds in lignin. Mtui [10] briefly elaborated on the process involving oxidative conversion of organic compounds and materials named oxidases. Dioxygen (O_2) acts as the co-factor (terminal for electron acceptor) that is needed by phenol oxidases. Therefore, the development of dark brown pigments is confirmed through the polyphenol oxidase (PPO) activity by fungal isolates.

Comparative studies are conducted on the ability of all *Trichoderma* isolates from Sabah and Antarctic. Antarctic isolates always exhibited lower capability in degrading lignin when compared to the Sabah isolates. They produced smaller brown pigments on TAM plates. Muratov and Kim [45] have performed an experiment on the degradation of lignin by using *Trichoderma viride*, *Trichoderma reesei*, and *Aspergillus niger* in supercritical CO_2. Similar studies have been conducted by Wu et al. [46] for *P. chrysosporium*, *P. ostreatus* and by Lozovaya et al. [47] for *Fusarium solani*. Nevertheless, there is a lacks of information about ligninolytic activities by other *Trichoderma* species.

Enzymatic degradation of cellulose

Fungi are the main cellulose-producing microorganisms. Nonetheless, only a few researchers found the microorganisms that are able to produce significant amounts of cellulase enzymes. Jahangeer et al. [48] have reported a few species of fungi, including *Trichoderma reesei*, *Humicola* sp., *Penicillium* sp., and *Aspergillus* sp. that have the ability to convert cellulose to glucose. Similar studies done by Khokhar et al. [49] reported that *Trichoderma* sp. exhibited the highest cellulase activity and consistency in producing cellulase when compared with *Penicillium* sp. and *Aspergillus* sp.

Carboxymethyl Cellulose (CMC) is chemically modified and used to resemble the cellulose in this research. Congo red can only colorize the cellulose and the area that is decolorized by the endoglucanases enzyme [25]. Mtui [10] specified hydrolases as the enzymes that are responsible for the catalyzation of chemical bonds by hydrolysis. For the degradation of cellulose, cellulases can be the hydrolases that catalyze cellulolytic reactions. Hydrolysis of cellulose, especially the endoglucanases enzyme, is crucial, as it initiates the next synergistic actions involving β-glucosidase and cellobiohydrolases [50].

Capability among all of the *Trichoderma* isolates in degrading cellulose is tested and shown in Figures 5 and 6. Antarctic *Trichoderma* isolates are found to exhibit lower capability in degrading cellulose toward Sabah *Trichoderma* isolates. As evident in the result, larger brown pigments are seen on plates containing CMC inoculated with Sabah *Trichoderma* isolates. According to Domsch et al. [51], by comparing all three activities, the hydrolysis of lignin exhibits lower activity while compared with cellulose and starch. On analysis of our

results, similar results have been found as previously mentioned by Domsch et al. [51]. Khokhar et al. [49] stated that the isolation and screening of filamentous *Trichoderma* fungi have performed the highest cellulolytic activities when compared with *Penicillium* and *Aspergillus*.

Cellulose decomposition in the various Antarctic regions has been studied by some researchers [20,22,23], and it has been found that cellulolytic fungi existed in the Antarctic regions and very slow rate decomposition occurred when compared with fungi in temperate environments. A similar result was found in our experimental results when testing *Trichoderma* isolates in tropical (Sabah) and very cold regions (Antarctic).

Enzymatic degradation of starch

Amylase enzymes are important and play key roles in the fermentation process and food industries, particularly related to starch hydrolysis. This enzyme can be produced in several microorganisms, including bacteria, fungi, as well as plants and animals. Starch is a glucose polymer that has the combination of α-1, 4 and α-1, 6 glycosidic bonds. On the basis of the facts stated by Bernfeld [52], starch is degraded by the amylases and other related polymers by hydrolyzing the glycosidic bonds between adjacent glucose units of the polymers to yield products that have characteristics of individual amylolytic enzymes.

The appearance of clearing halo zone and the absence of the blue-black indicator in the inoculation areas depict starch hydrolysis. Fungi releases α-amylase enzyme that later cleaves the bond in starch and leaves the starch in a simple form known as amylose. The amylase enzyme has a clear halo zone [53]. Amylase activities are found to be higher in Sabah *Trichoderma* isolates when compared with the smaller halo zone produced by Antarctic *Trichoderma* isolates.

Enzymatic degradation of starch by fungi has been studied by a few researchers such as *Aspergillus wentii* by Johnson et al. [54]; *Rhizopus* sp. by Adebiyi et al. [55]; *Aspergillus oryzae*, *Aspergillus flavus*, *Lichteimia* sp., and *Rhizopus oryzae* by Kim et al. [56]; *Saccharomyces cerevisiae*, *Aspergillus* sp., *Mucor* sp., and *Rhizopus* sp by Ominyi et al. [57]; and *Trichoderma* sp by Kausar et al. [41].

Conclusions

Most of the *Trichoderma* isolates from Sabah and Antarctic have exhibited ability in hydrolyzing three important categories: lignin, cellulose, and starch. In spite of this, Sabah *Trichoderma* isolates are found to exhibit higher ligninolytic, cellulolytic, and amylase activities when compared with Antarctic *Trichoderma* isolates. The best seven Sabah *Trichoderma* isolates (5D, 10L2, 10E, 1gB, SICCI, SICCII, and 11B) are chosen for further experimental work similar to the fiber decomposer based on their optimum capability displayed in degrading lignin, cellulose, and starch. In future, these *Trichoderma* isolates in the composting process are applied as biocontrol agents in controlling disease and increasing yield of production in the agricultural industry.

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Conflict of Interest

All authors have agreed to submit the prepared manuscript and declare to no interest conflict of patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company.

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