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# Potential of *trichoderma harzianum* as cellulose biodegrader in biocomposting of paddy straw

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Siti Zalina Yaacob[1,*] , Norhafizah Abdullah[1], Luqman Chuah Abdullah[1]

[1] Department of Chemical and Environmental Engineering, Universiti Putra Malaysia, 4300 Serdang, Selangor, Malaysia

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**ABSTRACT**

The present study was conducted to screen the significant cellulase (cellulolytic enzyme) produced by locally isolated fungi, *Trichoderma harzianum* using submerged culture system. Screening of the cellulase was conducted using carboxylmethylcellulase (CMC) plate assay to assist in determining of the microorganism potential as biodegrader. The highest diameter of inhibition zone around the *T.harzianum* colony which was grown on CMC agar was recorded at 1.9cm from a culture that used CMC supplemented with instant yeast. The culture with the best morphology was observed after 6 days incubation at temperature of 28°C and agitation speed of 150rpm. The biocomposting study on paddy straw showed that the waste is suitable for biocompost production with the lowest C:N ratio of 17.5 after 90 days of composting period.



## 1. Introduction

Malaysia as an agricultural based country produces a lot of lignocellulosic agrowastes per year. Much of these wastes are disposed by biomass burning, which is not restricted to developing countries alone, but it is considered as a global phenomenon. The biomass are not being treated and left to rot in the plantation due to accumulation of high organic content in the soil. Thus, biocomposting promises as the best option in agrowaste management through the utilization of effective microorganisms. Cellulolytic microorganisms are the key agents in depolymerizing the cellulose barrier in organic materials. Therefore, the selection of effective lignocellulolytic microbes is a crucial step leading to the success in acceleration composting process of any agrowaste. *Trichoderma harzianum* is a filamentous soil fungus that secrets a well-balanced cellulolytic complex, which efficiently hydrolyzes cellulosic substrates into monomeric glucose chain. Due to its elevated cellulolytic activity, *T.harzianum* is recognised to have considerable potential in biomass hydrolysis applications [3]. In this present study, the local fungal strain of *T.harzianum* was

* *Corresponding author.*
*E-mail address: sitizalina.upm@gmail.com (Siti Zalina Yaacob)*

examined for cellulase screening via submerged culture system for further biocomposting application.

## 2. Experimental

### *2.1 Inoculum Preparation*

*T.harzianum* was locally isolated and gradually subcultured and maintained at 4°C on potato dextrose agar slant. Cultures was grown on the potato dextrose agar plate for spore production about 10 to 14 days of growth in incubator at 28°C and stored at 4°C for regular subculturing.

### *2.2 Pretreatment of Substrate*

The pretreatment generally resulted in a decrease of hemicellulose and lignin contents. The paddy straw was shredded and soaked in the water for 24 hours before the composting process.

### *2.3 Culture Media Preparation*

The soymeal was prepared with distilled water in the ratio of 1:2. The soymeal solution was cooked on hot plate with continuous stirring at 80°C for an hour. Glucose was prepared separately at concentration of 20g/L. Then, both solutions were separatedly sterilized in different flasks at 121°C for 15 minutes. The 11g of instant dried yeast was deactived by soaking them in 110mL warm sterile distilled water for 10 minutes. 2%, 5%, 7% and 10% of the dried yeast solution were incubated for seven days on orbital shaker. Then, the incubated yeast solution were pipetted into the inoculated media and incubated for six days.

### *2.4 Screening of Cellulase*

The cellulase activity was determined by streaking the identified fungal cultures individually on carboxylmethylcellulose (CMC) agar plates and incubated at 28°C. After 5 days of growth, the zone was identified around the culture by treating the plate with iodine and sodium chloride. After incubation the plates were flooded with an aqueous solution of iodine (1% iodine in distilled water) and shaken at 50 rev/min for 15 min in shaking incubator. The iodine solution was poured off, the plates were further flooded with sodium chloride and shaken again at 50 rev/min for 15 min. The fungal growth was stopped by flooding with HCL onto the CMC agar plates which changes the dye colour to blue-violet to further inhibit the enzymatic activity. Cellulose degradation was visualized as a clear zone around the fungal colony. The diameter of the clear zone around colonies was used to assay the degree of cellulase activity [9].

### *2.5 Composting Process and Sampling*

The shredded paddy straw was soaked in the water for 24 hours prior to composting process. All the raw materials were analyzed for physiochemical, nutrients and heavy metal contents. In this experiment, there are two treatments, compost piles with *T.harzianum* ($C_1$) and compost piles without *T.harzianum* ($C_2$) for control. For piles inoculated with *T.harzianum*, a 5% inoculum was applied to constitute 20% of the amount of water to be added to a given mixture. Water was then added until the moisture content reached 60% of wet basis in each composting mixture. The moisture content was maintained at 50–60% with water addition throughout the composting

period with regular checking. The mixtures were composted for 90 days and were turned at 3-day intervals to maintain porosity. The temperature was daily measured using digital thermometer at random depths. The sampling were taken from each treatment at 0, 15, 30, 45, 60 and 90 days of the composting and were analysed for changes in the physical and chemical properties.

## 3. Results and Discussion

### *3.1 Morphological growth of T.harzianum*

*T.harzianum* grew perfusely by producing branching mycelia on potato dextrose agar within 24 hours of inculation with aerial spores at concentration x 108. The fungus at its mycelial stage was whitish in colour. The color changed to greenish upon sporulation. Well-developed fruiting bodies with chlamydospores appeared after 6 days of incubation at 28°C on orbital shaker as shown in Figure 1.

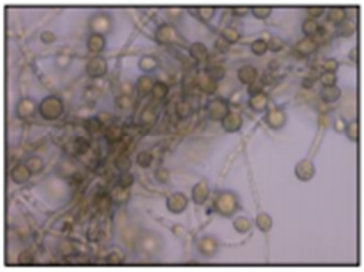

**Fig. 1.** Chlamydospores of *T.harzianum* (40x mag)

### *3.2 Screening of Cellulase*

The plate assay was adopted prior to optimization for cellulase production to explore the potential of *T.harzianum* for lignocellulolytic activity. The results were promising as there were cellulolytic activities on CMC plate assays, indicating the potential of T.harzianum as cellulose degrader. All *T.harzianum* isolates grew well on CMC media and produced a clear zone after iodine staining. The diameter of clear zone was measured in cm and it showed the increasing size of diameter as the concentration of Nsource increases (Table 1). The difference in cellulose excretion is closely related to nitrogen concentration as N-source takes vital role in providing protein for the cells building block. The plate assay with Gram's iodine in Figure 2 showed an inhibition zone around the fungal growth showing the presence of cellulose activities after overnight incubation. The unstained areas indicate where the CMC has been broken down.

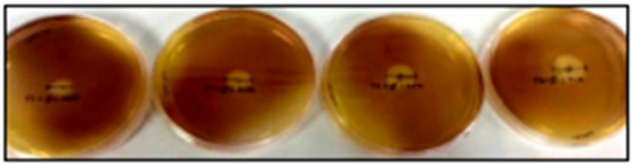

**Fig. 2.** Iodine Plate Assay

**Table 1**
Diameter of inhibition zone formed by T.harzianum on CMC media

| Treatment | Diameter of inhibition zone (cm) | | | |
|---|---|---|---|---|
| | T1 (2%) | T2 (5%) | T3 (7%) | T4 (10%) |
| A | 0.9 | 1.0 | 1.2 | 1.3 |
| B | 1.2 | 1.4 | 1.6 | 1.9 |

### *3.2 Physical and Chemical Properties of Substrate*

In general, most of the values for the parameters for paddy straw as in Table 2 are within the ranges that obtained by Iranzo *et al*., [8]. The pH value was also in a similar range as obtained in previous study [1] which indicated that the waste residue was non-acidic. Table 2 Chemical and physical properties of paddy straw (dry weight basin).

**Table 2**
Chemical and physical properties of paddy straw (dry weight basin)

| Parameter | Values |
|---|---|
| pH | 7.70 ± 0.08 |
| Total Organic Carbon (%TOC) | 39.10 ± 0.95 |
| C/N Ratio | 61.40 ± 2.61 |
| Moisture Content (%MC) | 11.42 ± 0.77 |
| Nitrogen (%N) | 0.65 ± 0.07 |
| Phosphorus (%P) | 0.25 ± 0.02 |
| Potassium (%K) | 1.21 ± 0.11 |

### *3.3 C:N Ratio Analysis*

Figure 2 shows the decrease in the value of C:N ratio in both treatment due to the mineralization of organic matter. The final C:N values after the 90 days of composting period were 17.5 for C1 and 28.7 for C2. According to Tumuhairwe *et al*. [15], they stated that a C:N ratio of less than 20 is considered as a mature compost and can be used without any restriction. Thus, the biocomposting of paddy straw took place progressively through the reduction of C:N ratio (Table 3) and showed the better reduction value in paddy straw treated with the inoculation of *T.harzianum*

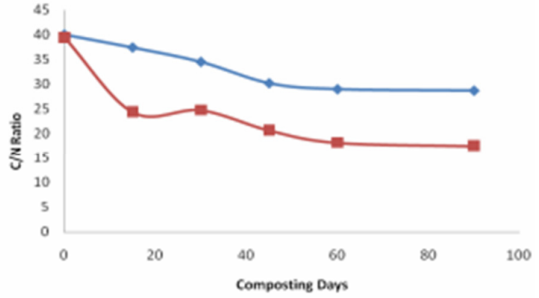


**Fig. 3.** Changes in C/N ratio with composting days (■) $C_1$ with *T.harzianum*; (◆) $C_2$ without *T.harzianum*

**Table 3**
Nitrogen, carbon content and C:N ratio of paddy straw treated by T.harzianum at various times

| Sampling (days) | N (%) | C (%) | C:N ratio |
|---|---|---|---|
| 0 | 0.97 | 38.3 | 39.5 |
| 15 | 1.44 | 35.2 | 24.4 |
| 30 | 1.43 | 35.4 | 24.8 |
| 45 | 1.65 | 34.1 | 20.7 |
| 60 | 1.81 | 33.0 | 18.2 |
| 90 | 1.84 | 32.2 | 17.5 |

Paddy straw is a plant material that consists of high carbon content. Diaz *et al*. [4], reported that during composting, carbon is an energy source for microorganisms for the cells growth. During the cells metabolism process, almost all the carbon is absorbed by the microorganisms and transformed to carbon dioxide ($CO_2$). The remaining carbon will be changed into membrane and protoplasm form. This organic matter is further decomposed by the microorganisms through the composting process in which the organic carbon will be oxidized to $CO_2$ gas to the atmosphere in aerobic condition and thus lower the C/N ratio. According to Viel *et al*. [16], the increase in total nitrogen content might be due to the net loss in dry mass as the organic carbon losses to $CO_2$ throughout the composting process. In addition, the increment of N values might also due to the microbial activity of nitrogen-fixing bacteria that commonly occurs at the end of composting period. Although a decrease of N can occur due to leaching of $NO^{-}_{3}$-N and ammonia volatilization, in this experiment, both piles were covered with plastic to retain the moisture content which could also lead to the result obtained.

## 4. Conclusion

Biocomposting is the most suitable option among the paddy straw 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. It also leads to final stabilized products, which can improve and sustain soil fertility. Cellulolytic microorganisms are the key agents in depolymerizing the cellulose barrier in most of organic materials. Therefore, the selection of effective lignocellulolytic microbes is a crucial step determining to the success of the acceleration of composting process of any agrowaste. Trichoderma sp. are well known as a lignocellulose decomposer as they are filamentous fungi that capable of producing prolific spores which can invade substrates quickly [14]. Various studies have shown that composting of lignocellulosic materials with potential Trichoderma sp. can reduce the biodegradation time [13]. Thus, this study has successfully shown the good potential of *T.harzianum* as cellulose biodegrader. Paddy straw was successfully composted in the presence of *T.harzianum* within 90 days with final C:N ratio of 17.5.

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