

Screening of Cultivable Indigenous Fungi which Responsible for Decomposing of Rice Straw

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Received 5 December 2010 / accepted 4 January 2012

ABSTRACT

The experiment was conducted to screen potentials indigenous fungi for rapid decomposing of rice straw. Seven isolates of dominant fungi were isolated from the burying rice straw on the 2.5 cm soil depth after 30 days incubation on the paddy fields. Five dominant isolates were tested for their potential to decompose rice straw by assessing their value of decreasing C/N ratio and dry weight of rice straw. Fungal inoculums treatments were arranged in a Completely Randomized Design with four replications. The results showed that the dominant cultivable fungi that isolated from decomposed rice straw were *Trichoderma* sp., *Fusarium* sp., *Mucor* sp., *Aspergillus* sp., and *Penicillium* sp. Among the tested fungi, *Trichoderma* sp. had the biggest ability to decompose rice straw compared to others indigenous fungi. The C/N ratio was reduced to 39.47 from an initial value of 73.33 of control treatment in 10 days of biodegradation process in laboratory scale, thus showing the potential of indigenous *Trichoderma* sp. for use in large-scale composting of rice straw.

Keywords: Cultivable, decomposer, indigenous fungi, rice straw

INTRODUCTION

Rice plant is the important crop in Lampung Province that produces large quantities of rice straw. Although rice straw have several nutrient, such as 0.38-1.01% N, 0.01-0.12% P, and 1.0-3.0% K (Ponnamperuma 1984), however, most of rice straw is burnt or removed after harvesting due to lack of knowledge of farmers. Removed out of rice straw from paddy field causes the loss of nutrients permanently from soils, but burying rice straw directly into soil also creates problems for farmer and soils. These rice straw cannot be applied or ploughed directly into the soil because of their high C/N ratio (Man and Ha 2006). To solve such problem the rice straw shall be decomposed before incorporated into soil.

Several studies have been done on decomposition of rice straw by many kind of point of view such as cellulolytic bacteria (Sirisena and Manamendra 1995); lignocellulolytic fungal (consortium Kausar *et al.* 2010). Decomposition of rice straws are responsibility of many type of

microorganisms, *i.e.* bacterial and fungi. Fungi as one of the main decomposer agents that can break down the rice straw (Nandi *et al.* 2000). Kind of cultivable microorganisms that are often used in order to accelerate reforms rice straw are *Aspergillus*, *Fusarium*, *Trichoderma*, *Chyptoga*, *Mucor* sp., and many others. The speed of decomposition of rice straw into compost is influenced by several factors in which composted plant materials, including plant type, plant age and chemical composition of plants; environmental factors, including aeration, temperature, humidity, pH and availability of the required elements of microorganisms decomposer; as well as the use of compost activator. The composting process can be accelerated by changed of composting conditions, such as physic of raw material, moisture, and invasion of microorganism

Fungi play a major role in the decomposition process of rice straw into compost, however the information on the decomposition of rice straw by different indigenous fungal inoculums on rice field is still limited. Although some recent progress in molecular techniques for identification of fungi responsible for decomposition process of rice straw have been published by Sugano *et al.* (2005); but conventional culture techniques for cultivation of

superior fungi for decomposing is still needed. In present study, the research was undertaken to study kind of genus of cultivable indigenous fungi that most rapid to decompose rice straw in a laboratory scale. So that it will be able to add information to Indonesian farmers in the utilization of rice straw through the process of composting using superior fungi as an agents of decomposing.

The purpose of this study was to elucidate the cultivable indigenous fungi which was developed in rice straw and to screen the indigenous fungal inoculums in the paddy field for acceleration of rice straw decomposing.

MATERIALS AND METHODS

Study Site

The field research site was conducted in Kedaloman Village, Tanggamus District on May to September 2008 and laboratory experiment in Laboratory of Soil Biology, Faculty of Agriculture, University of Lampung on March to May 2009.

Incubation of Rice Straw

Rice straws were collected just after harvesting from the rice field on Tanggamus District, Lampung Province. Rice straw were cut into short pieces of about 2 cm long. Samples of rice straw, each equivalent to 10 g dry wt, were buried in the soils (2.5 cm depth) inside nylon bags of 2 mm mesh size. Six replicates sample were selected 30 days after burying, carried to laboratory and isolated their indigenous fungi.

Isolation of Indigenous Fungi

Rice straw samples were gently picked and washed softly to free of extraneous soil and weighed into 3 g. Each sample was then placed aseptically on the potato dextrose agar (PDA) medium containing 0.1% antibiotics streptomycin. After 7 days incubation, the observations were done for fungi that grow in PDA. Each fungi then isolated on the new PDA medium and then keep in slant agar for using in the next experiment.

Morphological Observations

Colony characteristics such as colony appearance and sporulation pattern were examined from cultures grown in darkness at 30°C for 96 hr. The morphological identification of fungal strains was based on the morphology of fungal culture colony of hyphae, the characteristics of spores and

reproductive structures if these feature was discernible (Humber 1997).

Decomposition Experiment

The decomposition experiment was set up as a completely randomized design with four replications. The treatments applied were various fungi inoculums that isolation from the field experiment.

New rice straws were collected from the field and cut into 2 cm long and weighed into 6 g dry weigh based (w/w). Some of them were opened at a temperature of 40° C for 4 days to measure its water content. Five gram of dry based of rice straw were placed on dish that containing moist filter paper and were sterilized by steam in autoclave at a temperate of 120° C for 1 hr.

Indigenous fungal inoculums were inoculated to sterile rice straw above by picked 5 loop inoculation needle and lied into sterile rice straw. Incubation were keep into 10, 20, 30, 40 and 50 days at a room temperature. For observation of decomposition, after finish incubation, rice straw were picked up from the dish and autoclaved for stopping the growth of fungi and kept for further analyzing. The main variables were loss of rice straw weight, carbon total, and nitrogen total of inoculated rice straw.

Data Analysis

Data were analyzed by analysis of variance and followed by Least Significant Difference (LSD) at 5% significance level.

RESULTS AND DISCUSSION

Dominant Indigenous Cultivable Fungi

The growth of indigenous rice straw decomposing fungi were appeared completely after six days of incubation. By morphological observation, seven types of fungi grew in PDA media containing rice straw with different colony appearances (Figure1). Two fungi was unidentified with very low appearance. Five dominant fungi were identification as *Trichoderma* sp., *Fusarium* sp., *Mucor* sp., *Aspergillus* sp., and *Penicillium* sp. with appearances frequency shown in Table 1.

Total Nitrogen

The values of total nitrogen content of the rice straw over the period of decomposition are presented in Table 1. Total nitrogen content of rice straw was almost similar during incubation for all

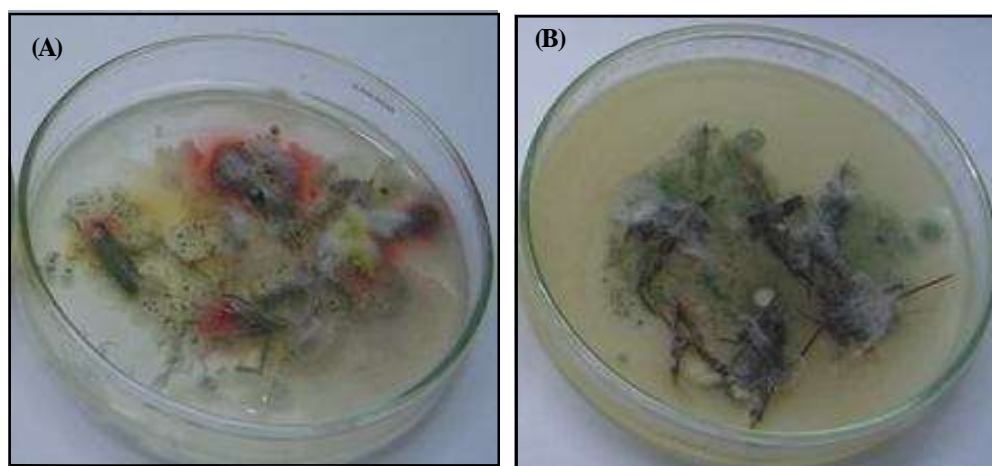


Figure 1. Colony of cultivable indigenous decomposer fungi in PDA medium. (A) high type of fungi and (B) low type of fungi growth.

Table 1. Occurrences of fungi on rice straw after burying in the floodwater paddy field.

| Fungi genera on decomposed rice straw | Appearance frequency |
|---------------------------------------|----------------------|
| <i>Trichoderma</i> sp. | ++++ |
| <i>Fusarium</i> sp. | ++ |
| <i>Mucor</i> sp. | ++ |
| <i>Aspergillus</i> sp. | + |
| <i>Penicillium</i> sp. | + |
| Others 1* | + |
| Others 2* | + |

* = unidentified, ++++ = high (> 67%), ++ = medium (< 67%, > 33%), + = low (< 33%).

fungi inoculums treatment, although the value increased from 0.59% to 64%. Slower increasing of nitrogen total was caused by the incubation condition that were isolated by others organisms except for pure cultures of inoculums treatment only.

Total Carbon

Total carbon content decreased during the decomposition of rice straw and treatments with *Trichoderma* sp resulted in a lower level of carbon total as compared to the control with-out inoculums and others fungal inoculums (Tabel 2). Thus, it can be described that organic matter consumed during rice straw decomposition by fungi. Fungi play an important role in decomposition of lignocelluloses from complex organic matter (Steffen *et al.* 2000).

C/N Ratio

The C/N ratio of the rice straw in all treatments significantly decreased with time (Figure 2). Fungal inoculums significantly affected the decrease in the C/N ratio, on observations of 10, 30, and 50 days after incubation (DAI). While on the observation of 20 and 40 DAI had no significantly affected on the decreasing of C/N ratio. In the observation of 10 DAI showed that the ratio of C/N in the fungal

Table 1. Nitrogen total changes during period of decomposition of rice straw by selected indigenous fungi.

| Fungi inoculums | Day after incubation | | | | |
|------------------------|--------------------------------|------|------|------|------|
| | 10 | 20 | 30 | 40 | 50 |
| | Nitrogen total (%) | | | | |
| Control | 0.53 | 0.56 | 0.57 | 0.58 | 0.57 |
| <i>Trichoderma</i> sp. | 0.60 | 0.59 | 0.63 | 0.68 | 0.69 |
| <i>Fusarium</i> sp. | 0.66 | 0.65 | 0.62 | 0.66 | 0.70 |
| <i>Mucor</i> sp. | 0.60 | 0.55 | 0.57 | 0.62 | 0.63 |
| <i>Aspergillus</i> sp. | 0.55 | 0.62 | 0.59 | 0.59 | 0.62 |
| <i>Penicillium</i> sp. | 0.63 | 0.59 | 0.60 | 0.62 | 0.64 |
| Average | 0.59 | 0.59 | 0.60 | 0.62 | 0.64 |

Table 1. Carbon total changes during period of ecomposition of rice straw by some indigenous fungi.

| Fungi inoculums | Day after incubation | | | | |
|------------------------|------------------------------|-------|-------|-------|-------|
| | 10 | 20 | 30 | 40 | 50 |
| | Carbon total (%) | | | | |
| Control | 39.38 | 37.04 | 37.84 | 35.72 | 33.76 |
| <i>Trichoderma</i> sp. | 34.19 | 26.88 | 24.84 | 24.83 | 24.99 |
| <i>Fusarium</i> sp. | 32.96 | 30.87 | 29.32 | 29.78 | 29.88 |
| <i>Mucor</i> sp. | 33.66 | 26.62 | 27.73 | 28.90 | 26.99 |
| <i>Aspergillus</i> sp. | 30.14 | 31.79 | 30.48 | 29.76 | 28.85 |
| <i>Penicillium</i> sp. | 33.39 | 31.37 | 30.01 | 30.89 | 29.38 |
| Average | 33.95 | 0.76 | 30.03 | 29.98 | 28.97 |

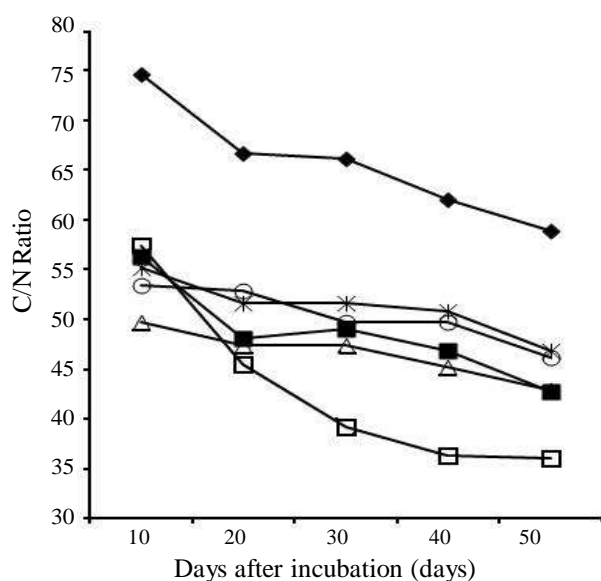


Figure 2. Decreasing C/N ratio of rice straw during decomposition process by ◆ = control (without fungi), ◻ = *Trichoderma* sp., ◻ = *Fusarium* sp., ■ = *Mucor* sp., * = *Aspergillus* sp., and ○ = *Penicillium* sp.. LSD 0.05 for 20 DAI = 6.66; 30 DAI = 8.04; and 50 DAI = 9.80.

inoculums was significantly different between control and treatments. Rice straws that were inoculated by *Trichoderma* sp., *Fusarium* sp., *Mucor* sp. and *Penicillium* sp. were not significantly different in the changes of C/N ratio. The lowest C/N ratio was found in rice straws which were decomposed by *Trichoderma* sp.. In the 20 and 50 DAI the decreasing of C/N ratio of rice straws were not significantly different between each fungi, but it was significantly different with control. Although, it was not different among fungi, but the lowest C/N ratio was found in rice straw treated by *Trichoderma* sp. inoculums. It was likely to decrease C/N ratio more quickly than other treatments.

This is likely that *Trichoderma* sp. produces variety of enzymes, including cellulolytic and chitins enzymes (Fioretto *et al.* 2005; Lemos *et al.* 2003; Nur *et al.* 2008). Therefore there cellulolytic enzymes *Trichoderma* sp. could grow directly on various types of substrate. Therefore, this fungi can be survived and were high competitiveness with other fungi. In addition, *Trichoderma* sp. can grow in a wide range pH, namely 2.5 - 9.5 (Rodiah and Madjid 2009; Nugroho *et al.* 2003).

Irawan *et al.* (2008) stated that *Trichoderma* sp. are microorganisms that can destroy a high level of cellulose and has the ability to synthesize a number of factors essential for dissolving the cellulose that bind strongly with hydrogen bonding. *Trichoderma* sp. is also the most efficient cellulose degrading fungi because it has an ability to break down cellulose into glucose for easily digested (Noverita 2009). In addition, *Trichoderma* sp. improves the functional protein feed stuffs, as well as, on cellulose materials it can stimulate the release of cellulose enzymes (Afrizal 2010).

Lemos *et al.* (2003) reported that microbes that release cellulolytic enzymes will hydrolyze cellulose and crystalline cellulose. There are three cellulose enzymes that play a role in hydrolysis, namely: (1) endo-1,4-β-D-glucanase (EG, EC 3.2.1.4), which works randomly along the cellulose chains to produce a new site for cellobiohydrolase, (2) exo-1,4-β-D-glucan (CBH, EC 3.2.1.91), which works as exoglucanase cellobiose as a major product release, and (3) 1,4-β-D-glucosidase (EC 3.2.1.21) that hydrolyzes cellobiose to glucose (Jorgensen *et al.* 2003).

Comparing this experiment with other research, slower decomposition of rice straw by each fungi that showed by high C/N ratio was caused by the condition of the experiment. In this experiment it was only one species of fungi that decomposed a sterile rice straw in petri dish. In field conditions,

there were many consortium decomposer microorganism decomposed together and the environment conditions were also support it.

Rice Straw Weight Loss

Weight losses were measured for decomposed rice straw by 5 isolates that caused weight loss of more than 5.0%. Fungal inoculums had no significantly affected on rice straw weight loss. Figure 2 showed that the weight of rice straw in all fungi treatments decreased during incubation periods. In general, the weight of rice straw in the treatment of fungal inoculums decreased more rapidly compared to control. Weight losses of rice straw by *Trichoderma* sp. were faster than other fungi. It mean that weight loss of decomposed rice straw mainly caused by *Trichoderma* sp. This study showed that the decreasing of C/N ratio had positive correlation with loss weight of rice straw with coefficient correlation of 0.83, 0.63, 0.80, 0.82, and 0.90 for 10, 20, 30, 40, and 50 DAI, respectively. This results in accordance with Isnaini *et al.* (2009a) and Isnaini *et al.* (2009b) who stated that the lost of organic carbon content is caused by the main constituent of rice straw which is carbon.

Organic carbon of rice straw will be converted into CO₂ and will be released into atmosphere. So the weight of straw will continue to decrease along with increasing time. A decreasing in C/N ratio can be interpreted as the decrease of organic carbon

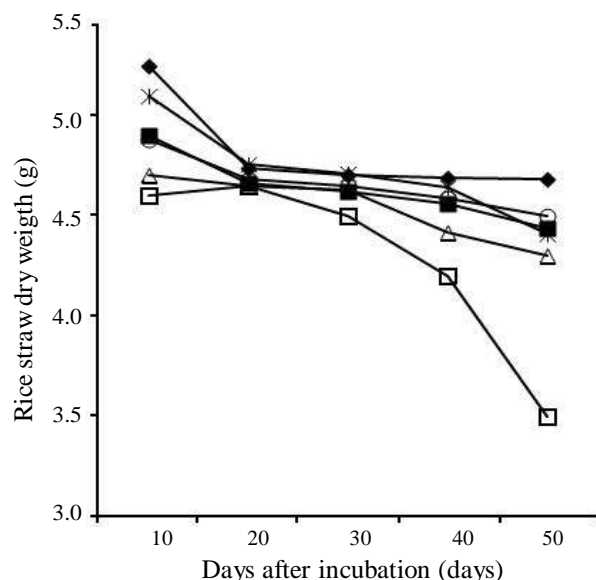


Figure 3. Decreasing C/N ratio of rice straw during decomposition process by ◆ = control (without fungi), ◻ = *Trichoderma* sp., △ = *Fusarium* sp., ■ = *Mucor* sp., * = *Aspergillus* sp., and ○ = *Penicillium* sp..

content from rice straw, this indicates that the decomposition takes place. Goyal *et al.* (2005) reported that during the composting, the organic materials are decreased. Furthermore, Atkinson *et al.* (1996) stated that C-organics are changed due to loss of C as CO₂. Carbon dioxide is released through oxidation in the composting by microbial activity (Barrington *et al.*, 2002).

CONCLUSIONS

From the results obtained it can be concluded that fungal inoculums can accelerate the decomposition of rice straw and *Trichoderma* sp. is the best indigenous fungi in the decomposition process of rice straw. After 50 day incubation C/N ratio in pure culture was still high, which ranged of 30-60. It can be recommended that for next research incubation time should be more longer up to 100 days.

ACKNOWLEDGEMENTS

The authors sincerely thank to Sisca Yunita for her helpful identification of fungi and laboratory analysis and to the Director General of Higher Education, Ministry of National Education and culture Republic of Indonesia that had funded this research in National Priority Based Research Grant Year 2009.

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