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Effects of pH on Inoculum Production of *Aspergillus tubingensis* on the Acid Rice Media

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Abstract
The addition of inoculum on the composting process is one of the topics widely studied by scientists. Therefore, construction of inoculum composition of fungal decomposer able to initiate and enhance the composting process is very interesting to study and this research was dealt with this matter. Compost is mostly used for acid dry soil in several plantation in Indonesia. Therefore preparing inoculum in an acid medium to understand their productivity is very important. The purpose of this research is to understand the influence of acid rice media on the production of inoculums including spore production and their viability of *A. tubingensis*, a xylanolitic fungus. The research was designed in Completely Randomized Design (CRD) with three replicates. The treatment is the acidity of the media consisting of pH 3.0, 3.4, 4.0, 4.4, 5.0, 5.4, and 6.0. The variables measured are number of spores and the Colony Forming Unit (CFU). The result showed that the production of spores of A. tubingensis and their viability are optimum at pH 6.0 with 4.63 x 104 spores / ml and 3.0 x108 CFU / ml respectively.
Keywords: *Inoculum, Xylanolytic Fungus, Aspergillus tubingensis,Composting.*

1. Introduction

The influence of pH on the relative importance of principal decomposer microorganism in compost, fungi, was investigated along a continuous pH gradient for inoculum preparation.

Compost is organic material from plant residues or agricultural waste and animal waste that has decomposed. Currently, composting is used as a major process of stabilizing agricultural organic wastes through the degradation of biodegradable components by microbial communities under controlled conditions. Bacteria and fungi species play significant roles in the decomposition and mineralization of agricultural organic wastes [1]

Decomposition of organic matter is completed by decomposing microorganisms possible to improve the soil properties because mature compost contains nutrient minerals essential for plants.

The problem is that to get the mature compost is still too long so it leads to the study of development of inoculums. Therefore the use of saprotrophic microorganisms that are environmentally friendly and effective for composting is interesting.

The major substrate for leaf litter composting is lignocelluloses difficult to decompose naturally and it has led to find a proper inoculum. One of the organic materials difficult to degrade is xylan.

The inoculum would be possibly performed by a superior fungus able to break down organic materials to make the time of composting shorter. Isolate potential to use to construct the inoculum is *A. tubingensis*. This fungi produce xylanase [2] useful for xylan degradation

Xylan is the largest component of the hemicellulose, the second largest component of the plant cell wall constituent after cellulose. Xylan is also found in plant cell walls of graminae and some monocots. Xylan constitutes 15-30% hemicellulose and some 30% on perennials [3]. Rice (Oryza sativa) is graminae members containing xylan possible used as a medium for xylanolytic fungi (A. tubingensis) for the inoculum compost construction.

The purpose of this study is to determine the effect of the acidic conditions of the rice media on

A. tubingensis inoculum production including spores and CFU numbers (viability).

2. Materials and Methods

The study was conducted in January to March 2017 in the Laboratory of Microbiology Department of Biology, the University of Lampung, Indonesia.

Isolation and screening of xylan-degrading fungi was performed using modification of [4] method. Isolates obtained were cultured in medium for fungi xylanolytic (xylan from beechwood 1 g, peptone 5 g, yeast extract 5 g, K2HPO4 0.2 g, agar 20 g, and distilled water 1000 ml). Confirmation of xylan-degrading ability of fungal isolates was performed by streaking on xylan enriched media. Media were 2 layer media (bilayer) with the bottom layer was a PDA of 1/5 recipes, agar 1.5, and distilled water 100 ml. The top layer consisted of xylan from beechwood 1%, agar 1.5 and distilled water 100 ml.

The research was designed in the completely randomized design (CRD) treated under acidic conditions varying in pH ranging from pH 3.0, 3.4, 4.0, 4.4, 5.0, 5.4 and 6. Firstly, fungal culture of *A. tubingensis* were rejuvenated in PDA. Inoculum development was made using modification of [5] method. Inoculum media were prepared using rice (*Oryza sativa*), coarsely ground as much as 30 grams and put in sterile glass bottles. Each bottle was added with $CaSO_4$ 4% (w / v) and 2% $CaCO_3$ 5 ml of each.

The media acidty adjustment was performed by adding acid pH buffer [6]. Rice media were sterilized for 15 minutes, cooled and inoculated with a loop of *A. tubingensis* hypha. The inoculum were incubated for 14 days at room temperature. The whole growth of each strain including mycelium, spores, and the grains were used as the inoculum. The inoculum was counted for the number of spores and viability by calculating CFUs. All analyses were performed at least in 3 replicates and the data were analyzed by uni- or multifactorial ANOVA and Least Significance Different (LSD).

3. Result and Discussions

3. 1. Xylanolytic Potential of Fungal Isolates

The xylanase production ability of fungi assessed by estimating zone around the colony formed due to ability of fungal isolates to hydrolyze xylan (Figure-1). Results showed that *A. tubingensis* enzyme activity indicated by diamter colony is 2.65 cm with the ratio of colony/halo 3.59 (Figure-1b). Screening of xylanolytic fungi is based on the size of the diameter of the clear zone indicating the ability of isolates to hydrolyze xylan [7]

Fig. 1 a.) Colony of *A. tubingensis*; b) Plate screening of xylanase by displaying clearing zone (halo).

These clear zone measurements are generally smaller than those of [7] which isolate fungi from weathered substrate, agricultural waste, agricultural land and lignocellulosic waste. They obtains a clear zone diameter ranging from 2.80-7.00 cm, but this result does not take into account the size of the colony. Another study by [2] also reinforced that *A. tubingensis* is a species able to produce xylanase.

3. 2. Number of Spores

The data indicated that treatment of acidic pH on the rice media did not significantly affect the number of fungal spores of *A. tubingensis*. Data retrieval is done after the fungal spores of *A. tubingensis* fungal inoculum were incubated for 14 days. The display of fungal spore number affected by media acidity are presented in Figure 2.

Figure 2 shows the fluctuation of fungal spore number of A. tubingensis on different pH. The number of fungal spores from the lowest to the highest are shown at pH 5.4, pH 3.4, pH 4.0, pH 3.0, pH 5.0, pH 4.4 and pH 6.0 respectively. It shows that the different number of spores are resulted from different pH of media. The highest

number of spores is 4.63 x 10^4 spores / ml at pH 6.0, while the lowest is 2.53 x 10^4 spores / ml at pH of 5.4. The data is interesting since it indicates that the fungi are able to grow at acid condition even as low at pH 3.0.

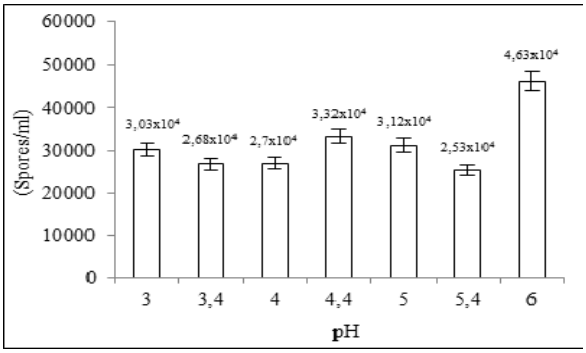

Fig. 2 Number of *A. tubingensis* spores in acid rice media

Another research about fungal biomass in acid soil showed that fungal biomass in soil increased from pH 4- 6 and dcreased from pH 6-9 [8]. This explaines that A. tubingensis may active in the acid condition and will be potential to use as composting inoculum. Most compost of any substratres usually turn to the acid environment due to the degradation of organic substances. Therefore the present of inoculum tolerant to the acid condition will be beneficial for composting process and increases compost quality..

3. 3. Number of CFU (Colony Forming Unit)

The data indicates that the treatment of acidic pH on the rice medium did not significantly affect the number of CFU. However the data also shows that A. tubingensis was able to grow in all low pH treatments even at the very acid condition (pH 3) and the maximum CFU was at pH 6 (Figure 3). Data retrieval was done after forteen days of incubation.

Figure 3 shows that CFU number order from lowest to highest, respectively, are pH 4.4, pH 5.4, pH 3.0, pH 3.4, pH 4.0, pH 5.0 and pH 6.0. Treatment pH 6.0 had the highest CFU numbers 3.0 x10^8 CFU / ml while the lowest number is 9.7x10^7 CFU CFU / ml at pH 4.4. CFU numbers data gives infrmation about spore viability. In fact the growth of colony do not only come from spores but also from hypha (sclerotia). Therefore the CFU is a representation of ability of fungi to grow.

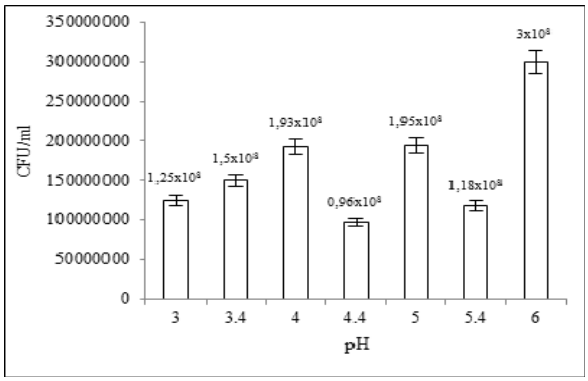

Fig. 3 CFU number of *A. tubingensis* in acid rice media

The data shows that the optimum pH for the highest spore and CFU number came from the same treatment which is pH of 6.0. The stastical analyses showed that all treatments do not affect the inoculum production. However, the data shows that A. tubingensis was able to grow at all pH treatment even at a very low pH (3.0). This indicated that the fungi are an acidic tolerant fungi. The acidity tolerant of *A. tubingensis* was also mention by other researcher and it could be caused by the present of beta glucosidases [9].

Spore production was the lowest at the treatment of pH of 5.4, while the lowest CFU numbers are at pH 4.4. In contrast, the highest number of spores and CFU are in the inoculum with the treatment of pH 6.0. This relates to the optimum pH for growth of fungi A. tubingensis. The results of this study are slightly different with statement of [10] which stated that the optimum pH for growth of fungi A. tubingensis is 3.5 to 8.0. However, the results obtained are consistent with a recent study of [11] which stated that the fungi *A. tubingensis* best growth in the medium with a pH of 5.5 to 6.5. Thus, treatment of pH 6.0 for the inoculum can produce spores and CFU at the highest number compared to others.

Observation to the number of spores and CFU was conducted to determine the level of productivity and viability of the fungi A. tubingensis. High and low limits of viability level are determined based on the extent of the average density of the colony isolates of fungi that grow.

Fungal isolates that showed high levels of viability had CFU numbers ≥ 1 x 10^7 CFU / ml and that has a level of viability is having a mean CFU ≥ 1 x 10^6 CFU / ml [12]. Based on this study, the level of viability of the fungi *A. tubingensis* in this study is relatively high because it has a mean CFU ≥ 1 x 10^8 CFU / ml. The data shows that inoculum which has the highest viability is at pH 6.0 with CFU number of 3.0 x 10^8 CFU / ml.

Effect of pH on the viability of fungal spores is associated with the activity of the enzyme, especially the dominant xylanolytic enzyme produced by fungi A. tubingensis. Fungi require enzymes to catalyze reactions that are associated with the growth. If the pH in a medium is not optimal it will interfere the enzyme activity and eventually interfere the growth of fungi [13]. Fungal cell activity is also influenced by pH of environment. Fungi are able to grow at extreme pH in the environment. Their pH affects the growth of fungi, especially against the total charge (net charge) of membrane proteins associated with nutrient absorption. Thus, compliance with environmental pH affects the absorption of nutrients by the fungus. Effect of pH on fungal growth affect the pH of the dissolution rate of mineral salts and the equilibrium of dissolved CO2 and bicarbonate ions [13].

This result demonstrates the potential of inoculum is very possible to be applied as Composting Starter Kit (CSK). CFU numbers of the inoculum was higher comparing to CFU of spores from decomposed leaf litter substrate in first isolation [14]. Viability of spores is very critical for CSK usage. The successful use of inoculum is largely determined by the level of spore viability, whereby if the spores have high viability it will produce a large fungal biomass. This will certainly give significant effect if this inoculum is applied for composting of organic material.

The production of the fungal inoculum of *A. tubingensis* on media rice are able to prepare at pH 3.0 to 6.0 and optimum production are at pH 6.0

4. Conclusions

The production of spores of *A. tubingensis* and their viability are optimum at pH 6.0 with 4.63 x 10^4 spores / ml and 3.0 x10^8 CFU / ml respectively.

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