Utilization of thermophilic cellulolytic bacteria isolates and *Trichoderma harzianum* fungi on rice straw composting

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Abstract. The thermophilic cellulolytic bacteria are potential organisms for applications such as in decomposition of organic materials and enzyme technology. One potential organic materials which is very abundant in Indonesia is rice straw, which have not been optimally utilized. The problem is, the composting process of straw in nature is used to take along time, so it needs to add effective decomposer microoroganisms which can adapt on compost termperature rise. The aims of this research were to isolated and selected thermophilic cellulolytic bacteria and *Trichoderma harzianum* and tested the isolates on rice straw composting. This research is executed in laboratory in faculty of agriculture, University of Alwashliyah Medan. Twenty thermophilic cellulolytic bacteria were isolated from several process composting of rice straw and corn waste at high temperature, from peat land and sidebu-debu hot spring, while *Trichoderma harzianum* fungi that used in this research was already got from previous research. Cellulolytic activity was determined by ability of bacteria and fungi to degrade *carboxymethyl cellulose* (CMC) substrate. Cellulolytic microorganisms was selected based on clear zone which form surrounding the colonies. Cellulase activity was measured by dinitrosalisilic acid (DNS) method. Results showed that there were 5 isolates (JG12, JG9, JG7 JR2, SD1) showing the best ability to degrade CMC. JG12 isolate had the higest diameter ratio of clear zone and enzyme activity (2,6 and 5,07 x 10^{-3} U/mL), while *T. Harzianum* had ratio of clear zone 1,22 and enzyme activity 4,02 x 10^{-3} U/mL).Then the selected bacteria and *T. Harzianum* were test on rice straw composting. This selection was used complete block Random Design Factorial, which consist of bacteria isolates as the first factor (S0=control, S1= Straw+JG7, S2=Straw+JR2, S3=Straw+JG9, S4=Straw+JG12, S5=Straw+SD1) and *T. Harzianum* as the second factor(T0=control, T1=Straw+*T. harzianum*). The result of this research are the highest decreasing of ratio C/N is caused by the treatment combination S4T1 which is consist of JG12 bacteria isolate that isolated from corn waste composting and *Trichoderma harzianum* (C/N = 17,16).
Key words: Thermophilic cellulolytic bacteria, Trichoderma harzianum, compost, rice straw

Introduction

Production activities in the agricultural sector, particularly in harvesting, post-harvest and processing of the product will always produce a large amount of waste that cause environmental problems and requires cost a lot to overcome it. One of the biggest agricultural waste is rice straw which has not been widely used in Indonesia. Farmer used to burned the rice straw on their land. However, air pollution and combustion resulting in the loss of certain nutrients. One of the ways that can be used to manage the waste rice straw is through composting. This compost can be used for a source of organic matter. Some of the problems on composting of rice straw are it has a high ratio of C/N and containing cellulose (37.71%), hemicellulose (21.99%) and lignin content (16.62%) (Dewi, 2002). The main problem now is that the cellulose material is stable in nature and require complex biodegradation before it can be used by microbes, plants or humans.

Cellulose decomposition can be performed using chemical or microbial agents. Microbes (bacteria, fungi, yeast, and actinomycetes) is a potential agent for cellulose decomposition. Cellulose decomposition process requires an enzyme called cellulase complex. Cellulase that was produced by bacteria and fungi can be used to speed up the composting of waste rice straw. But in the composting process usually through high-temperature phases, the increasing temperature is up to 55 ° C or up to 70°C . the habitat in material was not appropriate for the sellulolitik mesophilic microorganisms at that high temperature. Mesophilic microorganisms was eventually replaced by thermophilic microorganisms (microorganisms that can be alive at above 45°C). Therefore need to exploited of cellulase-producing microorganisms which is resistent at high temperature to accelerate the process of rice straw decomposition in composting.

In this reasearch, in addition to isolated and selected thermophilic cellulolytic bacteria from various organic materials, also this research tested *Trichoderma harzianum* fungi in composting rice straw. Pujioktari (2013) has showed that the use of *Trichoderma harzianum* on rice husk, showed significantly reduced the influence of dry matter, ash and crude fiber. Also according to Enari (1983), cellulase enzyme is also produced by *Trichoderma harzianum, Trichoderma viride, Trichoderma konigii*. This research aimed to isolate and selected some thermophilic cellulolytic bacteria and one isolate of *Trichoderma harzianum* so it can get high capability in rice straw composting.

Materials and Methods

Medium that is used to isolate cellulolytic bacteria is Mandels medium, which is consisted of KH_2PO_4 0.05 % (weight), 0.05 % K_2SO_4, (NH4) 2SO4 0.1 % , $MgSO_4.7H_2O$ 0.01 %, $CaCl_2$ 0.1 % and 0.6 % NaCl . As a source of carbon use carboxy methyl cellulose or CMC 0.5 % (for the formation of a clear zone around the bacterial colonies medium). To clarify the formation of a clear zone on each selection plate, 5 ml of Congo red solution (1 g / ml) was added to each plate. While the fungus of *Trichoderma harzianum* isolate was using an isolate collection from Department of agriculture, Alwashliyah University, which has been isolated from soil in previous studies.

Isolation of Thermophilic Cellulolytic Bacteria

Bacteria isolate was isolated from some organic material i.e. paddy and maize straw , peat soil and Sidebu -debu hot springs North Sumatra . Straw and corn sampling carried out during the composting takes place at high temperatures (45 - 50^{0}C). Isolation is done by dilution system. Isolation medium was prepared in a petri dish containing Mandels medium that comes with CMC.

Cellulolytic bacteria Selection Based on Medium Clear Zone Formation of CMC

Isolates were grown on Mandels medium with 0.5 % CMC carbon source. Then the Isolates was incubated for 4-5 days at a temperature of 50^{0}C, so the bacteria isolated is a thermophilic bacterium . In order for the formation of clear zone made clearer by using congo red staining. Furthermore the ratio between the diameter of the clear zone medium and the diameter of bacterial colonies was counted. From the test results based on that ratio was selected five best cellulolytic bacterial isolates, i.e. isolates that have the higher ratio between the diameter of the clear zone medium and the diameter of bacterial colonies. These five isolates were tested further their activity of cellulose quantitatively and tested its ability in decompotition rice straw with *Trichoderma harzianum* .

Quantitative Test Cellulase Activity
Bacterial cultures that were grown in a liquid medium containing 1 % CMC substrate were centrifuged for 15 minutes. Supernatant portion used for extracellular enzyme activity assays. A total of 1 mL of the supernatant was mixed with 1 mL of 1 % CMC at Mc Ilvaine buffer pH 7.2 . After that , the mixture was incubated at 50 °C for 60 min . The reaction was stopped by adding 3 mL of DNS reagent. then boiled for 15 minutes in a boiling water bath, then were cooled. After that, the absorbance was measured with a spectrophotometer at a wavelength of 540 nm .

Test Potential Bacterial Isolates and *Trichoderma harzianum* on Rice Straw Composting
Five bacterial isolates that have been chosen were then tested for their ability to decompose rice straw . This test used a factorial randomized block design with the first factor is a thermophilic cellulolytic bacterial isolates (S0 = hay without bacterial isolates , S1 = straw + bacteria 1 , S2 = straw + best bacteria 2 , S3 = straw + best bacterial 3 , S4 = straw + best bacteria 4 , S5 = straw + best bacteria 5) and the second factor is the fungus *Trichoderma harzianum* (T0 = hay without T. harzianum , T1 = straw + T. harzianum). Composting is carried out for 2 months. Observation paramater was the ratio of C / N each of the rice straw compost. Statistical data analysis performed by analysis of variance and the results which were significantly different were followed by Duncan Range Test .

Results and Discussion
Isolation of cellulolitic bacteria that has been done on medium carbon source at 50^0C has obtained 15 isolates of bacteria that can form a clear zone. This means that all of 15 isolates are able to produce cellulase enzymes that can decompose cellulosic substrates. Isolates of *T. harzianum* was also able to produce cellulase enzyme as evidenced by the formation of a clear zone on the fungus .

Tests based on the formation of a clear zone punctuated by congo red staining on CMC agar has results the Z/C was 1.00 to 2.60 (Table 1). Congo red interacts strongly with the CMC to form a reddish blue color, but if the CMC had decomposted by the cellulolytic microorganisms, the reddish blue color will turn out to be clear. The clear zone was formed by the bonding β - 1 ,4 - glucoside which unites monomers D - glucose into cellulose chain (CMC) has been lost by the activity of cellulase enzymes produced by the cellulolytic bacteria. The higher value of this ratio indicates the higher specific activity of the cellulose enzyme, especially the enzyme endo - β - 1 ,4 - glucanase or CMC - ase . In this study JG12 , JG9 , JG7 JR2 , and SD1 isolates are produced the highest ratio of clear zone.

Table 1. Testing the ability of Celullulolitic bacteria and *Trichoderma harzianum* based on the establishment of clear Zone

Kode isolat	Sumber Isolat	Diameter		Nisbah (Z/C)
		Zone (Z) (mm)	Colony (C) (mm)	
SD1	Air panas Sidebu-debu	12,85	8,57	1,50
JR2	Kompos jerami padi	13,59	7,55	1,80
JR3	Kompos jerami padi	9,42	7,03	1,34
JG1	Kompos jerami jagung	8,00	8,00	1,00
JG2	Kompos jerami jagung	8,11	8,11	1,00
JG3	Kompos jerami jagung	7,21	7,07	1,02
JG4	Kompos jerami jagung	8,32	8,00	1,04
JG5	Kompos jerami jagung	7,03	6,51	1,08
JG6	Kompos jerami jagung	9,59	8,64	1,11
JG7	Kompos jerami jagung	8,16	4,08	2,00
JG8	Kompos jerami jagung	4,83	4,56	1,06
JG9	Kompos jerami jagung	8,32	3,70	2,25
JG10	Kompos jerami jagung	7,10	7,00	1,01
JG11	Kompos jerami jagung	6,30	6,30	1,00
JG12	Kompos jerami jagung	13,00	5,00	2,60
TH1	Tanah	8,54	7,00	1,22

Enzyme activity that has obtained from the bacterial isolates in this research were still very low (Table 2.) as well as in isolates of *T. harzianum*. This is caused by the bacterial isolates still the wild type (wild type). Narasimha et al. (2005), mentions that the cellulase production by wild-type *Bacillus pumilus, biazotea Cellulomonas* and *Trichoderma aureoviride* in liquid media shall not exceed 1.5 U / mL. *Bacillus spp*. That has been isolated from Brassica compost has maximum cellulolitic activity of 1.90-2.33 U / mL (Chang et al. 2009). Another factor that causes low activity of the enzyme in this study was not carried out optimization of growth conditions for bacteria. Temperature and pH values used are appropriate with the current state of the bacteria were first isolated.

Physical condition is not used in the optimum conditions for bacteria to produce cellulase. Temperature and pH greatly affects the activity of enzymes that the bacteria must be grown under conditions of pH and temperature optimum for obtaining optimum cellulase activity. Enzyme activity is affected by pH. Changes in pH will cause the catalytic region and the enzyme conformational change. Changes in pH also causes denaturation of the enzyme and cause the loss of enzyme activity. Temperature also affects the enzymatic reaction. Enzyme reaction velocity and kinetic energy increases at the optimum temperature thus increasing the chances of enzymes and substrates react. Temperatures higher than the optimum temperature causes a conformational change and enzyme denatured (Girindra 1993). The substrate can be changed conformation at temperatures that do not fit so it can not fit into the active site of enzymes. Alam et al. (2004), mentions that the optimum pH, temperature, incubation period, the source of carbon and nitrogen sources are important factors that affect the maximum cellulase production and enzyme activity.

Table 2. Enzyme activity assay results quantitatively

Isolate	**Enzim activity (U/mL)**
SD1	$2{,}11 \times 10^{-3}$ U/mL
JR2	$4{,}26 \times 10^{-3}$ U/mL
JG7	$2{,}90 \times 10^{-3}$ U/mL
JG9	$4{,}81 \times 10^{-3}$ U/mL
JG12	$5{,}07 \times 10^{-3}$ U/mL
TH1	$4{,}02 \times 10^{-3}$ U/mL

Testing of bacterial and fungal isolates *T. harzianum* on composting rice straw showed significantly affect the results of the ratio C / N straw (Table 3.). Treatment S4T1 (JG12 isolates and isolates of T. harzianum) produces ratio C / N 17.16 and the lowest was significant compared to the control and compost were given other bacterial isolates.

Table 3.The Influence of Isolates Thermophilic cellulolitic Bacterial and *T.harzianum* towards C/N rice Straw

Treatment	C/N
S0T0	23,84[a]
S1T0	23,15[a]
S2T0	19,62[d]
S3T0	20,92[cd]
S4T0	18,21[e]
S5T0	20,12[d]
S0T1	22,01[ab]
S1T1	22,80[ab]
S2T1	20,29[d]
S3T1	21,96[bc]
S4T1	17,16[e]
S5T1	22,54[abc]

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