

# Ability of *Penicillium Griseofulvum* Inacc F 14 in Producing Cellulase Enzyme for Composting Media Plant of White Oyster Mushroom (*Pleurotus Ostreatus* Jacq. Ex Fr.) P. Kumm and Ear Mushrooms (*Auricularia Auricula* J.)

Y. S Soeka<sup>1</sup> and M Ilyas<sup>1</sup>

<sup>1</sup>Research Center for Biology, Indonesian Institute of Sciences (LIPI)  
Jl. Raya Bogor Km. 46, Cibinong 16911, Bogor, Indonesia

E-mail: ceuceu\_lipi@yahoo.com

**Abstract.** The aim of this study is to determine the characteristics of cellulase enzymes produced by the fungus LIPIMC selection. Results were then analyzed qualitatively and quantitatively. Enzyme activity includes incubation time, substrate concentration Carboxy Methyl Cellulose (CMC), temperature, optimum pH and the effect of the addition of some metal ions as activators or inhibitors were examined. Further molecular identification of fungus was examined through 16S rDNA sequencing. The optimum conditions for the enzyme activity were the conditions with an incubation period of two days, at pH 6, at the temperature 50°C, at substrate concentration 1.25 %. The cellulase enzyme was activated by cations CoCl<sub>2</sub>, CuCl<sub>2</sub>, and NaCl. Meanwhile MgCl<sub>2</sub> and ZnCl<sub>2</sub> were acted as an inhibitor. Results of molecular identification by 16S rDNA sequencing confirmed that LIPIMC 0575 was *Penicillium griseofulvum*. After identified, the tested supernatant of cellulase enzyme has the ability to inhibit the growth of *Bacillus cereus* and will be used for composting on the media plant of oyster mushrooms (*Pleurotus ostreatus* J.) and ear mushrooms (*Auricularia auricula* J.).

## 1. Introduction

Enzyme is a protein that has biochemical activity as a catalyst of a reaction. Because of a protein, this enzyme very susceptible to an environmental condition [1]. Enzyme could be isolated from various of source such as plants, animal tissues, and microbes [2]. At this time, microbe enzymes have been proven economically beneficial because microbe culture is far simpler, faster and can be genetically manipulated easier than plants and animals [3, 4]. Cellulase can be produced by fungi, bacteria or actinomycetes, but the most common producer is fungi. Fungi have the potential that good to be used in the cellulase production, considered to be the most active to degrade the polymers that abundantly available in the nature so that can decrease a pollution [5, 6, 7, 8]. Cellulase (EC 3.2.1.4) is one of the extracellular enzyme that can catalyze 1,4-β-D-glucoside bonds in a cellulose molecule glucose monomer [9, 10, 11, 12, 13]. Currently cellulase application is the most useful in industries include pulps and papers, textiles, and laundry [when cellulase enzyme added into detergent, then that detergent reacts with cellulose fibers to produce a bright color and can soften], the tannery, the biofuel production (such as bioethanol), the bread in food industry (cellulase enzyme can increase crumb structure to produce crumb that more regular and smooth to result increasing whiteness in foods and drinks, and animal food), beer production, and agriculture as organic fertilizers [14, 15, 16, 17, 18, 19,



20, 4, 21, 22]. Some fungi that used to cellulase industries were *Trichoderma*, *Humicola*, *Aspergillus*, and *Penicillium* [23, 24, 25].

The aim of this research was to characterize the cellulase enzyme activity of *Penicillium griseofulvum* InaCC F 14 which will be used for composting media plant of white oyster mushroom (*Pleurotus ostreatus* J.) and ear mushroom (*Auricularia auricula* J.).

## 2. Materials and Methods

Equipment and materials used were include water bath, oven, digital balance, incubator, centrifuge, vortex, shaker incubator, spectrophotometer, measuring pipette, measuring glass, test tube, erlenmeyer, magnetic stirrer, magnetic stirrer bar, micropipette, micropipette tips, plastic heater, test tube rack, petri dish, fungi strains of LIPIMC collections of Research Center for Biology - Indonesian Institute of Sciences Cibinong, Potatoes Dextrose Agar (PDA), paper disc, aquadest, yeast extract, bacteriological peptone, Carboxy Methyl Cellulose (CMC),  $\text{KH}_2\text{PO}_4$ ,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{CaCl}_2$ ,  $\text{NaCl}$ , bacto agar, 3,5-dinitrosalicylic acid (DNS) reagent, and Bradford reagent.

Fungi strains used in this experiment were LIPIMC 0544, LIPIMC 0546 a and LIPIMC 0575. LIPIMC 0544 collection was isolated from library air-borne of Research Center or Biology-Indonesian Institute of Sciences Cibinong. LIPIMC 0546 collection was isolated from Research Center for Biology - Indonesian Institute of Sciences Cibinong. LIPIMC 0575 collection was derived from *Magnolia condolii* rhizosphere of Taman Nasional Bodogol, Sukabumi District, West Java. Fungi was cultivated in Potatoes Dextrose Agar (PDA) slant media, disc paper 6 mm, *Bacillus cereus* InaCC B325.

Media used in qualitative selection were specific media contained CMC 1% and composed of 2.0 g of  $\text{KH}_2\text{PO}_4$  (Merck), 1.4 g of  $(\text{NH}_4)_2\text{SO}_4$  (Merck), 0.3 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck), 0.1 g of  $\text{CaCl}_2$  (Merck), 1.0 g of peptone (Difco) and 2.0 g of agar (Bacto). All materials were dissolved in aquadest 100 mL and prepared in accordance to Sohail et al [6].

Media used for microbe culture were the yields of strains selection that the biggest clear zone and have been grown in PDA slant media, then incubated in incubator until aged 3-4 days. Cellulase production media that used were prepared and composed similarly to the qualitative selection media as previously described in Sohail et al [6]. The supernatant were incubated in shaker incubator with 120 rpm velocity, at 37 °C for 5 days. They were centrifuged at velocity 8000 rpm, temperature 4°C for 10 minutes to separate enzyme solution (supernatant) from their substrate particles and cells. The acquired supernatant was saved in a freezer (-10°C) and was further measured for its cellulase activity. The cellulase activity of the culture was analyzed with spectrophotometer at  $\lambda$  540 nm everyday as in accordance to Jahangeer et al [26].

Cellulase enzyme activity was tested according to DNS method [27]. It was tested by measuring the quantity of reductor sugar that was produced by enzyme hidrolisis activity in CMC 1% substrate.

### 2.1. Qualitative examination of cellulase activity

Each of fungi strain was inoculated in selective agar media of CMC 1%. Each of fungi strains was grown in the surface of media, then incubated for 72 hours. Grown colony was dropped with a pipette congo red 0.1 % indicator and let it for 30-60 minutes, then it washed with  $\text{NaCl}$  2 %. The formed clear zone around the colony was measured in accordance Teather and Wood [28]. The formed clear zone around a colony was an indication of the existence of cellulase enzyme activity. Cellulolytic index was measured value of the diameter of the colony that divided by the diameter of the formed clear zone. Isolates that showed existence of cellulase activity with high relative value were then examined quantitatively.

$$\text{Cellulolytic index} = \frac{\text{clear zone diameter}}{\text{colony diameter}}$$

### 2.2. Quantitative examination of cellulase activity

In order to measure cellulase activity quantively, the reaction was prepared in accordance to Kar et al 2006 [29]. An equal amount of enzyme supernatant (i.e. 0.125 mL) was added into 0.125 mL of

substrate (i.e. phosphate buffer solution with pH 7.0). The reaction was incubated at 40°C temperature for 30 minutes. To stop the reaction, 0.25 mL of 3,5 dinitro salisilat (DNS) acid was added into reaction [29]. Then it was boiled for 5 minutes and was cooled under some flowing water. Aquadest was then added as much as 2.5 mL. The mixed solution was measured with spectrophotometer at  $\lambda$  540 nm.

Calculation of cellulase activity was carried out with a formula:

$$\text{Cellulose activity} = \frac{(\text{Glucose content} \times \text{dilution factor}) \times 1000}{(\text{Glucose molecular weight} \times \text{incubation time})}$$

One unit of cellulase activity is defined as enzyme quantity that produced 1  $\mu$ mol of glucose in 1 minute at examination condition. It is multiplied by 1000 as a conversion from mmol to  $\mu$ mol.

### 2.3. Characterization of cellulase enzyme

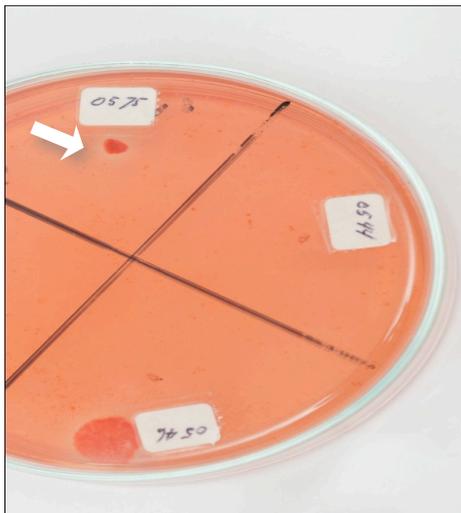
Characterisation of cellulase enzyme in this study was assessed by determining the optimum conditions for enzyme production, including incubation time, pH, temperature, CMC substrate concentration and metal ion addition consecutively. The effect of incubation time against enzyme activity was carried out by reacting enzyme supernatant in period of time, i.e. one to five days. The effect of pH against enzyme activity was carried out by reacting enzyme solution with the concentration of CMC 1%, then incubated in acetic buffer 0.05 N at pH 5, and phosphate buffer 0.05 N at various pH. The tested pH were 5.0, 6.0, 7.0, 8.0, and 9.0. The effect of temperature against enzyme activity was carried out by reacting enzyme solution with the concentration of CMC 1% in buffer solution with the obtained optimum pH from the previous analysis at various temperature, i.e. 35, 40, 45, 50, 60 and 70°C. The effect of CMC substrate concentration against enzyme activity was carried out by reacting enzyme solution with the concentration of 0.5%, 0.75%, 1.0%, 1.25% , 1.5% and 1.75% at the obtained optimum pH dan optimum temperature. The effect of metal ion with final concentration 1 mM was carried out by reacting enzyme solution with addition of various metal at the obtained optimum conditions pH, temperature and CMC concentration from the previous analysis. The tested ions were Na<sup>+</sup>, Co<sup>2+</sup>, Cu<sup>2+</sup>, Mg<sup>2+</sup> and Zn<sup>2+</sup> which were available in the form of NaCl, CoCl<sub>2</sub>, CuCl<sub>2</sub>, MgCl<sub>2</sub> and ZnCl<sub>2</sub> respectively. The samples were also compared with reacting enzyme without metal addition.

Examination of antibacterial activity from cellulase enzyme supernatant was assessed by measuring inhibition zone diameter that was formed around the tested disc [30]. The 6 mm diameter of tested disc was dipped into cellulase enzyme extract supernatant. For a positive control, the tested disc was dipped into Chloramphenicol (400  $\mu$ g/mL). Meanwhile for a negative control, the tested disc was dipped into steril water. The tested disc papers were placed in Nutrient Agar (NA) media that has previously inoculated with *Bacillus cereus* bacteria. The tested petri dish was incubated at 37°C, for 1-4 days.

### 3. Results and Discussion

Qualitative selection of cellulolytic microbe was aimed to identify isolates, which have cellulase enzyme activity or have not. Colony that can produce cellulase enzyme will be detected by the formation of a clear zone. The results of research showed that a clear zone was seen and existed at selective CMC 1% agar media around the colony of LIPIMC 0575 after incubated for 48 hours. Meanwhile, a clear zone was not seen and existed either around the colony of LIPIMC 0544 nor the LIPIMC 0546 isolates (**Figure 1**).

A clear zone around the colony of LIPI-MC 0575 isolate (**Figure 2**) is measured at the next research step. Cellulase enzyme can hidrolize a cellulose become a glucose. Different isolates can produce different clear zone because of their internal factor , such as gen, and external factors, such as nutrition, incubation time, pH, temperature, and supplementation of metal ion as activator.



**Figure 1.** LIPIMC 0554, 0546 and 0575 strains in selective media of CMC 1%



**Figure 2.** A clear zone of LIPIMC 0575

The ratio of a clear zone diameter of LIPI-MC 0575 isolate with its qualitative colony diameter is expressed as a cellulase index (semi qualitative) (**Table 1**). Enzyme activity depends on the content and structure of different substrates. Carboxy methyl Cellulose (CMC) substrate was much used as a substrate to examine the cellulase activity independently or dependently during cellulose enzymatic hidrolysis [31]. The CMC as a substrate produces enzyme activity that is far different compared with the other substrates, because of CMC is a material that contains a pure cellulose, and does not contain lignin that can inhibit enzyme activity to break down a substrate [32]. One of the qualitative test that commonly used is through staining with congo red solution 0.1%. Congo red solution is a acidic stain that has a negative charge that can not bind with a negative charge in cell wall, cytoplasm and a membran of microorganism cell, so that this stain does not color microorganism, but color the background of preparations (culture media) [33]. pH of congo red solution influences on media color (**Figure 1** and **Figure 2**). The formation of a clear zone around a colony caused by interaction with (1, 4) -  $\beta$  - D glucan and (1, 3)  $\beta$  D-glucan with a cellulose hidrolysis reaction by a fungus strain that produce cellulase enzyme [34]. Determination quantitatively was carried out by measuring index of a colony diameter and a clear zone diameter that were formed [35, 36].

**Table 1.** The results of cellulase activity examination qualitatively and semi qualitatively

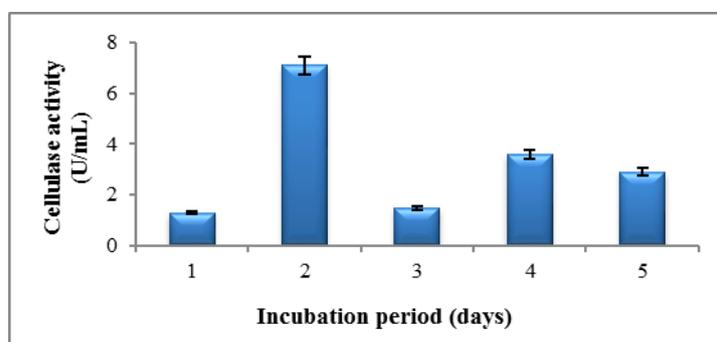
No. of Collection	Source of Isolate	Incubation (h)	Diameter of colony (mm)	Diameter of a clear zone (mm)	Cellulolytic Index
LIPIMC 0544	Air-borne of Library of Research Center For Biology -LIPI Cibinong	48	-	-	-
LIPIMC 0546	Air-borne of Library of Research Center For Biology -LIPI Cibinong	48	-	-	-
LIPIMC 0575	<i>Magnolia condolii</i> Rhizosphere of Taman Nasional Bodogol, Sukabumi Jawa Barat	48	0.8	0.3	2.67

The qualitative testing result was continued with quantitative analysis. Analysis of quantitative is a confirmation and the result of quantitative is not necessarily accurate. Qualitative hidrolysis activity is

a picture of cellulolytic microbe ability to form a clear zone around an isolate that was grown in cellulose-contained media and measured with method of 3,5 dinitro salicylic (DNS) acid by calorimetry technique. It is measuring the reductor sugar content of enzyme hidrolisis yield against substrate [37-41].

Characteristic of microbe cellulose enzyme could be carried out by measuring the optimum condition in several different parameters such as pH, temperature, substrate concentration and the existence of metal ion cofactor as activator or inhibitor [42].

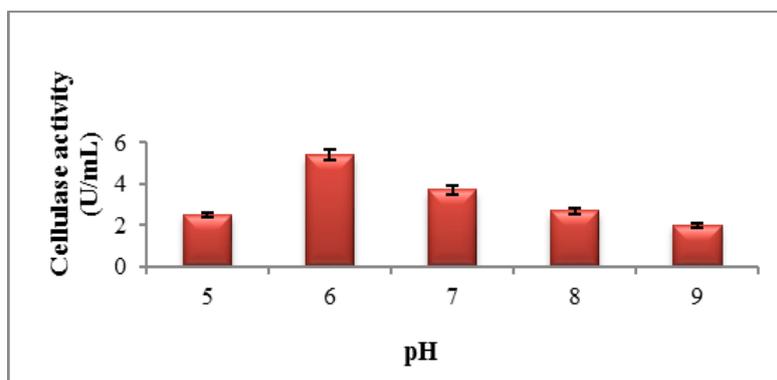
The effect of incubation against cellulase enzyme activity was determined by examining the cellulase activity for 5 days incubation period. Enzyme activity against incubation time of *P. griseofulvum* ranges between 1.3- 7.1 U/mL (**Figure 3**). The optimum time of incubation was at the second day. At this period, the enzyme activity was at its optimum activity (i.e. 7.1 U/mL) compared to the activity at other days of incubation period. At the first day, the cellulase activity was the lowest at 1.3 U/mL. At the third day until the fifth day, the cellulase activity was 1.5 U/mL, 3.6 U/mL and 2.9 U/mL respectively.



**Figure 3.** The effect of incubation time against the cellulase enzyme activity

The optimum incubation time of *P. griseofulvum* is 2 days to produce enzyme with the optimum activity. This incubation time to reach optimum enzyme activity is more faster than the optimum activity of *Penicillium* sp. that was obtained after 7 days of incubation period [43]. According to the result of research of Sharma 2012 [44], the optimum activity of cellulase enzyme could be different when using different isolate though the medium was the same.

The examination against pH was carried out at enzyme from supernatant that incubated at optimum incubation time (i.e. 2 days, **Figure 4**). The supernatant enzyme has a varied activity at different pH. The optimum activity of enzyme was reached at pH 6.0 (i.e. 5.4 U/mL.) Meanwhile, the enzyme activity at pH 5.0, pH 7.0, pH 8.0 and pH 9.0 were 2.5 U/mL, 3.7 U/mL, 2.7 U/mL and 2.0 U/mL respectively.

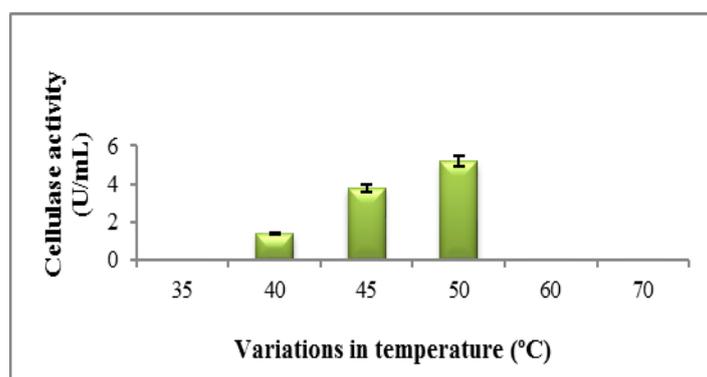


**Figure 4.** The effect of pH against the cellulase enzyme activity

Availability of the concentration change of substrate or environmental pH will result the enzyme activity. Also undergo changes although it can influence the enzyme activity, for example temperature or media composition. Every enzyme has a particular pH and the particular temperature will cause the enzyme activity reaches the optimum condition. Activity of enzyme will reach optimum condition influenced by certain pH and temperature. At the optimum pH, an enzyme will undergo the ionization changes of ionic group on the active side that cause the active side become more effective in binding with substrat [45]. The result of research of Nagah et al [43] showed that pH 5.0 was an optimum pH optimum of *Penicillium* sp. The result of research of Lu et al [46] showed that *Penicillium citrinum* can produce cellulase that tolerant to the alkali condition and resistant to the high temperature so that have a potential to be applied in detergent industry as an additive material. The changes of pH was not only influence the enzyme activity, but can also change the form or the characteristic of charge of its substrate [1].

The optimum condition of pH and temperature will support the enzyme to catalyze the reaction properly. Whereas the poorly temperature and pH will damage the result or protein is not becoming active in the enzym, so the function and activity of the enzyme will be decreased.

The examination against the effect of temperature was carried out at enzyme from supernatant that incubated with optimum time against temperature that is 2 days and at optimum pH 6.0 (**Figure 5**). The enzyme activity started increasing at 40°C (x U/mL). The activity was optimum at 50°C (5.2 U/mL). It was decreasing at a particular point of the increased temperature. It was x U/mL at 60°C and was not detected at 70°C. By this increased temperature, the enzyme was damaged or denaturated.

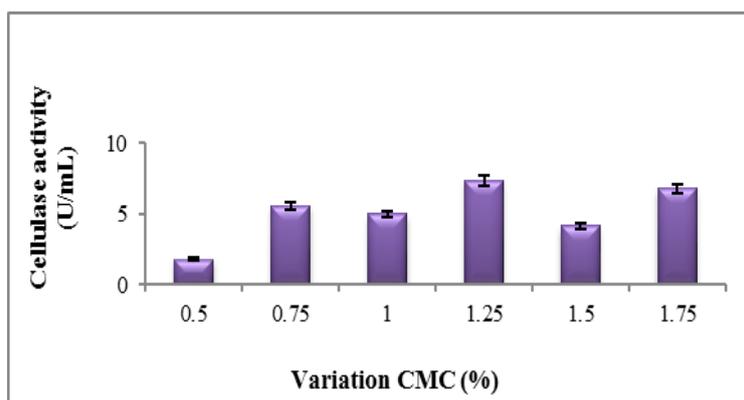


**Figure 5.** The effect of temperature against the cellulase enzyme activity

Temperature greatly influences on the enzyme activity. Every enzyme has optimum activity and stability at a particular temperature. Temperature really influences the growth and enzyme activity of microorganism. The enzyme activity increases in line with an increasing temperature until it reaches the optimal temperature. In this case, the optimum temperature of *P. griseofulvum* was 50°C (Figure 4). At the optimal temperature, the collision between enzyme and substrate is really effective so that the formation of the enzyme-substrate complex is easier and the formed product increases [43]. According to the result of research of Nagah et al [43] the cellulase of *Penicillium* sp. has the optimum temperature at 30°C. The result of research of Martins et al [47] showed that the optimum temperature to produce cellulose depend on a variation of isolate of its microorganism. According to Sethi and Gupta [48] cellulase production from *Penicillium chrysogenum* can be profitable because the level of enzyme production is higher than *Aspergillus niger*. The result of research of Gusakov and Sinitsyn [49] showed that *Penicillium echinulatum* was identified as a producer of potential cellulase at the process of bioconversion against CMC. In general, mesophylic microbe will produce the mesophylic enzyme too [50]. The enzyme activity increases in harmony with increasing temperature until reaches the optimum temperature. The optimum temperature of the enzyme depends on the characteristics of the isolates [51]. The activity of carboxymethyl on cellulase enzyme at low temperature and high temperature could be seen as the enzyme mayor physiologic adaptation [52, 53]. Low temperature or temperature under the optimum temperature will result in enzyme experiencing energy deficiencies that are unable to perform maximum activity. The increasing temperature until

reaches optimum temperature will increase the enzyme activity. After exceed optimum temperature then enzyme will undergo thermal denaturation and changes enzyme structure that cause denaturation so that enzyme activity decrease [54].

Examination against the effect of CMC substrate concentration was carried out at enzyme from supernatant that incubated within its optimum conditions, that are for 2 days incubation, at pH 6.0 and 50°C (**Figure 6**). At variation of CMC substrate concentration, the optimum enzyme activity was 7.4 U/mL with substrate concentration at 1.25 %.



**Figure 6.** The effect of concentration of CMC substrate

Concentration of substrate is one of the factor that can influence enzyme activity. Supplementation of the excess substrate concentration will result in decreasing velocity of enzymatic reaction [43]. This reaction takes place because of abundance CMC and its high viscosity could decrease the probability of substrate to bind with the active side of the enzyme [55].

Fermentation technique was carried out in the solid media fermentation with *Aspergillus niger* and *P. chrysogenum* according to Jayant et al 2011 [8] that incubation time, carbon source and the early pH of fermentation medium were maintained in optimum condition simultaneously. The condition was at pH 5.0 and temperature 40°C as it was the best to increase the cellulase production after incubated for 8 days.

Several species of *Penicillium* have shown the ability to produce the cellulase enzyme, lignocellulolytic and  $\beta$ -glucosidase, endoglucanase, and xylanase [56]. *Penicillium verruculosum* 28K mutant, *P. echinulatum* 9A02S1, *P. chrysogenum* and *P. funiculosum* [57, 58, 8, 59] can produce carboxy methyl cellulase (CMCase), xylanase and  $\beta$ -glucanase by using the submerged fermentation technique and the solid fermentation technique. Cellulase enzyme activity of *P. nalgiovense* SS240 on oil palm cluster substrate is as much 0.027 mol/mL [60].

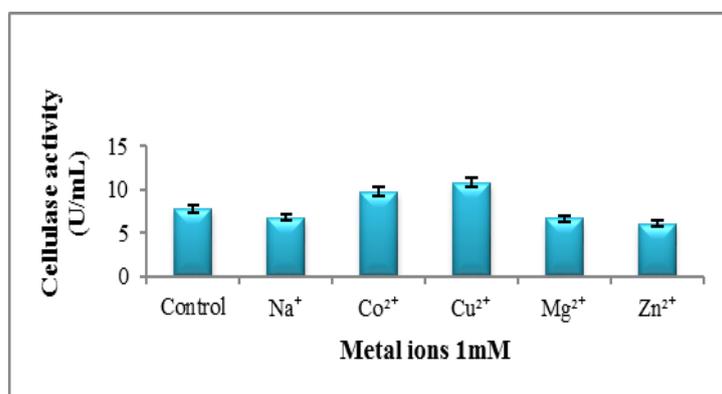
Examination against the effect of metal ion addition as activator was carried out at enzyme from supernatant that incubated within its optimum conditions that were for 2 days incubation, at pH 6.0, 50°C temperature and with 1.25% of substrate concentration (**Figure 6**). There is a particular chemical substance that can increase the enzyme activity (activator) and can inhibit the enzyme activity (inhibitor). LIPIMC 575 strain was activated by divalent cations of  $\text{CoCl}_2$  and  $\text{CuCl}_2$  by 25.64% and 38.46% respectively compared to the negative control (**Figure 6**). Whereas monovalent cation of  $\text{NaCl}$  and divalent cations of  $\text{MgCl}_2$  and  $\text{ZnCl}_2$  have decreased the enzyme activity by 12.82% and 14.10% and 21.79% respectively (**Figure 7**). They have acted as inhibitor. In the recent years, several of results of published research reported on commercially producing cellulase and  $\beta$ -glucosidase from *Aspergillus* sp. and from the genus *Penicillium* [61, 62, 63]. Those enzymes also increase their potential application in producing bio-energy and bio-fuel, in textile, pulp and paper industry [64]. Previous research on utilisation of potential indigenous bioresources diversity has been done to give impact on development of domestic-industry [65].

This fungus of LIPIMC 0575 derived from *Magnolia condolii* rhizosphere of Taman Nasional Bodogol, Sukabumi District, West Jawa. It can produce cellulase enzyme. According to Hanum and Kuswytasari [66], strain of member of the genus *Penicillium* produces high cellulase content, to isolate

virtually all the fibrous substrates investigated have found a *Penicillium* genus mold, including from a moldy woody substrate (obtained from pine wood), leaving garbage, sawdust and vegetable waste in isolating virtually all of the studied wood substrate found in the *Penicillium* genus, among them from the decayed wood substrates (obtained from *Pinus* wood), leaf litter, sawdust and vegetable waste. This indicates that the genus *Penicillium* has good cellulolytic and lignolytic capabilities that can grow directly on woody substrates.

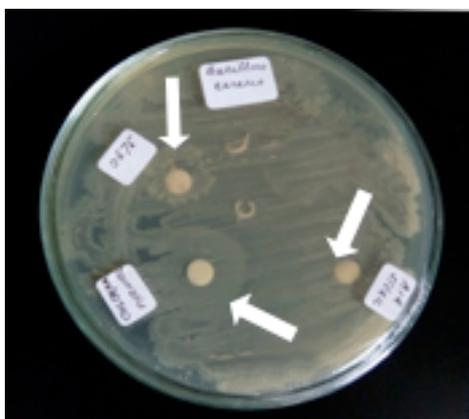
According to Prasanna et al [42], there are several metal ions that could have positive or negative effect against cellulase enzyme activity (**Figure 7**). The positive effect happens when enzyme can interact with metal ion as cofactor (activator) so that the enzyme activity can increase. Whereas the negative effect can decrease the enzyme activity as metal ion is acted as an inhibitor. According to Gandjar et al [67] the phosphate requirement in the fungus growth process is not much explained, but the balance between manganese, zinc, and phosphate is one of the determining factors that can provide the advantage as a cofactor in regulating the amount of enzymes involved in the reaction.

In accordance with the research of Pereira et al [68], that numerous metal ions from monovalent, divalent, and trivalent such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Fe}^{3+}$  were generally examined as inhibitor and activator in cellulase characterization.



**Figure 7.** The effect of metal ion against the enzyme activity

After LIPIMC 0575 strain was identified and known as *P. griseofulvum* with the collection number of InaCC F 14, its cellulase supernatant was further examined whether it has the ability as an antibacteria. Supernatant of cellulase enzyme was examined into media contained *Bacillus cereus* bacterium. **Figure 8** shows the formation of a circle around the disc with cellulase enzyme 0575 and around positive control of Chloramphenicol solution with relative values of 2 mm and 2.33 mm respectively, whereas in the negative control of the sterile water disc does not form circle.



**Figure 8.** Circles around supernatant disc of 0575, chloramphenicol and the negative control of sterile water discs are not formed circle

Circles formed around the paper disc, supernatant cellulase *P. griseofulvum* and around the paper disc with Chloramphenicol solution, it is possible that the enzyme and antibiotic supernatant concentrations are still low so that they are bacteriostatic and possibly if at high concentrations are bactericidal may form a clear zone around paper disc. According to Pelczar and Chan [69] the higher the concentration of extracts of antimicrobial active compounds contained more and more so that the ability to inhibit the growth of microbes is also higher. The cellulase enzyme supernatant of *P. griseofulvum* InaCC F 14 may inhibit bacterial growth and will be used in composting sawdust as one of the mixed ingredients on the medium of white oyster mushroom (*Pleurotus ostreatus* J.) and ear mushroom (*Auricularia auricula* J.).

According to Saskiawan [70] the addition of cellulolytic microbial inoculants can be used in composting rice straw for white oyster mushroom media (*Pleurotus ostreatus* J.) can affect the full grown time and the weight of the oyster mushroom harvest.

#### 4. Conclusion

The result of semi qualitative analysis showed a clear zone around colony of LIPIMC 0575 fungus with cellulolytic index of 2.67. The optimum enzyme activity consecutively with incubation time for 2 days, at pH 6.0, 50°C temperature and with 1.25 % of substrate concentration were 7.1 U/mL, 5.4 U/mL, 5.2 U/mL and 7.4 U/mL. On concentration of 1 mM of cellulase enzyme extracted from LIPIMC 575 fungus, divalent cations of  $\text{CoCl}_2$  and  $\text{CuCl}_2$  have activated and increased the enzyme by 25.64% and 38.46% respectively. Whereas, monovalent cation of NaCl and divalent cations of  $\text{MgCl}_2$  and  $\text{ZnCl}_2$  were acted as inhibitors. They have decreased the enzyme activity to 12.82% and 14.10% and 21.79% respectively. The LIPIMC 0575 strain was identified as *Penicillium griseofulvum* by molecular identification through 16S rDNA sequencing. Its supernatant found to be inhibited the growth of *B. cereus* bacterium. The LIPIMC 0575 is registered in the culture of Microbiology collection with the collection number of InaCC F 14.

#### 5. Recommendation

Necessary further research is needed to apply the cellulase enzyme produced from *P. griseofulvum* InaCC F 14 which has the ability to inhibit bacterial growth used for composting sawdust used as a medium material for planting white oyster mushroom (*Pleurotus ostreatus* J.) and mushroom (*Auricularia auricula* J.) so that the production of mushrooms can be accelerated and abundant.

#### Acknowledgment

This research was funded by DIPA of thematic of Research Center for Biology- Indonesian Institute of Sciences.

#### 6. References

- [1] Robinson PK 2015 Enzymes: Principles and Biotechnological Applications *Essays Biochemistry* **59** 1–41
- [2] Acharya S and Chaudhary A 2012 Bioprospecting thermophiles for cellulase production: a review *Brazilian J. Microbiol* **43**(3) 844–856
- [3] Demain AL and Vaishnav P 2009 Production of recombinant proteins by microbes and higher organisms *Biotechnology Advances* **27** 297–306
- [4] Adrio JL and Demain 2014 Microbial Enzymes: Tools for Biotechnological Processes. *Biomolecules* **4**(1) 117–39
- [5] Ariffin H, Abdullah N, Kalsom MSU, Shirai Y and Hassan MA 2006 Production and Characterization of Cellulase by *Bacillus pumilus* EB3 *Int. J. Engineer. Tech.* **3**(1) 47-53
- [6] Sohail M, Siddiqi R, Ahmad A and Khan SA 2009 Cellulase production from *Aspergillus niger* MS82: effect of temperature and pH *New Biotechnology* **25**(6) 437–41
- [7] Sharada R, Venkateswarlu G, Venkateshwar S and Rao MA 2013 Production of cellulase – A Review *Int. J. Pharma. Chem. Biol. Sci.* **3**(4) 1070-90

- [8] Jayant M, Rashmi J, Shailendra M and Deepesh Y 2011 Production of cellulase by different co-culture of *Aspergillus niger* and *Penicillium chrysogenum* from waste paper, cotton waste and baggase *J. Yeast and Fungal Res* **2**(2) 24-7
- [9] Morana A, Maurelli L, Ionata E, Cara FL and Rossi M 2011 Cellulases from fungi and bacteria and their biotechnological applications In: *Cellulase: Types and Action, Mechanism and Uses* 1-79 Nova Science Publishers, Inc.
- [10] Demers A, Doane R, Guzman S and Pagano R 2012 Enzymatic Hydrolysis of Cellulosic Biomass for the Production of Second Generation Biofuels A Major Qualifying Project Report Submitted to the faculty of Worcester Polytechnic Institute In: *Partial fulfillment of the requirements for the Degree of Bachelor of Science*
- [11] Mtui GYS 2012 Lignocellulolytic enzymes from tropical fungi: Types, substrates and applications *Sci. Res. Essays* **7**(15) 1544-55
- [12] Kim DM, Cho EJ, Kim JW, Lee YW and Chung HJ 2014 Production of cellulases by *Penicillium* sp. in a solid-state fermentation of oil palm empty fruit bunch *African J. Biotechnol* **13**(1) 145-55
- [13] Bianco L and Perrotta G 2015 Methodologies and Perspectives of Proteomics Applied to Filamentous Fungi: From Sample Preparation to Secretome Analysis *Int. J. Mol. Sci.* **16**(3) 5803–29
- [14] Bhat MK 2000 Cellulases and related enzymes in biotechnology *Biotechnol. Adv.* **18**(5) 355-83
- [15] Dienes D 2006 Effect of cellulase enzymes on secondary fiber properties *Ph.D. Thesis* Budapest University of Technology and Economics, Budapest, Hungaria
- [16] Vynios DH, Papaioannou DA, Filos G, Karigiannis G, Tzialis T and Lagios G 2009 Enzymatic production of glucose from waste paper *BioResources* **4**(2) 509-21
- [17] Whitehurst RJ and van Oort M 2010 *Enzymes in Food Technology* Second edition Blackwell Publishing Ltd. A John Wiley & Sons, Ltd., Publication
- [18] Hasan F, Shah AA, Javed S and Hameed A 2010 Review Enzymes used in detergents: Lipases *African J. Biotechnol.* **9**(31) 4836-44
- [19] Kuhad RC, Gupta R and Singh A 2011 Microbial cellulases and their industrial applications *Enzyme Res* Volume 2011 Article ID 280696 10
- [20] Biswas R 2014 Production of Cellulolytic Enzymes In: *Bioprocessing of renewable resources to commodity bioproducts* First Edition Bisaria VS and A Kondo (Eds.) pp 105-32. John Wiley & Sons, Inc.
- [21] Irvan, Prawati P and Trisakti B 2015 Pembuatan bioetanol dari tepung ampas tebu melalui proses hidrolisis termal dan fermentasi: pengaruh pH, jenis ragi dan waktu fermentasi *J. Teknik Kimia* **4**(2) 27-31(in Indonesian)
- [22] Imran M, Anwar Z, Irshad M, Asad MJ and Ashfaq H 2016 Cellulase production from species of fungi and bacteria from agricultural wastes and its utilization in industry: A Review *Adv. Enzyme Res* **4** 44-55
- [23] Sukumaran RK, Singhania RR and Pandey A 2005 Microbial cellulases—production, applications and challenges *J. Sci. Indust. Res.* **64**(11) 832–44
- [24] Zhang Z, Liu JL, La JY, Dua CJ, Ma QS and Feng JX **2014** Predominance of *Trichoderma* and *Penicillium* in cellulolytic aerobic filamentous fungi from subtropical and tropical forests in China, and their use in finding highly efficient  $\beta$ -glucosidase *Biotechnol. Biofuels.* **7** 107
- [25] Padmavathi T, Nandy V and Agarwal P 2012 Optimization of the medium for the production of cellulases by *Aspergillus terreus* and *Mucor plumbeus* *European J. Exp. Biol.* **2** 1161-70
- [26] Jahangeer S, Khan N, Jahangeer S, Sohail M, Shahzad S, Ahmad A and Khan Shakeel A 2005 Screening and Characterization of Fungal cellulases isolated from the native environmental source *Pak. J. Bot.* **37**(3) 739-748
- [27] Miller GL 1959 Use of dinitrosalicylic acid reagent of determination of reducing sugar *Anal. Chem.* **31** 246-48
- [28] Teather RM and Wood PJ 1982 Use of Congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen *Appl. Environ. Microbiol.* **43**(4) 777-80

- [29] Kar S, Mandal A, Mohapatra PK, Mondal KC and Pati BR 2006 Production of cellulase-free xylanase by *Trichoderma reesei* SAF3 *Brazilian J. Microbiol.* **37**(4) 462-64
- [30] Ratnam J, Syed JI, Singh SK 2014 Comparative Study on Production of Penicillin by *Penicillium Notatum* and *Penicillium Chrysogenum* & its Antimicrobial Property *Int. J. of Bio Trends and Techno* **6**(1) 28-30
- [31] Zhou X, Chen H and Li Z 2004 CMCase activity assay as a method for cellulase adsorption analysis *Enzyme and Microbial Technology* **35** 455- 59
- [32] Sonia NMO and Kusnadi J 2015 Isolasi dan Karakterisasi Parsial Enzim Selulase dari Isolat Bakteri OS-16 Asal Padang Pasir Tengger-Bromo *J. Pangan Agro.* **3** (4) 11-9 (in Indonesian)
- [33] Yosmar R, Suharti N and Rasyid R 2013 Isolasi dan Uji Kualitatif Hidrolisat Jamur Penghasil Enzim Selulase dari Tanah Tumpukan Ampas Tebu *J. Farmasi Andalas* **1**(1) 5-12 (in Indonesian)
- [34] Vasana PT 2012 Chapter-1 Isolation of Cellulolytic Bacteria [http://shodhganga.inflibnet.ac.in/bitstream/10603/56215/7/07\\_chapter%201.pdf](http://shodhganga.inflibnet.ac.in/bitstream/10603/56215/7/07_chapter%201.pdf)
- [35] Florencio C, Couri S and Farinas CS 2012 Correlation between Agar Plate Screening and Solid-State Fermentation for the Prediction of Cellulase Production by *Trichoderma* Strains *Enzyme Res* Volume 2012 7
- [36] Yu SS, Latt ZK, Kyaw EP and Lynn TM 2017 Bioconversion of agricultural cellulose waste to valuable product by cellulolytic nitrogen-fixing bacterial isolates *Sky J. Microbiol. Res.* **5**(2) 010–017
- [37] Bintang M 2010 *Biokimia Teknik Penelitian* Penerbit Erlangga (in Indonesian)
- [38] Kamble RD and Jadhav AR 2012 Isolation, identification and screening of potential cellulase-free xylanase producing fungi and its production *African J. Biotechnol* **11**(77) 14175-81
- [39] Gautam SP, Bundela PS, Pandey AK, Jamaluddin, Awasthi MK and Sarsaiya S 2012 Diversity of cellulolytic microbes and the biodegradation of municipal solid waste by a potential strain *Int. J. Microbiol.* Volume 2012 Article ID 325907 12
- [40] Rathore SS, Mannivannan A and Narendhirakannan RT 2014 Screening of cellulase producing microorganisms from lake area containing water hyacinth for enzymatic hydrolysis of cellulose *J. Adv. Sci. Res.* **5**(3) 23-30
- [41] Yao G, Wu R, Kan Q, Gao L, Liu M, Yang P, Du J, Li Z and Qu Y 2016 Production of a high-efficiency cellulase complex via  $\beta$ -glucosidase engineering in *Penicillium oxalicum* *Biotechnology for Biofuels* **9** 78.
- [42] Prasanna HN, Ramanjaneyulu G and Reddy BR 2016 Optimization of cellulase production by *Penicillium* sp. 3 *Biotech* **6** 162
- [43] Nagah M, Abdel-Aziz MS, El-Sherbiny GM, Moghannem SAM and Shawky BT 2016 qualitative and quantitative screening of cellulases from different local egyptian fungal strains middle east *Journal of Applied Sciences* **6**(3) 579-587
- [44] Sharma R 2012 Enzyme Inhibition and Bioapplications *IntechOpen*, DOI: 10.5772/39273 <https://www.intechopen.com/books/enzyme-inhibition-and-bioapplications/enzyme-inhibition-mechanisms-and-scope>
- [45] Soliman SA, El-Zawahry YA and El-Moughith AA 2013 *Fungal Biodegradation of Agro-Industrial Waste* **Chapter 4** 75-100
- [46] Lu W, Li and Wu Y 2003 Influence of water activity and temperature on xylanase biosynthesis in pilot-scale solid-state fermentation by *Aspergillus sulphureus* *Enzyme and Microbial Technol.* **32**(2) 305–11
- [47] Martins LF, Kolling D, Camassola M, Dillon AJP and Ramos LP 2008 Comparison of *Penicillium echinulatum* and *Trichoderma reesei* cellulases in relation to their activity against various cellulosic substrates *Bioresource Technol.* **99**(5) 1417–24
- [48] Sethi S and Gupta S 2014 Optimization of Cultural Parameters for Cellulase Enzyme Production from Fungi *Biolife* **2**(3) 989-96

- [49] Gusakov AV and Sinitsyn AP 2012 Cellulases from *Penicillium* species for producing fuels from biomass *J. Biofuels* **3**(4) 463-77
- [50] Sigres DP and Sutrisno A 2015 Enzim Mananase dan Aplikasi di Bidang Industri: Kajian Pusaka *J. Pangan dan Agroindustri* **3**(3) 899-908
- [51] Feller G and Gerday C 2003 Psychrophilic enzymes: hot topics in cold adaptation *Nature Rev. Microbiol.* **1**(3) 200-08
- [52] Carrard G, Koivula A, Soderlund H and Beguin P 2000 Cellulose-binding domains promote hydrolysis of different sites on crystalline cellulose *Proceedings of the National Academy of Sciences United State of America* **97**(19) 10342-47
- [53] More N, Daniel MR and Petach HH 1995 Research Communication The Effect of low Temperatures on Enzyme Activity *Biochemi. J.* **305** 17-20
- [54] Jørgensen H and Olsson L 2006 Production of cellulases by *Penicillium brasilianum* IBT20888—effect of substrate on hydrolytic performance *Enzyme Microbial Tech.* **38** 381-90
- [55] Smith, H A. 2014. Production of antimicrobials and antioxidants from filamentous fungi. *A thesis* The National University of Ireland
- [56] Solov'eva IV, Okunev ON, Vel'kov VV, Koshelev AV, Bubnova TV, Kondrat'eva EG, Skomarovskii AA and Sinitsyn AP 2005 The selection and properties of *Penicillium verruculosum* mutants with enhanced production of cellulases and xylanases *Mikrobiologiya* **74**(2) 172-178
- [57] Camassola M and Dillon AJP 2007 Production of cellulases and hemicellulases by *Penicillium echinulatum* grown on pretreated sugar cane bagasse and wheat bran in solid-state fermentation *J. Appl. Microbiol.* **103**(6) 2196-204
- [58] Maeda RN, Barcelos CA, Anna LMMS and Pereira JrN 2013 Cellulase production by *Penicillium funiculosum* and its application in the hydrolysis of sugar cane bagasse for second generation ethanol production by fed batch operation *J. Biotechnol.* **163**(1) 38-44
- [59] Nugraha R 2006 Produksi enzim selulase oleh *Penicillium nalgiovense* ss240 pada substrat tandan sawit *Skripsi* Program Studi Biokimia Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Pertanian Bogor, Bogor (in Indonesian)
- [60] Krogh KBR, Morkeberg A, Jorgensen H, Frisvad JC and Olsson L 2004 Screening genus *Penicillium* for producers of cellulolytic and xylanolytic enzymes *Appl. Biochemis. Biotechnol.* **113-16** 389-401
- [61] Sørensen A, Ahring BK, Lübeck M, Ubhayasekera W, Bruno KS, Culley DE and Lübeck PS 2012 Identifying and characterizing the most significant beta-glucosidase of the novel species *Aspergillus saccharolyticus* *Canadian J. Microbiol.* **58**(9) 1035-46
- [62] Liu D, Zhang R, Yang X, Zhang Z, Song S, Miao Y and Shen Q 2012 Characterization of a thermostable  $\beta$ -glucosidase from *Aspergillus fumigatus* Z5, and its functional expression in *Pichia pastoris* X33 *Microbial Cell Factories* **11** 25
- [63] Zhou J, Wang YH, Chu J, Zhuang YP, Zhang SL and Yin P 2008 Identification and purification of the main components of cellulases from a mutant strain of *Trichoderma viride* T 100-14 *Bioresour. Tech.* **99**(15) 6826-33
- [64] Suryanto D 2009 Prospek Keanekaragaman Hayati Mikroba (Microbial Bioprospecting) Sumatera Utara *Pidato Pengukuhan Jabatan Guru Besar Tetap dalam Bidang Mikrobiologi pada Fakultas Matematika dan Ilmu Pengetahuan Alam*, diucapkan di hadapan Rapat Terbuka Universitas Sumatera Utara Gelanggang Mahasiswa, Kampus USU, Universitas Sumatera Utara Medan (in Indonesian)
- [65] Vega K, Sarmiento VR, Ludeña Y, Vera N, Tamariz-Angeles C, Villena GK and Gutiérrez-Correa M 2015 Alkaline Cellulase Production by *Penicillium mallochii* LMB-HP37 Isolated from Soils of a Peruvian Rainforest *British Biotech. J.* **7**(4) 160-8
- [66] Hanum AM and Kuswyasari ND 2014 Laju Dekomposisi Serasah Daun Trembesi (*Samanea saman*) dengan Penambahan Inokulum Kapang *J. Sains dan Seni Pomits* **3**(1) 2337-3520 (in Indonesian)

- [67] Gandjar I, Syamsuridzal and Oetari A 2006 *Mikologi Dasar dan Terapan* Jakarta: Yayasan Obor Indonesia (in Indonesian)
- [68] Pereira J de Cassia, Giese EC, de Souza Moretti MM, dos Santos Gomes AC, Perrone OM, Boscolo M, da Silva R, Gomes E and Martins DAB. 2017 Effect of Metal Ions, Chemical Agents and Organic Compounds on Lignocellulolytic Enzymes Activities **Chapter 6** <http://dx.doi.org/10.5772/65934>
- [69] Pelczar, M and Chan ECS 1986 *Dasar-Dasar Mikrobiologi* Cetakan I Jakarta: UI-Press
- [70] Saskiawan I 2015 Penambahan Inokulan Mikroba Selulolitik pada Pengomposan Jerami Padi untuk Media Tanam Jamur Tiram Putih (*Pleurotus ostreatus J.J.*) *Jurnal Biologi Indonesia* **11**(2) 187-193 (in Indonesian)