Isolation and screening of local cellulolytic fungi from the digestive tract larvae of *Oryctes rhinoceros* L., North Sumatra

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**Abstract.** Cellulose, which is the main component of plant cell walls from higher plants, has been studied from different aspects. It is insoluble in a wide variety of solvents and is resistant to various chemicals treatments. Fungi are a group of cellulose-degrading microbes and plays major role in recycling of lignocellulosic material in nature. This study aimed to obtain cellulolytic fungi from the digestive tract of *Oryctes rhinoceros* L. larvae and to determine cellulolytic activity. Isolation and screening of cellulolytic fungi in the digestive tract of insects were carried out with specific medium Carboxymethyl Cellulose (CMC) and the Congo Red method to obtain potential cellulolytic isolates. Eleven fungal isolates showed positive results as cellulolytic fungi. The highest cellulolytic activity was obtained from isolate F05L with a cellulolytic index of 0.90 and isolate F10L of 0.66. The smallest cellulolytic activity was obtained from isolate F02L with a cellulolytic index of 0.14. All isolates would be identified to the species level and analyzed its potential applications. Our result can provide in addition to the environmental and industrial fields, cellulolytic fungi can a solution to the problem of pollution, namely reducing the amount of cellulose waste, and can be added value to the use of waste into processed organic fertilizers to be able to provide solutions to the problem of organic waste degradation.

1. **Introduction**

Indonesia is one of the largest palm oil-producing countries in the world. Palm oil is one of the main agricultural commodities traded, both for domestic and export industries. Indonesia has an area of 6,735,300 ha of oil palm plantations spread over 22 provinces with palm oil production of 31,070,000 tons per year. As much as 25-26% of total oil palm production is empty fruit bunches (EFB), a fairly large number but its utilization is still limited [1].

Oil palm EFB poses high organic content and composes of several important substances including cellulose (33.25%), hemicellulose (23.24%), lignin (25.83%), water, and other extractive substances (4.19%). The high content of cellulose causes EFB to be a waste that is difficult to degrade. One of the efforts to overcome organic waste is to utilize enzyme-producing microbes to accelerate the degradation process. Most of the enzymes produced in insects come from various microbes in the digestive tract. One type of insect that has a habitat on wood and wood as a food source is the larvae of *O. rhinoceros* L. [2].

*O. rhinoceros* L. larvae are commonly found in EFB of oil palm. They consume the EFB for their growth. This indicates there is symbiotic microbial association in the digestive tract of *O. rhinoceros* L. as reported in cellulose consuming organisms like termites. Research conducted by Tampoebolon
et al., showed that cellulolytic microbes isolated from the digestive tract of *Cryptothermes* sp. have high cellulase activity [3].

Based on the research of Jimenez and Hernandez, as many as 92 isolates of cellulolytic fungi were isolated from the larvae of the rotten wood-eating Coleoptera family in the tropical forest of National Park Costa Rica and most of the fungal isolates belonged to the phylum Ascomycota (89% of the total) [4]. The cellulolytic activity was tested using Carboxymethyl Cellulose (CMC) medium and it was found that 24 isolates of fungi showed cellulolytic activity. Sharma *et al.*, research as many as 5 isolates of cellulolytic fungi (S2F1, S2F2, S3F3, S1F1, S1F2) were isolated from the digestive tract of termites and showed cellulase activity [5]. The cellulolytic activity was tested by Congo Red screening test and fungal isolates with sample code S3F3 (*Aspergillus* sp.) showed the highest hydrolysis zone of 38 mm. Wahyudi *et al.*, reported that 363 fungal isolates were isolated from the buffalo intestine and 326 fungal isolates showed cellulolytic activity with an activity ratio of 5.60 [6]. Based on the background of the above problems, this study aimed to obtain cellulolytic fungi from the digestive tract of *Oryctes rhinoceros* L. larvae and to determine cellulolytic activity.

2. Methodology

2.1. Medium Preparation

The growth medium used in this study was the medium of Carboxymethyl Cellulose (CMC) – Sigma Aldrich. CMC medium 1% (per liter contains 1 g CMC; 0.02 g MgSO₄·7H₂O; 0.075 g KNO₃; 0.05 g K₂HPO₄; 0.002 g FeSO₄·7H₂O; 0.004 g CaCl₂·2H₂O; 0.2 g yeast extract; 1.5 g agar and 0.1 g glucose). These materials were put into an erlenmeyer and stirred evenly, then heated to boiling and after that, they were sterilized using an autoclave at 121°C for 15 minutes [7].

2.2. Preparatory Surgery

A total of 2 larvae of *O. rhinoceros* L. were prepared as a source sample of fungal isolates. The larvae of *O. rhinoceros* L. used were third instar larvae. The first step in surgery was to place a sample of *O. rhinoceros* L. larvae in a paraffin bath in a supine position. Then fixation (stab) the four sides of the larvae using pins so that the preparations are in a secure position and tied. Then cut a little part of the abdomen with a distance of 0.5 cm in front of the anal canal with a sterile tool and the internal organs of the sample are decomposed until the digestive tract is obtained.

The digestive tract was cut and then put into a test tube containing a sterile 0.9% NaCl solution. Then the dilution method was carried out, as much as 1 ml of the suspension was put into a tube containing 9 ml of distilled water (10⁻²) then homogenized using a vortex. Then 1 ml of the suspension at 10⁻² was put into a tube containing distilled water (10⁻³) and so on until 10⁻⁸ [8].

2.3. Isolation of Cellulolytic Fungi

The isolation technique uses the modified method [9]. A total of 0.1 ml of the suspension was spread on a petri dish containing a medium of PDA (Potato Dextrose Agar) - Merck KgaA and incubated at 37°C for 3-5 days. Purification of fungal isolates was carried out by taking colonies that grew separately and showed different morphological characters by inoculating isolates on new agar CMC media with the streak quadrant method to obtain a single colony. Then incubated at 37°C for 3-5 days. Single colonies on petri dishes were then inoculated onto inclined PDA medium as a fungal stock using a loop ose. Then incubated at 37°C for 3-5 days [10].

2.4. Screening of Cellulolytic Fungi

Fungal isolates to be tested for qualitative screening were obtained from PDA agar stock, then fungal isolates were inoculated on a CMC medium using a cockborer. Incubation was carried out at 37°C for 48 hours. Cellulolytic activity testing was carried out using the Congo Red method. Congo Red - Merck KgaA solution (0.1% w/v) was poured into the culture and left for 30 minutes. The solution was then discarded and rinsed with 0.2 M NaCl for 15 minutes three times. This washing aims to
remove Congo Red which is not bound to polysaccharides. Fungal isolates that were able to decompose CMC were indicated by the formation of a clear zone around the colony after being tested by the Congo Red method. The cellulase activity index can be determined by measuring the ratio of the diameter of the clear zone to the diameter of the colony [11].

3. Results and Discussion

Isolation of cellulolytic fungi from the digestive tract of *O. rhinoceros* L. larvae from the PTPN II Sampali, Desa Saentis, Kabupaten Deli Serdang, North Sumatra Province, Indonesia. The isolation results obtained as many as 11 fungal isolates, each of which has different morphological characteristics with isolate codes F01L, F02L, F03L, F04L, F05L, F06L, F07L, F08L, F09L, F10L, and F11L. The results showed that the isolated fungal isolates had a round shape, the edges of the colonies were flat with different mycelium colors between the upper and lower surfaces of the fungal colonies (Table 1).

Table 1. Morphological characteristics of cellulolytic fungus isolates.

| No. | Isolate code | Morphology | Form | Color | Colony edge |
|-----|--------------|------------|------|-------|-------------|
|     |              |            | Upper surface | Lower surface |             |
| 1.  | F01L         | Round      | White and green in the middle | White | Flat        |
| 2.  | F02L         | Round      | White | White | Filamentous |
| 3.  | F03L         | Round      | White | White | Flat        |
| 4.  | F04L         | Round      | White and dark green in the middle | White | Filamentous |
| 5.  | F05L         | Round      | White | Yellow | Flat |
| 6.  | F06L         | Round      | White | White | Flat        |
| 7.  | F07L         | Round      | White | White | Flat        |
| 8.  | F08L         | Round      | White | White | Flat        |
| 9.  | F09L         | Round      | White and dark green in the middle | Dark green | Flat |
| 10. | F10L         | Round      | White | White | Flat        |
| 11. | F11L         | Round      | White | Yellow | Flat |

Figure 1. Fungal mycelium isolated from the digestive tract of *O. rhinoceros* L. on PDA medium. (Left) F02L fungal isolate, (middle) F04L fungal isolate, and (right) F05L fungal isolate.
The characteristics of the 11 fungal isolates obtained were that most of the fungal isolates had white mycelium, two isolates had dark green mycelium in the middle (F04L and F09L) and one isolate had green mycelium (F01L). Some isolates produced spores (F01L, F03L, and F10L) and 8 other isolates did not produce spores (F02L, F04L, F05L, F06L, F07L, F08L, F09L, and F11L). Some isolates had flat colony margins while two isolates had filamentous colony margins (F02L and F04L) (Figure 1). Kokhar et al reported that the results of isolation from soil sources, industrial waste, fruit seeds, vegetables, bread, and wood obtained 17 types of fungal isolates with 8 fungal isolates identified that could degrade cellulose [12]. Cellulolytic microbes (fungi) are microorganisms that produce cellulase enzymes that can hydrolyze cellulose substrates into simpler products and can be found in a very wide habitat, both symbiotically in animals/plants, soil, water, litter, and air [13]. It has been reported [14] in the study isolated fungi from sawdust soil (plantations, beaches, and mud), rotting wood, and leaves reported that 21 isolates of fungi were isolated and 4 isolates had potential cellulolytic activity.

The results of the screening (Figure 2) carried out on 11 fungal isolates isolated from the digestive tract of *O. rhinoceros* L. larvae using CMC medium and given Congo Red solution showed that 11 fungal isolates could degrade cellulose with varying cellulolytic indexes ranging from 0.14 to 0.90. A total of 4 isolates had a high cellulolytic index are F05L (0.90), F10L (0.66), F08L (0.63), and F01L (0.57). The smallest cellulolytic activity showed by isolates F02L (0.14) followed by F06L (0.20) and F03L (0.29).

![Figure 2. Cellulolytic index of fungal isolates from the digestive tract of *O. rhinoceros* L.](image-url)
Cellulolytic fungal isolates with the most potential in degrading cellulose (Figure 3), namely isolates F05L (0.90) and F10L (0.66) showed high cellulolytic ability with the formation of a clear zone around the colony. The clear zone produced by the fungus on CMC medium given Congo Red indicated that the fungus was classified as a cellulolytic fungus or a cellulose-degrading fungus. CMC agar medium is a selective medium for cellulolytic microbial growth because cellulolytic microbes can hydrolyze cellulose as a carbon source. Artiningsih has been reported that qualitatively the enzyme activity of a type of fungus is said to be high if the species has a relatively small diameter of the colony, but produces a relatively large zone diameter [15]. Khokhar et al reported, that the results of cellulolytic fungus screening during the study showed relatively high cellulolytic activity on isolates of Aspergillus, Trichoderma, and Penicillium fungi [12]. Freitas et al also reported that the fungus Trichoderma reesei is one of the main producers of cellulase, and has the highest enzyme activity because its colony diameter is smaller than the diameter of the clear zone, as well as the fungi Aspergillus niger and Ganoderma spp [16].

4. Conclusion
The results of the study, 11 cellulolytic fungi were isolated with different characteristics. The results of screening using CMC medium given Congo Red showed that all isolates had different cellulolytic abilities. The ability of cellulolytic fungal isolate activity was characterized by the formation of a clear zone around the colony. The resulting clear zone indicates that the fungus is classified as a cellulolytic fungus or a cellulose-degrading fungus. Fungal isolate F05L showed the greatest cellulolytic activity with a cellulolytic index of 0.90, followed by isolate F10L with a cellulolytic index of 0.66, while isolate F02L showed the smallest cellulolytic index of 0.14. This study provides a variety of verification of the potential abilities of local cellulolytic fungi from the digestive tract of O. rhinoceros L. larvae that consume lignocellulosic materials and can provide solutions to industrial and environmental problems.

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