Isolation and Morphological Identification of Cellulolytic Fungi from Domestic Waste in South Toapaya Village, Bintan Regency

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Abstract. Domestic waste is one of the unresolved issues. The main challenge is the difficulty in degrading domestic waste due to its lignocellulosic content, which is hard to break down. One of the uses of cellulolytic fungi is identified as organisms capable of breaking down cellulose components in domestic waste and assisting in speeding up the degradation process. The aim of this research is to discover and identify the types of cellulolytic fungi present in household domestic waste in the South Toapaya Village. This research was conducted by collecting samples in South Toapaya Village and analyzing them at the Provincial Marine Service Laboratory of Riau Province from February to August 2023. The research methods included field exploration, laboratory analysis, identification with reference to the literature, as well as microscopic and macroscopic observations. The research results show that out of 14 fungal isolates isolated from domestic waste, 8 of them have significant cellulolytic activity. Among them, the isolate with code UMRCC 03 has the highest cellulolytic index of 3.24 and is identified as the Aspergillus sp. fungus, while the isolate with code UMRCC 05 has a cellulolytic index of 2.14 and is identified as the Trichoderma sp. Fungus.

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1 Introduction

The volume of waste in Bintan Regency from 2000 to 2022 has increased, with figures of 947,769 tons, 1,094,696 tons, and 1,196,438 tons [1]. This data indicates that Bintan Regency generates approximately 850 tons of waste per day. The accumulation of waste is a result of suboptimal waste management. Domestic waste or leaf litter naturally takes around 4 months to decompose [2].

Naturally, there are microorganisms that decompose cellulose in these wastes. In the fungi kingdom, most individuals are saprophytic and can efficiently degrade important polymers such as cellulose and lignin. Using fungi and their byproducts in papermaking and recycled materials can help eliminate a significant source of environmental pollution. Purified cellulolytic fungal enzymes have applications in commercial food processing, such as coffee production, where cellulose hydrolysis occurs during coffee bean drying. They are also widely used in the textile industry and detergents such as modern powder detergents containing fungal enzymes, and even in the fermentation of biomass to produce biofuels. In medicine, fungal cellulases are also used, for example, in the treatment of phytobezoar, a type of cellulose bezoar present in the human stomach [3]. It has also been demonstrated that the use of cellulolytic bacteria improves the compostability of cellulosic waste when the C:N ratio is not optimal and also increases the water retention capacity of the bacteria-inoculated samples [4].

Cellulose from plant residues and other organisms is broken down by microbes into glucose, carbon dioxide, and hydrogen, which are highly beneficial for plant growth. Generally, cellulolytic microbes inhabit soil layers at depths of 0-30 cm and are aerobic [1]. Some bacteria, yeasts, actinomycetes, and fungi can produce cellulase [2]. Fungi with the ability to degrade leaf litter include *Trichoderma*, *Aspergillus*, *Penicillium*, *Gliocladium*, *Paecilomyces*, *Gonatobotryum*, and *Syncephalastrum* [5].

Previous research has shown that *Trichoderma viride* has the highest waste degradation capability, followed by *Aspergillus niger* and *Fusarium oxysporum* [6]. Cellulolytic fungi successfully isolated from household waste can play a role in waste decomposition. Given the importance of cellulose-degrading fungi, this research aims to isolate and morphologically identify cellulolytic fungi from household waste. The study is expected to discover cellulolytic fungal isolates and their identities, making them valuable for waste decomposition purposes.

2 Materials and Methods

2.1 Location and Research Period

This research was conducted from February to August 2023. The sample collection was carried out in South Toapaya Village Bintan.

2.2 Sample Collection

Domestic waste, which would serve as a source of microorganism isolates, was obtained by collecting domestic waste in South Toapaya Village. The collection of domestic waste was done at various randomly selected points and combined in plastic containers. The samples were minced to reduce their size, then blended and ground using a mortar and pestle until fine. The finely ground domestic waste was then weighed at 10 grams and placed in an Erlenmeyer flask containing 90 ml of distilled water. The Erlenmeyer flask was tightly sealed, and the suspension was shaken for 15 minutes.

2.3 Serial Dilution and Fungal Isolation

The dilution used was a serial dilution. A 1 ml portion of the suspension was pipetted into a test tube containing 9 ml of physiological saline solution, resulting in a 1:9 ratio. Serial dilutions used for fungal isolation ranged from 10^{-2} to 10^{-7}.

2.4 Isolation Method

The method used was the Pour Plate method. Each suspension was poured into a Petri dish and Cellulose Congo Red Agar (CCRA) medium was added. Each isolate was given a code based on the dilution series used for that sample. The isolates were then incubated for 72 hours in an incubator at a temperature of 25°C. Subculturing of isolates was done on Carboxy Methyl Cellulose (CMC) medium and incubated for 48 hours at room temperature.

2.5 Qualitative Test

Isolate screening was performed qualitatively for the ability of fungi to degrade cellulose. The clear zones that formed were treated with 0.1% Congo red and then incubated for 15 minutes. After that, they were washed with 0.2 M NaCl and stored in a refrigerator for 24 hours. The measurement of the diameter of the clear zone formed around the fungal colony and the colony diameter was carried out. Selection was based on the ratio of the clear zone to the colony diameter on the CMC medium. Cellulose degradation index (CI) was determined by measuring the clear zone formed around the fungal colony. The ability to degrade cellulose was determined using the cellulose degradation index. The classification of the cellulose degradation index is considered low if the CI value is less than 1, medium if the CI value is between 1 and 2, high if the CI value is greater than 2, and the response is considered to be low if there is no clear zone [7, 8].

Cellulose degradation index (CI) can be calculated using the following formula:

$$\text{The cellulolytic index (CI)} = \frac{\text{Diameter of the clear zone} - \text{Diameter of colony}}{\textit{Diameter of colony}}$$

2.6 Fungi Morphological Identification

Fungi with a high cellulolytic index are further identified, while those with moderate and low indices are not identified. Identification is conducted using pure cultures based on macroscopic and microscopic characteristics. Cellulolytic fungi that have been purified are cultured on Potato Dextrose Agar (PDA) medium for macroscopic identification. Identification follows fungal identification guides such as "Pengenalan Kapang Tropik Umum," "Microfungi on Miscellaneous Substrates," "Introductory Mycology," and "Pictorial Atlas of Soil and Seed Fungi 3rd Edition" [9,10].

3 Result and Discussion

3.1 Isolation of Cellulolytic Fungi

The cellulose degradation test resulted in 14 fungal isolates that were obtained from household waste. The qualitative test was conducted to assess the fungi's ability to degrade cellulose, which is indicated by the presence of clear zones. The clear zones produced by cellulolytic fungi can be observed in Figure 1. CMC (Carboxy Methyl Cellulose) medium is a polymer with a high molecular weight, making it unable to penetrate microorganism cells [11].

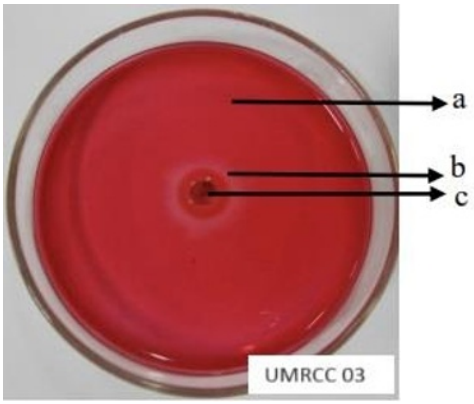

Fig 1. Cellulolytic Fungal Colonies: (a) CMC medium, (b) clear zone, (c) fungal colony

The results of cellulolytic fungal isolation from domestic waste samples are presented in Table 1. Three out of 14 fungal isolates were able to produce cellulase, as indicated by the formation of clear zones with high and medium criteria (Table 1). The clear zones formed around the colonies demonstrate the fungi's ability to degrade cellulose present in the 1% CMC (Carboxy Methyl Cellulose) medium. According to [12.13], the clear zones formed represent the breakage of β-1,4 glycosidic bonds connecting D-glucose monomers in CMC. CMC is an indicator of isolates with the ability to degrade cellulose. The cellulolytic index (CI) varied among the fungal isolates (Table 1). The different cellulolytic indices indicate variations in the ability of each isolate to produce cellulase for hydrolyzing cellulose in the CMC medium [14].

Out of the 3 fungal isolates, only 14,3% met the criteria for a high CI and 1 fungal isolate had medium CI criteria amount 7,14%, and the majority had low CI criteria by 78,56 % (Table 2). Two isolates had high cellulolytic indices, namely, UMRCC 03 and UMRCC 05, with CI values of 3.24 and 2.14, respectively (Table 1). Previous research successfully obtained fungal isolates from paddy fields with CI >3.00, including *Aspergillus niger, Aspergillus sp., Chaetomium murorum, and Trichoderma sp.* [12]. Fungal isolates with cellulolytic indices exceeding 3.00 indicate the highest potential for cellulase production [10]. Differences in cellulolytic indices among the isolates can be attributed to the ability of each fungus to produce cellulase. The fungi's ability to degrade CMC supports mycelial growth because simpler forms of cellulose can be easily hydrolyzed [12].

3.2 Morphological Identification

Two isolates with the highest cellulolytic indices were further identified: the isolate with the code UMRCC 03 (dilution 10^{-6}) and the isolate with the code UMRCC 05 (dilution 10^{-5}). Macroscopic observations revealed that isolate UMRCC 03 had grayish-green colonies, greenish-gray spores, a soft, convex shape, septate and branching hyphae, and conidiophores arising from the foot cell or swollen, thick-walled mycelium carrying sterigmata. Conidia grew in chains and were green in color (Figure 2a and 2b). The isolate UMRCC 03 was identified as Aspergillus sp. This fungus forms fluffy, soft, convex colonies with colors ranging from grayish-green, greenish-brown, black, and white. The color of the colony influences the color of the spores [15].

Aspergillus sp. is a type of eukaryotic fungus in the class Ascomycetes. It is characterized by septate, branching hyphae, conidiophores emerging from the foot cell or swollen, thick-walled mycelium carrying sterigmata, and conidia growing in chains, which can be green, brown, or black. Aspergillus sp. is capable of thriving in media with high acidity and sugar content. This fungus can cause the decay of fruits and vegetables. Research by [10], demonstrated that *Aspergillus sp*. M2P1, when grown on media containing CMC, exhibited the highest cellulase enzyme activity among the tested fungi, enabling it to grow better.

The isolate with the code UMRCC 05 exhibited flat, circular, fibrous, and rough-surfaced colonies with a smooth edge in macroscopic observations. Initially, the colony had a white center, a light green middle, and a clear, dark green circular boundary. The colony's color changed to dark green after 7 days following isolation. In microscopic observations, isolate UMRCC 05 displayed green-colored hyphae, short phialidic stems, and round, greenish conidia growing at the tips, as well as clustered greenish conidia on the conidiophore's surface (Figure 2c). The phialides were approximately 11.3 µm long, and the conidiophore branches were about 13.5 µm long. Many pyramidal-like conidiophore branches were observed, with longer branches below, and phialides were arranged in different groups, with 2-3 phialides in each group. The isolate UMRCC 05 was identified as *Trichoderma sp* (Figure 2c).

Table 1. Cellulolytic Index (CI) of Cellulolytic Fungi from Domestic Waste of Toapaya Selatan Village.

Isolate Code	Diameter (mm)		CI (mm)	Description
	Clear Zone	Colony		
UMRCC 01	22,5	11,7	0,92	Low
UMRCC 02	35,4	18,80	0,88	Low
UMRCC 03	28,00	6,66	3,24	High
UMRCC 04	38,3	11,68	1,28	Medium
UMRCC 05	22,30	7,10	2,14	High
UMRCC 06	15,30	9,20	0,66	Low
UMRCC 07	29,70	14,90	0,99	Low
UMRCC 08	17,60	9,80	0,80	Low
UMRCC 09	29,6	17,9	0,65	Low
UMRCC 10	13,00	7,80	0,67	Low
UMRCC 11	22,50	11,70	0,92	Low
UMRCC 12	28,2	16,11	0,71	Low
UMRCC 13	34,2	17,60	0,94	Low
UMRCC 14	27,60	20,40	0,35	Low

Table 2. The Percentage of Cellulolytic Index (CI) From 14 Isolates of Cellulolytic fungi

Criteria	CI (mm)	Isolate Amount	%
High	>2	2	14.3
Medium	$1 \geq x \leq 2$	1	7.14
Low	< 1	11	78.56

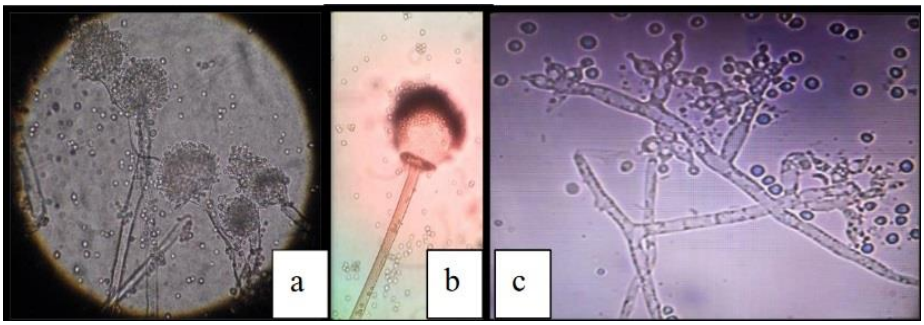

Fig 2. Microscopic morphology of fungi on PDA medium: (a) Genus Aspergillus sp. magnification 100x, (b) Genus Aspergillus sp. magnification 400x, and (c) Genus Trichoderma sp. magnification 400x."

Trichoderma sp. is a soil microorganism that acts as a saprophyte, naturally preying on pathogenic fungi and benefiting plants. *Trichoderma sp*. is commonly found in various types of soil and habitats and is known for its potential as a biological agent for controlling soilborne pathogens. This fungus can proliferate rapidly in the root zones of plants. Species of *Trichoderma sp.* play a role as decomposers and biological agents. Another benefit of *Aspergillus sp.* and *Trichoderma sp.* is their potential use in the processing of livestock feed from solid bioethanol waste [11]. Both fungi exhibit significant cellulolytic activity, which can enhance protein content, energy, dry matter, and the degradation of coarse fiber in solid bioethanol waste, thereby improving the quality of the waste for animal consumption [12,16].

4 Conclusion

A total of 14 isolates were obtained from the isolation of domestic waste in South Toapaya Village, and 2 of them indicated the ability to degrade cellulose in CMC medium, as indicated by the formation of clear zones. Among them, 2 isolates had high cellulose index (CI), namely isolate UMRCC 03 with a CI value of 3.24 and UMRCC 05 with a CI value of 2.14. Isolate UMRCC 03 was identified as *Aspergillus sp.,* while UMRCC 05 was identified as *Trichoderma sp*.

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