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Isolation and identification of cellulolytic fungi under *Swietenia macrophylla*, *Mimusops elengi*, and *Polyalthia longifolia* stands at the Universitas Sumatera Utara campus, Indonesia

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ABSTRACT
The Universitas Sumatera Utara has various types of trees scattered in the surrounding environment. *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* are the three most common tree species found on campus. To maximize the potential for utilizing tree species on the Universitas Sumatera Utara campus, several efforts can be made, one of which is by exploring the presence of cellulolytic fungi. Cellulolytic fungi are fungi that are able to hydrolyze cellulose which can produce cellulase enzymes. This study aims to obtain the potential and identify cellulolytic fungi from the soil under *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* stands. Sampling was taken by making 3 plots on each type of stand measuring 20 m x 20 m with a soil depth of 0 – 20 cm. In each plot, 5 sampling points were made. The soil taken from each type is then composed. Isolation of cellulolytic fungi was carried out using Carboxy Methyl Cellulose (CMC) media. The potenstial of cellulolytic fungi is obtain by calculating the cellulolytic index. All isolates obtained were identified morphologically down to the genus level. The result showed that the potency of the cellulolytic fungi ranged from 0.05 to 1.36. The identification results that the isolates included the genus *Aspergillus* and *Trichoderma*.
Keyword: Cellulolytic Fungi, *Mimusops elengi*, *Polyalthia longifolia*, *Swietenia macrophylla*

1. Introduction

Microbes as components of natural habitats have an important role and function in supporting the implementation of environmentally friendly agriculture through various processes, such as decomposition of organic matter, mineralization of organic compounds, nutrient fixation, nutrient dissolution, nitrification and denitrification [1]. Microbes both in and on the soil surface have the ability to reproduce in a variety of ways. The presence of microbes in the soil can act as soil fertilizer because it produces nutrients in the soil [2].

Fungus is one of the microorganisms that has an important role in the decomposition process of cellulose content in a material. Fungi decompose cellulose by producing cellulose so that cellulose will be broken down into simpler molecules. Cellulolytic fungi can be isolated from materials with high cellulose content. Cellulose is the most abundant glucose in plant bodies; this substance is the main constituent of each cell wall [3].

Cellulolytic fungi are microbes capable of hydrolyzing cellulose which can produce cellulase enzymes. In ecosystems, cellulolytic fungi play an important role in the decomposition of organic matter. The remains of dead organic matter are decomposed into elements that can be returned to the soil (N, P, K, Ca, Mg and others) and atmosphere (CH4 or CO2) as nutrients in the soil that can be reused into the soil plant environment.

Cellulolytic fungi also have the ability to hydrolyze cellulose through their activity. Cellulolytic microbes are capable of producing high cellulase enzyme activity which is important for plants [4].

The Universitas Sumatera Utara (USU) campus area is one of the green open spaces in the urban area of Medan city with an area of 120 ha which provides ecological, social, cultural and aesthetic benefits for campus residents and the surrounding community. There are several areas of green open space spread across the USU campus with different combinations of trees and plants [5]. The stands that dominate the campus area of the USU are *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* [6].

The existence of cellulolytic fungi capable of producing high cellulase activity is very important for the purpose of decomposing organic matter. There is no information regarding of cellulolytic fungi under *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* stands at the USU campus. Therefore, research related to cellulolytic fungi under these three stands is necessary considering that these three species are the most dominant species at the USU campus. This study aims to calculate populations and identify cellulolytic fungi from the soil under stands of *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia.*

2. Method

2.1. Soil Sampling

The location for taking soil samples is in the campus area of the Universitas Sumatera Utara. Soil sampling was carried out under *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* stands. For taking soil samples, 3 plots were made with a size of 20 m x 20 m each. Soil sampling was carried out in a composite manner from 5 sampling points diagonally, with a soil depth of 0-20 cm in each soil sampling hole. Soil samples taken at each point were then composed and placed on labeled plastic.

2.2. Soil Chemical Analysis

Soil chemical analysis is carried out before isolating and identifying the fungus. Chemical analysis aims to determine the amount of soil chemical elements that play a role in soil microbial life. Soil chemical analysis included: soil pH using the pH meter method, C-organic using the Walkley and Black method, available P using the Bray-I method and KTK using the NH4OAc pH 7 extraction method to determine the chemical properties of the soil at the study site [7].

2.3. Isolation of Cellulolytic Fungi

Isolation of cellulolytic fungi was carried out following method [8]: 10 g of soil, put into a 250 ml erlenmeyer volume containing 90 ml of sterile physiological solution, shaken with a shaker for 30 minutes. Dilutions were made with a concentration of 10-3 until 10-5, then 1 ml of the solution from each dilution was pipetted, and poured into a petri dish. Each petri dish was poured with Carboxyl Methyl Cellulose (CMC) medium added with 1% Congo red. The cup is allowed to stand until the media is solid and incubated at 28-30°C in an incubator for 7 days. Observations were made every day, fungi colonies surrounded by clear zones were the desired colonies. Prior to purification, the total fungal population in each petri dish was counted. Furthermore, the fungal colonies were purified by transferring them to new CMC media. The purified fungal isolates were stored for further testing.

2.4. Cellulolytic index calculation

The presence of cellulolytic fungi was indicated by the presence of clear zones formed around cellulolytic fungal colonies grown on Carboxyl Methyl Cellulose (CMC) media with 0.1% congo red. Colonies of cellulolytic fungi obtained were then measured for the diameter of the colony and the diameter of clear zone. After which the cellulolytic index was calculated using the following formula [9]:

$$Cellulolytic\ Index\ (IS) = \frac{Clear\ zone\ diameter - Colony\ diameter}{Colony\ diameter} \quad (1)$$

2.5. Identification of Cellulolytic Fungi

Identification of the fungus was carried out morphologically down to the genus level with macroscopic and microscopic observations. Macroscopic observations observed were the color of the colony, the shape of the colony, and the diameter of the colony. Microscopic observations were conidia or spores, conidia shape, and

color of conidia. Morphological observations were made after the fungi was first grown on Potato Dextrose Agar (PDA) media for 7 days. The results obtained were adjusted to the fungi identification manual [10].

3. Result and Discussion

3.1. Soil Chemical Properties

Soil chemical properties are one indicator to determine the level of fertility of a soil. Soil chemical analysis is important to determine the nature and characteristics of the soil at the research site. The results of the analysis of the chemical properties of the soil under the *Swietenia macrophylla*, *Mimusops elengi,* and *Polyalthia longifolia* stands are presented in Table 1.

Table 1. Results of Soil Chemical Properties Analysis

Stands	pH (H_2O)	C-Organic (%)	P-Available (ppm)	CEC (me/100 g)
Swietenia macrophylla	4.91 ± 0.00	2.55 ± 0.03	4.27 ± 0.16	27.30 ± 0.14
Mimusops elengi	4.57 ± 0.01	2.27 ± 0.02	4.83 ± 0.12	14.11 ± 0.02
Polyalthia longifolia	4.78 ± 0.01	2.23 ± 0.16	5.44 ± 0.07	11.64 ± 0.04

The results of soil pH analysis showed that soil pH values in *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* stands were 4.91, 4.57 and 4.78 respectively. The pH values in the three stands are classified as acidic. An acidic pH indicates that the soil is rich in hydrogen ions. The higher the level of hydrogen ions (H+), the more acidic the soil is. Soil pH describes the availability of nutrients in the soil, indicating the presence of elements that are toxic to plants and affect the development of microorganisms in the soil. At acidic pH, fungi are generally more dominant than bacteria and actinomycetes [11]. The results of [12] research found that the pH of the soil under a *Swietenia macrophylla* stand in Sa'dan Matallo Village, Sa'dan District, North Toraja Regency was 6.18 (including slightly acidic criteria). Differences in the location where it grows and possibly in climate will affect soil pH from one place to another. According to [13], the pH range suitable for the growth of *Swietenia macrophylla* plants is 6.1 to 7.4.

The C-organic value of the soil under the three stands was moderate, namely 2.55% in the *Swietenia macrophylla* stand, 2.27% in the *Mimusops elengi* stand and 2.23% in the *Polyalthia longifolia* stand. Organic matter is needed by microbes as a source of energy and carbon, because most of the microbes in the soil are heterotrophs. The presence of trees in the study area maintains the presence of organic matter in the soil [13]. Meanwhile [12] found that the organic matter content under *Swietenia macrophylla* stands in Sa'dan Matollu Village, North Toraja Regency was 1.40 (classified as low criteria). The organic matter content of a soil is affected by the presence of litter on the soil surface. This will increase microbial activity in decomposing litter, thereby increasing soil organic matter content [13].

Phosphorus is one of the essential macronutrients for plants. Available P values in the soil under *Swietenia macrophylla* stands were 4.27 ppm, *Mimusops elengi* 4.83 ppm and *Polyalthia longifolia* 5.44 ppm. This value is included in the low criteria. The low available P in the soil is caused by low or acidic soil pH levels. The lower the soil pH value, the greater the amount of P bound by soil components so that P is not available for plant soil. Phosphorus uptake by plants is highest at pH 5 to 6 [14]. The available of P on soil under *Swietenia macrophylla* stands in Sa'dan Matollu Village, North Toraja Regency was 17.20 (including medium criteria). The difference in available P values obtained was caused by different soil pH.

The CEC value of the soil for *Swietenia macrophylla* stands was 27.30 me/100 g (high criteria), for *Mimusops elengi* stands was 14.11 me/100 g (low criteria), and for *Polyalthia longifolia* stands 11.64 me/100 g (low criteria). Differences in soil CEC values between stands were caused by differences in organic matter content in each stand. Organic matter and clay are active materials or colloids in the soil which play a role in cation exchange. The higher the organic matter content in the soil, the higher the colloid content in the soil. So that the cation exchange capacity in the soil also high [13].

3.2. Population of Cellulolytic Fungi

The number of populations of cellulolytic fungi illustrates the number of cellulolytic fungi at the soil sampling location, which are capable of assisting the process of decomposing cellulose into minerals and nutrients that can be reused by *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia*. The number of fungus populations has different results in each stand (Table 2). This difference is cause by differences in the content of organic matter and the content of soil nutrients. In addition, it is also caused by differences in root exudates produced by each stand. Plant species is one of the things that affect the amount and type of root

exudate produced by plant [15]. Differences in the amount, type and composition of exudate will affect the population and composition of the microbial community in the soil [16].

Table 2. Population of Cellulolytic Fungi in Each Stand (X 104 CFU/g)

Stands	Plot 1	Plot 2	Plot 3	Mean and StDev
Swietenia macrophylla	33.39	32.49	34.52	33.45 ± 0.83
Mimusops elengi	26.46	17.42	23.73	22.53 ± 3.78
Polyalthia longifolia	26.52	18.96	30.67	25.38 ± 4.84

3.3. Cellulolytic Index Calculation

The degradation power of cellulose was measured based on the resulting cellulolytic index (CI). The cellulolytic index is low if the CI is less than 1, moderate if the CI value is between 1 to 2 and high if the CI value is more than 2 and there is no reaction if there is no clear zone [17]. The results of calculating the cellulolytic index can be seen in Table 3, Table 4 and Table 5. The cellulolytic index obtained range 0.05 to 1.36. The cellulolytic index showed the ability of fungal isolates to produce cellulase enzymes to decompose organic matter. Cellulase enzymes play an important role in decomposing materials containing lignocellulose. The higher the cellulolytic index value, the qualitatively the greater the ability of the isolate to decompose organic matter. Based on Table 3, Table 4 and Table 5, there are differences in the cellulolytic index produce by each stand. This is caused by differences in cellulase enzymes produced by each isolate. So the effectiveness of each isolate is also not the same.

Table 3. Cellulolytic Index of Fungal Isolates on *Swietenia macrophylla* Stands

Isolate Code	Colony Diameter (cm)	Clear Zone Diameter (cm)	Cellulolytic Index	Criteria
SM1	0.90	1.40	0.55	Low
SM2	2.80	3.10	0.11	Low
SM3	0.80	1.30	0.62	Low
SM4	3.25	3.60	0.11	Low
SM5	4.20	4.75	0.13	Low
SM6	4.60	5.20	0.13	Low
SM7	0.95	1.40	0.47	Low
SM8	1.35	1.95	0.44	Low
SM9	3.30	3.60	0.10	Low
SM10	0.85	1.75	1.06	Moderate
SM11	1.70	2.00	0.18	Low
SM12	4.50	5.15	0.15	Low

Table 4. Cellulolytic Index of Fungal Isolates on *Mimusops elengi* Stands

Isolate Code	Colony Diameter (cm)	Clear Zone Diameter (cm)	Cellulolytic Index	Criteria
ME1	2.45	2.90	0.18	Low
ME2	2.20	2.30	0.05	Low
ME3	2.70	2.95	0.09	Low
ME4	3.35	3.55	0.06	Low
ME5	0.60	1.35	1.25	Moderate
ME6	2.30	2.65	0.15	Low
ME7	2.30	2.75	0.20	Low

Table 5. Cellulolytic Index of Fungal Isolates on *Polyalthia longifolia* Stands

Isolate Code	Colony Diameter (cm)	Clear Zone Diameter (cm)	Cellulolytic Index	Krieria
PL1	2.90	3.30	0.14	Low
PL2	3.05	3.30	0.08	Low
PL3	1.15	1.50	0.30	Low
PL4	3.55	3.80	0.07	Low
PL5	0.53	1.25	1.36	Moderate
PL6	1.05	1.30	0.24	Low
PL7	1.40	2.00	0.43	Low
PL8	2.05	2.40	0.17	Low
PL9	1.30	1.95	0.50	Low

Of the 28 isolates tested for potency, only 3 (three) isolates had a medium category cellulolytic index, while 25 isolates included the low criteria. This showed that not all isolates have the same ability to decompose organic matter. Isolates with a cellulolytic index belonging to the medium category are isolates SM10 (isolated from the soil under *Swietenia macrophylla*), ME5 (isolated from the soil under *Mimusops elengi* stand) and PL5 (isolated from the soil under *Polyalthia longifolia*). These three isolates have the potential to be developed further into biological fertilizers as decomposers.

3.4. Identification of Cellulolytic Fungus Isolates

The number of isolates obtained from the *Swietenia macrophylla*, *Mimusops elengi* and *Polyalthia longifolia* stands is presented in Table 6. Overall, there were 28 isolates, namely 12 isolates in the Swietenia macrophylla stand, 7 isolates in the *Mimusops elengi* stand and 9 isolates in the *Polyalthia longifolia* stand. The results of morphological identification obtained 2 genera, namely *Aspergillus* and *Trichoderma*. *Aspergillus* fungi usually have round to semi-spherical conidia heads. As a result of microscopic observation, the *Aspergillus* genus shows the characteristics of a fungus with non-septate hyphae and insulated conidia. Meanwhile, fungi from the genus *Trichoderma* have conidiophores that can branch like pyramids and have oval-shaped conidia [10]. Based on these differences, 27 isolates belong to the genus *Aspergillus* and 1 (one) isolate to the genus *Trichoderma*.

Table 6. Number of Isolates and Genera Obtained From Each Stand

Stands	Genus	Number of Isolates			Total
		Plot 1	Plot 2	Plot 3	
Swietenia macrophylla	*Aspergillus*	4	3	5	12
Mimusops elengi	*Aspergillus*	1	4	2	7
Polyalthia longifolia	*Aspergillus*	2	3	3	9
	Trichoderma	1	-	-	

There were 3 different species of fungi belonging to the *Aspergillus* genus, namely 17 isolates of *Aspergillus* sp1, 9 isolates of Aspergillus sp2, and only one isolate of *Aspergillus* sp3. For the genus *Trichoderma*, only one isolate was found, namely *Trichoderma* sp1. The two genera have a wide distribution because the fungi from the *Aspergillus* and *Trichoderma* genera can live in tropical and subtropical areas. *Aspergillus* is currently known as one of several living things that has the widest and most abundant distribution area in nature, besides that this type of fungus is also a common contaminant on various substrates in tropical and subtropical regions. *Trichoderma* is a genus of asexually reproducing fungi that is most commonly found in tropical to subtropical soils [10]. According to [18,19], *Aspergillus*, *Trichoderma* and *Fusarium* genera are microbes that have the potential to produce cellulase enzymes.

3.5. Aspergillus

Aspergillus is included in the Ascomycetes class which is easy to find in nature, is saprophytic. *Aspergillus* reproduces by forming hyphae and conidiophores which form spores. Colonies are yellowish white to black, colonies in the form of round chains [10]. *Aspergillus* that had reported as cellulolytic fungi are A. *niger*, A. *nidulans*, A. *orizae*, [20,21], A. *fumigatus* [22] and A. *ochraceus* [23]. *Aspergillus* observations can be seen in Figure 1.

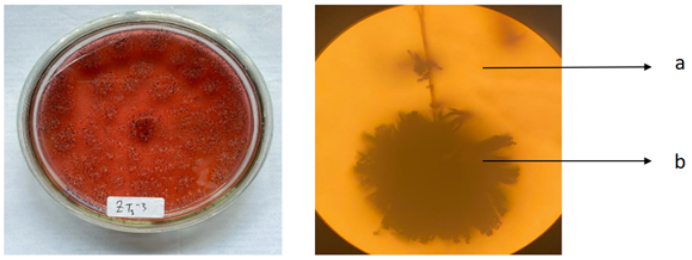

Figure 1. (A) *Aspergillus* sp1 colonies after 3 days old on CMC media (B) Observation of *Aspergillus* sp1 under a microscope (40x magnification) (a) conidiophores (b) conidia

The different types of *Aspergillus* fungi found in this study were distinguished from the macroscopically visible colony color and diameter of the colony, the shape of the conidia and the color of the conidia which were visible microscopically. The general characteristics of the *Aspergillus* fungus can be seen in Table 7.

Table 7. General characteristics of the *Aspergillus* fungus

Genus	Colony color and colony diameter	Conidia shape and color of conidia
Aspergillus sp1	The color of the colony is dark green to black, 5- 6 cm in diameter, the opposite color of the white colony	The heads of the conidia are black, round to semi-spherical in shape
Aspergillus sp2	The color of the colonies is yellow to green, diameter 4-5 cm, the opposite color of the yellow colonies	The heads of the conidia are yellow. The conidia are round to semi-spherical in shape
Aspergillus sp3	The color of the colony is light green to yellow with the diameter of the colony is 5-6 cm, the opposite color is from the yellow to brown colony	The heads of the conidia are green, the conidia are round to semi-spherical in shape

3.6. Trichoderma

Trichoderma fungus can be found in various types of soil. This fungus grows quickly and infects plant roots. The advantage of *Trichoderma* infection is that it can be used as a biocontrol agent that can help inhibit the growth of disease-causing organisms in plants. The *Trichoderma* fungus will develop around the surface and tips of the roots thereby inhibiting contact between plant roots and pathogenic organisms in the soil. *Trichoderma* fungus is a soil saprophytic microorganism that naturally attacks pathogenic fungi and is beneficial to plants [24].

Trichoderma was obtained from the soil under stands of *Polyalthia longifolia*. Based on observations made for 3 days on CMC media, the fungi were round in shape and has a dim green color. The heads of the conidia are round and the conidiophores are branched. *Trichoderma* fungus is characterized by greenish-colored colonies, circular colonies with clear boundaries. *Trichoderma* spp. it has branched conidiophores, short phyllids, and round green conidia [10] (Figure 2).

Figure 2. (A) *Trichoderma* colonies after 3 days old on CMC media (B) Observation of *Trichoderma* under a microscope (a) conidia (b) conidiophores

Cellulolytic fungi have the potential as litter composting inoculums, because fungi obtain nutrients from dead organisms. *Aspergillus* and *Trichoderma* fungi can accelerate litter composting, so the use of cellulase enzyme-producing microorganisms as effective composting agents will improve the composting process and support environmentally friendly technologies [25-27].

4. Conclusion

The isolation results obtained 28 isolates that have the potential to decompose cellulose with a cellulolytic index ranging from 0.55 to 1.36. Based on morphological identification, 27 isolates belonged to the *Aspergillus* genus and 1 isolate belonged to the *Trichoderma* genus.

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