Isolation and identification of fungi associated with natural forest land and post-mining areas of PT. Vale Indonesia: Preliminary study

by Siti Halimah Larekeng

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S W Jufri[1], Iswanto[2], S H Larekeng[2] and A Arif[2]

[1]Student, Graduate School of Faculty of Forestry, Universitas Hasanuddin, Makassar
[2]Lecturer, Faculty of Forestry, Universitas Hasanuddin, Makassar

Email: sitih5h.82@gmail.com

Abstract. This study aimed to determine the types of fungi that can provide information on microbes associated with the post-mining forest land area of PT. Vale Indonesia. This study used a descriptive method by recording and describing the phenomena obtained. Sampling in the field was done by taking 500 g of soil/point (with three replications). Soil samples were taken around the roots or rhizosphere areas with 10 - 30 cm depth in natural and reclamation forest areas. Isolation of microbial growth was conducted by using the dilution technique. The purification of the obtained isolates was carried out by inoculation in PDA media with the point method, then incubated for ± 5 days at 27°C, and their growth was observed. Characterization of microscopic fungi isolates was identified based on the characteristics of the morphological structures. Based on preliminary research results of fungi soil samples originating from reclamation and natural forest areas, 11 rhizosphere fungi isolates were obtained. The fungi colonies' color at the top and bottom had various colors and different textures. Greenish and whitish colors dominated the colony color, but some isolates had white, brown, cream, gray, yellowish, whitish, greenish colors, and some had spots. Colony texture was dominated by velvet, but some isolates had the texture of fine cotton and coarse cotton, presumably *Aspergillus niger, Aspergillus sp, Fusarium oxysporum, Penicillium sp., Trichoderma harzianum*, and *Trichoderma viride*. This research will be continued to ascertain the type and function of each isolate.

1. Introduction

Mining is one of the businesses that has become a controversial issue because apart from being a foreign exchange source for national and regional development, it provides benefits for entrepreneurs and the community. On the other hand, mining also has a negative impact. Mining activities' negative impact is a decrease in post-mining land conditions (tailings) [1]. PT. Vale Indonesia Tbk. is one of the nickel ore mining companies in Indonesia since 1968. One of the exploration areas in the Sulawesi region is Sorowako, East Luwu Regency, South Sulawesi. Mining activities cause post-mining land to have marginal soil characteristics, damaged structure, texture, porosity, soil compaction, lack of essential nutrients such as nitrogen and phosphorus, metal toxicity, soil acidity (low pH), and degradation on the number of species of both flora, fauna and land microorganisms [2].

Soil contamination in post-mining land generally refers to the accumulation of various heavy metals and other related compounds. This soil contamination can cause changes in diversity and number of microbial populations in soil. This critical matter occurs because of soil microorganisms' role in plant growth and development. The rate of microbial degradation to heavy metals depends on several factors

such as microbial activity, nutrients, degree of acidity, and environmental factors. Management includes physically transferring contaminated materials to a location to further handle and direct treatment of contaminants at the contaminated site. The microbes have a role in helping provide a better soil environment to support plant growth. Soil microbes are also actively associated with plants to be more resilient, thus growing in post-mining areas with high metal content. In this case, the microbes prevent plants from absorbing metals by holding metals in the roots; microbes produce certain enzymes that can reduce metal or microbial toxicity and even help plants accumulate metal in larger amounts without getting poisoned.

Soil quality is primarily determined by management level [3]. According to the International Soil Reference and Information Center (ISRIC), 46.4% of land in Asia has been degraded and decreased in productivity and quality, 15.1% have experienced the deterioration of biological function [4]. Through a microbial approach, improvement of soil quality and health can be accelerated [5]. Other supporting aspects such as soil nutrient status must also be adequately observed; thus, recommendations for microbial types can be analyzed to be used for various land uses. The land improvement approach through information on types of microbes is an environmentally friendly concept since it is based on renewable biological resources. Therefore, based on this description, research on the identification of fungi associated with natural forest land and post-mining areas of PT. Vale Indonesia was conducted.

2. Material and methods

The study was conducted for three months from August 2020 to September 2020. Survey activities, field sampling from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village. Laboratory analysis and data processing conducted at the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Universitas Hasanuddin for isolation and identification of microbes.

The culture media used was PDA (potato dextrose agar) isolation process. The tools used in this study were Erlenmeyer flask, pipette, test tube, petri dish, shaker, vortex, autoclave, laminar air flow cabinet, ose needle, cork borer Ø 5 mm, Bunsen burner, oven, beaker, measuring cup, scale, incubator, hoe, refrigerator, cool box.

2.1. Soil Sampling

Sampling in the field was done by taking 500 g of soil/point (with three replications). Soil samples were taken around roots or rhizosphere areas with 10 - 30 cm depth in natural and reclamation forest areas.

2.2. Isolation and Purification

Microbial isolation was performed with a dilution method by making a series of dilutions. The 10^{-1} and 10^{-2} dilutions as well as the medium used were PDA media with the addition of antibiotic modification to grow and isolate the fungi. The incubation process was conducted at room temperature 27°C ± five days, and the growth was observed. The selection of purified microbial colonies was based on differences in the appearance of colony morphology, both in terms of color, elevation, and surface texture, to obtain pure isolates. The purification of fungi isolates was done by removing the fungi using the point method on media.

2.3. Isolate Identification

Fungi identification refers to the key determinant of the mushroom identification book, *Illustrated Genera of Imperfect Fungi* (Barnett and Hunter, 1972). Fungi isolate identified with the descriptive method by recording and describing the phenomena obtained.

3. Results and discussion

The microbes that grow during the isolation process commonly form colonies overlapping one another. Therefore, a purification step is needed to separate each colony according to the macroscopic morphological appearance differences. Based on the purification results, five isolates were found in the natural forest area, and six isolates were detected from the reclamation area.

3.1. Morphological characteristics

The morphological characteristics of the 11 isolates growing on PDA media showed that the color of the fungi colonies at the top and bottom had various colors and different textures. Greenish and whitish colors dominated colony color, but some isolates had white, brown, cream, greenish colors, and some isolates had spots. The colony texture was dominated by coarse cotton, but some isolates had a fine cotton and velvety texture (Table 1).

Table 1. Macroscopic characteristics of growth of fungal isolates.

Isolate	Color of fungi colony		Texture
	Top	Bottom	
ORI 1	White	White	Fine cotton
ORI 2	Dark green, white margin	Dark green, white margin	Velvety
ORI 3	White	White, Cream	Coarse cotton
ORI 4	Yellow at the middle, white margin	Brown at the middle, white margin	Coarse cotton
ORI 5	Dark green	Orange	Velvety
ANOA 1	Cream at the middle, white margin	Cream at the middle, white margin	Coarse cotton
ANOA 2	Yellowish white	Yellow	Fine cotton
ANOA 3	Brownish green at the middle, white margin	Brown at the middle, white margin	Coarse cotton
ANOA 4	Green white	Greenish white	Fine cotton
ANOA 5	Putih, krem	Brown at the middle, white margin	Fine cotton
ANOA 6	White	Creamish white	Fine cotton

Notes: ORI (natural forest area) and ANOA (reclamation area)

3.2. Isolate identification

Observations were done using the descriptive method and the book of *Illustrated Genera Of Imperfect Fungi* [6] as the key determinant to fungi identification. Thus, we could eventually identify the fungi until their genus level.

Table 2. Identification of fungi isolates

Natural forest area		Reclamation area	
Isolate code	Conjectured Genus	Isolate from Mining Area	Conjectured genus
ORI 1	*Fusarium*	ANOA 1	*Aspergillus*
ORI 2	*Penicillium*	ANOA 2	*Trichoderma*
ORI 3	*Fusarium*	ANOA 3	*Gliocladium*
ORI 4	*Aspergillus*	ANOA 4	*Trichoderma*
ORI 5	*Penicillium*	ANOA 5	*Aspergillus*
		ANOA 6	*Fusarium*

Aspergillus is saprophytic conidia and releases many spores in its reproduction process. This observation was in line with the results [7], which stated that the Aspergillus genus has several species characterized by varying mycelium colors (yellowish green, black, and dark green) (Figure 1). The other hands *Aspergillus tamarii* repors suggested that use of pectinases substantially replaces the chemicals usage and will be considered as a suitable bio-agent in the current scenario on green process technology development [8].

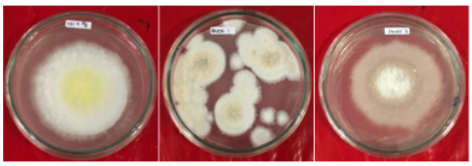

Figure 1. Macroscopic identification of Aspergillus from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village

Trichoderma observed by microscopic observation has many conidiophores and branches resembling a pyramid. This observation is similar to the results by [9] which reported that the genus Trichoderma has a different morphological appearance (Figure 2). Thus, it also has different types (white-dark green, and yellowish white-light green). Fertilizer with enrichment Trichoderma applied in *Capsicum annuum* showed a positive respons for growth and production [10].

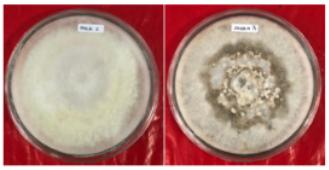

Figure 2. Macroscopic identification of Trichoderma from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village

Fusarium is a genus of filamentous fungi. Its presence is widespread in soil and associated with plants, and its numbers are relatively abundant in the soil microbial community. Spores are oval-shaped (Figure 3). Fusarium fungus has white mycelium at its first formation, and the abundant number of spores that cause it will have cream or orange in color [7]. The function of Fusarium on plant roots has been carried out so many times that it needs to be optimized for its use [11–13].

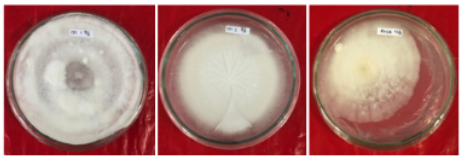

Figure 3. Macroscopic identification of Fusarium from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village

Penicillium is grouped in the Deuteromycetes class. Based on identification, the macroscopic form of Penicillium colonies is a fast growth, flat, stringy, and velvety, wool or cotton-like textures (Figure 4). Several new Penicilliums were discovered using morphological and molecular identification approaches [14,15].

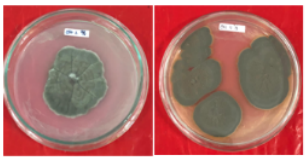

Figure 4. Macroscopic identification of Penicillium from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village

Gliocladium is a soil fungus with a saprophytic character. The colony color varies from white, pink to blackish gray produced by large amounts of phialides (Figure 5). This observation was in line with the results [16] under mahoni stand. The endophytic fungus *Gliocladium cibotii* is able to regulate metabolic and antioxidant formation systems in soybeans and sunflowers under heat stress [17].

Figure 5. Macroscopic identification of Gliocladium from area in nickel post-mining land owned by Vale Indonesia in Sorowako Village

Applications of beneficial microbes are useful toward plant growth-promoting strategies. Accordingly, we are optimistic that it can play potential roles in supporting plant growth in practical applications. This research provides important information relevant to the application of beneficial fungi to promote plant growth. This will have critical implications for the development of agriculture, industry, restoration ecology and forestry going forward.

4. Conclusion

Based on the preliminary research results, it can be concluded that isolation results have obtained 11 pure isolates of the fungi, presumably associated with genus types in natural forest areas (*Aspergillus, Fusarium, Penicillium*), and reclamation areas of PT. Vale Indonesia (*Aspergillus, Fusarium, Penicillium, Trichoderma, Gliocladium*). This research will be continued to ascertain the type and function of each isolate.

Acknowledgement

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