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EXPLORATION OF ENDOMYCORRHIZAL FUNGUS IN AREAS CONTAMINATED WITH HEAVY METAL

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ABSTRACT

Environmental researchers have focused themselves on the use of an endomycorrhizal fungus that is able to adapt and are resistant to environments contaminated with heavy metals for Phytorhizoremediation activities. The aim of the study was to explore Endomycorrhizal fungus from areas contaminated with heavy metals to be used as starter biological agents in the phytorhizoremediation program. This research was carried out in two phases, i.e., rhizosphere sampling of *Polypodium glycyrrhiza*, *Sumasang* sp (local name) and *Spathoglottis plicata* at Sumasang I. Rhizosphere of *Chromolaena odorata*, *Melastama affine* and *Nephrolepis exaltata* at Sumasang II, Sorowako, Indonesia; While the other phase is isolating and identifying Endomycorrhizal spores in the Microbiology Laboratory, Research and Development Center for Environment and Forestry in Makassar, Indonesia. The results showed that three genera of endomycoriza were able to adapt and resistant in areas contaminated with Fe, Mn, Cr, Co, and Cu, i.e., 60.15% *Acaulospora* sp; 26.98% *Gigaspora* sp and 11.21% *Glomus* sp, which can be used as symbionts for endemic plants in the phytorhizore mediation program which is faster, cheaper and easier.

Keywords: Endomycorrhizal; fungus; heavy metal; mutualistic symbiosis.

INTRODUCTION

The concentration of heavy metals that exceed the threshold becomes a source of pollutants in post-mining land. Heavy metal pollution is very harmful to the environment [1–3]. Heavy metal pollution has occurred in post-mining areas of gold [4], nickel [5], tin [6], and coal [7]. High concentrations of heavy metals will inhibit growth [8], change morphology [9], and disrupts an organism's metabolism [10]. However, each type of organism also has a defense strategy against heavy metal pressure to carry out the adaptation process [11]. Phytoremediation, the plant-mediated reclamation of polluted soils, is receiving increasing attention because of its lower costs in comparison to more traditional approaches, its consensus in public opinion, and the possibility to restore the biological features of the soil and especially the microbial soil community [12].

Heavy metals can cause changes in the community of microorganisms, so microorganisms are more resistant to pressure from heavy metals [13], essential and non-essential heavy metals show toxicity if they are above a certain concentration [14]. This stress toxicity is limited by the threshold value [15], which varies depending on many factors, including the type of microorganism, physicochemical properties, and metal concentration, and soil conditions (edafic) and the environment [11].

An endomycorrhizal fungus is one of the obligatory soil microorganisms. These fungi have ability mutualistic symbiosis with 80% of plant species [16]. However, very determined by the type of endomycorrhizal fungus, plant species, and environmental conditions [17]. Endomycorrhizal fungus, which has extensive adaptability, will have the ability to survive in a variety of environmental conditions, especially in soils contaminated with heavy metals [18]. However, the tolerance level of heavy metals varies between various groups of fungus [19]. Several strains of endomycorrhizal fungus can tolerate heavy metal stresses, including, *Glomus intraradices*, *Glomus mosseae,* and several other important *Glomus species*. Therefore, the selection of endomycorrhizal fungus that is tolerant of heavy metals is an important step in obtaining healthy plants in areas contaminated with heavy metals [20].

Evaluating the tolerance level of microorganisms in heavy metal contaminated soil, experts have adopted the concept of community tolerance caused by pollutants [21]. This perspective stipulates that over time, in an ecosystem, microorganism communities exposed to heavy metal contaminants will increase tolerance [22]. Tiwari and Lata [23] also argued that in a long time, heavy metal exposure would put pressure on soil microorganisms and increase tolerance. Long exposure to heavy metals will provide an opportunity for microorganism communities to adapt. This adaptation has been linked to two factors, namely, the availability of metals that decrease gradually due to the immobilization reaction in the rhizosphere region, while the second factor is the structure of microbial communities changing gradually, based on changes in the profile of fatty acid phospholipids [24] which results in more tolerant organisms. Apart from these two things, Endomycorrhizal fungi such as organisms accumulate heavy metals in vesicles, hyphae, and arbuscular fungi [25].

Native endomycorrhizal fungus isolate that has been adapted to environments that have abiotic stress can be potential biotechnology tools to be inoculated in plants for the successful restoration of degraded ecosystems. So that an activity is needed that aims to explore indigenous endomycorrhizal fungus from areas contaminated with heavy metals to be used as a starter for biological agents.

METHODOLOGY

The study was conducted in two phases. The first phase, the taking rhizosphere of *Polypodium glycyrrhiza*, Sumasang sp (local name) and *Spathoglottis plicata*, *Chromolaena odorata, Melastama affine,* and *Nephrolepis exaltata* at areas contaminated with heavy metal, Sorowako, South Sulawesi, Indonesia, use method from Krishnamoorthy [26] and Toh et al. [27]. Another phase, endomycorrhizal fungus spores in isolated from host plant of rhizosphere followed wet sieving techniques [28] using multilevel sieves (mesh size of 325, 40, and 50 µm) in Microbiology Laboratory, Center for Research and Development for Environment and Forestry, Makassar, Indonesia.

The concentration of heavy soil metals was measured while in the laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using a manual book of X-Ray Florence Spectrophotometer/Bruker/S2 Ranger, Heavy metal concentration in the soil can be seen in Table 1.

Table 1. Concentration of Fe, Mn, Cr, Co and Cu in Land Sumasang, Sorowako, South Sulawesi, Indonesia

Heavy metal	Location		Critical limit	
(ppm)	A	B	Soil	Plant
Iron (Fe)	45.463	92.036	3000[e]	20[b]-50[a]
Manganase (Mn)	41.334	10.739	2000[d]	200[b]
Chrome (Cr)	26.458	38.754	60[d]-3950[c]	1.5[b]
Cobal (Co)	1.578	3.005	50[d]	100[a]
Copper (Cu)	87.3	221	100[d]	10[b]-50[a]

Note: [29][a], [30][b], [31][c], [32][d]

RESULTS AND DISCUSSION

Laboratory test results show that post-mining land has been contaminated with Fe, Mn, Cr, Co, and Cu metals which exceed the critical limit for soil and plants (Table 1), this will give stress to macroorganisms and soil microorganisms to complete their life cycle, but some organisms are able to adapt and tolerant to the environment contaminated with the metal.

A series of "classical" ecological principles that examine the process of increasing tolerance or resistance of the microorganism community have been widely studied. The microorganism resistance refers to the ability to resist the effects of pollutants, which are usually effective against them, while microorganism resistance refers to the ability to adapt to persistent pollutants [33]. Tiwari and Lata [23] suggest that tolerance and resistance to the toxic effects of heavy metals depend on the mechanisms involved. In short, tolerance to heavy metals can be defined as a phenomenon where resistance microorganisms increase to again heavy metal stress, which can lead to poisoning.

All organisms, including microorganisms, can achieve resistance to heavy metals by "avoidance" when organisms are able to limit metal absorption, or by "tolerance" when organisms survive in high internal metal concentration [34,35]. The first mechanism involved is reduced absorption or increase in efflux, formation, and release of organic acids outside the cell complex. The second mechanism, metal is chelated intracellularly through the synthesis of ligands such as metallothionein, polyphosphate, and/or compartments in vacuoles [36,37]. Individuals who are tolerant and sensitive to heavy metals can be distinguished by their growth performance on substrates contaminated with heavy metals [38], [37].

Gherghel & Krause [12] dealing with the diversity and functional strategies of endomycorrhizal communities and population on metal-contaminated forest sites in trees established on heavy metal-contaminated areas in different stages of succession. New knowledge gained from investigation of the endomycorrhizal community in a former uranium mining area and an undisturbed site is included. In addition, molecular biological investigation of the endomycorrhizal fungus Tricholoma vaccinum demonstrates changed gene expression profiles after contact with heavy metals [39].

Identification result of native endomycorrhizal fungus spore obtained from various rhizosphere of host plants inland contaminated with heavy metals was found three genera of endomycorrhizal fungus spore that were adaptable and resistant in the land with high concentrations of heavy metals, i.e., *Acaulospora* sp, *Gigaspora* sp, and *Glomus* sp (Fig. 1). Adaptability and resistance of fungus, possibly following the mechanism of metal-chelating processes intracellularly through the synthesis of ligands such as metallothionein, polyphosphate, and / or compartments in vacuoles, so that become tolerant.

Glomus sp. is the genus endomycorrhizal from family *Glomeraceae*. Some of the characteristics of this genus are spores formed singly or in pairs in the terminals of the nongamethangium hyphae, which are undifferentiated in the sporocarp. When the adult spores are separated from the adhesive hyphae by a partition, spores are globose, sub-globose, ovoid, or obovoid with spore walls consisting of more than one layer, hyaline to yellow, brownish, brown, and black, measuring between 20 - 400 µm [40,41].

Acaulospora sp. is the genus endomycorrhizal which belongs to the family of *Acaulosporaceae*. This genus has several characteristics, including having 2-3 spore walls, spores are formed on the side of the sporiferous saccule neck, shaped spores of globose up to ellipse, colored hyaline, yellow, or yellow-red, measuring between 100-400 µm [40,42].

Gigasporaceae is a family of mycorrhiza, which belongs to the genus *Gigaspora* sp. This genus has a characteristic, that is, spores are produced singly in the soil, spore does not have an inner layer wall, be found of bulbous suspensor, shaped of globose or sub-globose, colored of cream to yellow, size spore 125-600 µm [40,43].

Germination and formation process of endomycorrhizal fungus spore, through 3 stages, i.e., (1) germination of spores in the soil, (2) penetration of hyphae into root cells and (3) development of hyphae in the root cortex [44] and these third of stages possibility also be carried out by endomycorrhizal fungus in the land contaminated heavy metals in to multiply themselves. The calculation results of the number of endomycorrhizal fungus spores in 100 g of heavy metals contaminated rhizosphere found in different amounts and dominated by *Acaulospora* sp. (Table 2). This allegedly due to heavy metal stresses, also influenced by other abiotic factors. According to Jamiołkowska et al. [45] that abiotic factors that determine the abundance and development of endomycorrhizal fungus spores, among other temperature, pH, soil organic matter, and soil water content.

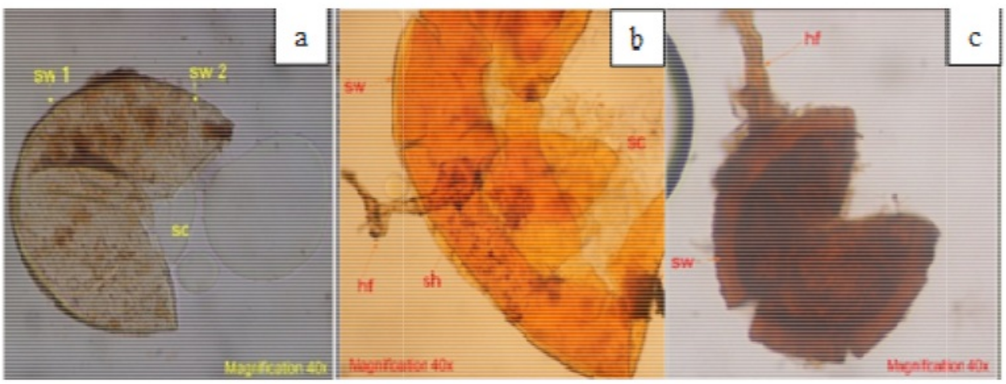

Fig. 1. Morphology of native endomycorrhizal fungus spores isolated from land contaminated Fe, Mn, Cr, Co, and Cu. (a) *Acaulospora* sp., (b) *Gigaspora* sp., and (c) *Glomus* sp.

Table 2. Number of endomycorrhizal fungus spores per 100 g of Rhizosphere samples before propagation activities

Family	Hots plant rhizosphere	Number of spora		
		GLS	GSS	ACS
Polypodiaceae	*Polypodium glycyrrhiza*	2	-	7
-	*Sumasang sp* (local name)	2	2	8
Orchidaceae	*Spathoglottis plicata*	-	1	1
Asteraceae	*Chromolaena odorata*	-	-	24
Melastomataceae	*Melastama affine*	-	1	1
Nephrolepidaceae	*Nephrolepis exaltata*	-	-	13

Note: GLS, *Glomus* sp; GSS, *Gigaspora* sp; ACS, *Acaulospora* sp

Optimum soil temperature for germination of endomycorrhizal fungus spores is very diverse and depends on the type. The best temperature for the development of endomycorrhizal fungus is at an ambient temperature of 30°C, but for the best colonization of mycelia is at ambient temperature 28°C - 35°C [18]. *Gigaspora* sp can grow and germinate well at ambient temperatures of 25°C - 35°C, while *Glomus mosseae* originates from cooler regions. The best germination is an ambient temperature of 18°C - 20°C. Some scientific literature also suggests that colonization of plant roots by endomycorrhizal fungus still occurs at soil temperatures as low as 5°C [46].

The fungus is generally more resistant to changes in soil pH. However, the adaptability of each species of Endomycorrhizal fungus to soil pH varies greatly. Soil pH can also affect germination, development, and the role of mycorrhiza to plant growth. The optimum pH for the development of endomycorrhizal fungus is ranging from pH 5.6-7 for *Glomus* sp. pH 4-6 for *Gigaspora* sp. pH 4-5 for *Acaulospora* sp, [47]. According to Bertham [48], *Glomus mosseae,* usually in alkaline soils, can germinate well at pH 6-9. *Gigaspora corallodea* and *G. heterogama* of more acidic species can germinate well at pH 4-6.

Soil organic matters also play a role in increasing the number of endomycorrhizal fungus spores. The maximum number of spores found in soils containing organic material from 1 to 2 percent, while in soils containing organic matter is less than 0.5 percent number of spores found is very low [49].

Groundwater content also affects germination and the period of dormancy of endomycorrhizal fungal spores. In wet soil, the dormancy period for *Glomus* sp and *Gigaspora* sp spores is longer than in dry soil. Whereas for *Acaulospora* sp spores, the period of dormancy is generally not affected by soil water content [50,51].

An endomycorrhizal fungus is very important in the heavy metal phytostabilization program that exceeds the critical limit. Plants that have been symbiosis with endomycorrhizal fungus accumulate and store heavy metals in vesicles and hyphae of fungus on their roots, so that metal pollutants do not move and do not inhibit the growth and absorption of phosphorus and other micronutrients [20,52]. Endomycorrhizal fungus also releases various organic acids, which increase the solubility of phosphate compounds, which are insoluble in soil [53,54]. Endomycorrhizal fungus release glomalin, which is a particular sorble metal glycoprotein that increases the immobilization of toxic metals [55]. The metallothionein protein released by a certain endomycorrhizal fungus also reduces the toxicity of heavy metals in the soil [20]. Certain external endomycorrhizal myceliums also produce a type of protein called glycoprotein (Glomalin), which has a heavy metal-binding area [56,57].

Several reports and reviews show that endomycorrhizal from areas contaminated with heavy metals has developed tolerance to heavy metal toxicity and adapted well. Endomycorrhizal has been proven to evolve tolerance to heavy metals, as stated by Lingua et al. [58] and [59], some strains of Endomycorrhizal fungus tolerant develop in one or two years. However, to date, the potential interaction mechanism between endomycorrhizal fungus and heavy metal, as well as cellular and molecular mechanisms about the tolerance of heavy metals by an endomycorrhizal fungus, is still poorly understood because endomycorrhizal fungus cannot be cultivated without a host plant, so more difficult to showed absorption of metals intrinsically by hyphae.

Isolate of native endomycorrhizal fungus in an area contaminated with heavy metals is more tolerant than isolate from the non-polluted area, and it has been reported that native endomycorrhizal fungus efficiently affects plant root growth in heavy metal stressed environments [60,16]. Thus, it is important to sieve native isolate that tolerant of heavy metal to ensure the effectiveness of symbiosis between an endomycorrhizal fungus and plant roots at area recovery programs contaminated heavy metal. Furthermore, it suggested that phytoremediation potential for the contaminated areas can be increased by inoculated of hyperaccumulator plant roots with endomycorrhizal fungus, which is most suitable for contaminated sites. Therefore, very important for combining endemic plants with an isolate of native Endomycorrhizal fungus that adjusted for the type and concentration of heavy metals in future studies for the Phyto-rhizoremediation program.

CONCLUSION

The found three genera of native endomycorrhizal fungus that able to adapt in the area contaminated Fe, Mn, Cr, Co, and Cu, which could be used as a source of inoculum in phytorhizoremediation program combined with plant endemic locations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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