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Identification and Abundance of Indigenous Endomycorrhiza Isolated from Nickel Post-Mining Plantation in Sorowako

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ABSTRACT

Endomycorrhiza or arbuscular mycorrhiza has received tremendous attention due toits ability to form a mutualistic symbiotic with 80-96% of plant species. Therefore, the objectives of this study were to identify and to determine the abundance of indigenous endomycorrhiza spores isolated from the nickel post-mining plantation area in Sorowako. Quantitative-qualitative research was conducted in two stages, which were; (1) Rhizosphere sampling of *Morus alba* and *Cyperus rotundus* at nickel post-mining in Sumasang 1 area, rhizosphere of *Wedelia trilobutu* and *Melostoma affine* in Sumasang 2 block A area, rhizosphere of *Gleichenia linearis* and *Equisetum gigatum* in Sumasang 2 block B area, Sorowako village, East Luwu Regency, South Sulawesi Province, Indonesia; (2) Isolation and identification of spores *Glomus* sp., *Acaulospora* sp., and *Gigasporas* sp. were performed at the Microbiology Laboratory, Research and Development Center for Environment and Forestry Makassar, South Sulawesi.Based on the results, it had been found 41.6-63.6% *Acaulospora* sp. spores with two layers of spore wall and average diameter of 71.6µm; 26.6-50.0% *Gigaspora* sp. spores with one layer of spore wall and average diameter of 232.7µm, and 8.3-13.3% *Glomus* sp. spores with hypha remnant to spore wall and average diameter of 85µm in Sumasang nickel post-mining plantation area.

Introduction

Plantation area of nickel (Ni) post-mining conducted by Vale Indonesia in Sorowakohas reached 3,975.9 ha in 2014 and 3,985.5 ha in 2015 with the total accumulation of planted trees was 1,859,107 trees (Vale, 2015) which consists of several types of plants namely *Paraserianthes falcataria*, *Eucalytus urograndis*, *Enterolobium macrocarpum*, *Melochia umbellata*, *Sandoricum kacappeae*, *Elmerelia* sp, *Ceiba pentandra*, *Acacia mangium*, *Mangifera indica*, *Adenanthera speciosa*, *Aleuritus molluccana*, *Cassia siamea* and *Casuarina* sp with average growth reached 97% (Abubakar, 2009).

High percentage of plant growth can not be separated from the support of microorganisms symbiosis with plant roots, one of which is endomycorrhiza or arbuscular mycorrhiza (AM). Recently, AM has gained tremendous attention due to its ability to form a mutualistic symbiosis with 80% - 96% of plant species although the effectiveness of its capabilities differs for each species (Wu et al., 2011; Prayudyaningsih and Sari, 2016).

A species of AMcan be symbiotic with a variety of plant species, *vice versa*, a plant species can be symbiotic with a variety of AM species (Tesitelova et al., 2013).

The symbiotic relationship between AM and plant root benefits to both, AM gains carbohydrates and energy from plant, while plant benefits from increased nutrient uptake especially P (Mau and Utami, 2014; Hosseini and Gharaghani, 2015), water level improvement and plant defence against drought stress (Sowmen et al., 2014; Augé et al., 2015), heavy metal stress (Bano and Ashfaq, 2013; Emamverdian et al., 2015), salinity stress (Yu et al., 2012; Fileccia et al., 2017), pathogenic diseases (Hemavani and Thippeswamy, 2014; Fauziyah et al., 2017), puddles (Marin's and Carrenho, 2017), and soil structure and aggregate improvement (Kimura and Scotti, 2016 ; Zhang et al., 2017). The role is strongly related to the formation of AM structures both in the roots and outside of plant roots (Smith and Smith, 2011; Smith et al., 2011).

Setiadi and Setiawan (2011) showed that *Glomus* sp. spores can be found in therhizospheres of *Stemonurus celebicus*, *Calophyllum* sp., *Vitex coffasus*, *Weimannia blumei*, *Dillenia serrata*, *Eucalyptus urograndis*, *Ficus* sp., *Syzygium* sp., *Tectona grandis*, *Cassia siamea*, *Casuarina equisetifolia*, *Alstonia spectabilis*, *Maranthes corymbosa*, *Filicium decipiens*, *Malotus* sp., *Palaquium obovatum*, *Adenanthera pavonia*, *Paraserianthes falcataria*, *Enterolobium macrocarpum* and *Elmerillia tsiampacca*. Spores of *Acaulospora* sp. can be found in the rhizospheres of *Vitex coffasus*, *Dillenia serrata*, *Eucalyptus urograndis*, *Cassia siamea*, *Casuarina equisetifolia*, *Malotus* sp., *Paraserianthes falcataria*, *Enterolobium macrocarpum* and *Elmerillia tsiampacca*; and spores of *Gigaspora* sp. are found in the rhizospheres of *Ficus* sp., *Alstonia spectabilis*, *Eucalyptus urograndis*, *Cassia siamea*, *Casuarina equisetifolia*, *Paraserianthes falcataria* and *Enterolobium macrocarpum* which grown in Ni post-mining in Sorowako. Furthermore, Setiadi and Setiawan (2011) showed that the average spore density of rhizosphere per 50 g samples from the nickel post-mining plantation area with dusty clay structurein the Fiona block is 253 spores, 122 spores in the Konde block, 50 spores in Deby block, 234 spores in the Petea block, and 209 spores in the Manggali block, whereas in the Nursery block with its sandy clay structure, it has been found 46 spores. However, this information does not indicate the abundance of AM spores of each genus.

In the gold post-mining location in West Lombok, Prasetyo et al. (2010) reported that few spores were found in tailing sites with clay particle content of 26.9% and 29.5% sand with pH of 5.4, which were *Glomus mossaeae* and *Scutellospora calospora* from the rhizospheres of *Acacia* sp.; *Glomus leptotichum* and *Glomus mossaeae* from the rhizospheres of *Gmelina* sp.; *Glomus deserticola*, *Glomus aggregatum*, *Glomus geosporum*, and

*Glomus geosporum*from the rhizospheres of *Leucaena* sp.; *Glomus intraradices*, *Glomus mosaeae*, and *Glomus deserticola* from the rhizosphere of *Cassava*.

In the lead mining area Angguran polluted by lead (Pb) and zinc (Zn) in Iran with clay particle content of 25.0 - 33.2%, dust 45.0 - 59.0%, sand 16 - 26% with pH of 7.5 – 7.9; *Glomus versiforme*, *Gintraradices*, *G. mosseae* in the rhizosphere of *Veronica rechingeri* had been found (Zarei et al., 2008). Similarly in the gold mining area of Morro do Ouro Brazil, which has pH of 3.9 to 6.6 with Arsenic (As) content of 10.2 - 1046.4 mg dm-3.23 species of mycorrhizas were identified: 10 species of genus *Acaulospora* sp., 4 species of genus *Scutellospora* sp., 3 species of genus *Racocetra* sp, 4 species of genus *Glomus* sp., 1 species of genus *Gigaspora* sp and *Paraglomus* sp. *Glomus clarum*, *Paraglomus occultum* and *Acaulospora morrowiae*are the most common species found in areas contaminated by As. These three species together with *Acaulospora mellea* are the most abundant species in plant's rhizosphere grown in mining areas (Schneidera et al., 2013).

This study aimed to identify and to determine the abundance of indigenous endomycorrhiza in Ni post-mining in Sorowako as one of natural resources that can be utilized as biological agent in rehabilitation program.

Materials and Methods

This quantitative-qualitative study was conducted in two stages. First, rhizosphere sampling of *Morus alba* and *Cyperus rotundus* in nickel post-mining of Sumasang 1 (2O31'48,7'' S, 121O22'40,9'' E), rhizospheres of *Wedelia trilobata* and *Melostama affine* in the Sumasang 2 block A (2O31'58,6'' S, 121O22'23,1" E) and rhizospheres of *Gleichenia linearis* and *Equisetum gigantum* in Sumasang Block B (2O31'58,1" S, 121O22'20,4" E), Sorowako village, East Luwu district, South Sulawesi, Indonesia, employing methods from Kumar (2012), Estrada et al. (2013) and Krishnamoorthy, (2015), as the first phase.

Sample soil analyzed in the Laboratory of Chemistry and Soil Fertility, Department Soil Science Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. Soil texture determinate with hydrometer method, pH of soil was measured with pH meter, C-organic with Walkley and Black method, base cation (BC) with ammonium acetate extracted in pH 7 then was measured with an atomic absorption spectrometer, Cation exchange capacity (CEC) with 1 M NH4OAc, base saturation calculated with % BS=(bases cation/CEC)x100%. Concentration of soil nutrition was measured while in laboratory of chemistry, Polytechnic of Ujung Pandang, Makassar, using manual book of X-Ray Florence Spectrophotometer/Bruker/S2 Ranger. Characteristic of soil can be seen in Table 1.

The second phase was conducted at Microbiology Laboratory, Research and Development Center for Environment and Forestry, Municipality of Makassar, South Sulawesi, Indonesia. Arbuscular mycorrhiza (AM) spores were isolated from rhizosphere samples using pour and wet filtration technique by Gerdemann and Nicolson (1963) and sucrose centrifugation technique by Daniel and Skipper (1982). The number of spores per 100 g rhizosphere sample and spore diameter was measured using biomolecular microscope while spore morphological identification was determined using manual book from International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM, 2016). The results of identification and abundance of AM spore population from each sample are shown in the form of tables and diagrams.

Results and discussion

The nickel post-mining plantation area in Sumasang has various plant vegetation which are different from one another, Sumasang 1 planting area is dominated bygrass vegetation, shrubs and naturally grown ferns and has shallow roots, prone to erosion and drought. As for the planting area of Sumasang 2 block A and block B, in addition to vegetation grass, shrubs and ferns, it has been dominated by tree vegetation aged 5 to 10 years (Fig. 1). This strongly supports the development of indigenous AM which can not be separated from the development of the roots of host plants where the symbiotic association occurs. Abubakar (2009) reported that plant growth in Ni post-mining plantation has reached a greater percentage of success with more than 80% which is dominated by local plants that have adapted to marginal environment. The laboratory analysis results indicated that soil conditions in nickel post-miningof Sumasang showed that the soil physical and chemical characteristics were not ideal. The rhizosphere sampling site is silty clay to clay loam with different percentages of sand, clay and dust content, slightly acidic soil pH due to very low Al elements (Table 1). However, according to Costa et al. (2013) and Bertham (2003), the optimum pH for spore development of *Glomus* sp. is 5.6 to 7, *Gigaspora* sp. is 4.0 to 5.0 and *Acaulospora* sp. is 4 to 6 (Bertham, 2003), yet the optimum pH for the spore development is highly dependent on adaptability in high Ni concentration environment.

The content of C and organic material (OM) in nickel-post mining of Sumasang 2 belongs to medium criteria (Table 1). Organic material released by the roots is *root exudate*. Root exudate is requiredby microorganisms in its metabolism as source of energy or substrates in its activity, ingredients removed from living root cell activity such as sugars, amino acids, organic acids, fatty acids and sterols, nucleotides, flavonon, enzyme, and miscellaneous (Wihardjaka, 2010).

Several factors affect the amount and composition of exudates released by plants are plant species, plant age, environmental conditions (temperature, irradiation, soil moisture, soil type, plant nutrients, and stresspressure on plants), growing plants environmental conditions, and microorganisms presence (Budiyanto, 2015). Root secretion is material which actively pumped out of the roots (Sari, 2015), e.g., the mechanism of organic acid secretion (external mechanism) as a form of physiological response to Al in rice plants (Fajarwati, 2007). Root lycate is material passively pumped out while root cells autolysis occur (Sari, 2015) such as proteins, fats and amino acids. Root mucilage is the material of polysaccharides synthesized in the Golgi apparatus root cap cells move in vesicles through cytoplasm to plasmalema (Takehisa et al., 2012), e.g., root secretion material, epidermal cell remnant, root cap cells mixed with cell microbe remnant, metabolite products, organic colloids and inorganic colloids (Sari, 2015). The major enzymes produced by roots are oxidoreductase, hydrolase, liase, and transferase. The enzymes produced by microbes in the rhizosphere are cellulase, dehydrogenase, urease, phosphatase and sulfatase (Sudana, 2005).

Morphological identification of AM spores indicated the difference in shapes, colors and sizes of spores, which are divided into three groups of spores namely *Acalauspora* sp., *Gigaspora* sp., and *Glomus* sp. (Table 2), it is suspected that these three genera have wide adaptability in environment contains high nickel concentration. This results are similar to result findings of Setiadi and Setiawan (2011) that there are three major genera of AMin nickel post-mining plantation area namely *Acaulospora* sp., *Glomus* sp., and *Gigaspora* sp. The morphological identification results also show that the size of spores found on post mining land generally has a relatively small average diameter. According to INVAM (2016) the spores size for *Acaulospora* reaches average diameter of 74μm at *Acaulospora rugosa* to 289 μm at *Acaulospora foveata*, *Gigaspora* reaches average diameter of 206μm at *Gigaspora vosea* to 358μm at *Gigaspora decipiens*, whereas for *Glomus* it reaches average diameter of 70μm on *Glomus hoi* to 220μm on *Glomus lacteum*.

The result of AM spore counting indicated that the diversity of three dominant AM generain percentage in Sumasang 1 area was 60.0% *Acaulospora* sp., 26.6% *Gigaspora* sp., and 13.3% *Glomus* sp.; in Sumasang 2 block A area was50.0% *Gigaspora* sp., 41.6% *Acaulospora* sp., and 8.3% *Glomus* sp.; while in Sumasang 2 Block B area was 63.6% *Acaulospora* sp., 27.3% *Gigaspora* sp. and 9.0% *Glomus* sp. (Table 3).

Fig. 1: Vegetation in nickel post-mining plantation Sumasang, Sorowako, South Sulawesi, Indonesia. [(A) = Sumasang 1, (B) = Sumasang 2 block A, (C) = Sumasang 2 block B]

Table 1. Characteristic of soil and nutrient elements in nickel post-mining plantation area in Sumasang, Sorowako village, East Luwu district, South Sulawesi, Indonesia.

Characteristics	Location		
	Sumasang 1	Sumasang 2 block A	Sumasang 2 block B
Pasir (%)	10	39	34
Debu (%)	45	38	37
Liat (%)	45	23	45
pH (H_2O)	5.59	6.01	5.85
C (%)	1.97	2.05	2.07
BO	3.40	3.53	3.57
KTK (cmol (+)kg^{-1})	10.14	14.63	15.21
KB (%)	64	48	42
Al (ppm)	<1	<1	<1
K (ppm)	<8	<8	<8
P (ppm)	<1	<1	<1
Ni (ppm)	9.207	23.859	267

Table 2. Morphology of AM spores isolated from nickel post-mining plantation area in Sumasang, Sorowako, South Sulawesi, Indonesia.

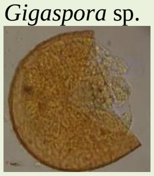
Location	Spore Genera	Morphology description	Spore Diameter (µm) Identified	Spore Diameter (µm) INVAM
Sumasang 1	*Glomus* sp. 	Round-shaped spore, has hypha remnant, hialin colored, one layer of spore wall.	77.5 × 77.5	70 to 220
	Gigaspora sp. 	Round-shaped spore, light yellow colored,one layer of spore wall, smooth surface unornamented.	203 × 203	206 to 358
	Acaulospora sp. 	Round-shaped spore, yellowy brown colored, two layersof spore wall with inner wall, the color of its center is darker then the outer	60 × 60	74to 289
Sumasang 2 Block A	*Glomus* sp. 	Round-shaped spore, has hypha remnant, hialin colored, one layer of spore wall.	82.5 × 82.5	70 to 220
	Gigaspora sp. 	Round-shaped spore, cream yellow colored, one layer of spore wall,smooth surface unornamented.	260 × 260	206 to 358
	Acaulospora sp. 	Round-shaped spore, yellowy brown colored, two layersof spore wall with inner wall, the color of its center is darker then the outer	80 × 80	74 to 289
Sumasang 2 Block B	*Glomus* sp. 	Round shape spore, has no hypha remnant, hialin colored, one layer of spore wall	95 × 95	70 to 220
	Gigaspora sp. 	Round-shaped spore, dark yellow colored, one layer of spore wall, smooth surface, has Bulbous Suspensor trace	235 × 235	206 to 358
	Acaulospora sp. 	Round-shaped spore,two layersof spore wall with inner wall, the color of its center is darker then the outer	75 × 75	74 to 289

Table 3. Number of mycorrhizal spores per 100 g of rhizosphere samples.

Location	Rhizosphere	Family	Number of spores		
			GLS	**GGS**	**ACS**
Sumasang 1	*Morus alba*	Moraceae	1	3	6
	Cyperus rotundus	Cyperaceae	1	1	1
Sumasang 2 Block A	*Wedelia trilobata*	Asteraceae	1	1	2
	Melostama affine	Melastomataceae	0	5	3
Sumasang 2 Block B	*Gleichenia linearis*	Gleicheniaceae	0	1	5
	Equisetum gigantum	Equisetaceae	2	5	9

Note: GLS, *Glomus* sp.; GGS, *Gigaspora* sp.; ACS, *Acaulospora* sp.

There are two hypotheses to support the dominance of *Acaulospora* sp abundance in the high nickel concentration (Table 1), first: *Acaulospora* sp. has two layers of cell wall, which can become aberrant and spore defense organ against the nickel heavy metal stress. According to Galli et al. (1993), most heavy metals are bound and stored in spore cell wall components such as chitin, cellulose, cellulose derivatives and melanin. Stronger defence allows the spore tolerance of *Acaulospora* sp. to be higher than spores of *Gigaspora* sp. and *Glomus* sp. It provides wider opportunity for faster adaptation process of *Acaulospora* sp. to reach domestication stage, is another hypothesis. As for *Gigaspora* sp. and *Glomus* sp. which have only one layer of cell wall, it is suspected that the hypha spore is place where heavy metals are bound, Andrade and Silveira (2008) and Aloui et al. (2011) stated that metals can be precipitated or chelated in the soil matrix through glycoproteins production or produce phosphatemamin complexes in hypha, yet hypha growth is strongly influenced by environmental factors. Suharno et al. (2013) suggested that the adaptation is related to the gradual changes of microbial community structures, based on gradual changes in community structures over fatty acid phospholipid profile affects more tolerant organisms.

The development and utilization of the more dominant indigenous mycorrhizas is unexploited natural resource for nickel post-mining rehabilitation program in Sorowako, South Sulawesi, Indonesia. Syib'li et al. (2013) suggested that high abundance of mycorrhiza in field can be used as the phytoremediation of soil fertility, since AM has high interaction with organic material, total phosphorus, and cation exchange capacity.

The number of spores in nickel post-mining plantation area at Sumasang showed 55.1% *Acaulospora* sp., 34.6% *Gigaspora* sp. and 10.2% *Glomus* sp., with diameter of 60 µm × 60 µm to 260 µm × 260 µm. The dominant indigenous mycorrhiza was *Acaulospora* sp which has two layers of spore wall as heavy metal storage organ. Therefore, this species has higher tolerance in high nickel concentration environment which is the natural wealth resource of Sorowako village which can be developed as biological agent for nickel post-mining land rehabilitation program.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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