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Research Article

A Preliminary Study of Soil Microbial Abundance in Succulent Plant Rhizospheres

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Abstract

Plant host symbiosis is close related to soil microbial abundance. Soil microbial abundance will affect soil quality and fertility, thus will improve plant growth. Studies on soil microbial abundance in succulent plant rhizosphere, especially in Indonesia, are scarce. This study aims to observe soil microorganisms' existence and their abundance on succulent plant rhizosphere. This research used two primary methods to isolate Arbuscule Mycorrhizal Fungi (AMF) and actinomycetes. The spore extraction (soil separating) method was used to obtain AMF spores, followed by the root staining method to observe AMF infection on the plant roots. Serial dilution and pour plate method were used for isolation of Actinomycetes. The isolation results showed a high number of Actinomycetes distribution up to 3.3 x 10^6 Actinomycetes CFU/g from the plant Echinocactus grusonii, while AMF spores displayed the most elevated number up to 47 spore/25g from the plant Deuterocohnia sp. The percentage of AMF root infection is covered by 27,9% median. AMF identification was based on spore. The result showed an abundance presentation of Actinomycetes in each soil sample. AMF and Actinomycetes existence displayed symbiotic interaction between succulent plants and soil microorganisms. AMF and actinomycetes play the role of endophytes that help the growth of cacti, generally have a dry growing environment, and limited nutrition by this symbiotic interaction. Actinomycetes distribution in rhizospheric soil will increase plant growth-promoting factors activity. Eventually, the implications of research results are to explore the abundance and biodiversity of soil microorganisms from succulent plant rhizosphere that lacked exploration.

Keywords: Abundance, Rhizosphere; Soil Microbes; Succulent

1. Introduction

Soil microorganisms are an essential factor in the soil ecosystem. These microorganisms carry out various processes of decomposing organic matter, transforming elements, and recycling nutrients necessary for the growth of animals and plants. Some soil-dwelling microorganisms also act as pathogens that affect the host directly or release toxic substances in the soil ecosystem (Baliyarsingh et al., 2017). The five main microorganisms found in the soil are bacteria, actinomycetes, fungi, algae, and protozoa (Susilawati et al., 2013). Various groups of bacteria, fungi, and actinobacteria penetrate the roots of plants and live there or live near the root system. The concept of "rhizosphere" was introduced to show the close relationship between soil microorganisms and the root system of higher plants. The “root area” covers the root surface and rhizosphere, a zone of high microbiological activity (Baliyarsingh et al., 2017).

The diversity and activity of the rhizosphere microbial community depend on soil conditions and the conditions of the vegetation above. A better understanding of the diversity of rhizosphere microbes in the soil and their interactions with the environment and other organisms is essential for interpreting their impacts on agriculture and the

environment. Research on the abundance of rhizosphere microbes has been carried out previously. The studies looked at rhizosphere microbes in different plant and soil conditions (Ara et al., 2012; Ohiwal et al., 2017; Prayudyaningsih et al., 2015). Ohiwal et al., 2017 stated that in the rhizosphere of oil palm in Riau peatlands, there are functional microbes such as cellulolytic microbes, *Azotobacter*, phosphate solubilizing microbes, and White-rot fungi. Prayudyaningsih et al., 2015 stated that soil microorganisms are beneficial in the rhizosphere of tuber plants under the forest stands of the people of South Sulawesi, including the phosphate solubilizing bacteria genera, *Micrococcus* and *Clostridium*, and the non-symbiotic nitrogen-fixing bacteria genera, *Azotobacter. Aspergillus niger, Aspergillus fumigatus, Aspergillus oryzae, Rhizopus oryzae* dan *Rhizopus stolinifer* were isolated from the rhizosphere of tomato, *Lycopersicum Esculentum* (Shinkafi & Gobir, 2018).

Succulents are plants that live in habitats with little or no water. To improve survival and fitness in these habitat conditions, succulents develop adaptation tools and survival strategies both morphologically and physiologically. Citlali Fonseca-García (2016) reviewed that Cacti (Cactaceae), also known as cactus, is a xerophyte. It's grouped in the succulent plant, consisting of approximately 2000 species distributed around the world. The most recent research on cactus-microbial association focuses on one or two cactus species and discusses the microbiome, climatic aridity gradient effect, and bacterial community (Kavamura et al., 2013; Fonseca-García et al., 2016; Karray et al., 2020; De Lyra et al., 2021). Most of the previous research focuses more on microbiome, bacterial and succulent plant interactions. Endophytic fungi's existence and role on succulent plants are still rarely studied (Bezerra et al., 2017). These previous research on the microbial association to succulent plants highlighted that some bacterial phylum were correlated with cactus, such as actinobacteria and proteobacteria. Bacterial and fungal diversity in each succulent plant varied and displayed an opposite relation in abundance (Torres et al., 2012; Kavamura et al., 2013). These findings are essential to help improve and manage agriculture in arid and semi-arid regions (Fonseca-García et al., 2016). Moreover, as reviewed by previous researchers, endophytic soil microbial exploration is still an important field to study due to its significant ecological and biotechnological potential (Afzal et al., 2019; Singh & Dubey, 2018).

Studies on rhizospheric soil microbial associated with succulent plants, especially from Indonesian rhizospheric soil samples, are recent and scarce. Our study suggests that succulent plants may form associations with microorganisms, which may give an essential role in their adaptation and survival. Therefore, this study aims to determine the abundance of soil microorganisms in the rhizosphere of the succulent plant.

2. Material and Method

2.1. Sample Collection

Five soil samples were collected from different succulent plants rhizospheres at Melrimba Garden, Bogor, West Java. Soils were collected from 8 to 10 cm depth, transferred into sterile plastic bags, and stored in a refrigerator at 4°C before isolation.

2.2. Arbuscular Mycorrhyza Fungi Spore Isolation

Abuscular mycorrhyza fungi (AMF) spores were isolated using the spore extraction method (Soil separating method) by Nusantara et al. (2012). First, weigh the soil as much as 25g, then put it in a 100 ml beaker and then add water to a volume of 100 ml. Next, the soil was stirred to break up the clumpy soil aggregate by lightly pressing the stirring rod, and then it was allowed to stand for 15 minutes until the large particles settle. Then the soil suspension was poured into a multilevel sieve, the top part is a filter with a filter eye size of 32µm (coarse), and the bottom is a filter with a filter eye size of 325µm (fine). The soil was drained with the help of tap water (not too tight) to ensure that all particles were well filtered. Soil sediment in a 325µm filter was put into a beaker glass with the help of water from a spray bottle, 60% sugar solution, then added to the soil suspension twice the volume of the extract, followed by centrifugation at a speed of 2500 rpm for 5 minutes. The resulting supernatant was filtered with filter paper and then put into a petri dish for further observation under a microscope.

2.3. Root Tissue Staining and Arbuscular Mycorrhiza Fungi Infection Observation

Observation AMF infection in plant root tissue was carried out through the root staining technique (Nusantara et al., 2012). This method has several steps. First, the root sample to be used was washed thoroughly until the soil attached to the roots was washed away. Next, the roots were soaked in 10% KOH for 18 hours. Afterward, the roots were washed with running water until clean and soaked in alkaline H_2O_2 solution for 10-20 minutes until the root color turned pale or whitish. Once the roots changed their color, they were washed with running water until clean and soaked in a solution in 1% HCl, let stand for 3-4 minutes, then discard the acid solution. In the last step for coloring root tissues, the roots were soaked in a 0.08-0.1% trypan blue solution in lactoglycerol, then heated at 90°C in a fume hood for 1 hour. Trypan blue solution was then removed, and the roots were soaked with lactoglycerol solution. The roots were cut into small pieces ± one cm then placed in an object glass. Each object-glass consists of five to ten parts of roots, then covered with a glass cover. The AMF structure was observed under the microscope.

The percentage of AMF infection in the roots was calculated using the Giovannety and Mosse formula (Setiadi & Setiawan, 2011) as follows:

$$\text{Infected Roots (\%)} = \frac{\Sigma \text{ Infected Roots}}{\Sigma \text{ Observed Roots}} \times 100\% \quad \ldots\ldots (1)$$

2.4. Actinomycetes Isolation

Actinomycetes were isolated by the serial dilution method. A weight of 10 grams of each soil sample was diluted with phosphate buffer to seven-fold dilution. The last five dilutions were plated by pour plate method on HV agar (Humic acid-Vitamin agar: 1 g humic acid; 0,02 g $CaCO_3$; 0,01 g $FeSO_4$. $7H_2O$; 1,71 g KCl; 0,05 g $MgSO_4.7H_2O$; 0,5 g Na_2HPO_4; 50 g siklohesamida; 20 g agar). The plate was incubated at 25°C for 4 to 14 days (Saraswati et al., 2007).

3. Results and Discussion

3.1. Results

AMF spore abundance and root infection percentage are displayed in Table 1. AMF root infection and spore are present in all samples (Figure 1 and 2).

Table 1. Arbuscular Mycorrhiza Fungi Spore Abundance and Root Infection Distribution in succulent plants

Soil Sample	Cactus	Spore (per 25-gram soil)	Root infection
K1	*Euphorbia* sp. 1	39	22.5 %
K2	*Echinocactus grusonii*	42	24 %
K3	*Deuterocohnia* sp.	47	39.50 %
K4	*Euphorbia* sp. 2	44	31 %
K5	*Escheveria* sp.	42	22.50 %
	Median	42,8	27,9 %

Table 1 shows data of AMF spore and its infection in succulent plant roots. All soil samples collected have an AMF spore with a total median of 42.8, and in every root, AMF infection is present with a total median of 27.9%.

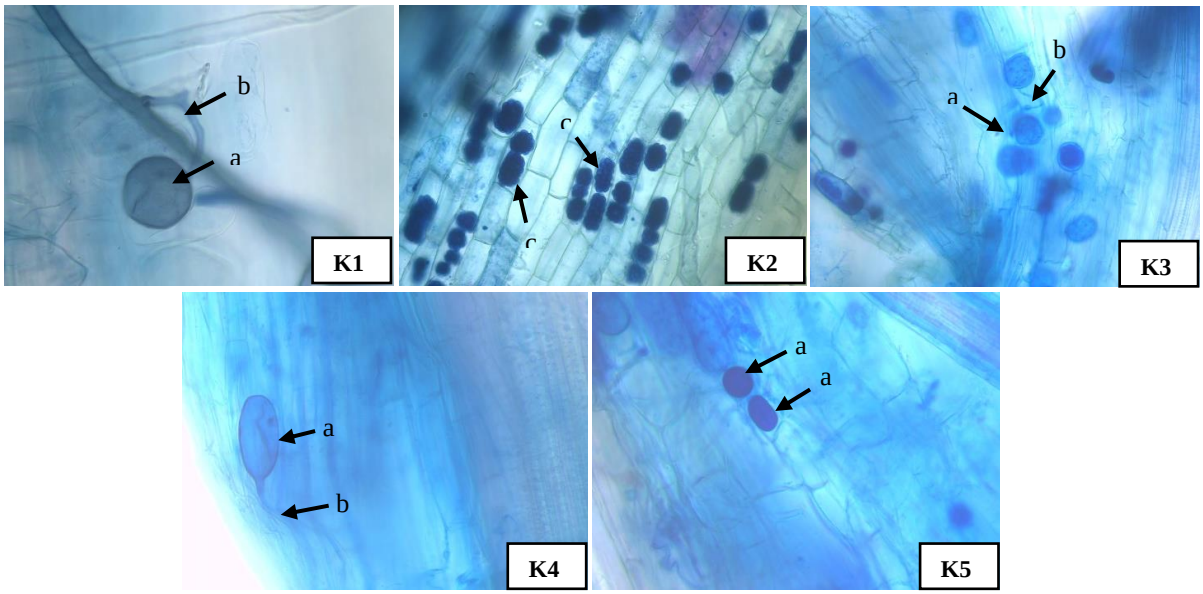

Figure 1. AMF infection on roots from K1, K2, K3, K4, and K5 soil samples; a. Vesicle, b. Internal hyphae, c. Arbuscules

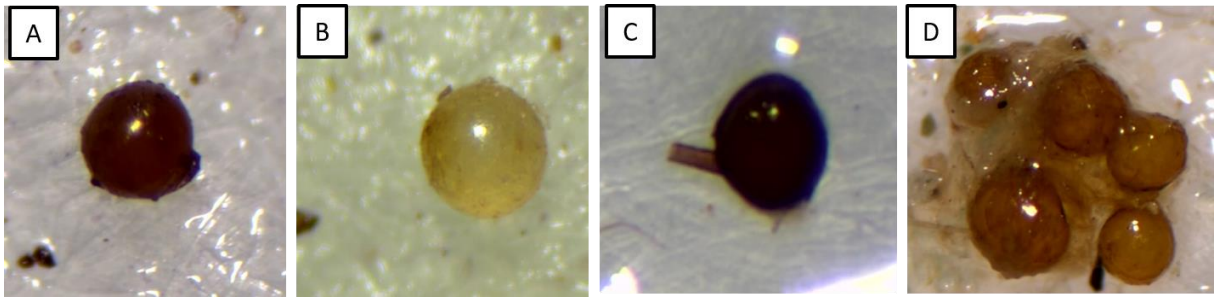

Figure 2. Spore Identification on Microscope(100x). A. *Acaulospora,* B. *Gigaspora,* C. *Glomus.* D. *Scutellospora*

Actinomycetes bacteria isolates from all samples as displayed in Table 2. The highest Colony Forming Unit (CFU) count is K2, *Echinocactus grusonii* rhizosphere 3.3 x 10^6 CFU/g. The second highest from K1, *Euphorbia* sp. 1 rhizosphere 3.0 x 10^6. The third highest from *Deuterocohnia* sp. rhizosphere 2.9 x 10^6.

Table 2. Actinomycetes abundance in each soil sample

Soil Sample	Succulent Plants	Actinomycetes CFU/g
K1	*Euphorbia* sp. 1	3.0 x 10^6
K2	*Echinocactus grusonii*	3.3 x 10^6
K3	*Deuterocohnia* sp.	2.9 x 10^6
K4	*Euphorbia* sp. 2	2.3 x 10^6
K5	*Escheveria* sp.	2.5 x 10^6

On HV agar, all soil samples showed various actinomycetes growth. Most of the colonies form aerial hyphae and represent small rounded colonies. Aerial hyphae color dominantly white mixed with soft color from grey to pinkish (Figure 3).

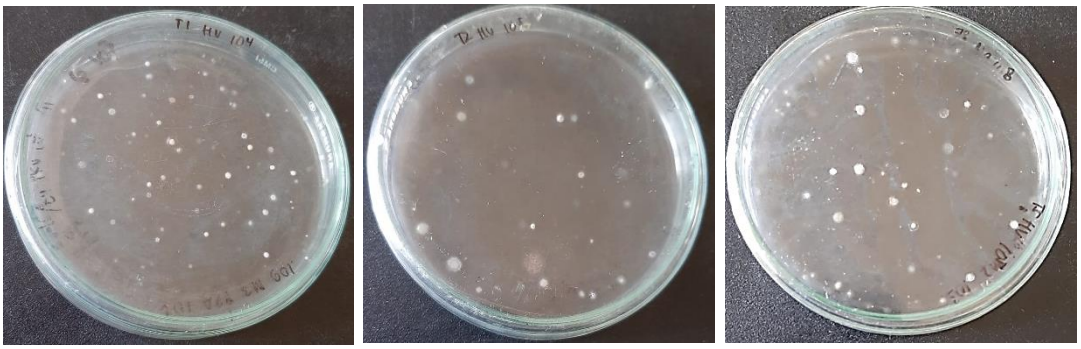

Figure 3. Isolation of Actinomycetes on HV agar

3.2. Discussion

The AMF spores were successfully isolated and identified from the succulent plant rhizosphere at Melrimba Garden. Our results showed AMF existence in Succulent plant rhizosphere soil. All soil samples showed AMF infection. AMF infection in every root is present with a total median of 27.9%. AMF infection structures present in the sample were vesicle, internal hyphae, and arbuscules. This structure indicates mutualistic symbiosis between succulent plants and AMF (Kobae et al., 2019). Arbuscules formation has been studied as a unique morphological feature. This feature is responsible for the nutrient exchange between the host plants and the AMF. (Smith & Smith, 2011). Arbuscules formation is also parallel with a specific cellular system expression that allows AMF accommodation in the root tissue and achieves nutrients such as phosphorus and nitrogen uptake via AMF mycelia (Bucking et al., 2012; Luginbuehl & Oldroyd, 2017). Some AMF roots infections may form Vesicles structure. This structure-function as fungal storage organs inside roots apoplast (Bucking et al., 2012). Further genetic studies have revealed a nutritionally beneficial relationship between plants and AMF (Choi et al., 2018; Lanfranco et al., 2018).

The AMF spores were identified to genus level based on their morphological structure. They belonged to four genera: Acaulospora, Gigaspora, Glomus, and Scutellospora (Nusantara et al., 2012; West Virginia University INVAM). *Acaulospora* spores are formed by the sporiferous saccule originating from an expansion of terminal hyphae. When the spores are fully developed, the contents of the saccule will move into the spore, thinning the saccule and, over time, degraded. The genus *Acaulospora* has around to round shape or irregular to oval with two spore walls. *Gigaspora* spores consist of bilayer spore walls without ornamentations and germ tubes that emerge from the inner surface layer. The Glomus spore is round to slightly oval with more than one

layer of spore walls. The color of the Glomus spores varies from yellowish-brown to blackish dark brown. *Scutellospora* spores are generally found with or without ornamentation. It has two layers of spore walls and two layers of flexible inner walls. Spore shape-round, slightly rounded, slightly oval, and sometimes irregular with yellow to brownish spore walls (West Virginia University INVAM, 2017).

Based on spore calculations and observations of mycorrhizal root infection, it is clear that AMF has a mutualistic symbiosis relationship with succulent plants. The high number of AMF spores found in the rhizospheric sample represents a strong symbiotic relationship between the succulent plants and AMF. As reviewed by (Fonseca-García et al., 2016), succulent plants are known to survive too much abiotic stress, including extreme temperature fluctuation, high ultraviolet radiation, low nutrient soils, and drought. Hence AMF infections are a piece of solid evidence on their role in promoting succulent plant growth.

AMF spores lack optimal environmental conditions, where general plant growth requirements such as soil moisture content, nutrients, pH, temperature, and light are disturbed or not available in sufficient quantities. With the formation of spores, AMF can last a long time even though environmental conditions are drought (Nusantara et al., 2012). AMF-Plant symbiosis relation roles are helping the plant absorb nutrients, plant defense, maintain plant growth in polluted or extreme environmental conditions such as drought, and supporting plant growth regulation to abiotic stress tolerance. Moreover, AMF also assists the absorption of soil nutrients with low mobility or immobile by increasing the rate of transfer of nutrients in the roots of the host plant (Suharno &; Wang et al., 2016; Ayub et al., 2020; Ceasar, 2020; Emmanuel & Babalola, 2020; Fonseca-García et al., 2016; Ravnskov et al., 2020; Solanki et al., 2021). AMF future promising role as biofertilizers and bioprotectors, gives them tools to biotechnological applications over environmental and agricultural challenges (Van der Heijden et al., 2015)

Actinomycetes are widely distributed in different habitats, especially in soil. Actinomycetes are one of the components of the rhizospheric microbial population. The richness and diversity of actinomycetes present in any specific soil is greatly influenced by the soil type, geographical location, cultivation, and organic matter (Arifuzzaman et al., 2010). The number and types of actinomycetes present in a particular soil are also influenced by physicochemical conditions such as temperature, type, pH, salinity, organic matter, cultivation, aeration, and moisture content (Zanane et al., 2018). Although the habitat of succulent plants is dry soil, our results show actinomycetes have been found in the succulent rhizosphere. The number of actinomycetes as a part of the rhizosphere microbial community overall showed similar abundance for the 5 succulent plants (*Euphorbia* sp. 1, *Echinocactus grusonii, Deuterocohnia* sp., *Euphorbia* sp. 2, *and Escheveria* sp.). Table 2 shows that the highest number is in *Echinocactus grusonii,* and the lowest is in *Euphorbia* sp. 2. A study by Lee & Seong in 2014 reported that A novel high G+C actinobacterium, designated strain OS1-21T isolated from the rhizosphere soil

of a cactus (*Opuntia fiscus*-indica var. sanboten) (Lee & Seong, 2014). A previous study on actinobacteria from *Cereus jamacaru* rhizosphere in the caatinga biome used taxonomic and statistical approaches. They summarized that actinobacteria existence on the caatinga biome is strongly related to dry seasons. Some genera found in the dry season suggest their tolerance to the extreme conditions at the caatinga biome. Moreover, they indicate that microorganisms play a role in managing tolerance over water stress or assisting plant's growth promotion (Kavamura et al., 2013).

The abundance and biodiversity of actinomycetes from succulent plants are poorly investigated and understood. Actinomycetes have been isolated from several succulent plats but regarding *Euphorbia* sp. 1*, Echinocactus grusonii, Deuterocohnia* sp*., Euphorbia* sp. 2*, and Escheveria* sp., rarely reported. Although AMF has been studied from various plants rhizospheres (Khastini, 2018; Kobae et al., 2019; Susilowati et al., 2019; Wijayanti & Turjaman, 2020), AMF research in regards to succulent plants is still scarce up to date. Our finding suggested a better understanding of Succulent Plant Actinomycetes and AMF mutualistic symbiosis.

Conclusion

There is an abundance of Actinomycetes in each soil sample, covering 4 genera of species identification and symbiotic interaction between succulent plants. Furthermore, arbuscular mycorrhizal fungi help the growth of succulent plants, which generally have a dry growing environment and limited nutrition by this symbiotic interaction.

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