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Morphological Diversity of Bacteria and Fungus of Rhizosfer Puspa Tree (*Schima wallichi* Korth.) at Turgo Forest, Mount Merapi Sleman Yogyakarta

Vidi Andany
Biology Department, Faculty of Science and Technology, UIN Sunan Kalijaga Yogyakarta
Jl. Marsda Adisucipto No 1 Yogyakarta 55281, Indonesia. Tel. +62-274-540971, Fax. +62-274-519739.
Email: vidi.andany@yahoo.com

Abstract. *Andany V. 2017. Morphological Diversity of Bacteria and Fungus of Rhizosfer Puspa Tree (Schima wallichi Korth.) at Turgo Forest, Mount Merapi Sleman Yogyakarta. Proc Internat Conf Sci Engin 1: 75-79.* The soil fertility aspect is characterized by the good biological properties of the soil. One of the important elements of the soil biological properties is the bacterial population present in it. This research has been conducted from March to April 2016 on the slopes of Mount Merapi Turgo Sleman Yoyakarta. This study aimed to determine the various characteristics of colonies and cells of microbial organisms that are on the sample soil area of rhizosphere in Puspa trees in the slopes of Mount Merapi and to know the microbes that dominated the slopes of Mount Merapi. The method used in this research is observation. The sampling method used is a composite method, which combines 3 children of soil samples taken from one sample point on the same plot of land on a diagonal basis. The result of purification of culture obtained 14 pure bacteria isolate and 5 isolate fungi. From bacteria, 11 gram positive isolates and 3 gram negative isolates. Further research is needed on the identification of bacteria up to the species level.

Keywords: bacterial morphological diversity, rhizosphere, puspa tree, fungus

INTRODUCTION

Merapi is the most active volcano in the world, in April 2006 has issued an eruption, and on October 26, 2010 erupted. Throughout the 20th century, the flow of hot clouds leads to the northwest, west and north, the eastern region free of hot clouds. The eruption of Mt. Merapi on October 26 - November 5, 2010 lava and hot clouds leads to the south and west (Wilson et al., 2007). Historically, mineral materials carried by hot clouds or lava are basal andesites in which they are calck-silicates. Volcanic ash Mount Merapi contains Al, Mg, Si, and Fe (Sudaryo & Sutjipto, 2009).

The physical condition of land affected by eruption of Merapi, causing agricultural land need to manage regularly. Soil management is needed to solve the soil layer containing moisture content. This can improve soil permeability. Knowledge and improvement of soil fertility and land productivity in Turgo area needs to be supported by data and information about the existing soil bacteria area especially in the area of Rizosphere.

Volcanic-exposed land can be encountered by some diversity of land and soil microbial populations. Microbes are seen visually when microbes form colonies. Microorganisms are often single-celled (unicellular) or multicellular (multicellular). However, some single-celled protists are still seen by the naked eye and some multicellular species are invisible to the naked eye. In addition to difficulty to observe, microbes form a colony that is mixed with other bacteria (Purwaningsih, 2005).

Organisms classified as microbes are bacteria, fungi, and actinomycetes. Microbial diversity in the soil, depending on the level of soil fertility. Conklin (2002) states that in one gram of soil there are 108-109 species of bacteria, 107-108 types of actinomycetes and 105-106 species of fungi. These three groups of microbes are large enough to be in the soil. The main problem in soil microbiology is that 99% of soil microbes cannot be grown in artificial media (Kirk et al., 2004; Gofar et al., 2007) in (Sudarma et al., 2012), therefore to describe the true microbial diversity in in the soil should use a molecular approach. The location of microbial habitat is of various kinds, but depends on a mixture of various biochemical cells (Purwaningsih, 2005).

Soil fertility rate is affected by several factors such as soil microbial uniformity, climatic factors such as temperature, rainfall, humidity, nutritional factors, and environment and microbes which is an indicator of soil fertility. Soil fertility can be predicted from the number of microbial populations living in it. High microbial count is a sign of the high level of soil fertility, because microbes serve as a remodel of organic compounds into nutrients available to plants (Dwidjoseptro, 1992).

There is a uniqueness towards a group of microorganisms that live around the roots of plants. This relates to a high-level root system that is not only associated with organic and inorganic compounds. The presence of microorganisms is not abrupt, but it is a natural trait. Rizosphere is a habitat dominated by plants and not land (Hieronymus, 2010). Knowledge of the type of microbial diversity in the plant rhizosphere needs to be isolated and identified. It can be ascertained that in Mount Merapi is very rich diversities of

microbial diversity considering the environment after the eruption.

MATERIALS AND METHODS

The sampling was conducted in Dusun Turgo Slope of Mount Merapi Sleman Yogyakarta for one month from 20 March to 22 April 2016. Isolation and identification was done at Microbiology Laboratory of Department of Biology of State Islamic University of Sunan Kalijaga Yogyakarta.

The tools used in this research were autoclave, elenmeyer, dark bottle, dropper drops, plastic bag, reaction tube, petri dish, microscope, LAF, glass object, glass cover, needle inoculation, tweezers, tissue paper, hair dryer and spritz lamp. While the materials used in this study are medium PDA (Potato Dextrose Agar), SGA (Saboraound Glucose Agar), aquades, 70% alcohol, and gram A (metylene blue), gram B (iodine), gram D (safranin).

Research Procedure

Soil Sampling

Soil sampling is done in the village of Turgo precisely on the slopes of Mount Merapi Sleman Yoyakarta. Soil sampling were done at depth 10 cm - 20 cm from rhizosphere area with distance from root 8 cm. This soil sample was then inserted in a dark labeled plastic container and placed on an ice box.

Medium Preparation

The media used in this research are medium PCA and SDA. For PDA media the composition of the material used was potato powder 4 g, dextrose 20 g, and agar 15 grams which then mixed in 1 liter of aquades flattened with spatula. While on SDA media composition used Tripton 5 g, Kamir Extract 1.5 g, Dextrosa 1 g, agar 15 g, aquades 1 liter, pH 7.0. Then all the ingredients were mixed.

Of all the ingredients that have been mixed then the solution sterilized by autoclave for 30 minutes with a temperature of 121^0C. After sterilization, poured in a petri dish and allowed to condense in room temperature 270 to 280C.

Isolation of Soil Microbes

A total of 1 gram of soil samples from each of the locations that have been taken are put into 9 ml of sterile distilled water and then in a corner to be homogenized. After homogeneous is taken 1 ml and put into 9 ml of distilled water until obtained by dilution 10-1 until 10-6. A total of 0.1 ml of suspension is pipetted from dilution 10-4, 10-5, 10-6, then by the method of suspension distribution is inserted into a petri dish containing solid media. PCA media is used for bacterial isolation, SDA media is used for mold isolation. After all is spread on subsequent media in incubation at room temperature. Growth of bacteria after incubation takes 72 hours, while for mold takes 96 hours.

The growing colony is then purified using a streak-plate method. This purification uses the same medium for each of the tested microbes. Each microbial growth takes the same time during isolation. After all in incubation continued calculation of colony count and morphological observation.

Morphological Observation

Observations of microbial morphology to be considered, including colony shape, colony color, colony edge, center of colony, and surface morphology of the colony. While in the mold observed include mold morphology, mold diameter, color, microscopically seen the color of spores, spores, and mycelium.

Observation of Morphology Cell Bacterial

Cell morphological observations and gram properties were performed by gram staining or staining and were observed with 200 and 400 magnification microscopy. Gram stain is done by first making preparatory pillow (smear) that has been fixed from the growing microbes. Then fixed by skipping over a bunsen flame. After that, the preparations are dropped with 1-2 grams of A and drop for one minute. Then washed with running water and allowed to air dried. Then add a gram of B and wait for a minute to rinse again with aquadest. After drying add 95% alcohol for penetration, added two grams of D and leave it for 30 seconds. Then washed with running water after air dried and then observed using a microscope.

Observation of Morphology Cell Mold

For observation morphology mold prepared used needle, cover glass, and glass objects then dried with tissue. The was heated on a bunsen flame and it has been taken captive culture using needle, then placed on the glass object. Once placed in glass object in tetesi with Lactofenol blue and put cover glass above glass object. After air dried, observed using a microscope.

Total microbe determination

Bacteria were count from petri dish that had 30-300 colony akteri dihitung hanya dari cawan petri yang mempunyai 30-300 koloni, and 10-100 colony for fungus.

$$Population\ total\ (CFU)\ g^{-1}\ dried\ soil = \frac{(amount\ of\ colony) \times (fp)}{bk\ soil}$$

Explanation:
fp : Dilution factor
bk : Weight of dried sample soil (g) = weight of soil x (1 – water content)

Microbe diversity index

The diversity of fungi and bacteria was analyzed using the Shannon (H ') index (Ludwig and Reynolds 1988) in (Bayu et al., 2014).

$$H' = -\sum_{i=1}^{S}(p_i)(log_2 p_i)$$

H ' : Shannon-Wiener diversity index,
S : number of species,
Pi : proportion of the number of individual types of i with total number of individual samples.

Table 1. Diversity index value.

Diversity Index Value	
H' < 1,0	(Low diversity)
1,0 < H' < 3,322	(moderate diversity)
H' > 3,322	(High diversity)

Analysis Data

Bacterial growth during the isolation was observed in number and morphology, then the data displayed in the table of number of occurrences and characteristics of bacterial isolates. Selection results are presented in the table, qualitative data of the growing number of colonies on the agar medium. It is also shown in quantitative data based on the index of uniformity.

RESULTS AND DISCUSSION

Using dilution method, bacteria and fungi have been successfully isolated from the five sample soil. All isolates showed fertile growth in PCA and SGA media. From the isolation of bacteria the number of population obtained from five samples obtained 14 pure isolates. with fungi, we obtained 5 pure isolates. This fact shows that the soil is covered with various plants must contain organic materials in sufficient quantities. Various kinds of organic materials are needed for the survival of bacteria and fungi. More organic material available, the higher bacterial population. This opinion is supported by the results of the research (Hoffman, 1914) in (Waksman, 1952) in (Purwaningsih, 2004) that 27 out of 30 surface soils and around roots have more microbial populations than roots.

Table 2. Some characteristic of colony and cell of soil bacteria in area Rhizosfer of Puspa tree (*Schima wallichii* Korth.).

Isolate Code	Total Appearance	Colony Characteristic	Cell Characteristic	∑ Cell cfu/mL
S1.2	1	Form: irreguler; Elevation: flat; Margin: lobate; Transparan: negative; color: white	Bacill (diplobaccil) 2-2 gram positive, average cell size 1,64 µm	$1x10^5$
S1.4	1	Form: irreguler; Elevation: flat; Margin: entire; Transparan: negative; color: white	Coccus (chained) gram positive, average cell size 0,4 µm	$1x10^5$
S2.1	1	Form: circular; Elevation: raised; Margin: entire; Transparan: positive; color: transparant	Baccil (monobacil) gram negative, average cell size 1,1 µm	$1x10^5$
S2.2	1	Form: irreguler; Elevation: flat; Margin: undulate; Transparan: negative; color: white	Baccill (chained) gram positive, average cell size 1,94 µm	$1x10^5$
S2.3	1	Form: circular; Elevation: flat; Margin: entire; Transparan: negative; color: white	Coccus (berantai) gram positive, average cell size 2,47 µm	$1x10^5$
S2.4	1	Form: circular; Elevation: flat; Margin: entire; Transparan: white; color: white	Baccill (diplobaccil) gram positive, average cell size 1,57 µm	$1x10^5$
S2.6	1	Form: circular; Elevation: raised; Margin: entire; Transparan: negatif; color: brown	Baccill (diplobaccil) gram positive, average cell size 2,4 µm	$1x10^6$
S2.8	1	Form: irregular Elevation: flat; Margin: entire; Transparan: negative; color: brown	Baccill gram negative, average cell size 2,7 µm	$1x10^6$
S2.9	1	Form: irregular; Elevation: raised; Margin: unddulate; Transparan: positive	Baccil gram positive, average cell size 2 µm	$1x10^6$
S2.11	3	Form: circular; Elevation: convex; Margin: entire; Transparan: negative; color: light brown	Coccus (monococcus) gram negative, average cell size 0,5 µm	$3x10^6$
S2.12	5	Form: circular; Elevation: convex; Margin: entire; Transparan: negative; color: yellow	Baccill gram negative, average cell size 0,74 µm	$5x10^7$
S3.3	1	Form: circular; Elevation: convex; Margin: entire; Transparan: negative; color: white	Baccill (chained) gram positive, average cell size 1,4 µm	$1x10^6$
S4.2	1	Form: irreguler; Elevation: flat; Margin: undulate; Transparan: negative; color: white	Baccill (monobaccill) gram positive, average cell size 1,5 µm	$1x10^6$
S5.1	1	Form: irreguler; Elevation: flat; Margin: undulate; Transparan: negative; color: yellow	Baccil (diplobacil) gram positive, average cell size 1,57 µm	$1x10^6$

Various kinds of microorganisms or bacteria grow in the soil. The complex nutrients for the growth of bacteria contained in the soil cause the bacteria grow very diverse. The first stage is the isolation of the soil followed by dilution. Dilution was continued by planting of bacteria on the specific media. Then incubate at 300C for 3-5 days seen bacterial isolates growing on the media. After growing purification proceed to purify bacteria with the same solid medium ie PCA. The time required to grow on both media is different, in the medium for isolation it takes 3-5 days to grow, but after purification it only takes 24 hours.

Groups of growing bacteria include gram-positive and gram-negative bacteria have a high content of G + C. Generally these bacteria are divided into two groups of Streptomyces and non-Streptomyces. Bacterial groups are generally abundant in nature such as soils that play an important role in the degradation of organic polymers. Obtained bacteria with 14 pure isolates, including gram positive bacteria amounted to 11 isolates and gram negative amounted to 3 isolates. With a variety of characteristics possessed both the characteristics of colonies and cell characteristics can be seen in Table 2.

Table 3. Several characteristic of soil fungi tanah in area Rhizosfer of Puspa tree (*Schima wallichii* Korth.).

Isolate Code	Number of Appearance	Diameter Isolate (cm)	Color	Spore Shape	Miselium
S2F.4	1	0,8 cm	brown	Spore: rounded ; color: green; dispersal distribution	No septum
S2F.2	1	2,1 cm	green	Spore: rounded; color: green; dispersal distribution	No septum
S4F.1	1	1 cm	green	Spora: rounded; color green; spore dispersal distribution	No septum
S5F.1	1	1,8 cm	white	Spore: rounded; color white; spore survival distribution	No septum
S5F.2	1	1,9 cm	black	Spore: rounded; color: black; spore survival distribution	septum

The various characteristics of colonies in fungi have many variant as there are those that have different colony sizes of each growth. After isolation and identification five fungi isolates were found. From Table 3, the number of occurrences of colonies is one of isolation and its blanche has a different color.

Table 4. Diversity Index of soil bacteria in area Rhizosfer of Puspa tree (*Schima wallichii* Korth.).

No	No isolate	Individual number	pi	ln pi	H'
1	S1.2	1	0,07	-2,64	0,19
2	S1.4	1	0,07	-2,64	0,19
3	S2.1	1	0,07	-2,64	0,19
4	S2.2	1	0,07	-2,64	0,19
5	S2.3	1	0,07	-2,64	0,19
6	S2.4	1	0,07	-2,64	0,19
7	S2.6	1	0,07	-2,64	0,19
8	S2.8	1	0,07	-2,64	0,19
9	S2.9	1	0,07	-2,64	0,19
10	S2.11	3	0,21	-1,54	0,33
11	S2.12	5	0,36	-1,03	0,37
12	S3.3	1	0,07	-2,64	0,19
13	S4.2	1	0,07	-2,64	0,19
14	S5.1	1	0,07	-2,64	0,19
				Total	**2,96**

Tabel 5. Indeks keaneragaman bakteri tanah pada area Rhizosfer pohon Puspa (*Schima wallichii* Korth.).

No	No Isolate	Individul Number	pi	ln pi	H'
1	S2F.4	1	0,20	-1,61	0,32
2	S2F.2	1	0,20	-1,61	0,32
3	S4F.1	1	0,20	-1,61	0,32
4	S5F.1	1	0,20	-1,61	0,32
5	S5F.2	1	0,20	-1,61	0,32
				Total	**1,61**

Keanekaragaman jamur dan bakteri dianalisis menggunakan indeks Shannon (H') (Ludwig dan Reynolds, 1988) dalam (Bayu *et al.*, 2014) dapat dilihat pada Tabel 3. dan Tabel 4. Dalam tabel tersebut didapat indeks keaneragaman bakteri terbilang sedang tetapi sudah akan naik dalam kondisi keaneragaman tinggi. Sedangkan dalam fungi indeks keaneragamannya terbilang sedang yaitu $1{,}0 < H' < 3{,}322$.

The diversity of fungi and bacteria were analyzed using Shannon (H ') index (Ludwig and Reynolds, 1988) in (Bayu et al., 2014) can be seen in Table 3 and Table 4. In the table, the index of bacterial diversity is fairly moderate but it will be rises in high uniformity conditions. While in the fungi index of the fairness is fairly moderate that is 1.0 <H '<3.322.

Microbial dominating were bacteria as seen in the calculation of the diversity index. According to (Dwidjoseptro, 1992) the high number of microbes is a

sign of the high level of soil fertility. Of the many isolates of bacteria that predominate were isolates S2.12. The number of these colonies is suspected to be very much in the land area of Rizosphere on puspa trees. While in the fungi isolates no one dominates because of its appearance there is only one colony of each isolation.

CONCLUSIONS

From the discussion above, the isolate bacteria that dominate were isolate S2.12 the number of these colonies allegedly very much in the land area Rizosphere on the tree puspa. While in the fungi isolates there were no domination.

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