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A STUDY OF MORPHOLOGY AND BIOCHEMIST OF BACTERIA FOUND IN SHALLOT (*Allium ascalonicum* L.) RIZOSPHERE WITH PB CONTAMINATION

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ABSTRACT

This research was to find out the effect of heavy metal accumulation in shallot field in Wanasari Subdistrict to the bacteria in shallot rhizosphere. This was a survey with Purposive Random Sampling, employing morphological and biochemical observations to collect the data. The qualitative descriptive method was applied to describe the data of testing results. Based on the results of morphology test, there are 27 various characterizations of bacteria on soil sample taken from the shallot (Allium ascalonicum L.) plantation in Kupu, Wanasari and Sisalem villages. Based on macroscopic, it has round, flat and non flat edges, convex elevation and their colors are all white and clear-white. From the microscopic observation, they have various sizes and their shapes are stem (basil) and round (coccus). The results of a biochemical test of bacteria on 27 isolates showed mixed results of positive and negative for all the tests of Methyl Red, Simon citrate, indole, catalase test, carbohydrate (gas), and a negative results for two tests of Vogeus-proskeur and H2S. The comparison of average Pb content in the soil had proved that the less Pb heavy metal contaminants in the soil means the more bacterial isolates

Keywords: *Bacteria, Rhizosphere, Shallot, Pb.*

I. INTRODUCTION

Shallot (*Allium ascalonicum L.*) is a multifunction tuber vegetable, which can be used as spices, vegetables, and flavoring [11]. According to [14] Wanasari District has the largest harvest area with the largest production in Brebes Regency, it is 6052 Ha with the production of 719,230,00 kg. The farmers there are accustomed to intensive cultivation practices with a high dependence on agrochemicals, especially fertilizers and pesticides. The volume of insecticide solutions used in each application ranges from 560 to 1,588 liters per Ha, meaning it has overdosed [1]. Pesticides used in agricultural cultivation can cause pollution of soil, water, seeds or fruits, and crops, because they contain heavy metals of Plumbum (Pb) [7]. The accumulation of metals in soils may lead to decreased soil microbial activity [8]. [6], in her research, argued that the increase of heavy metal Pb can inhibit bacterial growth. In turn, if the bacteria in the soil is reduced then the shallot production will surely decrease.

The quality of soil biology increases with the presence of soil microorganisms especially in the rhizosphere. According to [13], microbes growing in root areas (rhizosphere zone) are those having a great affect to the plant growth. The present research was to know the influence of heavy metal accumulation in the field of shallot planting in Wanasari District, especially its effect on the presence of bacteria in rhizosphere of shallot planting land.

II. METHOD & MATERIAL

This research was conducted for 4 months from February to May 2017 in two places, the shallot field of Wanasari District and the Integrated Laboratory of Muhammadiyah University of Purwokerto. This research employed a survey method by Purposive Random Sampling in determining and taking soil samples. Then it performed a descriptive analysis for the data results of the morphology and biochemical testing of the collected bacteria.

III. RESULT & DISCUSSION

1. The Pb Content Effect

The contamination of Pb in Kupu village, taken from 3 fields as the sample, is proved to have the highest contamination level of 18,253 ppm. The second is Wanasari village with a heavy metal contamination of Pb 17,154 ppmm and Sisalem village has contamination of heavy metal Pb of 17,094 ppm. The heavy metal accumulation in the soil can lead to decreased soil microbial activity [4]. It is found that in the sample of the Kupu village, there are only 7 isolates of bacteria, the same number is also indicated in the sample from Wanasari village. The sample from the Sisalem village with a least contamination has 13 bacterial isolates. From these results it is evident that an excessive heavy metal content in the soil can decrease soil microbial activity that can be detrimental to the soil. The average ratio of heavy metal contamination with the obtained isolates can be seen in Figure 4.10 below:

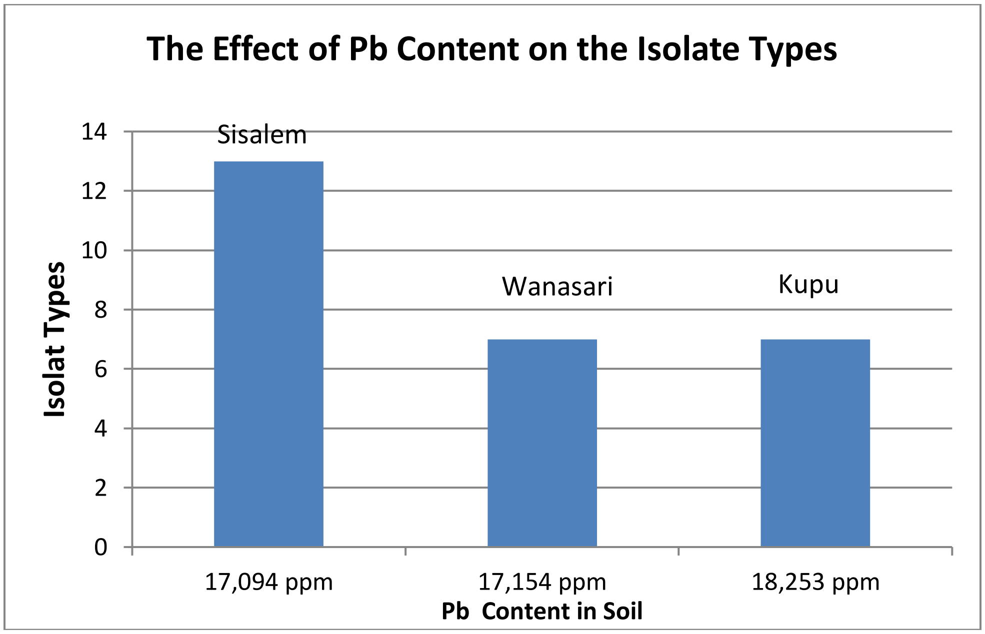

Figure 1. The Effect of Pb Content on the Isolate Types

2. Bacterial Isolates

Morphological test conducted was to identify the morphological appearances based on macroscopic and microscopic observations. From the macroscopic observations, the colony morphology of the 27 isolates are as follows:

Table 1. Morphological test conducted was to identify the morphological appearances based on macroscopic and microscopic observations

No.	Bacterial Isolatesi	Colony Shape	Colony Edge	Colony Color	Colony Elevation
1	B3-SSL-2	Round	Flat	All white	Convex
2	B2-SSL-1	Round	Flat	All white	Convex
3	B4-SSL-2	Round	Flat	All white	Convex
4	B6-SSL-2	Round	Flat	All white	Convex
5	B5-SSL-2	Round	Flat	All white	Convex
6	B1-SSL-2	Round	Flat	All white	Convex

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7	B2-SSL-3	Round	Flat	All white	Convex
8	B6-SSL-3	Round	Flat	All white	Convex
9	B1-SSL-1	Round	Flat	All white	Convex
10	B3-SSL-1	Round	Flat	All white	Convex
11	B4-SSL-3	Round	Flat	All white	Convex
12	B2-SSL-2	Round	Not flat	All white	Convex
13	B3-SSL-3	Round	Flat	All white	Convex
14	B5-WNS-3	Round	Flat	All white	Convex
15	B2-WNS-3	Round	Not flat	All white	Convex
16	B2-WNS-1	Round	Flat	All white	Convex
17	B3-WNS-1	Round	Flat	All white	Convex
18	B1-WNS-3	Round	Not flat	All white	Convex
19	B3-WNS-3	Round	Not flat	All white	Convex
20	B4-WNS-1	Round	Not flat	All white	Convex
21	B1-KP-2	Round	Flat	All white	Convex
22	B3-KP-1	Round	Not flat	All white	Convex
23	B2-KP-2	Round	Flat	All white	Convex
24	B3-KP-2	Round	Flat	All white	Convex
25	B1-KP-1	Round	Flat	All white	Convex
26	B2-KP-3	Round	Flat	All white	Convex
27	B3-KP-3	Round	Flat	White-Clear	Convex

Based on the microscopic observation of the 27 isolates, it was found out the results as follows:

Table 2. Morphological test conducted was to identify the morphological appearances based on microscopic observations

Name	Gram Test	Shape	Size
B3-SSL-2	Positive	Streptobasilus	1,7µ x 0,7 µ
B2-SSL-1	Positive	Streptobasilus	1,5 µ x 0,5 µ
B4-SSL-2	Positive	Streptobasilus	1,3µ x 0,8 µ
B6-SSL-2	Positive	Streptobasilus	1,5 µ x 0,6 µ
B1-SSL-2	Positive	Diplobasilus	1,9 µ x 0,8 µ
B2-SSL-3	Positive	Basilus	1,9 µ x 0,4 µ

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B6-SSL-3	Positive	Streptobasilus	1,6 µ x 0,5 µ
B1-SSL-1	Positive	Basilus	1,6 µ x 0,4 µ
B3-SSL-1	Positive	Streptobasilus	1,6 µ x 0,5 µ
B4-SSL-3	Negative	Basilus	1,6 µ x 0,4 µ
B2-WNS-3	Positive	Streptobasilus	2,1 µ x 0,9 µ
B2-WNS-1	Positive	Streptobasilus	1,6 µ x 0,5 µ
B3-WNS-1	Positive	Coccus	1,1 µ (d)
B1-KP-2	Positive	Streptobasilus	1,8 µ x 0,5 µ
B3-KP-1	Positive	Streptococcus	0,9 µ (d)
B2-KP-2	Positive	Streptobasilus	1,8 µ x 0,5 µ
B3-KP-2	Positive	Streptobasilus	2,1 µ x 0,8 µ
B5-SSL-2	Negative	Basilus	1,3 µ x 0,5 µ
B2-SSL-2	Negative	Diplobasilus	2,3 µ x 0,9 µ
B3-SSL-3	Negative	Diplobasilus	2,5 µ x 1 µ
B1-WNS-3	Negative	Basilus	1,2 µ x 0,5 µ
B3-WNS-3	Negative	Streptobasilus	2,1µ x 1,07µ
B4-WNS-1	Negative	Streptococcus	0,8 µ (d)
B5-WNS-3	Negative	Streptobasilus	1,4 µ x 0,8 µ

Name	Gram Test	Shape	Size
B1-KP-1	Negative	Streptobasilus	1,6 µ x 0,5 µ
B2-KP-3	Negative	Diplobasilus	1,4 µ x 0,4 µ
B3-KP-3	Negative	Streptococcus	0,6 µ (d)

The biochemical tests conducted include IMViC test, catalase test, H2S test, and carbohydrate test. The results of the biochemical tests on the bacterial cells are as follows:

Tabel 3. The results of the biochemical tests on the bacterial cells in the biochemical tests conducted include IMViC test, catalase test, H2S test, and carbohydrate test

Name	MR	VP	Indol	Citrate	Catalase	H_2S	Carbohydrate
B3-SSL-2	-	-	-	-	+	-	-
B2-SSL-1	-	-	+	-	+	-	-
B4-SSL-2	-	-	-	-	+	-	-
B6-SSL-2	-	-	-	-	+	-	-

B5-SSL-2	-	-	-	-	+	-	-
B1-SSL-2	-	-	-	-	+	-	-
B2-SSL-3	-	-	-	-	+	-	-
B6-SSL-3	-	-	-	-	+	-	-
B1-SSL-1	-	-	+	-	+	-	-
B3-SSL-1	+	-	+	-	+	-	-
B4-SSL-3	-	-	+	-	+	-	-
B2-WNS-3	-	-	+	-	+	-	-
B2-WNS-1	+	-	+	-	+	-	-
B3-WNS-1	+	-	-	-	+	-	-

Name	MR	VP	Indol	Citrate	Catalase	H_2S	Carbohydrate
B1-KP-2	-	-	-	+	+	-	-
B3-KP-1	+	-	-	-	-	-	Lactose (+)
B2-KP-2	+	-	+	+	+	-	-
B3-KP-2	+	-	+	-	+	-	-
B2-SSL-2	-	-	-	-	+	-	-
B3-SSL-3	-	-	-	-	+	-	-
B1-WNS-3	-	-	-	-	+	-	-
B3-WNS-3	+	-	-	-	+	-	-
B4-WNS-1	+	-	-	-	+	-	-
B5-WNS-3	-	-	-	-	+	-	-
B1-KP-1	-	-	-	-	-	-	-
B2-KP-3	+	-	-	-	+	-	-
B3-KP-3	-	-	-	-	+	-	-

The ability to grow living things in a contaminated environment is influenced by several factors including physicochemical processes such as sorption and desorption, diffusion and dissolution [2]. Indigenic microbes capable of growing in heavy metal contaminated media have the ability to accumulate heavy metals in their cell walls. [12] used Bacillus S1, SS19, and DA11 isolates isolated from Kalimas Surabaya which were contaminated with mercury stating that the highest bioaccumulation of mercury was Bacillus S1 of 89% (12 hours of incubation) and 90% (24 hours of incubation) Bacillus SS19 by 60% (12 hours incubation) and 37% (24 hours incubation), and Bacillus DA11 by 56% (12 hours incubation) and 32% (24 hours incubation). Metal ions are positively charged, so that electrostatically will be bound to the cell surface [9].

The mechanism of microbial tolerance to heavy metals by complexation includes the production of extracellular polysaccharides which have anionic properties that function as efficient bioaccumulators, production of organic metabolites which have chelating properties and form complexes with metals (Aspergillus niger, Penicilium spinulosum and Verticillium psalliotae), precipitation, and extracellular crystallization by sulfate reducing bacteria to form sulfide deposits which are rich in metals, and the formation of metal-ionin (a cysteine-rich protein in cells can bind metals) which functions to detoxify, store, and regulate metal ions in cells [4].

Microbes that are tolerant to heavy metals with a mechanism other than efflux are referred to as microbes which accumulate heavy metals. This bacterium can be used to overcome environmental pollution caused by heavy metals. Bacteria, molds, algae, and yeast can accumulate heavy metals Ag, Au, Cd, Co, Cu, Fe, Ni, U, Zn [5], [3]. Pseudomonas, Thiobacillus, Bacillus, and N2 fixing bacteria are reported to be able to accumulate heavy metals [10]. In the soil, microbial cells both dead and alive and their products can be very efficient heavy metal bioaccumulators.

IV. CONCLUSION

Based on the results of this study can be concluded are: 1.) the morphological tests of the bacteria in the soil samples of shallot (*Allium ascalonicum L.*) planting in the villages were done to 27 isolates collected. They show various characteristics. From their macroscopic aspect, they are round, flat (regular) or irregular shapes, have convex elevation, have a white and clear-white color. From their microscopic, they have diverse cell sizes and their shapes are stem (bacilli) or round (coccus); 2.) from the biochemical test of the bacteria, the 27 isolates having various results: the tests of Methyl Red, simon citrate, indole test, catalase test, and carbohydrate (gas) all show positive and negative, while the tests of Vogeus-proskeur and H2S indicate negative result; 3.) the result of average Pb content comparison in the three villages proves that the less Pb heavy metal contamination in the soil, the more bacterial isolates found there.

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