

1 Identification of Disease and Efforts to Protect Natural Orchid Plants Against 2 Bactria Infection in the Liwa Botanical Garden

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10 **ABSTRACT**

11
12 Liwa Botanical Garden is located in West Lampung Regency and is one of the conservation of ornamental plants that presents the rich
13 flora of the Bukit Barisan Selatan National Park. One of the flagship collections of this botanical garden is a natural orchid. Until now,
14 disease infections are still a major obstacle in efforts to preserve and develop the potential of natural orchids. Based on previous
15 research, it is known that several individual orchid collections from Liwa Botanical Garden show symptoms of bacterial infection. This
16 pathogen will infect orchids and cause soft rot disease. This research was conducted to determine the identification of diseases and
17 efforts to protect natural orchids against bacterial infections in the Liwa Botanical Garden through a collection of samples that showed
18 symptoms of infection, analysis of disease symptoms, and analysis of disease resistance levels. The results showed that the response of
19 the natural orchids of Liwa Botanical Garden to bacterial infections was to show symptoms of soft rot disease, i.e *Bulbophyllum* sp.,
20 *Dendrobium crumenatum*, *Dendrobium montanum*, *Pholidota* sp., and *Vanilla* sp. The type of orchid that shows the most symptoms of
21 bacterial infection is *Vanilla* sp. 11 samples, and *Pholidota* sp. and *Dendrobium crumenatum* with 3 samples each. The type of orchid
22 that shows the most symptoms is *Flickingeria* sp. a total of 8 samples. The natural type of the Liwa Botanical Garden which is most
23 susceptible to bacterial infections is *Pholidota* sp. and *Vanilla* sp. with disease intensity of 10% and 7.7%. The results of this activity are
24 expected to be basic information in efforts to protect plants against diseases to support the application of conservation of natural orchids
25 in Liwa Botanical Garden.

26 **Key words:** identification of diseases, bacterial infections, orchid bacteria, natural orchids, liwa botanical garden

27 **INTRODUCTION**

28
29 Liwa Botanical Garden is located in Pekon Kubu Perahu Village, Balik Bukit District, Liwa City, West
30 Lampung Regency. This botanical garden with the concept of Indonesian Ornamental Plants has become one of
31 the conservation of ornamental plants that presents the rich flora of the Bukit Barisan Selatan National Park
32 (Rosanti and Widianjaya, 2018), one of which is a natural orchid. The natural orchid collection at the Liwa
33 Botanical Garden was obtained from exploration and donation activities. As of November 2013, there were 384
34 numbers of exploration results and 41 numbers of donations from the Bogor Botanical Gardens (Solihah,
35 2015). Natural orchids are orchids that can grow naturally in nature without human assistance. However, the
36 presence of natural orchids in their natural habitat (forest) has been reported to be greatly reduced. This is
37 caused by several factors, including excessive exploration and forest destruction. In addition, disease infection
38 is still a major obstacle in the cultivation and development of natural orchid potential (Mahfut, 2019).

39
40 Based on previous research (Mahfut et al., 2019), it is known that several individual orchid collections in the
41 Liwa Botanical Garden show symptoms of being infected with bacteria. This pathogen will infect orchids and
42 cause soft rot disease. The spread of bacteria can occur through the media of water, soil, contact between
43 healthy and diseased plants, and contamination of equipment for vegetative propagation and harvesting flowers.
44 To facilitate bacterial control, data inventory is first carried out as preliminary information about the infection
45 of the disease. Recognition of symptoms as an infection response is the first data in disease identification. The
46 data is used to determine disease intensity, plant resistance, and disease control efforts. Based on this, the
47 research on "Identification of Disease and Efforts to Protect Natural Orchid Plants against Bacterial Infection in
Liwa Botanical Garden" is very necessary. The results of this study are expected to be fundamental data in
efforts to protect orchids to support the application of conservation of natural orchids in the Liwa Botanical

48 Garden. In addition, the results of this study provide a good communication medium between researchers and
49 managers of the Liwa Botanical Garden in their efforts to control disease.

50 MATERIALS AND METHODS

51 **Sample Collection.** Sample collection was carried out on orchid leaves in Liwa Botanical Garden which
52 showed symptoms of being infected with a bacteria. All samples were tabulated and documented with photos.

53
54 **Analysis of Disease Symptoms.** This analysis is done by matching the sample documentation with the
55 literature that has been previously reported. The literature used is Muharam et al., 2013; Mahfut and Daryono,
56 2014; Mahfut et al., 2017; Fery et al., 2018.

57
58 **Disease Intensity Analysis.** This stage is done to determine the severity of the disease (disease severity).
59 Disease intensity is the proportion of infected hosts to the total observed surface area of the host. Calculation of
60 disease intensity analysis is done using the method of Rahardjo and Suhardi (2008) and the scale of disease
61 intensity refers to Mahfut et al. (2019).

62
63 **Plant Resistance Level.** Determination of the level of resistance of orchids to disease follows the method of
64 Mahfut et al. (2019).

65 RESULTS AND DISCUSSION

66 Sample Collection

67 The sample collection stage is carried out in the second week of January to the second week of February
68 2020. Samples are randomly selected from individual orchids showing symptoms of bacterial infection at the
69 Liwa Botanical Garden green house. Based on the collection results obtained 21 samples from 5 types of
70 orchids, i.e. *Bulbophyllum* sp., *Dendrobium crumenatum*, *Dendrobium montanum*, *Pholidota* sp., and *Vanilla*
71 sp. The type of orchid that is most infected with bacteria is *Vanilla* sp. as many as 11 samples. Furthermore,
72 *Pholidota* sp. and *Dendrobium crumenatum* for 3 samples each. Overall data collection results are shown in
73 **Table 1.**

74
75 **Table 1.** Collection of orchid leaf samples at Liwa Botanical Garden which shows a bacteria infection
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No	Species of Orchid	Number of Samples	Symptoms of Infection
1	<i>Bulbophyllum</i> sp.	2	Soft rot on the edge of the leaf
2	<i>Dendrobium crumenatum</i>	3	Soft rot on the middle of the leaf
3	<i>Dendrobium montanum</i>	2	Soft rot on the middle of the leaf
4	<i>Pholidota</i> sp.	3	Soft rot on the middle and edge of the leaf
5	<i>Vanilla</i> sp.	11	Soft rot on the edge of the leaf

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78 The selection of sampling locations was based on previous research (Mahfut et al., 2019) which reported
79 bacterial infections in the natural orchid collection at Liwa Botanical Garden in August and December 2019.
80 The type of orchid infected with bacteria was *Calanthe* sp. and *Flickingeria* sp. with symptoms of a mixture of
81 viruses, fungi and bacteria. Several other studies also reported a bacterial infection with symptoms of soft rot in
82 the orchid *Phalaenopsis* sp. at Ridho nursery, Lembang, West Java; orchid *Onchidium* sp. at Mr. Nursery
83 Huang, Bogor, West Java; orchid *Paphiopedilum* sp. at the Global Orchid nursery, Lembang, West Java
84 (Hanudin et al., 2011); *Grammatophyllum*, *Dendrobium*, and *Catleya* sp. in D. I. Yogyakarta (Joko et al.,
85 2010).

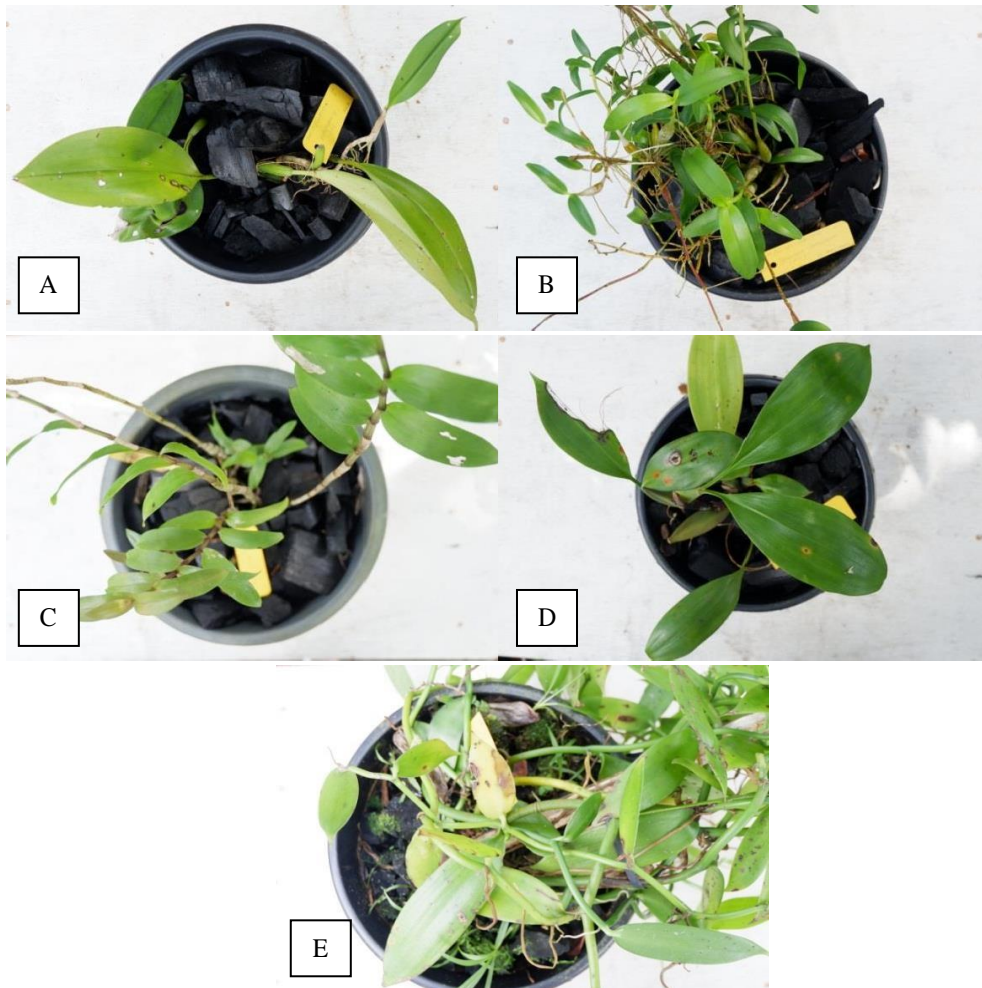
86 In this study, *Vanilla* sp. be the type of orchid that is infected with the most bacteria. This shows that this
87 type of orchid is the most vulnerable host infected with pathogenic bacteria. In addition to the orchids are

88 *Pholidota* sp., *Dendrobium crumenatum*, *Dendrobium montanum*, and *Bulbophyllum* sp. Joko et al. (2010)
89 reported that the orchid *Dendrobium* sp. also showed a fairly severe bacterial infection in D. I. Yogyakarta.

90

91 Analysis of Disease Symptoms

92 Based on the analysis of disease symptoms, *Vanilla* sp., *Pholidota* sp., *Dendrobium crumenatum*,
93 *Dendrobium montanum*, and *Bulbophyllum* sp. showing symptoms of bacterial infection that is rotten disease
94 characterized by leaves rot, runny, and cause a foul odor. Overall symptoms of a bacterial disease infecting
95 natural orchids in the Liwa Botanical Garden are presented in **Fig. 1**.



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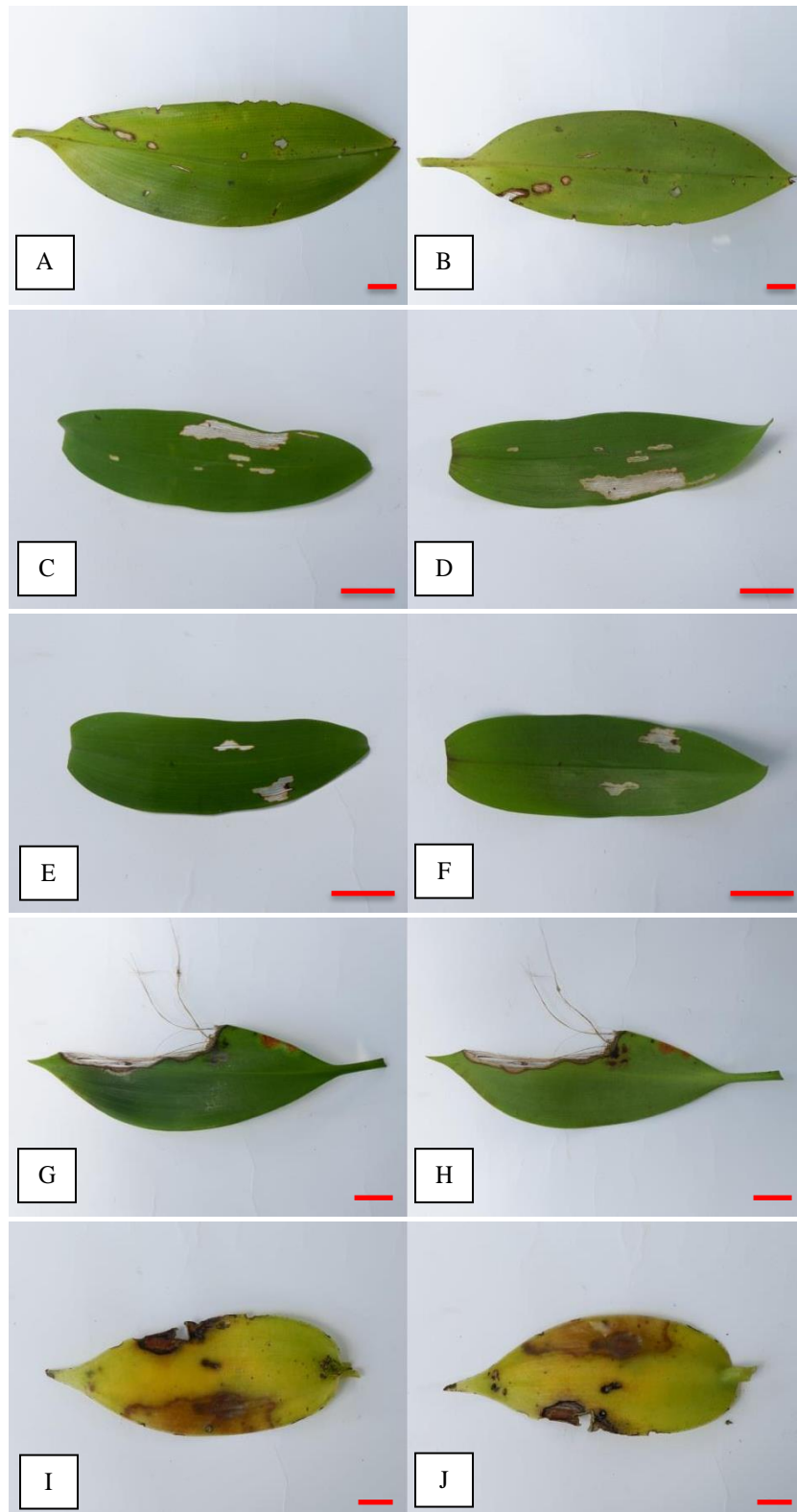
97 **Figure 1.** Symptoms of bacterial infection in orchids: (A) *Bulbophyllum* sp., (B) *Dendrobium crumenatum*,

98 (C) *Dendrobium montanum*, (D) *Pholidota* sp., and (E) *Vanilla* sp.

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100 Bacterial infections on samples *Bulbophyllum* sp., *Dendrobium crumenatum*, *Dendrobium montanum*, and
101 *Pholidota* sp., cause the formation of watery, cold-colored rot areas that resemble clear plastic. In contrast to
102 sample *Bulbophyllum* sp., *Dendrobium crumenatum*, and *Dendrobium montanum* which have more than one
103 spot on the leaf, orchids *Pholidota* sp. only has one wound area but with a large enough area. On *Pholidota* sp.
104 also shows the wound area that has begun to cause the formation of holes in the leaves.

105 Symptoms of bacterial infection on sample *Vanilla* sp. very different when compared with the other four
106 samples. But the symptoms of the infection are very similar when compared with the literature. Bacterial
107 infection of *Vanilla* sp. causing the formation of a soft, watery brownish wound area. In addition to causing the
108 formation of wound areas, bacterial infections in this type of orchid also cause some parts of the leaves to turn
109 yellow.



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Figure 2. Collection of samples of bacterial infection symptoms at Liwa Botanical Garden:
 (A, B) : Soft rot disease in orchid leaves *Bulbophyllum* sp.
 (C, D) : Soft rot disease in orchid leaves *Dendrobium crumenatum*
 (E, F) : Soft rot disease in orchid leaves anggrek *Dendrobium montanum*
 (G, H) : Soft rot disease in orchid leaves anggrek *Pholidota* sp.
 (I, J) : Soft rot disease in orchid leaves anggrek *Vanilla* sp.
 (A, C, E, G, I): Top surface of the leaf, (B, D, F, H, J): Surface under the leaf. Bar: 1 cm

118 The difference in symptoms that arise in the entire collection of this sample can be caused by several factors,
 119 including: the types of bacteria that infect the orchids are different, or the types of bacteria that infect the
 120 orchids are the same but the response of each species to bacterial infections are different (Hanudin and
 121 Rahardjo , 2012). The difference in these symptoms is not a problem if the plant protection measures taken are
 122 to use a bactericide as in this study (Huang, 2008). However, if the protective measures applied are to use
 123 antagonistic microbes, it is necessary to further identify the type of bacteria that infects orchids. This is done
 124 because antagonistic microbes work specifically with certain pathogenic bacteria. In other words, if the
 125 pathogenic bacteria that infect the orchid are different, the antagonistic microbes used will be different
 126 (Djatnika et al., 2011). Overall symptoms of a bacterial disease infecting natural orchids at Liwa Botanical
 127 Garden are showed on **Fig 2**.

128
 129 **Disease Intensity**

130 The results of the analysis of the intensity of bacterial diseases on natural orchids at Liwa Botanical
 131 Garden, obtained the percentage of intensity as shown in the **Table 2**.

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 133 **Table 2.** Analysis of bacterial infection on native orchids at Liwa Botanical Garden

No.	Species of Orchid	Disease Intensity
1	<i>Bulbophyllum</i> sp.	1,6%
2	<i>Dendrobium crumenatum</i>	1,39%
3	<i>Dendrobium montanum</i>	0,54%
4	<i>Pholidota</i> sp.	10%
5	<i>Vanilla</i> sp.	7,7%

134
 135 *Pholidota* sp. has the highest disease intensity. This is caused by several factors, including: the percentage
 136 of disease events is quite high at 30%, and as many as 3 samples from a total of 9 leaves show symptoms of
 137 bacterial infection. On 11 samples of *Vanilla* sp. samples showed symptoms of bacterial infection and the
 138 percentage of disease was 35%, but the intensity of the disease in this orchid was smaller when compared with
 139 *Pholidota* sp. Lower intensity of disease on *Vanilla* sp. this is caused by the total number of leaves in one plant
 140 that is 50 leaves. So that the ratio of the number of diseased and healthy leaves in this orchid is lower when
 141 compared with *Pholidota* sp. The lower the total ratio of the number of diseased and healthy leaves and the
 142 lower percentage of disease events will cause the results of the analysis of disease intensity to be lower
 143 (Rahardjo and Suhardi, 2008).

144 In contrast to *Pholidota* sp. and *Vanilla* sp. which shows the percentage of disease intensity is quite high,
 145 on *Bulbophyllum* sp., *Dendrobium crumenatum*, and *Dendrobium montanum* show a very low percentage of
 146 intensity that is less than 2%.

147
 148 **Plant Resistance**

149 Based on analysis resistance level that has been done, the results obtained as showed on **Table 3**.

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 151 **Table 3.** Analysis of resistance level to bacterial infection on native orchids in Liwa Botanical Garden

No.	Species of Orchid	Plant Resistance
1	<i>Bulbophyllum</i> sp.	Resistance
2	<i>Dendrobium crumenatum</i>	Resistance
3	<i>Dendrobium montanum</i>	Resistance
4	<i>Pholidota</i> sp.	Resistance
5	<i>Vanilla</i> sp.	Resistance

152
 153 Overall the sample has a rather resistant level of resistance because it has a disease incidence of less than
 154 40%. Mahfut et al. (2019) explain that the level of resistance is somewhat resistant is the level of resistance in
 155 orchids infected with the disease but the incidence of the disease is not more than 40%. This shows that the
 156 overall collection of natural orchid samples on Liwa Botanical Garden is capable of being infected by
 157 pathogens but a small portion of the cells supports the growth and development of pathogens so that they cause
 158 less disease.

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161 collaboration program with Universitas Lampung.

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