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Hemolysis Activity of Bacteria Isolates from Pelangi Forest of Ijen Geopark

Sattya Arimurti[1], Yeya Nepsan[1], Vivien Ajeng Pradita[1], Nuruli Fifi Anggraeni[1], Sutoyo Sutoyo[1], Siswanto Siswanto[1], Tantri Raras Ayuningtyas[2], Esti Utarti[*1]

[1]Biology Department, Jember University, City Jember, Indonesia
[2]Ijen Geopark, Bondowoso
* Correspondence Author: esti.fmipa@unej.ac.id

Abstract

Ijen geopark is one of Indonesia's geoparks, which is located in East Java. A total of 153 bacteria have been isolated from Pelangi Forest, which were then given the isolate code Isolate from Hutan Pelangi (IHP). These bacterial isolates can be utilized in industries, including organic matter decomposer agents, plant biocontrol agents, and probiotics. To ensure these bacteria are safe to use in various fields, they must be non-disease-causing (non-pathogenic). The hemolysis reactions offer simple tests to ease the analysis of pathogenic bacteria. The study aimed to evaluate the safety of bacterial isolates from Pelangi Forest based on their hemolysis reactions. Pathogenicity tests are performed through hemolysis reactions. The hemolysis test is performed by inoculating bacterial isolates on blood agar media after incubating for 48 hours. The results showed that as many as 123 (80.39%) bacterial isolates had type ß hemolysis thus being pathogenic, meanwhile as many as 30 (19.61%) bacterial isolates had type γ hemolysis so they were non-pathogenic.

Keywords: bacteria, hemolysis, Ijen geopark

Introduction

Ijen geopark, one of Indonesia's geoparks in Bondowoso East Java, has an area of 4,723 kilometers. In this geopark, the Pelangi forest is one of the biological sites that support natural biodiversity (Rahardjo et al., 2021). Pelangi Forest has a high vegetation diversity with a spot dominated by *Eucalyptus deglupta* (Rahardjo et al., 2021). The diversity of vegetation contributes to the high diversity of bacteria. Arimurti et al. (2022) reported a total of 153 bacteria isolates from Pelangi Forest with Isolate code based on the origin of from Hutan Pelangi (IHP). The 153 bacterial isolated from Pelangi Forest have the potential to be utilized in industries including organic matter decomposer agents (Arimurti et al., 2017; Sutoyo et al.,2019), plant biocontrol agents (Amaria et al., 2023), and probiotics (Paramita et al., 2019).

A biosafety assessment is a crucial first step in the management of microbial culture collection to screen and determine unexpected potential, so it must be non-disease-causing (nonpathogenic) (Tariq et al., 2022). This pathogenicity test is crucial so that the bacteria utilization will not be harmful to the environment and the researcher. The hemolysis test is a common method for the early step in evaluating their safety to humans (Thakkar et al., 2015, Sukmadewi et al. 2017). Bacteria capable of hydrolyzing red blood cells in blood media are indicated as pathogenic bacteria. The previous study by Amaria et al. (2019) showed that 3 out of 35 bacteria (9%) isolated from the Philippine-thung plant (*Reutealis trisperma* (Blanco) Airy Shaw) were presenting hemolysis reactions. Another study by Triwidodo and Listihani et al. (2021) showed of the 59 endophytic bacteria that were isolated from *Bambusa vulgaris*, *Gliricidia sepium*, and *Ocimum tenuiflorum*, 14 isolates (23.72%) underwent hemolysis. Furthermore, 68 out of 95 bacterial isolates from both rhizosphere and endophytes (71.57%) exhibited a positive hemolysis reaction, as reported by Amaria et al. (2023). The purpose of this study was to obtain the hemolysis activity of bacterial isolates from Pelangi Forest.

Materials and Methods

The research was conducted from May to August 2023 at the Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Jember University. This study was conducted using descriptive research methods. The hemolysis test was performed by Gilligan (2013). goat/sheep blood) using the streak method for 24–48 hours of incubation at 28 ºC. After an incubation time of 48 hours, observations were made of the formation of clear zones or changes in the colour of media around the bacterial colony to determine the hemolysis reaction that occurred. Different colours were noted to specify reaction types of hemolysis, i.e., a dark or greenish-grey coloured zone around the bacteria indicated partial lysis of the blood agar known as α hemolysis reaction, a distinct, clear zone referred to complete lysis of the blood agar or β hemolysis reaction, and if no zone or colour change observed around the bacterial colony indicated negative or γ hemolysis reaction.

Results and Discussion

There are 123 out of 153 bacterial isolates (80.39%) that showed positive hemolysis reactions (β), meanwhile, 30 (19,61%) bacterial isolates showed type γ hemolysis on the blood agar medium, (Table 1; Figure 1). The results of this study revealed that the bacterial isolates detected as positive hemolysis were all β hemolysis. The transparent clear zone surrounding the bacterial colony is a characteristic of β hemolysis formed due to the complete lysis of red blood cells in the medium. Lysis is caused by toxic hemolysin produced by bacteria to destroy red blood cells, causing the denaturation of haemoglobin to form a colorless product (Gilligan, 2013).

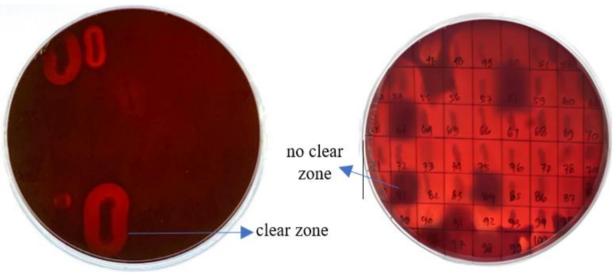

Figure 1. Clear zone surrounding colony indicated ß hemolysis activity of bacterial isolates in blood agar media

Type β hemolysis is considered a determinant or assesses the level of virulence and clinical relevance related to their potential as human and animal pathogens. Hemolysin compounds produced by bacteria are important virulence factors (Mogrovejo et al., 2020). The presence β hemolysis of bacteria in Pelangi forests shows that most indigenous Pelangi forest bacteria are potentially pathogenic. The presence of such bacteria can come from the plant rhizosphere or agricultural activities. Pelangi forest is a secondary forest with various types of plants for educational purposes. Based on Arimurti (2022), sampling locations other than Eucalyptus sp are also Dendrocalamus asper. According to Cruz et al. (2021), organic and conventional agriculture can be a reservoir for opportunistic microbes that are pathogenic to animals and humans.

The remaining only about 19.60% or about 30 isolates are bacteria that cannot lyse red blood cells (γ hemolysis) and are classified as negative hemolysis bacteria (non-pathogenic bacteria) (Figure 2). These bacteria can be used for further testing the potential of these bacteria.

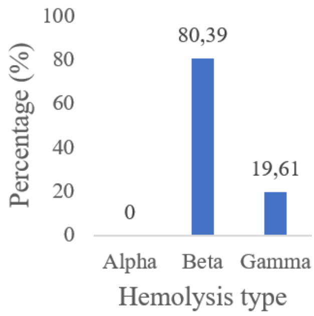

Figure 2. Percentage of hemolysis type of bacterial isolates from Rainbow Forest Ijen Geopark

Conclusion

A total of 123 (80.39%) bacterial isolates had type ß hemolysis thus being pathogenic, meanwhile as many as 30 (19.61%) bacterial isolates had type γ hemolysis so they were non-pathogenic.

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Table 1 Analysis of the pathogenicity of bacteria from Pelangi Forest soil litter

No	Isolate Code	Hemolysis Activity	No.	Isolate Code	Hemolysis Activity	No.	Isolate Code	Hemolysis Activity
1	IHP 1	ß	52	IHP 52	ß	103	IHP 103	ß
2	IHP 2	ß	53	IHP 53	ß	104	IHP 104	ß
3	IHP 3	γ	54	IHP 54	ß	105	IHP 105	ß
4	IHP 4	ß	55	IHP 55	γ	106	IHP 106	γ
5	IHP 5	ß	56	IHP 56	ß	107	IHP 107	γ
6	IHP 6	ß	57	IHP 57	ß	108	IHP 108	ß
7	IHP 7	γ	58	IHP 58	γ	109	IHP 109	γ
8	IHP 8	ß	59	IHP 59	ß	110	IHP 110	ß
9	IHP 9	ß	60	IHP 60	ß	111	IHP 111	γ
10	IHP 10	ß	61	IHP 61	ß	112	IHP 112	γ
11	IHP 11	ß	62	IHP 62	ß	113	IHP 113	γ
12	IHP 12	ß	63	IHP 63	γ	114	IHP 114	ß
13	IHP 13	ß	64	IHP 64	ß	115	IHP 115	ß
14	IHP 14	ß	65	IHP 65	ß	116	IHP 116	ß
15	IHP 15	ß	66	IHP 66	ß	117	IHP 117	ß
16	IHP 16	γ	67	IHP 67	ß	118	IHP 117	ß
17	IHP 17	ß	68	IHP 68	ß	119	IHP 118	ß
18	IHP 18	ß	69	IHP 69	ß	120	IHP 119	ß
19	IHP 19	ß	70	IHP 70	ß	121	IHP 120	ß
20	IHP 20	ß	71	IHP 71	ß	122	IHP 121	ß
21	IHP 21	ß	72	IHP 72	ß	123	IHP 122	γ
22	IHP 22	ß	73	IHP 73	ß	124	IHP 123	ß
23	IHP 23	ß	74	IHP 74	ß	125	IHP 124	ß
24	IHP 24	ß	75	IHP 75	ß	126	IHP 125	ß
25	IHP 25	ß	76	IHP 76	ß	127	IHP 126	ß
26	IHP 26	γ	77	IHP 77	ß	128	IHP 127	ß
27	IHP 27	γ	78	IHP 78	ß	129	IHP 128	ß
28	IHP 28	ß	79	IHP 79	ß	130	IHP 130	ß
29	IHP 29	ß	80	IHP 80	ß	131	IHP 131	γ
30	IHP 30	γ	81	IHP 81	γ	132	IHP 132	ß
31	IHP 31	γ	82	IHP 82	ß	133	IHP 133	ß
32	IHP 32	γ	83	IHP 83	ß	134	IHP 134	ß
33	IHP 33	ß	84	IHP 84	γ	135	IHP 135	ß
34	IHP 34	ß	85	IHP 85	ß	136	IHP 136	ß
35	IHP 35	ß	86	IHP 86	ß	137	IHP 137	ß
36	IHP 36	ß	87	IHP 87	ß	138	IHP 138	ß
37	IHP 37	ß	88	IHP 88	γ	139	IHP 139	ß
38	IHP 38	ß	89	IHP 89	ß	140	IHP 140	ß
39	IHP 39	ß	90	IHP 90	ß	141	IHP 141	ß
40	IHP 40	γ	91	IHP 91	γ	142	IHP 142	ß
41	IHP 41	ß	92	IHP 92	ß	143	IHP 143	ß
42	IHP 42	ß	93	IHP 93	ß	144	IHP 144	ß
43	IHP 43	γ	94	IHP 94	ß	145	IHP 145	ß
44	IHP 44	ß	95	IHP 95	ß	146	IHP 146	ß
45	IHP 45	ß	96	IHP 96	γ	147	IHP 147	ß
46	IHP 46	ß	97	IHP 97	γ	148	IHP 148	ß
47	IHP 47	ß	98	IHP 98	γ	149	IHP 149	ß
48	IHP 48	ß	99	IHP 99	ß	150	IHP 150	ß
49	IHP 49	ß	100	IHP 100	ß	151	IHP 151	ß
50	IHP 50	ß	101	IHP 101	γ	152	IHP 152	ß
51	IHP 51	ß	102	IHP 102	γ	153	IHP 153	ß

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