Isolation of Lignocellulolytic Bacteria From Erek-erek Geoforest Ijen Geopark, Indonesia

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Abstract. Ijen Geopark is one of the UNESCO Global Geoparks in Indonesia. The Erek-erek Geoforest (EEG) is one of the biosites in the Ijen Geopark. The EEG has an abundant biomass which is composed of lignocellulosic complex. The abundance of biomass has the potential as a source of lignocellulolytic microorganisms that can degrade lignocellulose material. The purpose of this research was to isolate lignocellulolytic bacteria from the Erek-erek Geoforest. This research method was soil sampling, physicochemical analysis of soil, isolation, and purification of bacteria, growth capability in lignocellulose media, and pathogenicity-test. The results showed that a total of 180 were isolated from EEG. There are 93 bacteria growth in lignin media and 142 bacteria growth in cellulolytic with the isolated code EIB (erek-erek Ijen Banyuwangi) under aerobic conditions which had various bacterial morphologies. To ensure the safety of using these isolates, it is necessary to do a pathogenicity test of these bacteria using a blood agar plate. Indicated pathogenic bacteria will form a clear zone around the bacterial colony on the blood media. The pathogenicity test of the highest lignocellulolytic bacteria showed that the 3 ligninolytic and 3 cellulolytic EIB bacteria isolates were non-pathogenic. Further, non-pathogenic bacteria from the soil litter of erek-erek forests can be tested for their potential as biological agents for the industry.

1 Introduction

Use Erek-erek Ijen geoforest is a primary tropical rainforest located in Licin, Banyuwangi, East Java, Indonesia. Erek-erek geoforest is located on the eastern slope of Mount Ijen and is part of the Ijen Geoforest. This forest is classified as a primary forest because it has complete stratification with a forest floor rich in biodiversity Marchantiophyta, Bryophyta, Pteridophyta, and several grass species such as reeds [1]. The diversity of these plants shows a high level of soil fertility which indicates that the decomposition process in the soil is going well, especially lignocellulose decomposition as part of the carbon cycle in nature. Lignocellulose is a constituent component of plant cell walls, so the abundance of biomass available in Erek-erek is positively correlated with its lignocellulose abundance. Litter decomposition in forests occurs as a form of degradation of lignocellulose components.

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Lignocellulose is composed of three main polymers, namely cellulose (35-50%), hemicellulose (20-35%) and lignin (10-25%) [2]. The structure of lignocellulose is very compact where the bond between lignin and cellulose and hemicellulose is inserted in the cellulose, making lignocellulose a very complex substrate for several enzymes. The bond between lignin with hemicellulose and cellulose plays a major role in composing plant cell walls, impermeability, and maintaining plant resistance to microbial attack and oxidative stress. Some factors that cause lignocellulose to be classified as a recalcitrant material are lignin content that protects cellulose, cellulose-hemicellulose complex, crystallinity, and high degree of polymerization of cellulose and low accessibility of cellulose due to fiber strength [3]. The fibril cellulose matrix complex is embedded in hemicellulose and lignin, which reduces the accessibility of cellulase and hemicellulase to bind to their substrate thereby reducing the degradation activity of plant biomass [4]. Therefore, it is necessary to research microbes that can degrade lignin and cellulose, both crystalline and amorphous, so that the utilization of lignocellulose waste as a carbon source can be optimal.

Bacteria are one of the microbes that play a role in lignocellulose biodegradation in forest floor litter. In the decomposition process, the advantage of bacteria compared to other microbes is a rapid growth rate and a relatively small enzyme molecule size compared to fungi so it is easier to diffuse into plant tissues [5]. Some bacteria have been reported to have lignocellulose degrading abilities including *Pseudomonas poae, Streptomyces thermoviolaceus, Klebsiella oxytoca, Paenibacillus polymyxa* ND25, *Pantoea ananatis* Sd-1, and *Sphingobacterium* sp. KSN-11. There are also several genera of *Bacillus, Paenibacillus, Actinobacteria, Pseudomonas, Arthrobacter, Pseudoxanthomonas* [6]–[11]. The research aims to characterize lignocellulolytic bacteria, especially lignin degradation or ligninolytic bacteria, and cellulose-degrading or cellulolytic bacteria from Erek-erek Ijen Geoforest, Banyuwangi, Indonesia.

2 Materials and Methods

2.1 Sampling

Soil samples (± 500 grams) were collected from Erek-erek Geoforest Banyuwangi using a soil drill. The samples underwent physicochemical analysis, including pH and temperature measurements.

2.2 Isolation and Screening of Lignocellulolytic Bacteria

2.2.1 Isolation of Bacteria

Bacterial isolation was carried out using Nutrient Agar (NA) media at dilution results stratified to 10^{-7} and incubated at 30 ° C for 24 hours. Bacterial colonies that have different morphologies were purified using the NA media quadrant method.

2.2.2 Screening of ligninolytic bacteria

Screening of ligninolytic bacteria is carried out by growing isolates on a minimum of M9 + 0.25% alkaline lignin media. The ligninolytic activity of bacterial isolates was shown by the ability of these bacterial isolates to grow on alkaline lignin media.

2.3 Pathogenicity test

Pathogenicity tests were carried out on 5 isolates of ligninolytic bacteria that can degrade dyes and 10 isolates of cellulolytic bacteria that had the best growing ability on seulose media. The pathogenicity test was performed by inoculating each isolate on blood agar media and incubating it at room temperature for 24 hours. Isolates that can hydrolyze blood agar indicated pathogenic.

2.4 Data analysis

This study used descriptive statistical analysis. Data was displayed in the form of tables and histograms. The best results were determined by ranking values from highest to lowest.

3 Results and Discussion

The isolation of lignocellulolytic bacteria was conducted by collecting litter samples from six different locations in Erek-erek Ijen Geopark Banyuwangi. Additionally, abiotic conditions of the sample location i.e. humidity, temperature, pH, and vegetation analysis were measured (Table 1). The environmental data was evaluated for optimisation on the isolation and screening process, while the type of vegetation was evaluated for the analysis of lignocellulolytic bacterial potential. The composition of biomass, which serves as a substrate for the activity of decomposer bacteria on the forest floor litter, is affected by the type of vegetation.

Table 1. Environmental conditions at the sampling location

Location	Geographical Coordinates	Humidity (%RH)	Temperature (°C)	Soil pH	Vegetation
1	8°03'35.71"S 114°04'16.73"E	79,6	22,5°C	5,4	*Selaginella* sp., *Selaginella plana, Cyathea orientalis*
2	7°59'51.73"S 114°15'20.24"E	89,2	22	5,1	*Selaginella plana*
3	8°09'51.75"S 114°09'00.17"E	93,9	19,9	6,2	*Selaginella* sp., *Begonia* sp.
4	8°11'20.69"S 114°16'30.54"E	89,2	20,1	6,0	*Begonia* sp
5	8°05'07.61"S 114°19'58.48"E	89,7	21,6	5,3	*Dyera* sp.
6	8°02'27.69"S 114°08'58.12"E	86,6	21,5	5,5	*Selaginella plana*

3.1 Isolation and screening of ligninolytic and cellulolytic bacteria

Plant diversity shows the level of soil fertility which indicates the decomposition process in the soil is going well. One of the microorganisms that play a role in the decomposition process of plant biomass on the forest floor of Erek-erek Ijen Geopark Banyuwangi is bacteria. The results showed that a total of 180 bacteria had been successfully isolated from six sampling locations. Bacteria isolation is performed using nutrient agar media, an enrichment medium. This method allows for obtaining a higher number of culturable bacteria.

These bacterial isolates become a source of bacterial isolate to obtain potentially lignocellulose-degrading bacteria. The results of lignocellulolytic activity tests are expected

to be additional material for culture collection of biodiversity of microorganisms. The diversity of bacteria from the Erek-erek forest floor plays an important role, especially in the process of lignocellulose decomposition as part of the carbon cycle in nature. The abundance of biomass available in Erek-erek is positively correlated with its lignocellulose content. Lignocellulose is a constituent component of plant cell walls.

Forest litter decomposition involves the degradation of lignocellulose components. To analyze lignocellulolytic degradation activity, bacteria were inoculated on alkaline lignin media for lignin degradation and on carboxymethyl cellulose (CMC) media for cellulose degradation. The initial screening involved testing the ability to grow bacteria that can degrade lignin and utilize cellulose as the sole source of carbon. In this study, a total of ninety-three bacterial isolates were positively degrading lignin based on the results of the ability-to-grow test on selective media. Additionally, a total of 142 isolates were able to utilize cellulose as their sole source of carbon. (Table 2, Table 3, and Figure 1).

Table 2. Screening of ligninolytic bacteria based on ability to grow on alkaline lignin media

Isolate code	Growth capability	Isolate code	Growth capability	Isolate code	Growth capability
EIB 1	(++)	EIB 61	(-)	EIB 121	(+)
EIB 2	(++)	EIB 62	(-)	EIB 122	(+)
EIB 3	(+)	EIB 63	(+)	EIB 123	(+)
EIB 4	(+)	EIB 64	(-)	EIB 124	(-)
EIB 5	(-)	EIB 65	(-)	EIB 125	(-)
EIB 6	(++)	EIB 66	(-)	EIB 126	(-)
EIB 7	(-)	EIB 67	(-)	EIB 127	(-)
EIB 8	(++)	EIB 68	(-)	EIB 128	(+++)
EIB 9	(+)	EIB 69	(-)	EIB 129	(+)
EIB 10	(-)	EIB 70	(-)	EIB 130	(+)
EIB 11	(+)	EIB 71	(-)	EIB 131	(-)
EIB 12	(+)	EIB 72	(-)	EIB 132	(+)
EIB 13	(-)	EIB 73	(-)	EIB 133	(+)
EIB 14	(+)	EIB 74	(+)	EIB 134	(-)
EIB 15	(+)	EIB 75	(+)	EIB 135	(-)
EIB 16	(+)	EIB 76	(+)	EIB 136	(-)
EIB 17	(+)	EIB 77	(+)	EIB 137	(-)
EIB 18	(+)	EIB 78	(++)	EIB 138	(+++)
EIB 19	(+)	EIB 79	(-)	EIB 139	(++)
EIB 20	(+)	EIB 80	(++)	EIB 140	(+)
EIB 21	(-)	EIB 81	(++)	EIB 141	(-)
EIB 22	(++)	EIB 82	(-)	EIB 142	(+)
EIB 23	(-)	EIB 83	(-)	EIB 143	(-)
EIB 24	(-)	EIB 84	(-)	EIB 144	(+)

Isolate code	Growth capability	Isolate code	Growth capability	Isolate code	Growth capability
EIB 25	(+)	EIB 85	(++)	EIB 145	(++)
EIB 26	(++)	EIB 86	(+)	EIB 146	(-)
EIB 27	(+)	EIB 87	(-)	EIB 147	(++)
EIB 28	(-)	EIB 88	(+)	EIB 148	(+)
EIB 29	(-)	EIB 89	(+)	EIB 149	(+)
EIB 30	(+)	EIB 90	(+)	EIB 150	(-)
EIB 31	(+)	EIB 91	(-)	EIB 151	(-)
EIB 32	(+++)	EIB 92	(++)	EIB 152	(-)
EIB 33	(+)	EIB 93	(++)	EIB 153	(-)
EIB 34	(-)	EIB 94	(++)	EIB 154	(+)
EIB 35	(-)	EIB 95	(++)	EIB 155	(++)
EIB 36	(++)	EIB 96	(-)	EIB 156	(-)
EIB 37	(+)	EIB 97	(+)	EIB 157	(-)
EIB 38	(+)	EIB 98	(-)	EIB 158	(-)
EIB 39	(-)	EIB 99	(+)	EIB 159	(+++)
EIB 40	(+)	EIB 100	(++)	EIB 160	(-)
EIB 41	(-)	EIB 101	(++)	EIB 161	(++)
EIB 42	(+)	EIB 102	(++)	EIB 162	(++)
EIB 43	(+)	EIB 103	(++)	EIB 163	(-)
EIB 44	(-)	EIB 104	(-)	EIB 164	(-)
EIB 45	(+)	EIB 105	(+)	EIB 165	(+)
EIB 46	(-)	EIB 106	(+)	EIB 166	(-)
EIB 47	(-)	EIB 107	(+)	EIB 167	(-)
EIB 48	(+)	EIB 108	(+)	EIB 168	(-)
EIB 49	(-)	EIB 109	(-)	EIB 169	(-)
EIB 50	(-)	EIB 110	(-)	EIB 170	(-)
EIB 51	(-)	EIB 111	(++)	EIB 171	(++)
EIB 52	(-)	EIB 112	(+)	EIB 172	(-)
EIB 53	(-)	EIB 113	(-)	EIB 173	(-)
EIB 54	(-)	EIB 114	(-)	EIB 174	(++)
EIB 55	(-)	EIB 115	(-)	EIB 175	(-)
EIB 56	(-)	EIB 116	(+)	EIB 176	(++)
EIB 57	(-)	EIB 117	(-)	EIB 177	(-)
EIB 58	(-)	EIB 118	(+)	EIB 178	(++)
EIB 59	(-)	EIB 119	(++)	EIB 179	(-)

Isolate code	Growth capability	Isolate code	Growth capability	Isolate code	Growth capability
EIB 60	(-)	EIB 120	(+)	EIB 180	(+++)

Description: (-) no growth, (+) grows rarely, (++) grows well, (+++) grows very well

Table 3. Screening of cellulolytic bacteria based on ability to grow on CMC media

Isolate code	Growth capability	Isolate code	Growth capability	Isolate code	Growth capability
EIB 1	(+)	EIB 61	(++)	EIB 121	(+++)
EIB 2	(+)	EIB 62	(+)	EIB 122	(+)
EIB 3	(++)	EIB 63	(+++)	EIB 123	(+)
EIB 4	(+++)	EIB 64	(+)	EIB 124	(+)
EIB 5	(-)	EIB 65	(+++)	EIB 125	(++)
EIB 6	(+)	EIB 66	(++)	EIB 126	(+)
EIB 7	(-)	EIB 67	(+)	EIB 127	(+)
EIB 8	(++)	EIB 68	(-)	EIB 128	(+)
EIB 9	(+++)	EIB 69	(-)	EIB 129	(+++)
EIB 10	(+)	EIB 70	(-)	EIB 130	(+)
EIB 11	(+++)	EIB 71	(-)	EIB 131	(++)
EIB 12	(+)	EIB 72	(-)	EIB 132	(+)
EIB 13	(-)	EIB 73	(+++)	EIB 133	(++)
EIB 14	(+)	EIB 74	(+++)	EIB 134	(+)
EIB 15	(+)	EIB 75	(+)	EIB 135	(+)
EIB 16	(+)	EIB 76	(++)	EIB 136	(++)
EIB 17	(-)	EIB 77	(+)	EIB 137	(+++)
EIB 18	(+++)	EIB 78	(+++)	EIB 138	(+)
EIB 19	(+)	EIB 79	(+)	EIB 139	(+)
EIB 20	(+)	EIB 80	(+)	EIB 140	(++)
EIB 21	(++)	EIB 81	(+++)	EIB 141	(+)
EIB 22	(+)	EIB 82	(-)	EIB 142	(+)
EIB 23	(++)	EIB 83	(-)	EIB 143	(+++)
EIB 24	(++)	EIB 84	(-)	EIB 144	(++)
EIB 25	(-)	EIB 85	(+)	EIB 145	(++)
EIB 26	(-)	EIB 86	(++)	EIB 146	(++)
EIB 27	(+)	EIB 87	(-)	EIB 147	(+)
EIB 28	(+)	EIB 88	(++)	EIB 148	(+)
EIB 29	(+)	EIB 89	(+)	EIB 149	(+++)
EIB 30	(+)	EIB 90	(+)	EIB 150	(+)

Isolate code	Growth capability	Isolate code	Growth capability	Isolate code	Growth capability
EIB 31	(-)	EIB 91	(-)	EIB 151	(+)
EIB 32	(+)	EIB 92	(+)	EIB 152	(++)
EIB 33	(+++)	EIB 93	(+++)	EIB 153	(+)
EIB 34	(+++)	EIB 94	(+)	EIB 154	(+)
EIB 35	(-)	EIB 95	(+)	EIB 155	(+)
EIB 36	(+)	EIB 96	(+)	EIB 156	(+++)
EIB 37	(+)	EIB 97	(+)	EIB 157	(+)
EIB 38	(+)	EIB 98	(+)	EIB 158	(-)
EIB 39	(-)	EIB 99	(+++)	EIB 159	(++)
EIB 40	(-)	EIB 100	(+)	EIB 160	(++)
EIB 41	(++)	EIB 101	(++)	EIB 161	(+)
EIB 42	(+++)	EIB 102	(-)	EIB 162	(+)
EIB 43	(+)	EIB 103	(-)	EIB 163	(-)
EIB 44	(+)	EIB 104	(-)	EIB 164	(-)
EIB 45	(+)	EIB 105	(++)	EIB 165	(++)
EIB 46	(-)	EIB 106	(+)	EIB 166	(++)
EIB 47	(-)	EIB 107	(+++)	EIB 167	(+)
EIB 48	(+++)	EIB 108	(+)	EIB 168	(+++)
EIB 49	(-)	EIB 109	(++)	EIB 169	(++)
EIB 50	(-)	EIB 110	(+)	EIB 170	(++)
EIB 51	(+)	EIB 111	(+++)	EIB 171	(+++)
EIB 52	(-)	EIB 112	(+)	EIB 172	(+)
EIB 53	(+)	EIB 113	(-)	EIB 173	(+++)
EIB 54	(+++)	EIB 114	(-)	EIB 174	(+++)
EIB 55	(+)	EIB 115	(-)	EIB 175	(+)
EIB 56	(+)	EIB 116	(-)	EIB 176	(+++)
EIB 57	(-)	EIB 117	(-)	EIB 177	(+)
EIB 58	(-)	EIB 118	(+)	EIB 178	(+++)
EIB 59	(++)	EIB 119	(+++)	EIB 179	(+)
EIB 60	(++)	EIB 120	(++)	EIB 180	(+)

Description: (-) no growth, (+) grows rarely, (++) grows well, (+++) grows very well

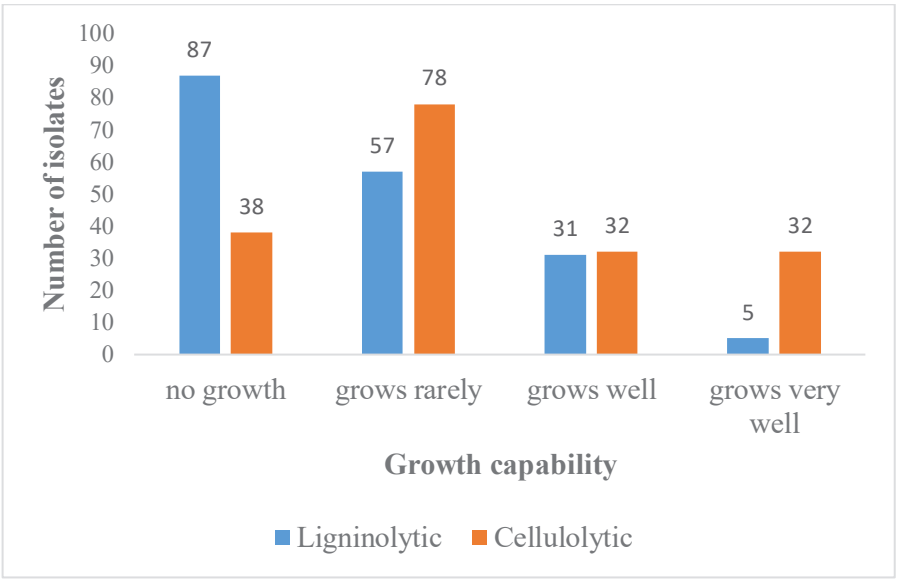

Fig. 1. The ability to grow bacteria on alkaline lignin media (ligninolytic bacteria) and CMC media (cellulolytic bacteria)

Bacteria degrade lignocellulose to obtain organic carbon both as an energy source and as a constituent of cellular components. Lignocellulose consists of 3 main components, namely lignin, cellulose, and hemicellulose. The three main polymers are bound in hetero matrix bonds with abundant presence varying depending on the type of biomass. Lignin, hemicellulose, and cellulose are bound to form a matrix that blocks hemicellulose and cellulose activity, where lignin is called glue by filling the gap between cellulose and hemicellulose. Microorganisms can degrade lignin to utilize lignin as a carbon source. However, some microorganisms carry out lignin degradation activity as a co-metabolism. The microorganism initially breaks down lignin to enhance the accessibility of its hydrolytic enzymes to cellulose substrates, making them both ligninolytic and cellulolytic. The study results showed that 85 bacteria had both ligninolytic and cellulolytic activity (Table 4).

Table 4. Bacterial growing ability of lignin and cellulose

Isolate code	Ligninolytic	Cellulolytic	Isolate code	Ligninolytic	Cellulolytic
EIB 1	(++)	(+)	EIB 98	(-)	(+)
EIB 2	(++)	(+)	EIB 99	(+)	(+++)
EIB 3	(+)	(++)	EIB 100	(++)	(+)
EIB 4	(+)	(+++)	EIB 101	(++)	(++)
EIB 6	(++)	(+)	EIB 102	(++)	(-)
EIB 8	(++)	(++)	EIB 103	(++)	(-)
EIB 9	(+)	(+++)	EIB 105	(+)	(++)
EIB 10	(-)	(+)	EIB 106	(+)	(+)
EIB 11	(+)	(+++)	EIB 107	(+)	(+++)
EIB 12	(+)	(+)	EIB 108	(+)	(+)

Isolate code	Ligninolytic	Cellulolytic	Isolate code	Ligninolytic	Cellulolytic
EIB 14	(+)	(+)	EIB 109	(-)	(++)
EIB 15	(+)	(+)	EIB 110	(-)	(+)
EIB 16	(+)	(+)	EIB 111	(++)	(+++)
EIB 17	(+)	(-)	EIB 112	(+)	(+)
EIB 18	(+)	(+++)	EIB 116	(+)	(-)
EIB 19	(+)	(+)	EIB 118	(+)	(+)
EIB 20	(+)	(+)	EIB 119	(++)	(+++)
EIB 21	(-)	(++)	EIB 120	(+)	(++)
EIB 22	(++)	(+)	EIB 121	(+)	(+++)
EIB 23	(-)	(++)	EIB 122	(+)	(+)
EIB 24	(-)	(++)	EIB 123	(+)	(+)
EIB 25	(+)	(-)	EIB 124	(-)	(+)
EIB 26	(++)	(-)	EIB 125	(-)	(++)
EIB 27	(+)	(+)	EIB 126	(-)	(+)
EIB 28	(-)	(+)	EIB 127	(-)	(+)
EIB 29	(-)	(+)	EIB 128	(+++)	(+)
EIB 30	(+)	(+)	EIB 129	(+)	(+++)
EIB 31	(+)	(-)	EIB 130	(+)	(+)
EIB 32	(+++)	(+)	EIB 131	(-)	(++)
EIB 33	(+)	(+++)	EIB 132	(+)	(+)
EIB 34	(-)	(+++)	EIB 133	(+)	(++)
EIB 36	(++)	(+)	EIB 134	(-)	(+)
EIB 37	(+)	(+)	EIB 135	(-)	(+)
EIB 38	(+)	(+)	EIB 136	(-)	(++)
EIB 40	(+)	(-)	EIB 137	(-)	(+++)
EIB 41	(-)	(++)	EIB 138	(+++)	(+)
EIB 42	(+)	(+++)	EIB 139	(++)	(+)
EIB 43	(+)	(+)	EIB 140	(+)	(++)
EIB 44	(-)	(+)	EIB 141	(-)	(+)
EIB 45	(+)	(+)	EIB 142	(+)	(+)
EIB 48	(+)	(+++)	EIB 143	(-)	(+++)
EIB 51	(-)	(+)	EIB 144	(+)	(++)
EIB 53	(-)	(+)	EIB 145	(++)	(++)
EIB 54	(-)	(+++)	EIB 146	(-)	(++)
EIB 55	(-)	(+)	EIB 147	(++)	(+)

Isolate code	Ligninolytic	Cellulolytic	Isolate code	Ligninolytic	Cellulolytic
EIB 56	(-)	(+)	EIB 148	(+)	(+)
EIB 59	(-)	(++)	EIB 149	(+)	(+++)
EIB 60	(-)	(++)	EIB 150	(-)	(+)
EIB 61	(-)	(++)	EIB 151	(-)	(+)
EIB 62	(-)	(+)	EIB 152	(-)	(++)
EIB 63	(+)	(+++)	EIB 153	(-)	(+)
EIB 64	(-)	(+)	EIB 154	(+)	(+)
EIB 65	(-)	(+++)	EIB 155	(++)	(+)
EIB 66	(-)	(++)	EIB 156	(-)	(+++)
EIB 67	(-)	(+)	EIB 157	(-)	(+)
EIB 73	(-)	(+++)	EIB 159	(+++)	(++)
EIB 74	(+)	(+++)	EIB 160	(-)	(++)
EIB 75	(+)	(+)	EIB 161	(++)	(+)
EIB 76	(+)	(++)	EIB 162	(++)	(+)
EIB 77	(+)	(+)	EIB 165	(+)	(++)
EIB 78	(++)	(+++)	EIB 166	(-)	(++)
EIB 79	(-)	(+)	EIB 167	(-)	(+)
EIB 80	(++)	(+)	EIB 168	(-)	(+++)
EIB 81	(++)	(+++)	EIB 169	(-)	(++)
EIB 85	(++)	(+)	EIB 170	(-)	(++)
EIB 86	(+)	(++)	EIB 171	(++)	(+++)
EIB 88	(+)	(++)	EIB 172	(-)	(+)
EIB 89	(+)	(+)	EIB 173	(-)	(+++)
EIB 90	(+)	(+)	EIB 174	(++)	(+++)
EIB 92	(++)	(+)	EIB 175	(-)	(+)
EIB 93	(++)	(+++)	EIB 176	(++)	(+++)
EIB 94	(++)	(+)	EIB 177	(-)	(+)
EIB 95	(++)	(+)	EIB 178	(++)	(+++)
EIB 96	(-)	(+)	EIB 179	(-)	(+)
EIB 97	(+)	(+)	EIB 180	(+++)	(+)

Description: (-) no growth, (+) grows rarely, (++) grows well, (+++) grows very well

Pathogenicity tests were conducted on 63 isolates of ligninolytic and cellulolytic bacteria based on their hemolytic characteristics on blood agar media. This was indicated by the presence of a clear zone produced around the bacterial colony. Beta-hemolysis and alpha-hemolysis bacteria are bacteria that can lyse red blood cells on blood agar media and classified as pathogenic bacteria. Based on pathogenic tests on 63 isolates of lignocellulose-

degrading bacteria, 39 were found to be pathogenic due to the formation of a clear zone, while the remaining 24 isolates were non-pathogenic lignocellulose-degrading bacteria, as indicated by the absence of a clear zone (Table 4). These 24 bacterial isolates were classified into gamma-hemolysis types or nonpathogenic bacteria. Therefore, these 24 bacterial isolates can be developed for use as organic material decomposer agents [13], plant biocontrol agents [14], and probiotics [15]. The isolate number EIB 180 presented alpha hemolysis type i.e. isolates were not able to form clear zones, but there was a change in the blood agar medium around the colony (Table 4). This bacterial isolate was indicated as a pathogenic bacteria because it can lyse red blood cells in blood media even though it undergoes incomplete lysis [16]. The proportion of non-pathogenic lignocellulolytic bacteria was about 38.1 % or 24 (Figure 2).

Table 5. Analysis of hemolysis of bacterial isolates of Erek-erek Forest Ijen Geopark

No.	Isolate code	Types of Hemolysis	No.	Isolate code	Types of Hemolysis	No.	Isolate code	Types of Hemolysis
1.	EIB 1	ß	22.	EIB 32	γ	43.	EIB 102	ß
2.	EIB 2	ß	23.	EIB 33	γ	44.	EIB 103	γ
3.	EIB 3	γ	24.	EIB 35	γ	45.	EIB 105	ß
4.	EIB 4	γ	25.	EIB 37	γ	46.	EIB 120	γ
5.	EIB 5	γ	26.	EIB 38	γ	47.	EIB 123	ß
6.	EIB 7	γ	27.	EIB 42	ß	48.	EIB 128	γ
7.	EIB 8	ß	28.	EIB 70	ß	49.	EIB 138	γ
8.	EIB 9	γ	29.	EIB 71	γ	50.	EIB 139	ß
9.	EIB 10	ß	30.	EIB 72	ß	51.	EIB 142	γ
10.	EIB 11	ß	31.	EIB 78	ß	52.	EIB 143	ß
11.	EIB 12	ß	32.	EIB 80	γ	53.	EIB 149	ß
12.	EIB 13	γ	33.	EIB 81	ß	54.	EIB 150	ß
13.	EIB 14	ß	34.	EIB 85	ß	55.	EIB 153	ß
14.	EIB 15	ß	35.	EIB 90	γ	56.	EIB 157	ß
15.	EIB 16	ß	36.	EIB 92	γ	57.	EIB 159	ß
16.	EIB 18	γ	37.	EIB 94	ß	58.	EIB 160	ß
17.	EIB 19	γ	38.	EIB 95	γ	59.	EIB 162	ß
18.	EIB 20	γ	39.	EIB 96	ß	60.	EIB 166	ß
19.	EIB 26	ß	40.	EIB 99	ß	61.	EIB 168	ß
20.	EIB 27	ß	41.	EIB 100	ß	62.	EIB 170	ß
21.	EIB 31	ß	42.	EIB 101	ß	63.	EIB 180	α

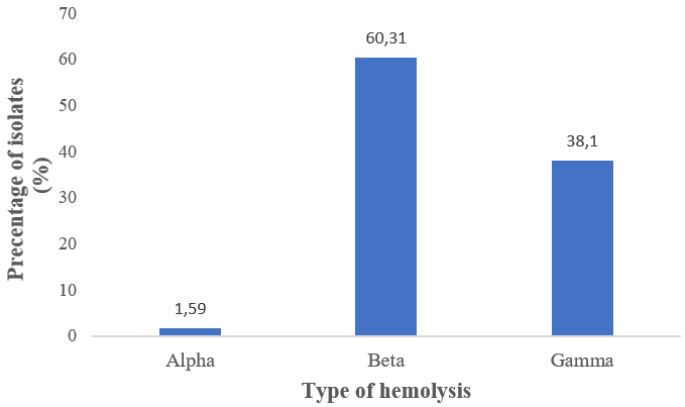

Fig. 2. Percentage of hemolysis type of bacterial isolates from Erek-erek Ijen Geopark

4 Conclusion

A total of 180 bacterial isolates had been isolated from the forest floor of Erek-erek Ijen Geopark Banyuwangi whereas a total of 93 bacterial isolates were ligninolytic, 142 bacterial isolates were cellulolytic and a total of 85 bacteria had both ligninolytic and cellulolytic activities or lignocellulolytic. Based on the results of the hemolysis test, a total of 3.68% of lignocellulolytic bacteria from Erek-erek forest soil litter were gamma-hemolysis which indicated the nature of non-pathogenic-bacteria.

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References

1. I. Geopark, Buku Pintar Ijen Geopark Wilayah Bondowoso, (Bondowoso, 2021).
2. P.-G. C., Y.-H. Choi, Y.-S. Choi, C.-N. Seong, S.-S. Cho, H. J. Lee, J. C. Yoo, A novel thermostable cellulase-free xilanase stable in broad range of pH from Streptomyces sp. CS428. Proc Biochem. **4**, 11881196 (2013).
3. M. Andlar, T. Rezic, N. Mardetko, D. Kracher, R. Ludwig, B. Santek, Lignocellulose degradation: an overview of fungi and fungal enzymes involved in lignocelluloses degradation. Eng Life Sci. **18**, 768778 (2018).
4. M. Dimarogona, E. Topakas, and P. Christakopoulos, Cellulose degradation by oxidative enzymes. Comput Struct Biotechnol. **2**, 18 (2012).
5. D.-A. Saropah, A. Jannah, and A. Maunatin, Kinetika Reaksi Enzimatis Ekstrak Kasar Enzim Selulase Bakteri Selulolitik Hasil Isolasi Dari Bekatul. Alchemy. **2**, 34–45 (2015).
6. Y. Chen, Q. Zhang, Y. Zhang, J. Chen, D. Zhang, and J. Tong, Changes in fibrolytic enzyme activity during vermicomposting of maize stover by an anecic earthworm Amynthas hupeiensis. Polym. Degrad. Stab. **120**, 167177 (2015).
7. O.-M. Buraimoh, M.-O. Ilori, O.-O. Amund, F.-C. Michel, and S.-K. Grewal, Assessment of bacterial degradation of lignocellulosic residues (sawdust) in a tropical estuarine microcosm using improvised floating raft equipment. Int. Biodeterior. Biodegrad. **104**, 186193 (2015).
8. M. Taha, K.-K. Kadali, K. Al-Hothaly, A.-T. Smith, A.-S. Ball, and E.-M. Adetutu, An effective microplate method (Biolog MT2) for screening native lignocellulosic-straw-degrading bacteria. Ann. Microbiol. **65**, 20532064 (2015).
9. L. Medouni-Haroune, F. Zaidi, S. Medouni-Adrar, S. Roussos, S. Azzouz, V. Desseaux, M. Kecha, Selective isolation and screening of actinobacteria strains producing lignocellulolytic enzymes using olive pomace as substrate. Iran. J. Biotechnol. **15**, 7477 (2017).
10. V. Bohra, N. A. Dafale, and H.-J. Paenibacillus polymyxa ND25: candidate genome for lignocellulosic biomass utilization. Biotech. **8** (2018)
11. K.-S. Neelkant, K. Shankar, S.-K. Jayalakshmi, and K. Sreeramulu. Optimization of conditions for the production of lignocellulolytic enzymes by Sphingobacterium sp. ksn-11 utilizing agro-wastes under submerged condition. Prep. Biochem. Biotechnol. **49**, 927934 (2019).

12. R.-C. Kasana, R. Salwan, H. Dhar, S. Dutt, and G. Arvind. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. Curr. Microbiol. **57**, 503507 (2008).
13. A. Mangungsong, Soemarsono, and F. Zudri. Pemanfaatan Mikroba Tanah dalam Pembuatan Pupuk Organik serta Peranannya terhadap Tanah Aluvial dan Pertumbuhan Bibit Tanaman Kakao. J. Agron. Indones. **47**, 318325 (2019).
14. A. Bonaterra, E. Badosa, N. Daranas, J. Francés, G. Roselló, and E. Montesinos. Bacteria as Biological Control Agents of Plant Diseases. Microorganisms. **10** (2022).
15. E.-S.-D. Dewi. Pertumbuhan Kultur Probiotik Hasil Isolat Bakteri Non Patogen dalam Berbagai Jenis Media. J. Bioma. **3**, 5365 (2014).
16. S. Darmawati, S.-I. Muchlissin, A.-R. Ernanto, A.-R. Sulistyaningtyas, H. Fuad, K.-M.-Z. Rahman, A. Sabdono, S.-N. Ethica. Pathogenicity Scoring System for Selection of Bacterial Consortium Formulated as Bioremediation Agent of Hospital Wastewater in Central Java. IOP Conf. Ser. Earth Environ. Sci. **701**, 1 (2021).