Evaluation of in vitro activity of Ganoderma-antagonistic bacteria from peatland under acidic condition

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Abstract. As of 2017, 2.05 ha of Indonesia's total oil palm area has been identified as peatlands. A large-scale peatland has caused oil palm commodities to be a global concern because they are thought to have contributed to the increase in greenhouse emissions that have triggered climate change. This research is an effort to resolve the main problems in the cultivation of oil palm on peatlands in a more environmentally friendly way. The main obstacle to oil palm cultivation on peatlands is Ganoderma fungi attacks that cause basal stem rot (BSR) disease, where the attack is higher than in mineral soils. Biological control is a prospective alternative way to control BSR disease. However, its development in peatlands is hampered by extremely low pH. The possible approach is to utilize appropriate biological control agents for peatlands. This study aimed to examine antagonistic bacteria's from peatlands to control Ganoderma under low pH conditions in vitro. The bacterial isolates from peatlands were tested for antagonism against Ganoderma and tested for their growth ability in 2-7 pH situations. The results showed that from the West Kalimantan peatlands, Ganoderma-antagonistic bacteria grew at pH 2-4, even though the growth rate had decreased significantly. The isolates were E4B6, E2B12, E2B13, B3B11, and E2B3. These results indicate that these bacteria can be used in controlling oil palm BSR disease caused by Ganoderma in peatlands.

1. Introduction
Oil palm is the most important plantation commodity in Indonesia [1]. The limited fertile land has caused the development of oil palm plantations to utilize peatlands that are infertile. Until 2017, it is recorded that around 14.58% of the total area of Indonesian oil palm was on peatlands which is equivalent to 2.05 million ha [2]. A large-scale peatland use has caused oil palm commodities to be a global concern because they are thought to have contributed to the increase in greenhouse gas emissions that have triggered climate change. This research is an effort to resolve the main problems in the cultivation of oil palm on peatlands in a more environmentally friendly way. Therefore, it can help reduce greenhouse gas emissions.

One of the threats to the sustainability and productivity of oil palm plantation on peatlands is the high incidence of basal stem rot (BPB) disease caused by Ganoderma [3]. In peatlands, BPB disease intensity is higher than on mineral soils, and appears earlier [4]. Until today, there is no effective way to control BPB disease [5]. Biological control is a prospective alternative for controlling BPB disease [6]. Besides being low cost and safe for the environment, biological methods are the most suitable way, considering...
that *Ganoderma* is mostly transmitted through the roots contact [7]. However, the development of biological control is constrained by the narrow spectrum of biological control agents. There are no commercial biological fungicides that are intended explicitly for peatlands.

A very low soil pH is the main obstacle to *Ganoderma* 's biological control in oil palm on peatlands. Accordingly, the most feasible approach is to utilize biological agents suitable for peatlands. Several studies have shown that acid-tolerant bacterial species can also be found with other special functional abilities [8][9]. It is estimated that in the peatlands of West Kalimantan, there are acid-tolerant bacteria that have special functional abilities as *Ganoderma*-antagonists in oil palm. This study aimed to test the ability of *Ganoderma*-antagonistic bacteria from peatlands under acidic conditions in the laboratory in vitro. The discovery of these bacteria is very important in developing a biological control strategy for the BPB disease of oil palm caused by *Ganoderma*.

2. Material and methods

2.1. The origin of *Ganoderma* isolate and their antagonistic bacteria

*Ganoderma* isolates were obtained from diseased oil palm trees in the PT. Bumi Pratama Khatulistiwa (PT. BPK) Plantation in Kubu Raya Regency, West Kalimantan Province (0° 2' 44.11" N, 109° 26' 6.20" E). *Ganoderma*-antagonistic bacteria isolates were isolated and collected from peat soil from 10 sample blocks in the oil palm plantation of PT. BPK and from mineral soils in the oil palm plantation of PT. Perkebunan Nusantara XII (PTPN) Gunung Meliau in Sanggau Regency, as a comparison. Soil samples were taken at a depth of 0-15 cm and 50-60 cm. Three soil samples from each block were made into a composite based on a depth of 0-15 cm and 50-60 cm.

Isolation was carried out by serial dilution using a sterile saline solution (NaCl 0.85%) in Nutrient Agar (NA) and Kings' B medium, identified based on colony morphological characteristics [10]. All isolated bacteria were tested for their antagonism ability against *Ganoderma* using a dual culture method [11]. Disc-shaped *Ganoderma* isolate pieces with a diameter of 5 mm, were placed opposite to 5 cm in a Petri dish and incubated for 1 night. After 24 hours, bacterial isolates were streak crossed 5 cm right in the middle between the two *Ganoderma* fungi isolates then incubated at room temperature and observed for 7 days. The percentage of inhibition of antagonistic bacteria against *Ganoderma* was calculated as equation (1). Isolates with inhibition of ≥ 60% were collected and treated in the NA medium for further testing. The pH value of each peat sample was measured by mixing 2.5 mL of fresh peat with 4 mL of 0.01 M CaCl$_2$ solution and allowed to stand for at least an hour and the pH was measured [12].

\[
\text{Inhibition zone} = \frac{C \times T}{C} \times 100
\]  

(1)

Where, $C$ is the *Ganoderma* mycelium radius leading to the Petri edge; $T$ is the *Ganoderma* radius leading to the bacterial colony.

2.2. Testing of antagonistic bacteria in low pH

Testing of antagonistic bacteria at low pH was carried out following Hayward's method [13] with modification. Medium Nutrient Broth (NB, Himedia) was diluted in distilled water and adjusted to pH 2, 3, 4, 5, 6, and pH 7 by adding HCl or KOH. A total of 10 ml of NB medium was put into a test tube then autoclaved for 15 minutes at 121 °C (1 atm). After cooling, 0.1 ml of the antagonistic bacterial isolate suspension was inoculated and shaken at 300 rpm for 48 hours. As a control, used 1 treatment of NB medium inoculated with 0.1 ml of sterile distilled water. After 48 hours, the culture was measured for turbidity using a spectrophotometer at a wavelength of 600 nm and the optical density (OD) value was recorded. Simultaneously, the antagonistic bacteria were also tested for hypersensitivity using tobacco as an indicator plant.
3. Results and discussion

3.1. The antagonistic bacterial isolates origin

The peatlands condition where *Ganoderma* isolates and their antagonistic bacteria were collected that tested in this study was described by Supriyanto et al [14]. Ten samples of oil palm plantation blocks in the peatlands were attacked by *Ganoderma* with varying intensities, ranging from 4.62% to 69.81%. Three blocks, namely 12A, 13A, and 13E, were intensively maintained with twice-a-year fertilization, including ammonium-phosphorus-potassium (NPK), Borate, and CuSO₄ fertilizers.

**Table 1.** Inhibition ability, hypersensitivity reaction, and soil pH origin of the *Ganoderma*-antagonistic bacterial isolates.

| Isolate | Blocks | Inhibition against *Ganoderma* (%) | Hypersensitive reaction | Soil pH (pH CaCl₂) At a depth of 0-15 cm | Soil pH (pH CaCl₂) At a depth of 50-60 cm |
|---------|--------|-----------------------------------|------------------------|------------------------------------------|------------------------------------------|
| F1B19   | F1 PlsI| 72.7                              | -                      | 2.6                                      | 2.3                                      |
| E4B11   | E4 PlsIII| 82                                 | -                      | 3.4                                      | 2.8                                      |
| E4B17   | E4 PlsIII| 66                                 | -                      | 3.4                                      | 2.8                                      |
| F1B30   | F1 PlsI | 94.4                              | -                      | 2.6                                      | 2.3                                      |
| B4B9    | B4 PlsI | 82                                 | -                      | 3.0                                      | 2.6                                      |
| B4B14   | B4 PlsI | 74                                 | -                      | 3.0                                      | 2.6                                      |
| C5B5    | C5 PlsIII| 66                                 | -                      | 3.4                                      | 2.8                                      |
| E4B12   | E4 PlsIII| 83                                 | -                      | 3.4                                      | 2.8                                      |
| 13EJ10  | 13E     | 73                                 | -                      | 2.9                                      | 2.4                                      |
| F1B18   | F1 PlsI | 76                                 | -                      | 2.6                                      | 2.3                                      |
| 13EB6   | 13E     | 64                                 | -                      | 2.9                                      | 2.4                                      |
| F1B22   | F1 PlsI | 88                                 | -                      | 2.6                                      | 2.3                                      |
| 13EB11  | 13E     | 75                                 | -                      | 2.9                                      | 2.4                                      |
| E4B16   | E4 PlsIII| 80                                 | -                      | 3.4                                      | 2.8                                      |
| 12AB4   | 12A     | 60                                 | -                      | 2.4                                      | 2.2                                      |
| F1B13   | F1 PlsI | 100                                | -                      | 2.6                                      | 2.3                                      |
| C5B2    | C5 PlsIII| 70                                 | -                      | 3.4                                      | 2.8                                      |
| MB5     | PTPN Meliau| 65                                 | -                      | 4.0                                      | 4.0                                      |

| (++) = positive reaction |
| (-) = negative reaction |

Meanwhile, 7 other blocks were maintained intensively until around 8 years; however, the maintenance was subsequently less intensive. Meanwhile, oil palm plantations at PTPN Gunung Meliau
has mineral soils with sloped topography. The oil palms were 18 years old and the intensity of *Ganoderma* attacks was 0.04%. From all soil samples, 25 bacterial isolates were obtained that potential as *Ganoderma* antagonists. The ability to inhibit *Ganoderma* for each isolate was varied, ranging from 60% to 100%. However, based on Pearson’s correlation, there was no relationship between each isolate’s ability to inhibit *Ganoderma* and soil pH. The antagonistic ability of the antagonistic bacteria obtained is shown in Table 1.

**Table 2.** The mean value of OD (optical density) of NB medium treated with pH and inoculated with antagonistic bacterial isolates after incubation for 48 hours.

| Isolate  | pH 2 | pH 3 | pH 4 | pH 5 | pH 6 | pH 7 |
|----------|------|------|------|------|------|------|
|          | Sig. 0.00 | Sig. 0.01 | Sig. 0.02 | Sig. 0.00 | Sig. 0.00 | Sig. 0.00 |
| F1B19    | 0.03a** | 0.05a** | 0.03a** | 0.45bc** | 0.63ab** | 1.43ghij** |
| E4B11    | 0.04ab  | 0.2a   | 0.12a  | 0.6cd  | 0.63ab  | 0.82abc  |
| E4B17    | 0.04abc | 0.04a  | 0.04a  | 0.94abc | 0.56a  |          |
| F1B30    | 0.05abcd | 0.05a  | 0.03a  | 1.12fgi | 1.09bdec | 0.89bcd  |
| B4B9     | 0.05abcd | 0.05a  | 0.03a  | 0.38abc | 0.39a  | 0.68ab   |
| B4B14    | 0.05abcde | 0.06a  | 0.72cde | 0.76abc | 0.67ab |          |
| C5B5     | 0.05abcde | 0.07a  | 0.05a  | 0.85def | 1.16bdec | 0.94bdec |
| E4B12    | 0.05abcde | 0.05a  | 0.19a  | 2.03jk  | 1.23cde | 1.31gfi  |
| 13EJ10   | 0.06bcdefg | 0.07a  | 0.05a  | 1.45ghi  | 1.28cde | 1.02cdef |
| F1B18    | 0.06bcdefg | 0.05a  | 0.05a  | 1.32ghi  | 1.15bdec | 1.07cdef |
| 13EB6    | 0.06bcdefg | 0.07a  | 0.1a   | 0.77abc  | 0.98bcdef|          |
| F1B22    | 0.06bcdefg | 0.06a  | 0.05a  | 1.35ghi  | 1.67ef  | 1.73jk   |
| 13EB11   | 0.06bcdefg | 0.06a  | 0.04a  | 1.46ghi  | 1.52efl | 1.53hij  |
| E4B16    | 0.06bcdefg | 0.08a  | 0.17a  | 1.39ghi  | 1.65ef  | 1.67j    |
| 12AB4    | 0.06bcdefg | 0.06a  | 0.12a  | 1.06efg  | 1.28cde | 1.43ghij |
| F1B13    | 0.06bcdefg | 0.06  | 0.05a  | 1.48hi   | 1.58ef  | 1.64j    |
| C5B2     | 0.06bcdefg | 0.06a  | 0.05a  | 1.09efgh | 1.52ef  | 1.89defg |
| MB5      | 0.07efgh   | 0.05a  | 0.05a  | 1.06efg  | 0.67ab  | 1.44ghij |
| 12AB3    | 0.07efgh   | 0.08a  | 0.05a  | 1.16ghij | 1.35de  | 1.60ij   |
| B3B11    | 0.07efghi  | 0.1a   | 0.09a  | 2.37kl   | 2.24gh  | 2.10lm   |
| E2B3     | 0.07ghi    | 0.09a  | 0.08a  | 2.32kl   | 2.62h   | 2.7n     |
| E2B13    | 0.08hi     | 0.1a   | 0.27a  | 1.9j     | 2.02fg  | 2.12lm   |
| E2B12    | 0.08hi     | 0.07a  | 0.06a  | 1.51i    | 1.49def | 1.25efgh |
| MBK1     | 0.08ij     | 0.07a  | 0.04a  | 1.34ghi  | 1.49def | 2.02k    |
| E4B6     | 0.09j      | 0.09a  | 0.57b  | 2.44kl   | 2.41gh  | 2.4m     |
| Control  | 0.04a      | 0.04a  | 0.04a  | 0.04a    | 0.05    | 0.04     |

*) The value that indicates the level of turbidity of the medium based on turbidity measurements using a spectrophotometer.

**) The same letter that follows the numbers shows no significant difference in the DMRT test at 5% level.

3.2. The effect of low pH on the growth of antagonistic bacteria

Table 2 shows that the medium's OD value had a large variation in the treatment of pH 2, 5, 6 and 7 while at pH 3 and 4 the variation was relatively narrow. Based on the variance analysis, all treatments showed a significant difference between the tested antagonistic bacterial isolates with a significance value of 0.00 to 0.02 at 95% confidence level. Table 2 shows that some isolates tend to have significantly
higher OD values than other isolates at various pH levels, especially in the pH 2 to pH 5 range. The isolates were E4B6, E2B12, E2B13, B3B11 and E2B3. In a liquid medium, the cloudier the medium, the higher the density of bacterial cells growing. It means that it has a higher rate of bacterial growth in the medium. This shows that the medium's OD value at pH 2, pH 3, pH 4 and pH 5 is acidic. In most of the isolates the value was still higher than the control.

Meanwhile, the control medium's OD value was not zero because it contains various nutritional elements that affect the medium's turbidity. Thus, based on this in vitro test, most of the antagonistic bacterial isolates from peatlands were still able to grow at low pH. However, the growth rate decreased significantly. This study's results are the same as the research results conducted by Goswami et al [15] in Assam, India. In tea plantations with a pH of 3.8-5.5, acid-tolerant bacteria could also be found to have the functional ability to support plant growth based on the production of Indole Acetic Acid (IAA), siderophore and HCN as well as dissolving phosphate, zinc and potassium. However, almost all functional abilities decreased when tested at low pH.

These results also indicated that there were several antagonistic bacteria from peatlands that can be developed as biological control agents on peat soils. However, their growth capacity will decrease. These bacteria, especially those that consistently showed the ability to always grow better than other isolates at various pH levels tested in this study, were thought to be acid-tolerant bacteria. Bacteria were classified as acid-tolerant if they prefer a pH above 5.5 but can still grow at a pH of 5.5 or below. While acidophilic bacteria grow optimally at a pH of 5.5 or below [8][16]. It is well known that certain bacteria can live as acid-tolerant bacteria by various mechanisms [17]. Hashidoko et al [9] reported acid-tolerant and well-developed bacterial species on cleared peatlands in Kalimantan.

4. Conclusion

This study showed several Ganoderma-antagonistic bacterial isolates from peatlands that could grow in low pH. Even though, their growth rate decreased significantly. The isolates were E4B6, E2B12, E2B13, B3B11 and E2B3. These isolates can be used as Ganoderma biological control agents in oil palm on peatlands. However, various further studies are still needed to find out in more detail about these isolates' potency. The ability to antagonize Ganoderma in low pH situations also needs to be studied carefully before making these isolates as biological control agents for Ganoderma in peatlands.

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