

Indigenous Peat Cellulolytic Bacteria and Its Potential as A Liberica Coffee Growth Promoter

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Abstract. Among the main microbes in peat are cellulolytic bacteria. The research aimed to select peat cellulolytic bacteria and identified its potential as a plant growth promoter bacteria (PGPB). The cellulolytic bacteria were isolated by serial dilutions and cellulase activity by the carboxymethyl cellulose (CMC) method, species types recognized by the sequencing method and P solubilization and phytohormones productions by Pikovskaya, and the high-performance liquid chromatography method, respectively. Research results found the 1st identified peat cellulolytic bacteria, *Comamonas testosteroni*, dissolved fixed P, 1.908 $\mu\text{g PO}_4^{3-}/\text{mL.day}$ and released phytohormones of indole acetic acid (IAA) 0.385 mg/kg, gibberellin (Ga.3) 2.989 mg/kg, zeatin 0.348 mg/kg, and kinetin 0.115 mg/kg. The 2nd identified bacteria, *Delftia lacustris*, dissolved fixed P from 1.107 $\mu\text{g PO}_4^{3-}/\text{mL.day}$ to 1.329 $\mu\text{g PO}_4^{3-}/\text{mL.day}$ and produced IAA from 0.775 to 1.161 mg/kg, Ga.3 from 2.551 to 4.429 mg/kg, and zeatin from 0.228 to 1.127 mg/kg and no kinetin. Adding both cellulolytic bacterial colonies on peat improved Liberica coffee seedling growth significantly. Keywords: cellulolytic bacteria, peat, phosphate solubilization, phytohormones, Liberica coffee.

1 Introduction

Various microorganisms grow and develop on peatlands due to their rich organic matter. Among the main microbes in peat are cellulolytic bacteria, which play a role in weathering lignocellulosic complexes such as cellulose, hemicellulose, and lignin [1]. The cellulolytic microorganisms produce cellulase enzymes, sometimes release some phytohormones, and solubilize fixed phosphate [2]; [3]. Hence, some cellulolytic bacteria are also plant growth-promoting bacteria (PGPB) [4]; [5]. Some phytohormones produced by cellulolytic bacteria are auxins, cytokinin, and gibberellin (Ga.3) [6]; [7]; [8].

The PGPB presence could reduce some chemical fertilizer roles and the risk of environmental damage due to land cultivation [9]. Some cellulolytic bacteria isolated from Indonesian soils had been identified as having potential as the PGPB because of their ability to dissolve P and fix N. They are *Bacillus stratosphericus*, *Bacillus amyloliquefaciens*, *Bacillus cereus*, *Pseudomonas pseudoalcaligenes*, *Pantoea dispersa*, and *Sinomonas atrocyanea* [8].

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The Liberica coffee plant is one of three commercial coffees in Indonesia besides Robusta and Arabica. Liberica coffee grows larger than Robusta and Arabica plants, of which the crown diameter is about 3.5 - 4 m and a height of about 5 m, and also has sizer cherries and beans and leaf area [10]; [11]. The Liberica coffee plantation is more suitable for the peatlands [12]; [13]; [14]. However, the fertility of peatland is low because of low nutrient content like available P [14].

In this study, cellulolytic bacteria isolated from peat were followed by identifying bacterial species, testing cellulase activities, the ability to dissolve fixed P and produce phytohormones, and then testing their application effect on available P and organic C of peat and the Liberica coffee seedling growth. So, the research was to select peat cellulolytic bacteria and to identify its potential as a plant growth promoter bacteria.

2 Materials and methods

2.1 Peat sample

For microbial isolation, peat was taken on a smallholder plantation of Liberica coffee on peatland in Tanjung Jabung Barat, Province of Jambi, Sumatra. The peat was sampled on the coordinates of -0°59'(11"- 27") S and 103°21'(0"- 42") E at a depth of 0-20 cm, mixed, and put into waterproof plastic bags, and stored in a cool box. The peat sample for the greenhouse experiment, approximately 600 kg, was also from the same peatland. The analysis results for several peat chemical properties such as pH (a glass electrode method), organic carbon, and ash [loss on ignition (LOI)], total N (Kjeldahl method), and P₂O₅ (Bray method) are in Table 1.

Table 1. Peat chemical properties of Tanjungjabung, Jambi Province

Parameters	Units	Values
pH	H ₂ O	3.61
pH	KCl	2.64
Organic C	%	50.91
Ash	%	12.23
N	%	1.37
P ₂ O ₅	mg/kg	24.58

2.2 Cellulolytic bacterial isolation

The method to isolate the cellulolytic bacteria was the serial dilutions method, started by putting ten grams of peat sample into 90 mL of physiological solution (0.85% NaCl solution) in an Erlenmeyer and rotated for 30 minutes at a speed of 150 rpm by a rotary shaker, and then diluted for a concentration of 10⁻² up to 10⁻⁵. Next, one mL of microbial cultures from dilutions of 10⁻³, 10⁻⁴, and 10⁻⁵ were grown in the Nutrient Agar (NA), incubated for three days at room temperature, and then the bacterial colonies formed on the plates were counted.

2.3 Cellulase activity

The cellulase activity of bacteria qualitatively was by the CMC method (the mixture of 0.4 g CMC (Merck), 0.5 g MgSO₄·7H₂O, 0.03 g KNO₃, 1.0 g K₂HPO₄, 0.0008 g FeSO₄·7H₂O, 0.08 yeasts, 2 g NaNO₃, and 18 g agar), which was all dissolved into a liter

of distilled water, into which the pure bacterial colonies were spread then for 24 hours was incubated at room temperature pre-drop it with 0.1% (w / v) Congo red solution. The presence of clear zones around the bacterial colony indicated a cellulolytic enzyme (cellulase activity) by which the most active bacteria was with the higher ratio of clear-zone diameter and bacterial colony diameter of cellulolytic bacteria (AI). The formula to calculate the qualitative cellulolytic index is:

$$\text{The activity index (AI)} = \frac{\text{Diameter of the clear zone (mm) in CMC}}{\text{Diameter of the bacterial colonies (mm) in CMC}}$$

(1)

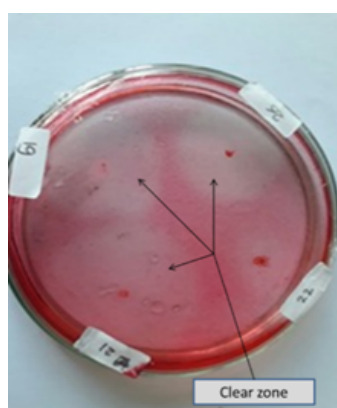


Fig. 1 The clear zone around the bacterial colonies by cellulase activity of bacteria in CMC

Then the Selected bacteria were grown in media consisting of a mixture of 2.0 g KH_2PO_4 , 1.4 g $(\text{NH}_4)_2\text{SO}_4$, 0.3 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g CaCl_2 , 1.0 g peptone, and 2.0 g agar and incubated for seven days on slant agar at 37 °C. Then, the enzyme solution (supernatant) was separated from the particle of substrate or bacterial cell by centrifugation of bacterial culture at 13,000 rpm and 4° C for 10 min and then kept in a freezer -10° C. Then the quantity cellulase activity measurement was by the DNS (dinitro salicylic acid) method [15]. The assay for the cellulase activity of the cellulolytic bacterial colonies was by Duplo analysis.

2.4 Cellulolytic bacterial genotypic identification

Identifying bacterial genotypes of some selected cellulolytic bacteria was done by sequencing primer 16sRNA. The process of identifying bacterial genotypes was by the nucleotide base sequencing technique by aligning the sequences with BioEdit 7.7 sequence alignment editor, then compared to the GenBank database using Basic Local Alignment Search Tool (nih.gov) (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>.)

2.5 Phosphate solubilization

The potential of cellulolytic bacteria as a phosphate solubilizer was assayed *in vitro* using Pikovskaya agar [16]; [17]. One ml of bacterial colony isolate was put into Pikovskaya in an Erlenmeyer and shaken at 100 rpm for two weeks. After two weeks, 20 ml of the

supernatant was filtered with Whatman paper no. 1 into the centrifugal tube and centrifuged at 1000 rpm for 15 minutes. Then was transferred 5 ml into a test tube, added 0.5 ml of concentrated dye reagent, shaken for several minutes, and incubated for 30 minutes. The standard solution is 1 ml of 1000 mg/kg K_2HPO_4 diluted to concentrations of 0, 2, 4, 6, 8, and 10 mg/kg, respectively. Add 0.5 ml of P reagent to each standard series, shake for a few minutes, and incubate for 30 minutes. The solution absorbance of each concentration series, and in Pikovskaya media inoculated by bacterial colonies isolates, was measured by a spectrophotometer at a wavelength of 693 nm. The PO_4 of the standard solution series graph was used to calculate the dissolved P of bacterial colony isolates, with the formula:

Bacterial isolate PO_4 (mg/kg) = curve mg/kg x fp - PO_4 control concentration (mg/kg) and fp is the dilution factor (if any).

2.6 Phytohormones production

The phytohormones' presence in the extract of cellulolytic bacterial colonies isolates quantitatively detected by high-performance liquid chromatography (HPLC) [18]. The sample of cellulolytic bacterial colony isolate was Duplo in analysis. Some phytohormones measured were Indole-3-Acetic Acid, gibberellin (Ga. 3), zeatin, and kinetin.

2.7 Assay the effect of cellulolytic bacteria enrichment on Liberica coffee seedling growth

It was a pot experiment conducted in a greenhouse (GH). Fresh peat enriched by peat indigenous cellulolytic bacteria, planted with 9-month-old Liberica coffee seedlings, were compared with fresh peat in pots with no bacterial enrichment. Cellulolytic bacterial colonies enrichment (10^8 CFU/mL) was applied as much as 25 mL to 5 kg of fresh peat for ten pots. After bacterial inoculation, mixed peat thoroughly and incubated for a week. There was no addition of chemicals and other manure to the peat. To keep the peat moist, water at a plastic dish at the pot bottom at a level of 5 cm was maintained. Observations were on the Liberica coffee seedling growth, like stem growth (height and diameter) and the number of leaves. A comparison of the variable data was analyzed using the T-test at a 5% significance level.

3 Result and discussion

3.1 Cellulolytic bacterial index and activity

By the serial dilutions method, 486 bacteria grew from peat samples and at CMC media, 31 of which formed clear zones. The bacterial colonies with sample codes BT-19, BT-21, and BT-26 had higher cellulase activity indexes than others (Table 2) and the highest cellulose enzymes released by the bacterial colony of sample code BT-21 (Table 3). The presence of cellulolytic bacteria in peat is in line with what was stated by [19], namely cellulolytic bacteria were among the microbes that are adaptive to the aquatic environment like peat, which is rich in carbon as the essential source of microbes' energy [20].

Table 2. Cellulolytic activities index (AI) and cellulase activity of selected cellulolytic bacteria

Bacterial colonies Sample codes	Cellulolytic activities index (AI)	Cellulase Activity (mU/mL)*
BT-19	3.2	100.62
BT-21	3.7	72.47
BT-26	3.0	74.44

Note: BT = Bacteria, *1 Unit (U) = 1 µmol product / minute

3.2 Cellulolytic bacterial index and activity

The results of the sequencing method showed the bacterial isolate of BT-19 is similar at 81% to *Comamonas testosteroni* strain NBRC 14951, while the similarity of the bacterial isolate of BT-21 and BT-26 are 91% and 88% to *Delftia lacustris* strain 332, respectively (Table 3). The consortia of *Comamonas* sp are able to degrade lignocellulose [21]; [22]. They live in an organic acid environment [23], wetland environments, activated sludge, and sediments in forest soils [24]; [25].

The bacterium *Delftia lacustris* strain 332 isolated from freshwater, was first identified in Denmark [26]. The *Delftia* sp was easily adaptive to aqueous/wet environments such as freshwater, marine, rhizosphere, soil, and so on [27].

Table 3. Bacterial species closely related to the cellulolytic bacteria of Jambi peat soils

Sample codes	Acc Number	Bacterial Species	Similarity (%)
BT-19	NR_113709.1	<i>Comamonas testosteroni</i> strain FB1	81
BT-21	NR_116495.1	<i>Delftia lacustris</i> strain 332	91
BT-26	NR_116495.1	<i>Delftia lacustris</i> strain 332	88

3.3 Phosphate solubilization

The analysis result showed that *Comamonas testosteroni* and *Delftia lacustris* could dissolve fixed P, of which the isolate of *Comamonas testosteroni* colonies' ability to dissolve 1.908 µg PO₄³⁻/mL.day) is better than *Delftia lacustris* namely from 1.107 µg PO₄³⁻/mL to 1.329 µg PO₄³⁻/mL.day (Table 4). The capability of *Comamonas testosteroni* to dissolve P was also previously reported [28], even though it solubilized potassium and fixes N as well. Another experiment reported the consortia of bacterial taxa, including *Comamonas testosteroni*, inoculated into inorganic fertilizers to mobilize soil bound-P increased the productivity of several crops such as Jalapeno and wheat up to twofold [29]. The ability of *Delftia lacustris* to solubilize P was also reported by Agafonova et al. [2] and Tamás et al. [30].

Table 4. The ability of cellulolytic bacteria in the dissolution of phosphate

Sample codes	Cellulolytic Bacteria	P dissolution ($\mu\text{g PO}_4^{3-}/\text{mL day}$)
BT – 19	<i>Comamonas testosteroni</i>	1.908
BT – 21	<i>Delftia lacustris</i>	1.107
BT - 26	<i>Delftia lacustris</i>	1.329

3.4 Phytohormones production

The cellulolytic bacterium *Comamonas testosteroni* a facultative anaerobic bacterium could release the plant growth regulator hormones such as auxins (IAA), gibberellin, zeatin, and kinetin, while *Delftia lacustris* produced IAA, gibberellin, and zeatin and no kinetin (Table 5). Previously the *Comamonas testosteroni* was identified in the banana rhizosphere, could produce siderophore [31], and had been used as a good bio-fertilizer [32]. In the USA, an experiment found that *Comamonas testosteroni* could be a bio-fertilizer for flax crops (*Linum usitatissimum* L), which were growing under salinity stress. Its ability to alleviate the salinity is threatening because it reduced photosynthesis pigments and enhanced carotenoids and anthocyanin [33].

Delftia lacustris is potential as a plant growth-promoting bacteria (PGPB) [34]; [35], including the ability to release phytohormone, produce siderophore, and help plant resistance to pathogens [36]; [2]. The phytohormones production by the *Delftia lacustris* supports the development of root, and plant production [37], and the gibberellin (Ga.3) hormone improves plant stem growth and fruit and also seed germination [7].

The IAA is a useful hormone for cell diffraction and extension. Zeatin is a type of cytokinin hormone be useful for improving plant resistance to abiotic and biotic stress [38], and extending the dormancy period of shoots, tubers, and seeds [39] and Kinetin is another kind of cytokine hormone that plays a role in the propagation and development of shoots, increasing chlorophyll synthesis, and root growth [40]; [41].

Table 5. The phytohormones produced by cellulolytic bacteria of peat

Sample codes	Cellulolytic bacteria	Phytohormones (mg/kg)			
		IAA	Ga.3	Zeatin	Kinetin
BT-19	<i>Comamonas testosteroni</i>	0.385	2.989	0.348	0.115
BT-21	<i>Delftia lacustris</i>	0.775	4.429	0.228	Nd
BT-26	<i>Delftia lacustris</i>	1.161	2.551	1.127	Nd

Notes: Nd = not detected

3.5 Liberica coffee growth on peat inoculated with cellulolytic bacteria

The average vegetative growth (plant height, stem circumference, and leave number) of the Liberica coffee seedlings growing on peat, inoculated, and uninoculated with cellulolytic bacteria was significantly different for all parameters (Table 7). On the other hand, the cellulolytic bacterial colonies inoculated on peat significantly influenced the growth of Liberica coffee seedlings. As mentioned before, the cellulolytic bacteria, *Comamonas testosteroni* and *Delftia lacustris* colonies enriched on peat could improve the availability of P by dissolving fixed P and also released some phytohormones such as auxins (IAA),

gibberellin, zeatin, and kinetin. That all improved the Liberica coffee seedling growth (Fig. 2).



Fig. 2 Performance of Liberica coffee seedling growing on inoculated peat (IN) by cellulolytic bacterial colonies of *Comamonas testosteroni* and *Delftia lacustris*, and uninoculated peat (UN)

Improving available P was essential for resistance to root, fruit, bean, and plant disease [42]. Phosphate stimulates root development and strengthens stalk and stem [43]. Whereas the release of IAA is essential for cell diffraction and extension, the gibberellin (Ga.3) for stem growth of plants, and seed germination [7]. Zeatin, a cytokinin hormone, improves plant resistance to abiotic and biotic stress [38], and kinetin, another cytokine hormone supports the propagation and development of shoots, synthesis of chlorophyll, and root growth [40]; [41].

Enrichment of peat with indigenous cellulolytic bacteria, which can improve the growth of Liberika coffee through its ability to dissolve fixed P and release some phytohormones, is a signal that the enrichment of indigenous cellulolytic bacteria on peatland is an alternative technology towards a sustainable peatland management system.

Table 6. Growth of stem (diameter and height), and leaves of Liberica coffee seedlings planted on inoculated and uninoculated peat by cellulolytic bacteria in the greenhouse experiment

Treatments	Improved Liberica coffee growth by adding		
	The diameter of the stem (mm)	The height of the stem (cm)	The number of leave
Inoculated peat	2.76 a	8.72 a	4.00 a
Uninoculated peat	2.15 b	3.33 b	1.83 b
<i>Sig.</i>	0.004	0.00	0.01

Note: Column mean followed by the different letters are significantly different at the 5% T-test

4 Conclusions

The cellulolytic bacterial colonies isolated from the peat Tanjung Jabung Barat with sample code BT-19 had the highest cellulase activity, 100.62 mU/mL, and similar 81% with *Comamonas testosteroni* species. Cellulolytic bacteria with sample codes BT-21 and BT-26 had cellulase activity of 72.74 mU/mL and 74.44 mU/mL, respectively, and each is similar

to 91 and 88% with *Delftia lacustris* species. The *Comamonas testosteroni* dissolved 1.908 µg PO₄³⁻/mL.day and released phytohormones of IAA, 0.385 mg/kg, GA₃, 2.989 mg/kg, zeatin, 0.348 mg/kg, and kinetin 0.115 mg/kg. The *Delftia lacustris* dissolved 1.107 µg PO₄³⁻/mL to 1.329 µg PO₄³⁻/mL.day and produced IAA, 0.775 to 1.161 mg/kg, GA₃, 2.551 to 4.429 mg/kg, and zeatin, 0.228 to 1.127 mg/kg and no kinetin. The indigenous peat cellulolytic bacteria enhancement significantly improved *Liberica* coffee seedlings' vegetative growth.

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