

# Exploration of bacteria associated with chili peppers' rhizosphere and their capacity to absorb and produce gibberellin hormone

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## Exploration of bacteria associated with chili peppers' rhizosphere and their capacity to absorb and produce gibberellin hormone

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**Abstract.** Plant *rhizospheric* zone is a narrow soil region were rich in microbes. Microbes are of capacity to dissolve phosphate. Phosphate (P) is the second essential component after N, which plays a crucial part in photosynthesis and root development. P is difficult to dissolve due to strong bonding with compounds in the soil. Here, we explored rhizospheric bacteria around chili peppers' roots area. The bacteria were isolated and purified on solid media. The bacterial isolates were then quantitatively assayed for their capacity to dissolve phosphate using Pikovskaya liquid medium consisted of Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> as Phosphate source. Moreover, the capacity to produce Gibberellin hormone was also evaluated using Borrow et al. method. The assays revealed that phosphate concentration produced by rhizospheric bacteria of chili pepper ranged from 19.0 to 58.8 µg/L. Meanwhile, gibberellin concentration ranged from 5.81 - 12.79 µg/L. Our results present that bacteria inoculated from roots and roots area of chili pepper are potential as Plant Growth Promoting Bacteria. vThe first section in your paper.

### 1. Introduction

Chili pepper is a vital daily commodity as Indonesian primary spice. Soil fertility strongly influences an increase in chili pepper production. Fertile soil contains components required by plants for the metabolism process. Such a crucial component is Phosphate. However, this component is difficult to provide because of the intense bonding with other compounds. Microorganisms can convert unavailable phosphate to be available for plants [1], such as bacteria which are abundant in plant rhizosphere [2]. Bacterial activity converts phosphate that previously bonding become unbound chemically and available to be absorbed by plants as a nutrient.

Bacterial activity produces a growth hormone in the form of gibberellin that is required by plants. This hormone plays an essential role in plant growth and physiological process. The process encompasses seed germination, seedling, stem, and leave growth, flower formation, and flower and fruit growth. Gibberellin involves root and hairy root development and inhibition and differentiation of



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flower bud on angiosperm [3]. It indicates that the rhizospheric bacterial colony is potential as Plant Growth Promoting Bacteria.

## 2. Methods

### 2.1 Bacteria isolation and purification

Samples were gathered in Takalar Regency by pulling out a healthy and vigorous chili pepper plant from the soil and then collected the soil attached to the plant roots. Roots were washed and grounded using mortar and pestle. Grounded roots and soil were then diluted using aqua distillate until 10-6 dilution. Bacterial isolation was conducted by dripping the sample on Nutrient Agar (NA) medium and flattening it using a spatula. The medium was incubated for two days and then purified and re-isolated on NA medium using zig-zag streak method.

### 2.2. Phosphate Dissolution Assay

The purified isolate was quantitatively assayed for its capacity to dissolve phosphate using Pikovskaya liquid medium fortified with  $\text{Ca}_3(\text{PO}_4)_2$  as phosphate source. Pikovskaya broth medium consisted of 10 g glucose, 5 g  $\text{Ca}_3(\text{PO}_4)_2$ , 0,5 g  $(\text{NH}_4)_2 \text{SO}_4$ , 0,1 g  $\text{MgSO}_4 \cdot 2\text{H}_2\text{O}$ , 25 mg  $\text{MnSO}_4$ , 25 mg  $\text{FeSO}_4$ , 0,2 g KCl, 0,5 g yeast extract, and 15 g agar. Those compositions were then dissolved until 1.000 ml with sterile water [4].

The 30 ml pipette of suspense was placed in the Erlenmeyer flask contained Pikovskaya broth medium and then incubated on a rotary shaker at 150 rpm for seven days. 20 ml of culture was filtered using Whatman 42 filter paper. The filtration was centrifuged at 1.000 rpm for 15 minutes. 5.0 ml supernatant was pipetted into a tube and added 0.5 ml P reagents (12 g ammonium molybdate, 0.277 g potassium antimonyl tartrate) and dye reagent (0.53 g ascorbic acid). The solution was stirred briefly and allowed to stand for 30 minutes. The absorbance of the solution was measured using spectrophotometer with a wave-length set at 693 nm. The same treatment was also done on the Pikovskaya broth medium without fungi inoculation as the control.

### 2.3. Production of Gibberellin Acid (GA3)

GA3 hormone production by weathering-fungi isolate was measured using method [5]. As many as five pieces of isolates from PDA medium were taken using cork borer and cultivated on PDB medium. They were incubated at room temperature for seven days. Afterward, culture was centrifuged at 8.000 rpm for 10 minutes and replaced into 15 ml reaction tube and added 2 ml of zinc acetate. After two minutes, the culture was added 2 ml potassium ferrocyanide and then re-centrifuged at 8.000 rpm for 10 minutes. 5 ml supernatant was added 5 ml of 30% hydrochloric acid and incubated at room temperature for 75 minutes. The cuvette was prepared using 5% hydrochloric acid. The absorbance was measured with 254 nm wavelength using a spectrophotometer. GA concentration was compared to a GA3 standard curve (Sigma-Aldrich) at ranged from 0,25 to 2,25 ppm.

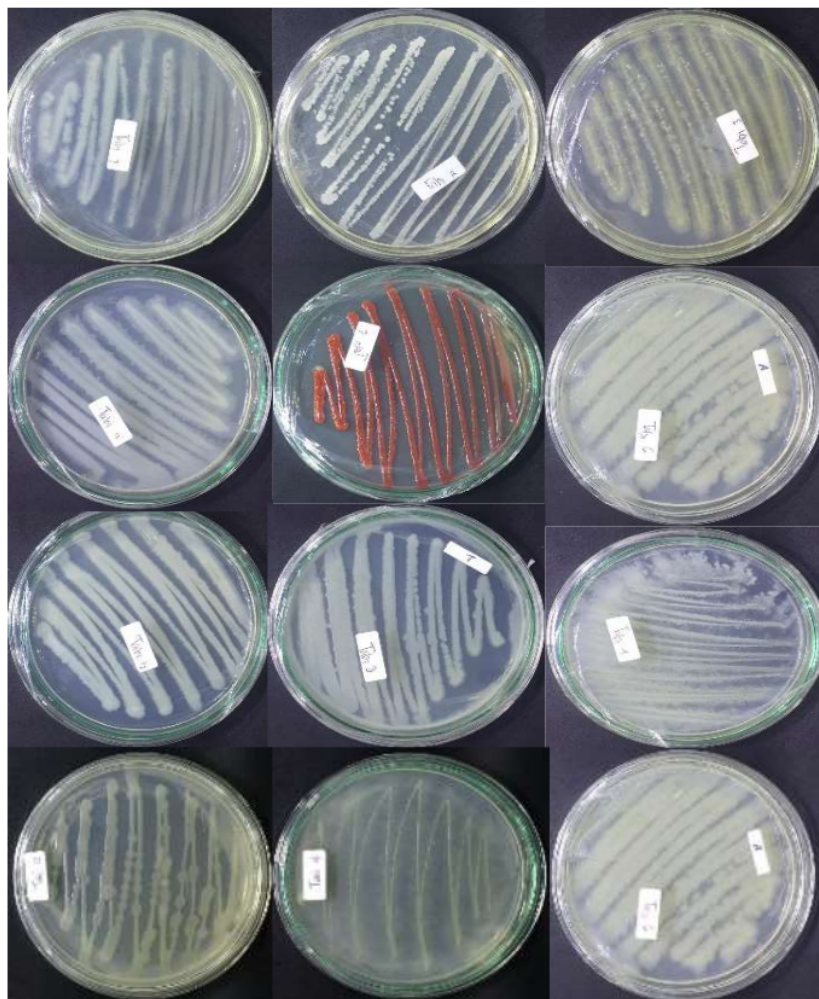
## 3. Results and discussion

### 3.1. Isolation and purification of bacteria

Bacteria isolation and purification generated 17 bacterial isolates from roots and soil around chili peppers' roots area. Meanwhile, 13 isolates were from rhizosphere soil. Isolation is performed to obtain pure culture using NA medium. Even though NA medium is a general medium consists of non-selective nutrients [6], it can grow bacterial colonies from roots and rhizosphere of chili pepper.

The isolate colors were white, yellowish, and red, but the milky white color was more dominant. Suryanto and Munir (2006) stated that round shape and milky white dominate bacterial colony [7]. A similar result was also observed by Fitri and Yasmin (2011) [8], they isolated chitinolytic bacteria from river water, pond water, and seawater and derived 13 milky white and five yellowish isolates.

Morphological characterization on the margin of bacteria isolates showed entire, undulate, filamentous, and rhizoid. Moreover, colony elevations were flat, raised, and pulvinate (Figure 1).

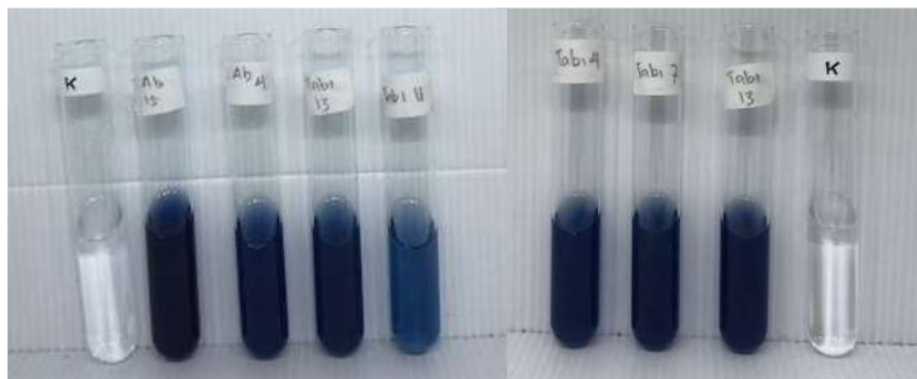


**Figure 1.** Bacterial isolate isolated from chili pepper's roots and rhizosphere

Many study about morphological study and this very important for confirmation about the other species [9–11].

### 3.2. The capacity of phosphate absorption of Rhizosphere bacteria from chili pepper

Phosphate absorption was qualitatively and quantitatively measured. Qualitative measurement presented the difference of color compared to control (transparent in color). The higher phosphate concentration is the bluer supernatant of the bacterial isolates (Figure 2).



**Figure 2.** The concentration of supernatant phosphate of a bacterial isolate from chili pepper's roots and rhizosphere. The bluer the supernatant, the higher concentration of phosphate compared to control (transparent).

All bacterial colonies isolated from roots and roots area of chili pepper were able to dissolve phosphate that could be seen on isolate supernatant cultured on Pikovskaya liquid medium and incubated for three days. The qualitative assay showed a difference in supernatant color as compared with control. Figure 2 presents the variation of blue color on supernatant (light blue to dark blue).

The pH measuring test on the suspension of each isolate colony ranged 4.00 to 5.97 (Table 1). This acid condition supported bacterial colonies growth. Optimum pH of the medium for growing fungus varies based on its strain and species [12]. Organic acids produced by fungi cause a decrease in pH on Pikovskaya liquid medium. Those organic acids are crucial for increasing phosphate absorption. The higher organic acid production is the higher phosphate absorption [13].

Here, quantitative assay for phosphate dissolve capacity was carried out by measuring the absorbance of supernatant from weathering-fungi isolate using a spectrophotometer. It detected variation in concentration of phosphate dissolution, 19.0 – 58.8 mg l<sup>-1</sup>. AB15 from bacterial colony isolate showed the highest phosphate concentration, whereas isolate TB11 was of the lowest phosphate concentration (Table 1). Isolate AB15 was isolated from roots, whilst TB11 was from roots area of chili pepper. However, a study claimed that fungi are better agents for dissolving phosphate than bacteria [14], yet bacterial colonies obtained in this study are potential to enrich phosphate by inoculating seeds and soils in order to prevent phosphate deficiency. Isolate AB15 was of the highest phosphate concentration than other bacteria colonies (Table 1), as consequent it becomes the source of phosphate provider in the soil.

**Table 1.** The concentration of phosphate produced by a bacterial isolate from chili peppers' roots and rhizosphere

No.	Isolate Code	pH	Absorbance	Phosphate Concentration ( $\mu\text{g/L}$ )
1	AB1	4.28	1.45	34.9
2	AB2	4.26	1.47	35.4
3	AB3	4.74	1.09	27.3
4	AB4	4.29	1.56	37.3
5	AB5	4.31	0.8	21.1
6	AB6	4.21	1.39	33.7
7	AB7	4.44	1.45	34.9
8	AB8	4.26	1.4	33.9
9	AB9	4.82	1.48	35.6
10	AB10	4.21	1.37	33.2
11	AB11	4.68	1.37	33.2
12	AB12	4.31	1.34	32.6
13	AB13	4.40	1.45	34.9
14	AB14	4.85	1.48	35.6
15	AB15	4.26	2.57	58.8
16	AB16	4.72	1.46	35.1
17	AB17	5.90	0.89	23.0
18	TB1	4.21	0.77	20.5
19	TB2	5.97	1.33	32.4
20	TB3	4.13	0.83	21.7
21	TB4	4.25	1.40	33.9
22	TB5	4.15	1.24	30.5
23	TB6	4.00	0.70	19.0
24	TB7	4.13	1.41	34.1
25	TB8	4.20	1.32	32.2
26	TB9	4.25	1.37	33.2
27	TB10	4.26	1.35	32.8
28	TB11	4.87	0.70	19.0
29	TB12	4.67	0.97	24.7
30	TB13	4.90	1.53	36.6

The capacity of phosphate absorption from chili peppers' rhizosphere was higher than that of isolated from potato var Hartapel from Buru Island, Maluku (4.457 – 14. 237 mg l-1) [15].

### 3.3. Gibberellin concentration of bacterial isolate

GA<sub>3</sub> concentration contained in the bacterial isolates ranged 5.81 – 12.79 µg/L. Bacterial colony isolates from roots and roots area of chili pepper could produce gibberellic acid, except for AB11 whose measurement was turbid and unreadable (table 2).

The best gibberellin concentration observed on isolate TB11 and the lowest was on isolate AB16. The isolates grew in PDB liquid medium and incubated for three days. During that time, bacteria adapted to medium and utilized nutrients in the medium. The culture was then centrifuged at 8.000 rpm for 10 minutes and added zinc acetate and Potassium ferrocyanide solution after two minutes. It was then re-centrifuged at 8.000 rpm for 10 minutes. This treatment induced isolate to produce secondary metabolite, for instance, gibberellin acid, which influenced the growth of cacao seedlings as stated by Rahim *et al.* (2018). Cacao seedlings planted at compost medium inoculated with rot fungi performed higher Leaf Area Index (LAI), root-shoot ratio, Net Assimilation Rate (NAR), and Crop Growth Rate (CGR) than that of a medium without microbes.

**Table 2.** The concentration of Phosphate GA<sub>3</sub> of a bacterial isolate from chili peppers' roots and rhizosphere

No.	Isolate Code	pH	Absorbance	Concentration of GA <sub>3</sub> µg/L
1	AB1	4.28	1.45	10.65
2	AB2	4.26	1.47	10.72
3	AB3	4.74	1.09	10.42
4	AB4	4.29	1.56	10.40
5	AB5	4.31	0.8	10.36
6	AB6	4.21	1.39	10.45
7	AB7	4.44	1.45	10.60
8	AB8	4.26	1.4	9.61
9	AB9	4.82	1.48	Keruh
10	AB10	4.21	1.37	10.51
11	AB11	4.68	1.37	10.45
12	AB12	4.31	1.34	10.36
13	AB13	4.40	1.45	10.22
14	AB14	4.85	1.48	10.47
15	AB15	4.26	2.57	10.40
16	AB16	4.72	1.46	5.81
17	AB17	5.90	0.89	10.33
18	TB1	4.21	0.77	10.33
19	TB2	5.97	1.33	10.45
20	TB3	4.13	0.83	10.51
21	TB4	4.25	1.4	10.72
22	TB5	4.15	1.24	11.21
23	TB6	4.00	0.70	10.54
24	TB7	4.13	1.41	10.18
25	TB8	4.20	1.32	10.02
26	TB9	4.25	1.37	10.40

27	TB10	4.26	1.35	10.74
28	TB11	4.87	0.70	10.38
29	TB12	4.67	0.97	10.47
30	TB13	4.90	1.53	12.79

#### 4. Conclusion

The isolated bacteria colonies from chili peppers' roots and rhizosphere had sufficient capacity to absorb Phosphate that ranged 19.0 – 58.8  $\mu\text{g l}^{-1}$ . Similar to phosphate absorbance, the capacity to produce gibberellin ranged 5.81 – 12.79  $\mu\text{g/L}$ . It indicates that the colonies demonstrate potency as Plant Growth Promoting Bacteria. Hence they present promise as bio-fertilizer.

#### Acknowledgement

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