Effect of sound treatment on phosphate solubilizing microbial activity

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Abstract. Many soils have the problem of high phosphate retention values (> 85%) thus P is not available for plants. The activity of phosphate solubilizing microbes (PSM) is able to facilitate the availability of phosphorus in the soil. The ability of each microbe to dissolve inorganic phosphate varies and depends on the type of microbe and the microbial growth factors. In nature, almost all forms of life are surrounded by various types of sounds and they also interact with these sounds. Previous research found that the soil that had been applied with the murottal Al-Qur'an sound for 2 hours per day for 7 days increased the total population of soil microbes 23.08% more than without applying murottal sound. This research was carried out in the laboratory of the Faculty of Agriculture, University of Muhammadiyah Sumatera Utara from April to August 2019. This treatment was sounds on 9 rhizosphere of corn, were put into the box. The sounds were treat every morning during 2 hours at 08.00 -10.00 o'clock and compared with 9 rhizosphere of corn was not treated with sounds. The result showed that sound treatment affected the growth of phosphate solubilizing microbes and all microbes that were not pathogenic to plans.

1. Introduction
The common problem in colloidal mineral soil such as Andisols, Ultisols, and Histosols is the high phosphate retention value (>85%) which causes low P fertilization efficiency. It has been widely reported that the role of phosphate solubilizing microbial (PSM) activity is able to facilitate the availability of phosphorus in the soil. Furthermore, among all microbial populations in the soil, phosphate solubilizing bacteria (PSB) were 1 to 50%, while phosphate solubilizing fungus (PSF) were only 0.1 to 0.5% with the potential to dissolve phosphate [1].

The ability of each microbe to dissolve inorganic phosphate varies and depends on the type of microbe and the optimal environment for microbial growth. Environmental factors that affect PSM were temperature, humidity, pH, and others. One such environmental factor is the sound that can be heard and has a wide distribution in nature [3]. In nature, almost all life forms are surrounded by various types of sounds and they also interact with these sounds, but not enough effort has been made to find out the interaction between sounds and biological systems [4].

Sound (in the form of music) affects microbial growth and metabolism [4]. The movement of antibiotics or their degradation products across cell membranes, the flow of calcium and potassium and the concentration of intracellular proteins in bacteria is also influenced by the treatment of music and
shows that sound waves have an influence on the growth of *Aspergillus* spp. *in vitro*. The sound that was played on *Escherichia coli* in both jelly and nutrient media obtained the best results at the 5 kHz frequency treatment compared to the 1 kHz and 10 kHz treatments [6].

From the findings of preliminary research, it was found that the land that was applied with the murottal Al-Qur’an sound for 2 hours per day for 7 days increased the total microbial population of the soil 23.08% higher than the land without murottal sound. The concentration of P available in the soil increased significantly (α 5%) in the soil-applied by the murottal Al-Qur’an sound from 77.1 ppm to 86.7 ppm (an increase of 11.07%) [8]. Murottal produced sound consisting of waves or vibrations that moved in the air. The number of vibrations or the number of waves produced per second was called frequency. This research was a study to compare microbes, the potential of isolates and the activity of phosphate solubilizing bacteria between the soils which were applied with murottal Al-Qur’an and those which were not.

2. **Material and methods**

2.1. **Place and time**
This research was conducted in the laboratory of the Faculty of Agriculture, University of Muhammadiyah Sumatera Utara, from April to August 2019.

2.2. **Materials and tools**
The materials used were: Pikovskaya media (Ca₃(PO₄)₂, NaCl, KCl, MgSO₄·7H₂O, MnSO₄·7H₂O, FeSO₄·7H₂O, (NH₄)₂SO₄, glukosa, yeast extract, aquadest, jelly, physiological solution NaCl 0.85%, KCl, and soil from Tanjung Anom.

The tools used in the study included hoes, gauges, rulers, measuring cups, sprayer, petridishes, shaker, spectrophotometers, transparent mica, incubators, Erlenmeyers, micro pipettes, measuring cups, inoculation needles, bunsen lamps, laminar airflow, incubator, excicators, centrifuge, autoclave, thermometer and laboratory instruments for analysis.

2.3. **Methods**
This research was sounds on 9 rhizosphere of corn, were put into the box. The sounds were treat is murottal al Quran which done or every morning during 2 hours at 08.00 -10.00 o'clock and compared with 9 rhizospheres of corn without sounds treatment.

2.4. **Parameters**

2.4.1. **Dissolution index**. The dissolving index is measured with a crossbar and then each potassium dissolving index (DI) was calculated to determine the ability of bacteria to dissolve potassium using the following formula:

\[ DI = \frac{a - b}{a} \]

Description :
DI = Dissolution Index
a = Colony diameter and clear zone
b = Colony Diameter

2.4.2. **Hypersensitivity test on tobacco leaves**. Hypersensitivity test was carried out with tobacco leaves (*Nicotiana tabacum* L.) which was 3 months old, by injecting selected microbes to the lower surface of the tobacco leaves in the mesophyll. If the site which had been injected, bacterial culture turns yellow or symptoms of necrosis after 48 hours means that the test bacteria were categorized as plant pathogens.
Aquades was used as negative controls and *Xanthomonas oryzae* pv. *oryzae* was used as a positive control.

3. Results and discussion

3.1. Isolation of phosphate solubilizing bacteria

After the soil had been treated with sound the murottal Al -Qur’an for 2 hours per day for 7 days, then isolation was carried out and 10 microbes were obtained consisting of 6 fungi and 4 bacteria that are able to dissolve phosphate in Pikovskaya medium and soil that had been not applied to sound obtained 5 bacterial isolates which were marked by the presence of holozone around the colony (Figure 1). The holozone showed the ability of microbes to dissolve phosphate.

![Figure 1](image1.png)

Description: (A). phosphate solubilizing bacteria without sound, (B). Solubilizing phosphate bacteria with Murottal sound, (C). phosphate solubilizing fungus with sound

Figure 1. Growth of bacterial colonies on Pikovskaya medium after 4 days

From the isolation that had been carried out, it was found that the sound treatment affected the growth of phosphate solubilizing microbes. The phosphate solubilizing fungus was not found in the soil which was not treated with sound. Phosphate solubilizing microbes that grew and formed the clear zones were then purified on a petridish by the quadrant scratch method (Figure 2), then stored on pikovskaya slanted agar as a collection.

![Figure 2](image2.png)

Figure 2. Bacterial isolates in Pikovkaya medium

3.2. Dissolution index

That isolated solubilizing microbes from the treat soil with sound and untreated were tested qualitatively through the formation of holozone around the colony by calculating the dissolution index (DI). The highest DI microbe from the treated soil with sound found, was 4 bacteria and 6 fungus meanwhile from the untreated was 5 bacteria and 3 base soil bacteria. Dissolution index data can be seen in Table 2.
Table 1. Results of microbial testing in phosphate dissolving after 4 days

| No | Primary Vegetation Isolates | Treatment | Isolate Code | Dissolution Index |
|----|-----------------------------|-----------|--------------|------------------|
| 1  | Corn                        | Murottal Sound | SM.B1       | T                |
| 2  | Corn                        | Murottal Sound | SM.B2       | 1.17             |
| 3  | Corn                        | Murottal Sound | SM.B3       | 2.16             |
| 4  | Corn                        | Murottal Sound | SM.B4       | 1.4              |
| 5  | Corn                        | Murottal Sound | SM.B5       | 0.23             |
| 6  | Corn                        | Murottal Sound | SM.B6       | T                |
| 7  | Corn                        | Murottal Sound | SM.B7       | 0.56             |
| 8  | Corn                        | Murottal Sound | SM.B8       | 0.12             |
| 9  | Corn                        | Murottal Sound | SM.B9       | 0.15             |
| 10 | Corn                        | Murottal Sound | SM.B10      | 0.67             |
| 11 | Corn                        | No Sound    | TS.B1       | 0.42             |
| 12 | Corn                        | No Sound    | TS.B2       | 0.59             |
| 13 | Corn                        | No Sound    | TS.B3       | 0.15             |
| 14 | Corn                        | No Sound    | TS.B4       | 0.29             |
| 15 | Corn                        | No Sound    | TS.B5       | 0.27             |

Based on Table 1, it could be seen that the sound treatment gave a very good influence on the activity of solubilizing phosphate microbial solubilizings, the dissolution index for bacteria is 1.17 to 2.16 and, the dissolution index for fungus is 0.12 to 0.67. Whereas, the land which was not applied to sound showed the dissolution index is 0.15 to 0.59. The research that had been done also stated that the sound was played to *Escherichia coli* influenced the growth of these bacteria which were inoculated in agar medium and nutrient broth [6].

![Figure 3. Microbial growth in the Pikovskaya medium](image)

Note: (A) PSB isolates (B) PSF isolates on pikovskaya medium
3.3. Hypersensitivity test on tobacco leaves

Hypersensitivity test results on tobacco leaves from the 17 selected isolates were negative hypersensitivity (not pathogenic to plants). Tobacco leaves injected each isolate did not show symptoms of necrosis after incubation for 48 hours.

![Image](image_url)

Description: (A). Bacterial isolate SM.B2 (B). The leaves were injected with the bacterium *Xanthomonas oryzae* pv *oryzae* as a positive control (C). Isolate TS.B4, (D). Negative control (aquadest)

**Figure 4.** Observation results of hypersensitivity test on tobacco leaves

The results of this test reacted negatively to hypersensitivity because the leaves injected with isolate did not show symptoms of necrosis in tobacco leaves and a positive hypersensitive reaction (positive control) was indicated by the tobacco leaf area injected with the bacterium *Xanthomonas oryzae* pv *oryzae*. This bacterium was able to induce hypersensitivity reactions in tobacco leaves which were indicated by the presence of yellow spots or experiencing symptoms of necrosis in the injection area. Negative hypersensitive reactions were also shown by leaves injected with distilled water (aquadest). Hypersensitivity reactions were rapid and localized cell death programs. This reaction occurred in infected plants when introducing pathogens and this was an attempt to inhibit the growth of pathogens [7].

4. Conclusions

Sound application affected the growth process of microbial phosphate solubilizing as indicated by the discovery of phosphate solubilizing fungus in the soil that was applied to the sound. The sound treatment gave a very good influence on the activity of phosphate solubilizing microbes as seen from the potential of microbes in forming holozone through the dissolution index test. The best bacterial dissolving index was SM.B4 (2.17) and in fungus was SM.J6 (0.67) and all microbes were are not pathogenic to plants.

References

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