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RESEARCH ARTICLE

Isolation and identification of phosphate solubilizing microbes in the rhizosphere of maize by sound exposure

Asritanarni Munar[1,4], Mariani Sembiring[2], Ahmad Rafiqi Tantawi[3], Tengku Sabrina[2]*

[1]1Doctoral Program of Agricultural Sciences, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia, [2]Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia, [3]Department of Agrotechnology, Faculty of Agriculture, Universitas Medan Area, Jl. Kolam No. 1 Medan Estate, 20223, Indonesia, [4]Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Jl. Kapten Mukhtar Basri No. 3 Medan 20238, Indonesia

ABSTRACT

Phosphorus (P) is a macronutrient requirement by plants. P elements applied to the soil are quickly deposited into an insoluble form. Insoluble P in the soil can be converted into available P by phosphate solubilizing microbes. One of the factors that affect the activity and growth of phosphate solubilizing microbes is sound. This study aims to isolate and identify phosphate solubilizing soil microbes in the rhizosphere of maize by sound exposure. The research was conducted by sound treatment and without sound of Al-Quran recitation in the rhizosphere of maize, then isolated and identified the phosphate solubilizing microbes. The research results obtained 11 isolates consisting of three isolates of fungi and eight isolates of bacteria. Fungi isolates found on sound-exposure soil. Subsequently, the highest microbes of phosphate dissolution index and growth curve after the sound exposure will be identified. The results of identification using the PCR-16S rRNA sequencing method showed the phosphate solubilizing bacteria isolate TSB1 was *Burkholderia contaminans*, TSB4 was *B. latens*, SMB2 was *B. cepacia*, and SMB4 was *Burkholderia* sp. Phosphate solubilizing fungi SMJ3 isolate was *Talaromyces muroii* and SMJ6 was *Talaromyces* sp. The growth curve of the phosphate solubilizing bacteria showed a different pattern, likewise phosphate solubilizing fungi.

Keywords: Isolation; Identification; Phosphate Solubilizing Microbes; Sound

INTRODUCTION

Phosphorus has important an essential role in various cellular processes, including maintenance of membrane structures, synthesis of biomolecules, and the formation of high-energy molecules. Phosphorus also helps in cell division, activation or inactivation of enzymes, and carbohydrate metabolism (Razaq et al., 2017). The availability of P for soil is relatively low. Therefore, there is a lack of phosphate elements that can be absorbed by plants. Fertilization containing P elements required to maintain crop production. However, P fertilizers applied to the soil are quickly deposited into insoluble forms of $CaHPO_4$, $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$, so they cannot be absorbed by plants (Wahbi et al., 2016).

Phosphate solubilizing microbes (PSM) such as phosphate solubilizing bacteria (PSB) and phosphate solubilizing fungi (PSF) help to dissolve phosphate and make it available to plants. PSB is present in all soils, and the amount varies depending on the type of soil. The PSB population range is around 1–50% of the total population of each microbe in the soil (Liu et al., 2015). The percentage of PSM population to total soil bacteria is 0.03% to 0.11% (Paul et al., 2018).

The capability of PSM dissolving inorganic phosphate varies and depends on the type of PSM and the optimal environment for microbial growth. Environmental factors have to affect PSM include temperature, humidity, pH, and sound. Audible sound has a wide distribution in nature (Mortazavian, 2012). Various kinds of sounds surround almost all life forms. All organisms interact with the sounds that exist. However, there is insufficient evidence to exhibit the interaction between sound and biological systems (Rillig et al., 2019).

The study of Sarvaiya and Kothari (2017) argue that sound (in the form of music) can affect the growth of various

***Corresponding author:**
Tengku Sabrina, Departement of Agrotechnology, Universitas Sumatera Utara, Padang Bulan, Medan 20155, Indonesia.
E-mail: t.sabrina@usu.ac.id

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types of microbes and metabolism. According to the results of research by Banerjee et al. (2018) on *E. coli*, it shows that exposure to sound at a frequency of 432 Hz can increase bacterial growth by 17.94%. Sound waves can affect the development of Escherichia coli. The influence of classical music with a frequency range of 38-689 Hz can affect the growth of all bacteria become great bacteria (3.15-40.37%) except *Serratia marcescens*. Furthermore, music treatment can affect the increase in antibiotic susceptibility as well (3.81-18.69%) compared to no-sound exposure (Sarvaiya and Kothari, 2015). The Al-Qur'an recitation has the type of sound and it has a positive effect on the activity of all organisms, including phosphate-solubilizing microbes. However, there is insufficient scientific evidence to explain it. Therefore, it is necessary to isolate and identify phosphate solubilizing microbes on soil treated sound and without the sound of Al-Quran recitation.

MATERIAL AND METHODS

Research methods

This study used the descriptive method. The stages of activities carried out included microbial isolation, P solubility testing, genetic identification of microbes using the PCR-16S rRNA Sequencing method, and observing the growth of phosphate solubilizing microbes. The observational data for the dissolution index were tested using the Welch test (T-test).

The research site conducted in Lau Bekeri Village, Kutalimbaru District, Deli Serdang, Indonesia, on the coordinates: N: 3o28'09; 3o28'17 and E: 98o31'13; 98o31'12 on June-September 2019.

Soil sampling

Rhizosphere soil samples were taken randomly from corn plants aged 30 days, with 18 sampling points using water pipes. Furthermore, cutting the maize until the stems are 5 cm above the ground. A water pipe with a height of 8 cm and a diameter of 8 cm is buried in the soil. The second stage is cleaning the dirt on the edge of the pipe to lift the tube from the ground. In the last stage after flattened the soil in the tube then inserted the pipe into the chamber for sound exposure.

Sound exposure

Water pipes containing rhizosphere soil are inserted into two chambers, each chamber containing nine water pipes. One partition used as sound exposure (SE) and another no-sound exposure (NS).

Sound exposure applied in the morning for the duration of 2 hours starts at 8-10 am. Exposure sound uses Al Qur'an recitation sound MP3 player, Surah Ar Rahman at a sound pressure level (SPL) of 85 ± 5 dB, an amplitude of 25 Hz (Fig. 1). The rhizosphere soil was treated by sound exposure for seven days. The distance between the first squares and second squares is 6 m (Fig. 2).

Microbial isolation and purification

After the sound exposure, weighed each soil around 10 g and put it into 90 ml of 0.85% NaCl physiological solution. Then, shaken for 1 hour at a speed of 120 rpm and categorize a series of dilutions starting from 10-1 to 10-6 dilutions. Each dilution of 10-4, 10-5, and 10-6 was taken as much as 0.1 ml and spread on Pikovskaya medium, incubated at 28 ° C for seven days (Temraleeva et al., 2016). After the colony is visible, it is followed by a purification phase. Microbial colonies that grow and form clear zones on Pikovskaya media are taken according to shape and colour as much as one loop. Subsequently, it was raised back on Pikovskaya medium to get pure culture results and also put the isolated code into each pure culture.

Phosphate dissolution index measurement

The pure phosphate solubilizing microbial isolates were grown on Pikovskaya medium by placing them in the center and incubated for seven days at room temperature into the incubator and observed the clear zone formed around the microbes using the formula:

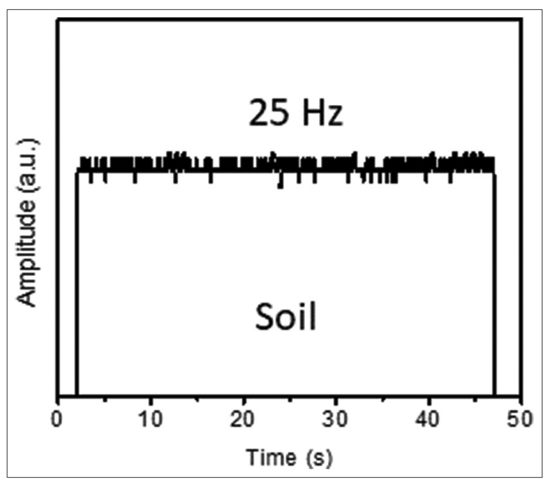


Fig 1. The sound amplitude of Surah Ar-Rahman recitation in the soil.

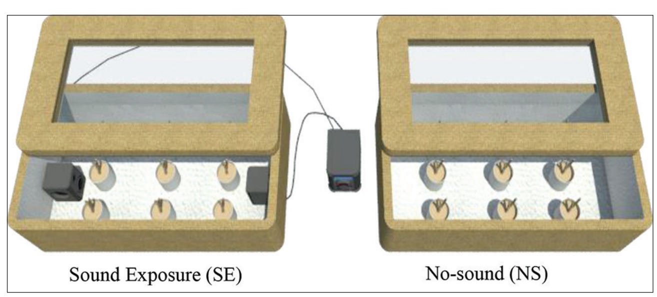


Fig 2. Sound exposure to rhizosphere soil in the laboratory.

$$\text{Phosphate Dissolution Index} = \frac{A}{B}$$

where,
A = Clear zone diameter
B = Colony diameter
Source: Paul and Sinha (2017)

Measurement of the phosphate dissolution index aims to see the capability of microbes to dissolve phosphate, through the formation of a clear zone. In other words, the larger the clear zone formed, the more P can be dissolved. After measuring the phosphate dissolution index, the phosphate solubilizing microbes were selected, which showed the highest index of phosphate dissolution. After the selection, it will select two isolates that have the highest phosphate dissolution index based on the type of microbe and sound exposure to be identified.

Genetic identification

Genetically identification of selected isolates that show the highest phosphate dissolution index conducted at the Environmental Biotechnology Laboratory, PT. Biodiversity Biotechnology Indonesia in Bogor, Indonesia. Identification using the PCR-16S rRNA sequencing method by following the Clarridge (2004).

Bacterial growth curve

First, measure the absorbance of bacterial growth for every hour to create the curves. Then, bacterial isolates were incubated into 50 ml of Nutrient Broth liquid medium. The next stage is shaking it at 90 rpm for 24 hours. Every hour, the absorbance was measured with a spectrophotometer with a wavelength of 620 nm.

Fungi growth curve

Fungal growth curves were obtained by measuring the area of fungal colonies on solid Pikovskaya medium in Petri dishes. Measurements conducted by drawing the location of the colony using transparent plastic and replicating it on millimeter paper and calculating the area. Moreover, measurements conducted once a day up to the Petri dishes were full of fungal colonies.

RESULTS

Isolation and purification of phosphate solubilizing microbes

Soil exposed sound or no sound was incubated on the jelly nutrient. The results obtained different colony morphological shows (Fig. 3). The appearance of the colony is circular, irregular, filamentous and rhizoid with colours varying from clear, creamy, and broken white.

The purification stage carried out separating each colony according to its macroscopic morphological appearance on Pikovskaya medium. The results of purification obtained six bacterial isolates through no-sound exposure and two bacterial isolates of sound exposure. For soil with sound exposure, there are three isolates of phosphate solubilizing

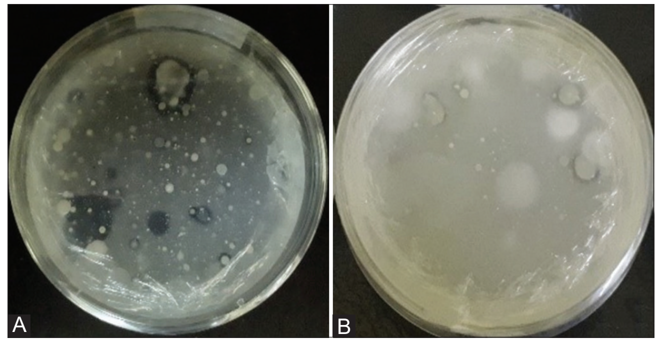
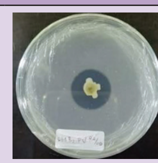
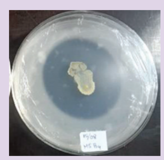
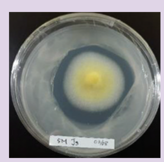
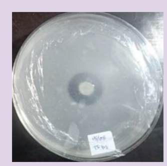
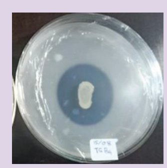
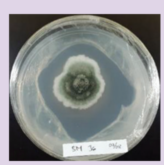


Fig 3. Growth of bacterial colonies on Pikovskaya medium; A= Isolation result with sound exposure (SE), B = Isolation result of no-sound (NS).

Table 1: The results of the isolation of phosphate solubilizing microbes in sound exposure

Isolate Code	Microbes	Isolate Code	Microbes
SMB2		TSB1	
SMB4		TSB2	
SMJ3		TSB3	
SMJ4		TSB4	
SMJ6		TSB5	
		TSB6	

fungi, whereas soils with no-sound exposure were not found isolates of phosphate solubilizing fungi (Table 1).

Dissolution index

The dissolution index of 11 pure isolates incubated on Pikovskaya media for seven days show in Table 2.

Table 2: The measurement results of the phosphate dissolution index by phosphate solubilizing microbes on the seventh day of incubation

No	Treatment	Isolate Code	Microbes	Dissolution Index
1.	Qur'an Recitation	SMB2	Bacteria	0.91±0.83
2.	Qur'an Recitation	SMB4	Bacteria	2.34±0.83
3.	Qur'an Recitation	SMJ3	Fungi	0.3±0.83
4.	Qur'an Recitation	SMJ4	Fungi	0.25±0.83
5.	Qur'an Recitation	SMJ6	Fungi	1.03±0.83
6.	No-sound	TSB1	Bacteria	2.31±0.84
7.	No-sound	TSB2	Bacteria	0.46±0.84
8.	No-sound	TSB3	Bacteria	1.00±0.84
9.	No-sound	TSB4	Bacteria	2.26±0.84
10.	No-sound	TSB5	Bacteria	0.49±0.84
11.	No-sound	TSB6	Bacteria	0.92±0.84

Identification of phosphate solubilizing bacteria and fungi

The identification of isolates selected, namely TSB1, TSB4, SMB2 SMB4, SMJ3 and SMJ6. The results of the microbial isolates category explained in the phylogenetic tree in Fig. 4 and Fig. 5.

Phosphate solubilizing bacteria growth curve

Phosphate solubilizing bacteria that have been identified as *B. contaminans B. latens, B. cepasia* and *Bulkhorderia,* sp. shows the growth curve as follows (Fig. 6)

Phosphate solubilizing fungi growth curve

The phosphate solvent fungi that have been identified are *Talaromyces muroii* and *Talaromyces* sp. Growth curve as described in Fig. 7.

DISCUSSION

The microbial colonies obtained through isolation and purification based on the fungi and bacteria groups. The

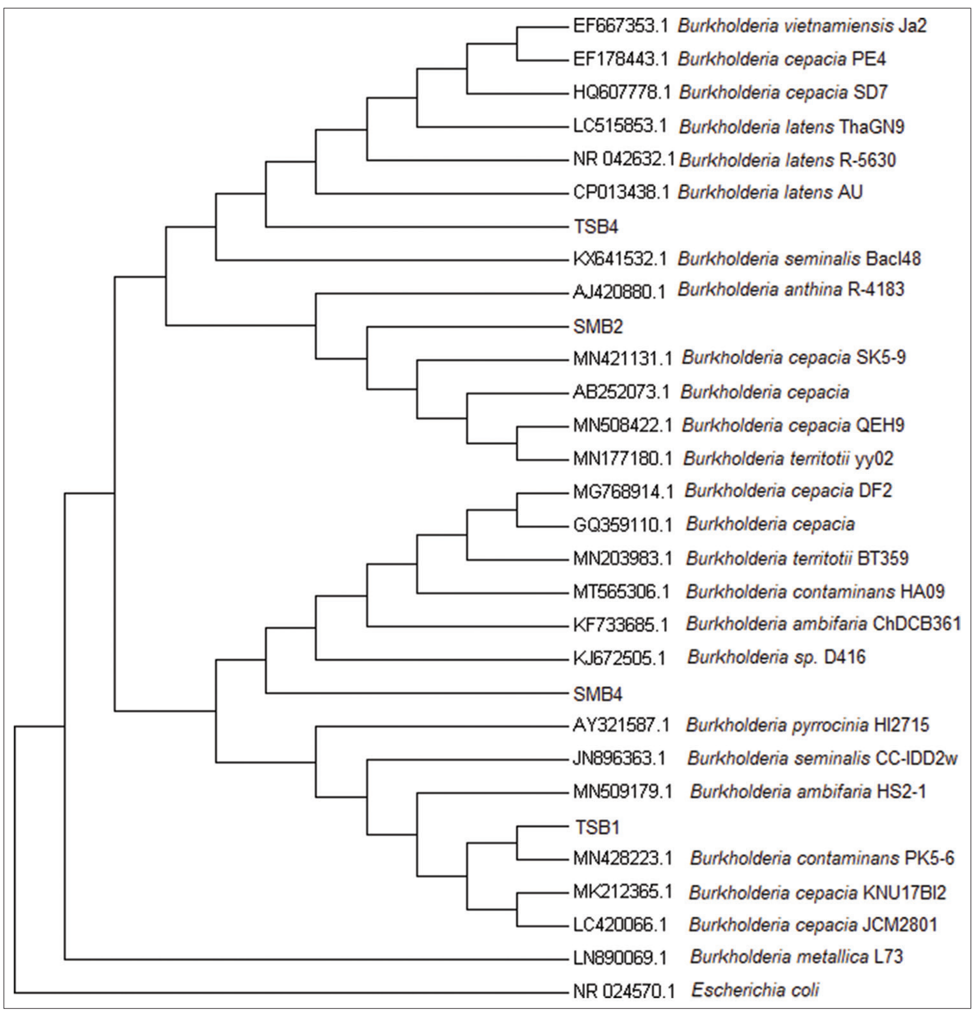


Fig 4. Phylogeny tree of bacterial isolates. Construction of a phylogenetic tree using Maximum Likelihood method as of Jukes-Cantor model. The scale corresponds to the number of changes per 200 nucleotides. Phylogenetic analysis performed using software MEGA 7.

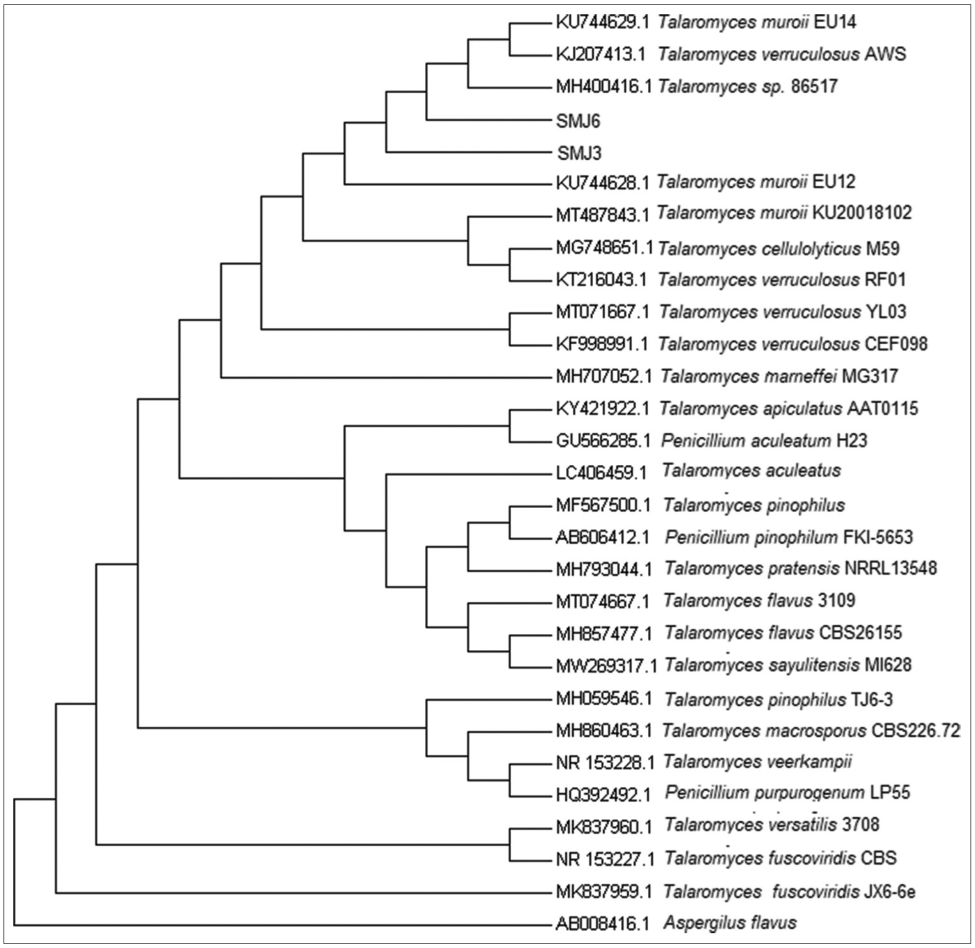


Fig 5. Phylogeny tree of fungal isolates. Construction of a phylogenetic tree using Maximum Likelihood method as of Jukes-Cantor model. The scale corresponds to the number of changes per 200 nucleotides. Phylogenetic analysis performed using software MEGA 7.

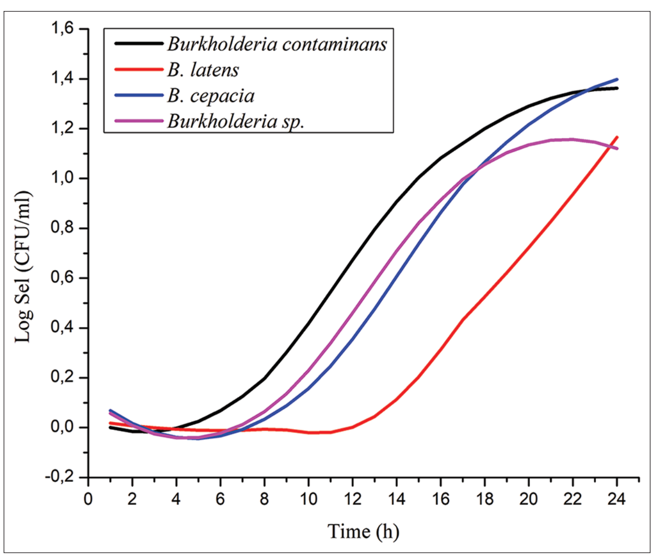


Fig 6. Growth curves for isolates bacteria of TSB1 *(B. contaminans),* TSB4 *(B. latens),* SMB2 *(B. cepacia)* and SMB4 *(Burkholderia* sp.*)*

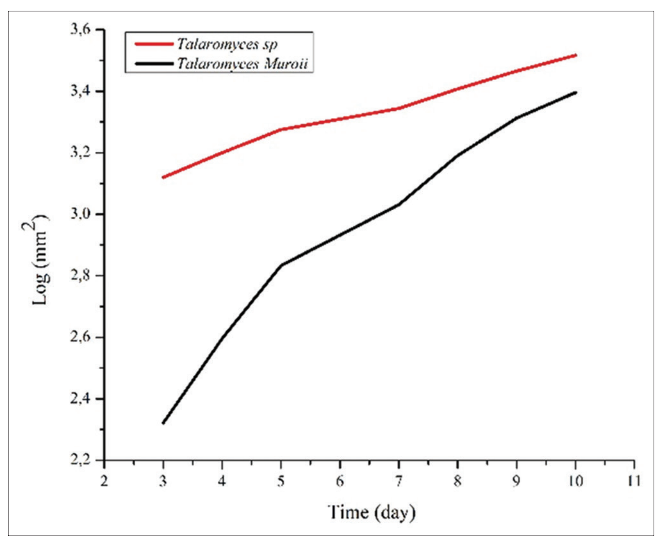


Fig 7. Growth curve of *Talaromyces* sp. and *Talaromyces muroii.*

appearance of the colonies seen on jelly media varies according to the type of microbe (Fig. 3). The activity of each microbe is totally dependent on many factors, including sound exposure (Sarvaiya and Kothari, 2017; Munar et al., 2022). For rhizosphere soils with no-sound exposure, there were six isolates of phosphate solubilizing bacteria, and no phosphate solubilizing fungi. While rhizosphere soils with sound exposure, there were two isolates of phosphate solubilizing bacteria and three isolates of phosphate solubilizing fungi (Table 1). Phosphate solubilizing fungi were not found in soils with no-sound exposure because they failed to form the clear zone. According to (Pardo et al., 2006), the growth of phosphate solubilizing microorganisms is strongly influenced by soil acidity. In acid soils, the activity of microorganisms is dominated by fungal groups due to optimum fungal growth is at pH 5-5.5. Soil pH at the research location ranged at 5.04 to 5.06. This pH range is the optimum pH for fungal growth. Phosphate solubilizing fungi are found in sound exposure soils due to the sound of Al Quran recitation of surah Ar Rahman with an amplitude of 25 Hz produces sound waves that can stimulate microbial activity to produce organic acids. The resulting of organic acid was able to dissolve P. Therefore, the Pikovskaya medium formed a clear zone and was detected as a phosphate solubilizing microbe. Tarigan et al. (2023) revealed that most colonies could grew within 2–6 days of incubation at 30°C.

Moreover, Sound affects the growth process of phosphate solubilizing microbes. The results of the study Sarvaiya and Kothari (2017) on the effect of Indian classical music (Raag Malhar) on microbial growth, showed that all bacteria and yeast used as test organisms showed better development under the influence of music except Serratia marcescens. Likewise, the results of the research by Shaobin et al. (2010) which explained that sound could affect the growth and metabolic activity of *E. coli* organisms. For acterial cells, when stimulated by sound waves, it can affect the stimulation of their development. It is similar with the results of research by Sarvaiya and Kothari (2015) argue that the application of Indian classical music Raag Kirwani in the culture of several bacteria *Chromobacterium violaceum* increases calcium by 24.13%. Giving voice to *E. coli* bacteria also affects the growth of these bacteria, which are inoculated on nutrient media to make them better (Ying et al., 2009). The effect of two different music patterns Ahir Bhairav (172-581 Hz) and Piloo (86-839 Hz) of audible sound significantly to influence microbial growth and metabolite production (Shah et al., 2016). The identification results showed that the phosphate solubilizing bacteria found were all genus *Burkholderia* with a homology level of 99.49% to 100% and query cover 99% to 100% (Fig. 6). Pande et al. (2019), isolates and identifies phosphate solubilizing bacteria from the rhizosphere of sweet corn *cv.* Golden Bantan to get *B. cepacia* (99%) and *B. contaminans* (94-99%) as phosphate solubilizing bacteria. According to Ghosh et al. (2015) *B. cepacia* can dissolve phosphate from $Ca_3 (PO_4)_2$ as much as 484.18 ± 14.23 $mg.L^{-1}$ and is an excellent phosphate solvent to release phosphate from $FePO_4$, $AlPO_4$ and four different phosphate rocks. Sembiring and Fauzi, (2017) also found *B. cepacia* as a phosphate solubilizing bacteria which was effective in increasing available P in the soil.

Besides, it has role into phosphate dissolution, *B. cepacia* bacteria also participate into potassium dissolving. Isolates of *B. cepacia* bacteria isolated through soil samples around the Cirebon limestone mining area were able to dissolve potassium (Mursyida et al., 2015). *Burkholderia* sp. is useful into forming endophytic relationships with maize plants. It can be able to increase plant growth. Moreover, it also raises available P, as evidenced by rhizosphere phosphatase activity (Young et al., 2013). *Burkholderia latens* obtained from oil palm rhizosphere in Colombia can dissolve 1143 mg/L (42%) of phosphate (Acevedo et al., 2014).

The results of the molecular identification of phosphate solubilizing fungi in this study were SMJ3 which has a 100% similarity with *T. muroii*. The results of molecular identification from culture SMJ6 have 100% similarity with the fungus *Talaromyces* sp. (Fig. 4). Following several studies that have found that *Talaromyces* sp. is a fungus that can dissolve P in soil (Wakelin et al., 2010). This statement similar with the results of Hegde's research (2017) exhibit that *Talaromyces* sp. showed maximum extracellular pectinase activity in both soil and liquid media. Environmental conditions such as pH and temperature are known to alter expression and activity of exoenzymes.

The phosphate solubilizing bacteria with the highest growth was *B. contaminans,* and the lowest was *B. latens* (Fig.6). Gu et al. (2015) supported that *E. coli* k-12 exposed to sound waves had higher biomass and a faster growth rate compared to the control group. The average length of cells *E. coli* k-12 also increased by 27.26% longer. Fungi *Talaromyces* sp. and *Talaromyces muroii* started the logarithmic phase on the third day and failed to show the stationary phase until day 10. The growth curve of *Talaromyces* sp fungi was higher than *Talaromyces muroii*. The growth and number of different microbes are caused by several factors, namely nutrition, oxygen, carbon dioxide, pH, and temperature. Similar to the opinion Pelczar (2019) that energy sources can influence time differences, carbon sources, pH, environmental temperature, O_2 and the incubation period or the nature of the organism.

CONCLUSION

The sound has been affected the growth and metabolism of microbes. Phosphate solubilizing fungi only found in sound exposure soils. The results of molecular identification showed that all bacteria were include Bulkholderia genus with their respective species: *Bulkholderia contaminans, B. cepacia, B. latens and Burkholderia* sp., while the fungi were *Talaromyces muroii* and *Talaromyces* sp. The growth of phosphate solubilizing bacteria and phosphate solubilizing fungi showed different growth curves.

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Authors' contributions

Asritanarni Munar, conducted experiments and wrote the manuscripts; Mariani Sembiring, assisted the isolation and identification of phosphate solubilizing microbes; Ahmad Rafiqi Tantawi, helps microbial growth; Tengku Sabrina, designed the research, provided guidance and proofread the manuscript. All authors played a role in the completion of the final manuscript.

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