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# Screening of Phosphate Solubilizing Microorganisms Collected from Post-Tin Mining

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***Abstract***

*Tin mining produced massive tailings which dispersed across the land in the post-tin mining area, transforming the landscape into a hilly or basin landscape. The dominated white silica sand in this area is prone to erosion from water and wind, resulting in soil nutrition deficiency, meanwhile, heavy metal levels were found to be elevated. This condition makes it difficult for organisms to live in such a degraded post-tin mining area. Phosphate solubilizing microorganisms (PSM) contribute to phosphate availability by dissolving both fertilizers and bounded P in soil. This study aimed to select indigenous PSM collected from post-tin mining areas. Zea mays spp., the tested plant, was grown in sterilized sand to avoid the possible nutrient content, particularly phosphate, in the soil. Rock phosphate Granufos (containing 20% $P_2O_5$) was applied in sterilized sand (v: v= 1:100) as the source of limited and insoluble P. The 17 indigenous PSM were tested by inoculating them into Zea mays spp. growing in sterilized sand. Non-inoculated Zea mays spp. were prepared as well as control. Inoculation of 17 indigenous PSM significantly had higher soil available phosphor in comparison to control. Two PSM of P7 and P15 significantly had higher shoot concentrations among treatments. The isolate of P5 had the highest shoot dry weight among treatments. Overall, all PSM inoculations improved growth performance due to increased soil available P and improved shoot P uptake. These findings suggest that indigenous PSM potentially fills nutrient deficiencies, particularly P as a macronutrient needed to rehabilitate degraded post-tin mining areas.*

***Keywords:*** *nutrient deficiency, phosphate solubilizing microorganisms, post-tin mining*

## 1. INTRODUCTION

Indonesia is the world's second largest producer of tin, trailing only China. In Indonesia, the main areas for tin mining are Bangka Island and Belitung Island. In 2020, Indonesia exported approximately 65.35 thousand metric tons of refined tin. This value was one of the lowest in the previous ten years, encouraging more mining of this mineral in the future.

Continues tin mining has created massive land degradation, resulting in a large number of tailings, mixing soil layers, and transforming the landscape into hilly or basin landscapes. This mining activity created a new landscape of white quartz sand stretching as far as the eye can see. The fragile characteristics of white quartz sand to wind and water erosion encourage nutrient leaching including organic matter as the source of soil nutrients is missing. It is reported that tin mining has a significant impact on soil physicochemical properties. The soil transforms into quartz sand soil, damaging the soil structure (Sukarman *et.al.,* 2020), resulting

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in a decrease in organic carbon, total nitrogen (N), available phosphorus (P), exchangeable potassium (K), and cation exchange capacity (CEC) (Wulandari *et.al*., 2022). The concentration of magnesium (Mg), calcium (Ca), and sodium (Na) were found very low (Oktavia *et.al.*, 2014). Based on these harsh conditions of soil after tin mining, organisms will struggle to survive due to a large number of limiting factors. To overcome the problems in the post-tin mining area, rehabilitation using a cost-effective friendly method is required.

Phosphorus (P) is one of the most important major nutrients for plant growth, which accounts for 0.2% (w/w) of plant dry weight (Maharajan *et.al.*, 2018). As a vital component, P is found in every living cell serving as an energy unit as ATP in plants (Carstensen et al., 2018). Phosphorus is a component of plant nucleic acid structure that regulates protein synthesis (Raven, 2013), allows energy transformation via photosynthesis (Thuynsma et al., 2016), forms ATP during photosynthesis (Carstensen et al., 2018) and assists plants in converting other nutrients into usable building blocks. Adding P to deficient soil promotes healthy root growth including its emergence which finally influence on promotion of healthy plant growth (Kim and Li, 2016). One of the most common soil problems in tropical areas is that the total P in soil is high, but the available P is very low. This is due to the fact that P can be chelated by Al, Fe, and Ca, rendering it unavailable to plants (Penn and Camberato, 2019). Based on this fact, it is necessary to meet the requirement of P in the soil in order to aid plant growth for the rehabilitation of degraded land.

Phosphate solubilizing microorganisms (PSM) are beneficial soil microorganisms having the potential to increase soil P levels by converting unavailable P compounds into available P compounds via bacteria and fungi (Li et al., 2016; Abdelgalil et al., 2022). Because of this ability, these microorganisms can mobilize and increase P uptake (Atekan et al., 2014), thereby improving the plant-microbe interaction in the rhizosphere (Ehaissoufi et al. 2020), which determines plant health and, as a result, soil productivity. Because additional PSM improves the efficiency of phosphate fertilizer, this application of PSM may act as a plant growth-promoting microorganism.

Research about the application of PSM into plants has been confirmed by previous studies, however, understanding of the variation of indigenous PSM ability collected from degraded tropical post-tin mining is still limited. Therefore, in this study, we aimed to select and

determine the ability of indigenous PSM collected from a post-tin mining area in increasing soil available P and its effect on plant growth.

## 2. MATERIALS AND METHODS

### 2.1. Study site and soil sampling

The research was carried out in Sungailiat, Bangka Regency, Bangka Belitung Province, Indonesia. According to Indonesian statistics, this region receives between 2000 and 3000 mm of rain per year. Tin mining that had been abandoned was subjected to soil sampling on degraded land. The research was conducted in the Rambak Sungailiat Bangka Regency, Province of Bangka Belitung Island. As a replication, three post-tin mining areas within a 7-10 km radius were studied. For analysis, the soil was collected at a depth of 0 - 20 cm for about 1- 2 kg per soil. This depth of soil was chosen for sampling because the depth of a plant's root and topsoil is reached at this depth, which means that biological activity including soil microorganisms is active.

### 2.2. Isolation and inoculums preparation of Phosphate Solubilizing Microorganism (PSM)

There were 17 isolates of PSM collected from the soil of the post-tin mining area, called P1 – P17. There were 15 bacteria and 2 fungi (P13 and P17). Pikovskaya agar was used as the selected media to isolate PSM. Isolated microorganisms growing in pikovskaya agar forming halozone were selected and determined as PSM. Each colony of isolates of PSM was recultured in order to obtain a pure culture of PSM. Isolates of PSM were recultured every two months.

In this study, pure cultures of PSM were used as inoculum. The 17 isolates of PSM were cultured in nutrient broth supplemented with 5 g/L tricalcium phosphate at 30$^{o}$C in an orbital shaker (150 rev min−1) for 24 h. Cultures were centrifuged in 50 ml sterile plastic tubes at 6000 × g for 15 min. The pellets were resuspended in PSM to obtain a final concentration of 106 ml/colony forming units (CFU). The liquid cultures of each PSM isolate were used for individual inoculations. The inoculums were prepared with each individual culture of PSM and used as pure inoculums.

### 2.3. Growth media preparation

Sand was used to grow plant for screening PSM. Preparation of sand is started by washing the sand with water and immerse it in HCl 5% for one night. The sand was washed till the pH close to 6. Sand was dried and sterilized by autoclave at 121$^{o}$C for 5 minutes. This sterilized sand was used as the growth media to minimize the nutrient content in the media. Rock phosphate Granufos (contains 20% P2O5) was applied in sterilized sand (v : v= 1:100). Sterilized soil contained rock phosphate was weighed 500 grams and put it onto 600 ml pot.

### 2.4. Seedling preparation

*Zea mays* spp. was used as a testing plant because this plant is sensitive to P deficiency. The seeds was scarified by immerse the seeds in alcohol 70% to avoid possible contamination brought by the seeds. Flying seeds were discharged and determined as bad seeds. There were 3 seeds of Zea mays spp put into pot containing sterilized sand at 1.5 cm depth. Water was applied once in two days. After the seeds were germinated, one healthy seedling per polybag was selected and allowed to grow. This selected seedling was used as the testing plant.

### 2.5. Inoculation of Phosphate Solubilizing Microorganism

Inoculation of PSM for phosphate solubilizing experiment was carried out by using *Zea mays* spp as the host plant. Seeds of Zea mays spp. and Brassica campestris were sterilized before inoculation. First inoculation was carried out by immersed sterilized seeds into test tube contained PSM inoculums for 20 minutes. Sterilization and inoculation of seeds were carried out aseptically. Inoculated seeds were sown into sterilized sand contained rock phosphate that already prepared. Each pot received five seeds. Two weeks old germinated seedlings were allowed to grow one seedling per pot. There were seven replications for each treatment. Three weeks after germination, the seedlings received second inoculation of PSM (~200 ml/L of substrate) suspension contained 106 CFU (cell forming unit) which was poured onto the rhizosphere of the seedlings. Sterilized pipette is used for inoculation. Non-inoculated seedlings were prepared as well as control. Plants are allowed to grow for three months under greenhouse conditions. Fertilizer of modified Hoagland solution with free phosphate was applied once in every two days.

### 2.6. Parameter measurement and statistical analysis

Three months after growing under greenhouse conditions, seedlings of *Zea mays* spp. were harvested. Seedlings were observed for shoot dry weight, shoot P concentration, and soil available P concentration. Statistical significance of the data was analyzed by comparing

seedlings with or without inoculation of PSM using analysis of variance (ANOVA). Post-hoc analysis was performed using the Tukey HSD test ($P<0.05$, $n= 5$).

## 3. RESULTS AND DISCUSSION

Inoculation of PSM resulted in better seedlings growth performance than control, particularly isolates P4, P5, P6 (Figure 1A), P7, P8, P9, P10, P11 (Figure 1B), P14, P16, and P17 (Figure 1C) one month after inoculation under greenhouse conditions. This good growth performance persisted in the majority of inoculated seedlings until harvest time of three months old seedlings.

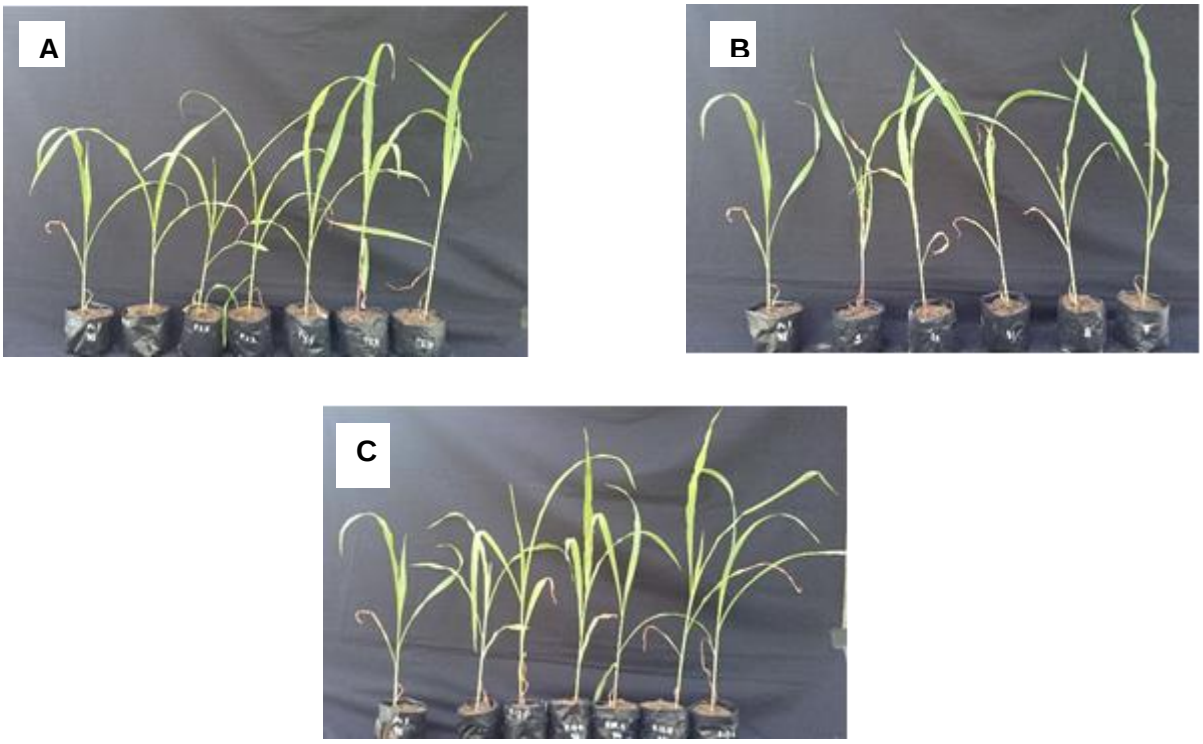


Figure 1. Growth performance of one month old *Zea mays* spp with and without inoculation of PSM collected from post tin mining area under greenhouse condition. From left to right: A: control; P1, P2, P3, P4, P5, P6; B: Control, P9, P10, P11, P8, P7; C: Control, P12, P13, P14, P15, P16, P17.

Figure 2 depicts soil available P with and without PSM inoculation. The addition of all PSM increased soil available P significantly. The best soil available P concentration was found in isolates P1, P2, P8, P15, P16, and P17. Isolates P3, P4, P5, P7, P9, P11, P12, P13, and P14 were the second best isolates PSM in terms of producing soil available P. The remaining isolates P6 and P10 came in third place in terms of producing soil available P. The lowest soil available P produced by PSM was approximately 270 mg/kg, while the highest

soil available P produced by PSM was approximately 500 mg/kg. In control soil, the available P concentration was only about 25 mg/kg.

Shoot P concentration of Zea mays spp with and without inoculation of PSM is presented in Figure 3. Inoculation of PSM into Zea mays spp. majority resulted in higher shoot P concentration than control. Isolate P 15 gave the highest shoot P concentration among treatments, followed by isolate P7. Isolates P2, P3, P4, P5, P6, P8, P9, P10, P14, and P16 tended to have higher shoot P concentrations than control. Meanwhile, isolates P1, P11, and P13 did not give a higher shoot P concentration than the control. Isolates P13 resulted in the lowest shoot P concentration among treatments. PSM of P15 resulted in the highest shoot P concentration of approximately 730 mg/kg. Meanwhile, P13 produced the lowest shoot P concentration, which was approximately 480 mg/kg. Seedlings without inoculation of PSM resulted in a shoot P concentration of approximately 550 mg/kg.

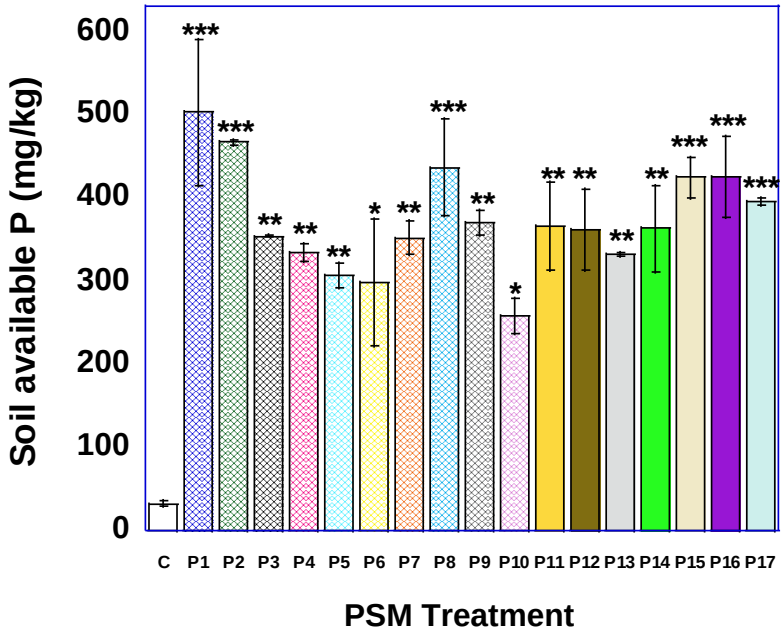


Figure 2. Soil available P (mg/kg) with and without inoculation of phosphate solubilizing microorganism (PSM) grow in sterilized sand for 3 months under greenhouse condition. *) indicates statistically significant different by Tukey HSD ($P<0.05$, $n=5$). C: control; P1 – P17: inoculation of PSM.

The inoculation of PSM on the growth parameter is presented in Figure 4. All inoculation of PSM tended to have a higher shoot dry weight of three months old Zea mays spp. Inoculation of P5 significantly gave the highest shoot dry weight among treatments.

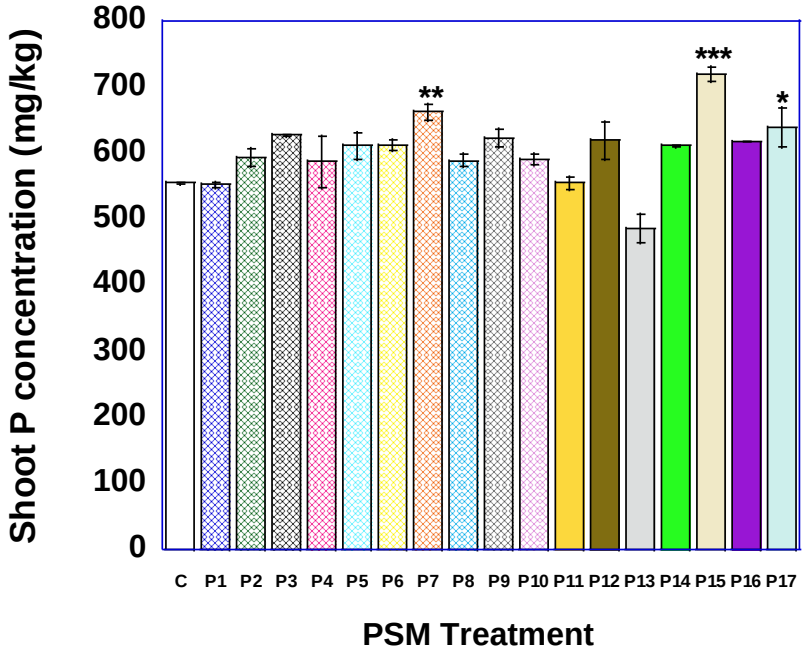


Figure 3. Shoot P concentration of *Zea mays* spp (mg/kg) with and without inoculation of phosphate solubilizing microorganism (PSM) grow in sterilized sand for 3 months under greenhouse condition. *) indicates statistically significant different by Tukey HSD (P<0.05, n=5). C: control; P1 – P17: inoculation of PSM.

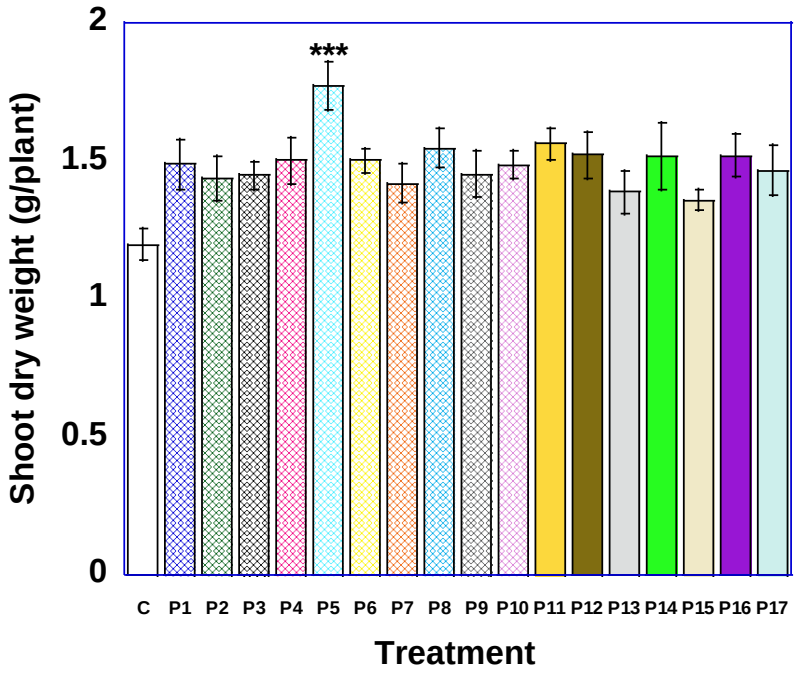


Figure 4. Shoot dry weight of *Zea mays* spp (mg/kg) with and without inoculation of phosphate solubilizing microorganism (PSM) grow in sterilized sand for 3 months under greenhouse condition. *) indicates statistically significant different by Tukey HSD (P<0.05, n=5). C: control; P1 – P17: inoculation of PSM.

The ability of PSM to solubilize P was demonstrated to influence positively *Zea mays* spp. seedling development (Figure 1; Figure 4). The final study revealed that inoculation indigenous PSM supports the hypothesis of good plant growth performance of *Zea mays* spp. Similarly, PSM improved the growth of wheat (El Habil-Addas et al., 2017; Adnan et al., 2022), *Hevea brasiliensis* Muell. Arg. (Promwee et al., 2014), *Zea mays* L. (Viruel et al., 2014), *Carthamus tinctorious* L (Zhang et al., 2019), and *Vigna radiate* (Lelapalli et al., 2021).

Even though most indigenous PSM collected from the post-tin mining area gave better results on plant growth, however, there were variations in their ability to influence seedlings' growth (Figure 4). This could be due to the variation of each PSM in solubilizing P as shown in Figure 2, or the effectivity in P uptake of the seedlings (Figure 3). This result corresponds to Schoebitz et al. (2013) who reported a different effect of PSM on shoot dry weight and shoot P concentration. Different shoot dry weights and soil available P were also reported by Ehaissoufi et al. (2020). It is well-known that P has a vital role in plant growth. It is involved in energy transfer, photosynthesis, including nutrient movement within the plant. Therefore, an improvement in soil available P is related to the plant's growth performance.

The variation concentration of soil available P is influenced by the concentration of organic acid produced by PSM. Previous research found that PSM bacteria and fungi produced a variety of organic acids, including glyoxalic acid, malic acid, citric acid, oxalic acid, acetic acid, isobutyric acid, isovaleric acid, itaconic acid, lactic acid, and succinic acid, which chelated cations or reduced the pH to release P (Seshachala and Tallapragada, 2012). The periplasmic space produces organic acids through the direct oxidation pathway (Zhao et al., 2014). Filamentous fungi may increase P solubilization by producing organic acid (Li et al., 2016) and siderophore (Karmakar et al., 2018), whereas bacteria may produce enzyme phosphatase (Abdelgalil et al., 2022), organic acid (Chen et al., 2016), and siderophore (Nailwal et al., 2014).

In addition to organic acid production, the success of PSM colonization to the root of the hostplant also influences the success of P solubilization. The colonization, establishment, and performance of PSM are affected severely under stress environments, such as temperature, pH (Suleman et al., 2018), and carbon, N source (Nahas, 2007). Because all of those factors were well controlled under greenhouse conditions, it is likely that all of the seedlings were

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successfully colonized by PSM based on the findings of this study. While the presence of solubilized P in control soil seedlings was most likely due to the role of root exudates. It has been reported that root exudates play a role in phosphorus acquisition (Gerke, 2015), and assimilation in plants (Pantigoso et al., 2020).

The addition of PSM majority increased seedling biomass production. This finding suggested that seedlings inoculated with PSM had a higher phosphate use efficiency than the control. This study discovered that seedlings treated with PSM absorb and utilize P more effectively for maximum yield production. In agreement to our result, the application of PSM improves P uptake and P use efficiency, resulting in a high grain yield of upland rice (Rawat et al., 2022). A study on nutrient use efficiency in plants have been previously reported (Galatro et al., 2020; Nieves-Cordones et al., 2020).

## 4. CONCLUSION

Inoculation of phosphate solubilizing microorganisms (PSM) from post-tin mining areas resulted in all potential PSM isolates due to their ability to significantly produce extremely high soil available P. There were 3 (P7, P15, P17) isolates that showed the most superior in absorbing P into plant tissue. Meanwhile, there was one isolate (P5) significantly increased seedlings` biomass. Overall, it is possible to conclude that PSM inoculation has the potential to be used as a bioremediation effort, particularly to meet the nutrient requirements for revegetation purposes in post-tin mining areas.

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