Utilization of selected microorganisms in enhancing the growth of selected plant in ex gold mining soil

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Abstract. Gold mining is an essential economic booster in enhancing the foreign exchange of a country. However, the gold mining activities resulting in contaminated soil. Phytoremediation, using plants for environmental remediation, has been intensively investigated in last decade. Phytoremediation has fewer side effects than conventional physical and chemical methods. This remediation technique embraces an array of low-cost plant-based technologies that could be potentially advantageous for remediation of the former gold mining site. This research aimed to assess the potency of selected microorganisms to support the growth of the plant in the soil of the former gold mining area. The application of selected microorganisms of P solubilizer, N fixing, growth-promoting bacteria, and arbuscular mycorrhizal fungi showed the best result on yield. This study showed that sengon (Falcataria) yields the better one compared to that of sorghum both in a medium consisting of ex gold mining soil and compost and medium with the composition of the soil, compost, and zeolite. It can thus be concluded that sengon inoculated with selected microbe is a potential method for remediating ex gold mining area.

Keyword : sorghum, sengon, microorganism, ex gold mining

1. Introduction
Gold mining is an economic booster in many countries around the world. In general, mining leads to economic growth in transparent conditions, with proper management due to employment and opportunities for migrants to work. Besides, there is creativity both directly and indirectly contributes to the economy of a country. In Indonesia, gold production reaches 59 and 60 tons in 2013 and 2014, respectively. The country still has 3,000 tons of reserves [1]. In 2014, Indonesia is among the top 10 gold producers, producing 4% of world gold production of 89 tonnes and providing a national gross income of $ 1.188 billion. In 2013, the value of gold reached $ 764.3 million (0.5% of domestic gross domestic product). In 2014, gold production was $ 732.66 million [2].
Plant restoration is the most widely known method for restoring degraded land, such as ex-mining areas [3]. The plant species planted are tolerant unfavorable environment and toxic chemicals. It also means that phytoremediation uses green plants to remove and degrade contaminants in soil, sludge, sediment, surface water, and soil surface. Depending on the process, phytoremediation can be grouped into phytodegradation using plants to absorb storing and degrade of contaminants in the tissues, phytoextraction using plants to absorb, translocate and store toxic contaminants from the soil particles to root and shoot tissues. Fitostimulation or rizodegradation uses an association of plants and symbiotic microbes in the rhizosphere to degrade the pollutants.

Revegetation uses fast-growing crops to stimulate the growth of new plants and facilitates the closure of land that plays primary crops to grow on degraded land [4]. Crop tolerance to trace elements [5], dry, acid, and deep root growth capabilities are suitable for revegetating of ex-mining areas.

The beneficial microorganisms, i.e. arbuscular mycorrhizal fungi, Rhizobium sp., and P solubilizing, are well known for their ability to provide nutrients, which may increase the plants' growth under extreme conditions. Soil microorganisms can induce plant growth through different mechanisms, i.e., by symbiosis, endophytes, and even as free-living microorganisms. Mycorrhizal fungi have a role in nutrient cycles through specific activity in mycelium or hyphae in absorbing soil nutrients, especially P and transfer to plants (6). (7) emphasizes the ability of mycorrhizal fungi to obtain N from organic matter. Nitrogen becomes a barrier to terrestrial ecosystems that mycorrhizal fungi may be helpful in providing P and N nutrients. These results are reported by (8), where mycorrhizal fungi increase Albizia saman growth and Paraserianthes falcatoria in the ex-coal mine area in Indonesia. In the Philippines, rehabilitation of acid minerals using Eucalyptus sp. Alnus and Casuarina equisetifolia inoculated with mycorrhizal fungi led to better survival compared to control plants [9]. The use of mycorrhizal fungi and Rhizobium increased the plant growth, plant growth resistance, and physical properties of soil N and P of Centroclena coriaceum after 48 months grown in ex-mining areas (Matias et al., 2009). The growth of Jatropha curcas in the Mogpog, Marinduque, Philippines mines is better on the use of mycorrhiza, lime, and compost [10].

Rhizobium can contribute directly to nutrient balance and affect plant hormones [11]. With nitrogenase, Rhizobium can reduce N atm and convert to amino acids and proteins through ammonia [12]. The P solubilizer microorganism (PSM) may alter the solubilizers of an insoluble form of P due to the chelation of Al,P4O10, Ca3PO4, or Fe3PO4 into a soluble form of P and therefore becomes available for the plant. The low molecular weight organic acids released by P-solubilizers microorganisms have been known to dissolve P (13). Biological treatments is increasingly being studied since they are environmentally friendly, efficient, and highly viable in both process and energy terms (14). At present, there are no specific inoculants for mining land either in Indonesia or in the Philippines. The purpose of this study is to investigate the effectiveness of microorganisms isolated from the gold mining area in improving the growth of test plants growing in ex gold mining soil under greenhouses condition.

2. Materials and Methods

Abandon tailing from the gold mining was used as a planting grow medium to investigate the effectiveness of microbial isolates in increasing plant growth. That soil chemical characteristic used is presented in Table 1. It showed that the soil is acid and the carbon content is low, either the N, P, K Ca and Mg. The content of Hg 0.1 ppm while the CEC is 2.9 cmol(+)/kg. It showed that the soil is infertile.
Table 1. Soil chemical characteristics used as planting medium

| Analyses | C organik (%) | N (%) | P₂O₅ Total (%) | K₂O Total (%) | CaO (%) | MgO (%) | P Bray (ppm) | Hg (ppm) | KTK Cmol(+) /kg |
|----------|---------------|-------|----------------|---------------|---------|---------|-------------|---------|----------------|
| pH 6.4   | 0.54          | 0.02  | 0.057          | 0.007         | 8.06    | 1.00    | ttd         | 0.1     | 2.90           |

The compost characteristics used are presented in Table 2. It is shown that the carbon content is 8.18% while the N, P, and K levels are 0.99, 0.64, and 0.79%. Compost reacts neutrally to alkaline. The ratio of C/N is 8.26. It showed that the ratio of C/N of compost was relatively low.

Table 2. Chemical characteristics of compost used

| Analyses | Concentration | Method |
|----------|---------------|--------|
| C organik | 8.18          | %      |
| N        | 0.99          | %      |
| P₂O₅     | 0.64          | %      |
| K₂O      | 0.79          | %      |
| pH       | 7.5           |        |

2.1 Preparation of planting media

To get optimum condition of planting medium, optimization composition of plant medium was done. For this purpose two experiments were performed using sorghum as planting material. In the first experiment, soil mine, and soil were used as planting medium (M1). In contrast, in the second experiment, in addition to soil and zeolites, we also used compost as components of medium (M2).

2.2 Seedling preparation and maintenance

*Parasenierantes falcata* (sengon) was used as a tested plant due to its characteristics as a fast growing plant and tolerant to degraded land conditions. The seeds of this plant were immersed in 40°C overnight before planting to increase seed germination. Immersed seeds were spread on sterilized sand. Uniform two-weeks old seedlings were used for planting material.

2.3 Inoculation of selected microbe (Rhizobium, PSM, Azotobacter, PGPR, AM fungi)

As planting material, two weeks old of *P. falcata* seedling was transferred into 300 gram two best media (M1 and M2). One week after transferred, all selected microorganisms were inoculated. While AM fungi were inoculated at the same time with transferred the plant to the pot. The inoculums of PSM were selected and decided based on their ability of its capability in forming halo zone as an indicator in P solubilizing activity. While the *Azotobacter* isolates used was the high ability to produce IAA. These all bacteria were prepared by propagating one colony of bacteria into each broth media. The broth media was incubated until the population of PSM reach $10^8$. When the inoculums were ready, inoculation was started by injecting it into the rhizosphere of the seedling. Seedlings were allowed to grow for three months under greenhouse condition. No fertilizer was applied. Water was applied every day. Seedling height, diameter, and leaf number were measured each week after transferred.

3. Result and Discussion

3.1. Optimization of media composition

Medium plants were selected to get an optimum condition; two experiments were performed. First experiment, the medium composition consisted of ex gold mining soil and compost. Our result
indicates that media 1 (M1) produced the highest dry weight of the sorghum plant though the tallest plant was observed in plant planting in soil medium (Fig. 1). These results showed that there is a synergy between ex-mining soil and compost in affecting the growth of sorghum. The similar result has been reported [15], which used 10% husk charcoal, and 2% of fertilizer resulted most significant effect on the total dry weight of Acacia mangium seedlings. Healey showed that composting of organic material increase the growth of plant compared to co composting. The results of the second experiment showed that the medium with the composition of soil+zeolite+compost, with a ratio of 2: 3: 3 produced the highest sorghum biomass. However, the medium with soil composition, zeolite, compost 6:1:1 also yielded relatively high sorghum plant weight (Fig 2). Additional ingredients of soil on that second composition medium, less when compared to the first medium. For this, the composition of 6:1:1 (M2) is selected for the medium in the microbial effectiveness test in addition to the medium with soil compost composition (1: 1, v / v).

Figure 1. Height and biomass weight of sorghum in the first experiment

Figure 2. Plant height and biomass weight of sorghum in the second experiment

3.1. Effect of inoculation of selected microorganisms and type of media on the growth of sengon and sorghum
The results showed that the composition of soil : compost 1: 1 (v / v) (M1) produced the highest dry weight of sorghum plant (Fig. 1). The use of compost alone results in higher plant weight than that of soil alone. These results indicate that there is a synergy between ex-mining soil and compost in affecting the growth of sorghum.

The results of the second experiment showed that the medium consisting of soil, zeolite, and compost, with a ratio of 2: 3: 3 (v:v:v) produced the highest sorghum biomass. However, the medium with composition of soil+zeolite+compost 6:1:1 (v:v:v) also yields relatively high sorghum plant weight (Fig 2). Since the soil content was less in the second medium compared to the first medium, the composition of 6:1:1 (v:v:v) (M2) is selected also for the planting medium in the microbial effectiveness test, in addition to the medium with soil compost composition (1: 1, v / v).

The result of the experiment to tested the effectiveness of microbial isolates in increasing plant growth showed that in medium 1, inoculation increase the height of the plant as well as the number of leaf of sengon (Fig 3). In addition, inoculated sengon looks green while the non-inoculated plants are yellow. (17) also repoted the similar result. However, the opposite result was occured in the sorghum plant (Fig 3). Microbial inoculation, inhibited the growth of plant. The same result was also obtained on M2 as a medium. Microbial inoculation in sengon plants produces higher growth than those non inoculated plants. Leaf color looks greener while the non inoculated plants was yellow. These results indicate that, sengon plant more responses to the provision of microorganisms compared to those sorghum.

Figure 3: Sengon (Paraserianthes falcataria) and sorghum seedling in the assessment of effectiveness of selected isolates. Non inoculated plant (left) and inoculated plant (right)

In the M1 medium, the height of the sengon inoculated (red line) was higher compared to those non-inoculated one (blue line) (Fig 4). The similar result was shown in leaf number parameter. It showed that inoculation yield the positif response in sengon.
Sorghum has different response to microbial inoculation. Inoculated sorghum had higher height than non-inoculated sorghum. There was no different in leaf number between inoculated and non-inoculated plant (Fig 5).

The growth of sengon in the M2 medium showed in Figure 6. The height of the inoculated plant was higher compared to those non inoculated plant. Moreover, the leaf number of inoculated plant resulted the higher leaf number compared to those non inoculated. It showed that in M2 medium the respons of sengon to inoculation was similar with those in M1 medium.

Figure 4: Effect of microbial inoculation on height and leaf number of sengon plant in M1 medium

Figure 5: Effect of microbial inoculation on height and leaf number of sorghum in M1 medium

Figure 6: Effect of microbial inoculation on height and leaf number of sengon in M2 medium

The growth of sorghum in M2 medium was relatively similar between inoculated and non inoculated in term of height. Though the leaf number has different pattern of leave growth but in week 6, the number of inoculated and non inoculated plant was similar (Fig 7). These result showed that sorghum was not response to microbial inoculation. It supposed that sorghum is the tolerant plant to the minimal condition of the medium. Microbe is the living system and posses higher adaptability to...
thrive in adverse conditions. The microorganism plays an important role in the soil system, especially in extreme ecosystem as such gold mining soil [18].

![Figure 7. Effect of microbial inoculation on height and leaf number of sorghum in M2 medium](image)

4. Conclusions and recommendations

*Rhizobium*, *P* solubilizing microbe, *Azotobacter*, *Pseudomonas*, AM fungi isolated from the land of ex gold mining, have the potency to enhance the growth of the sengon in the fresh extracted soil. The assessment showed that medium with the composition of that soil and compost and medium with the composition of that soil, compost and zeolite could be use as growth medium of plant, however sengon was more responsive to microbial inoculation than sorghum in term of growth of plant, and the number of leaf. The result could be continued by testing the effectiveness of the microbe in the field.

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