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# ISOLATION OF BACTERIA AS A BIOREMEDIATION AGENT FOR RECLAMATION OF MERCURY-CONTAMINATED SOILS

## Isolasi Bakteri Sebagai Agen Bioremediasi Dalam Mereklamasi Tanah Tercemar Logam Merkuri

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***ABSTRACT***
*Gold mining commonly involves the use of mercury, which generates hazardous and toxic mercury-based waste, leading to soil contamination and adverse effects on human health. Bioremediation has been explored as a potential solution to this issue. This study aimed to identify indigenous microbial species with potential as bioremediation agents, assess their ability to influence chemical properties, and evaluate their effectiveness in reducing mercury content. The research was conducted in five stages: isolation of native bacteria, testing the potential of microorganisms, assessing the ability to reduce mercury, conducting soil tests, and identifying bacterial characteristics through microscopic and biochemical analyses. The results revealed three types of indigenous microbes, namely* Pseudomonas, Neisseria*, and* Klebsiella *bacteria, with the highest potential as bioremediation agents. These bacterial isolates were found to enhance the availability of phosphorus in the soil, maintain soil pH, but had no effect on total soil nitrogen. Furthermore, the bacterial isolates exhibited the ability to reduce mercury content after treatment with NA isolates.*

***Keywords:*** *indigenous bacteria, isolation, mercury, reclamation, polluted*

**ABSTRAK**
Penambangan emas umumnya menggunakan merkuri sebagai aditif dalam prosesnya, yang menghasilkan limbah berbasis merkuri yang berbahaya dan beracun yang merusak kualitas tanah dan berdampak buruk pada kesehatan manusia. Oleh karena itu, bioremediasi telah dieksplorasi sebagai solusi potensial. Penelitian ini bertujuan untuk mengidentifikasi jenis mikroba asli yang memiliki potensi sebagai agen bioremediasi, menentukan kemampuan spesies mikroba ini untuk mempengaruhi sifat kimia, dan mengevaluasi kemampuan mikroba dalam mengurangi kandungan merkuri. Penelitian dilakukan dalam lima tahap yaitu: isolasi bakteri asli; pengujian potensi Mikroorganisme; pengujian kemampuan pengurangan merkuri; tes tanah; dan mengidentifikasi karakteristik bakteri melalui mikroskopik dan tes biokimia, Hasil penelitian menunjukkan bahwa ada tiga jenis mikroba asli dengan potensi terbesar sebagai agen bioremediasi, yaitu bakteri *Pseudomonas, Neisseria,* dan *Klebsiella.* Isolat bakteri ini ditemukan dapat meningkatkan ketersediaan P di tanah, menjaga pH tanah, tetapi tidak dapat meningkatkan total N tanah. Selain itu, isolat bakteri dapat mengurangi kandungan merkuri setelah diolah dengan isolat NA.

**Kata Kunci:** bakteri indegenous, isolasi, merkuri, reklamasi, tercemar

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## INTRODUCTION

Environmental damage, whether biotic or abiotic and affecting water, occurs for various reasons, including pollution caused by mining activities (Smith 2010). In general, gold mining uses mercury as an additional ingredient in its processing, producing mercury-based waste (Johnson et al.15). This waste is the largest contributor of hazardous toxic waste (B3) and disrupts ecosystem stability, posing serious health risks to humans (WHO17). Mercury can accumulate in the brain and kidneys, leading to neurological diseases (Clark et al. 2012). Unfortunately, land damage caused by mining activities in Indonesia has not been fully resolved, with land reclamation efforts still ongoing (Nurhayati et al. 2018). Research shows that mining activities have caused a decrease in agricultural production by 0.4-0.6 tons per year, as well as a decrease in the quality of rice fields in the surrounding areas of Banjar Regency (Demmallino 2018). It is crucial to restore soil quality to its pre-mining state. One promising solution is bioremediation, an environmentally friendly method that employs microbes (Nuryana 2017). In addition to appropriate growing media, plant growth and development are influenced by several factors, such as pH, light, air, water, temperature, nutrition, humidity, and soil (Fauziah et al. 2022).

Microbes have the ability to thrive in environments with high concentrations of heavy metals and can degrade toxic wastes (Smith et al. 2018). Using indigenous microbes in agriculture holds great potential since the best microorganisms often originate from the environment (Anggriany 2018). The utilization of indigenous microbes from mining waste benefits the environment, both biotic and abiotic, and the community.

Indigenous microbes, particularly heavy metal-degrading bacteria, can potentially improve soil conditions by dissolving heavy metals (Rahadi 2020). BRM (mercury-resistant bacteria) is a term used to describe bacteria resistant to mercury stress (Rahayu 2022). These bacteria can reduce or decompose heavy metals like mercury (Hg) by converting $Hg^{2+}$ to $Hg^{0}$ with the help of the merA gene code and the mercury reductase enzyme (Putri 2021). The use of mercury-reducing bacteria is a basic technique in environmental biotechnology for reducing pollutants through bioremediation, an approach for rehabilitating polluted or improperly managed ecosystems (Smith et al. 2019). Bioaugmentation, which involves adding native bacterial cultures to the soil, is one of several bioremediation techniques. The effectiveness of indigenous bacteria degradation depends on proper management practices (Alori et al. 2022).

Bacteria that have the ability to degrade heavy metals can also enhance soil productivity (Purnomo et al. 2021). Research findings indicate that indigenous bacteria can reduce harmful substances in polluted soil, improving soil conditions. In addition, alongside changes in pH, indigenous bacteria can provide nutrients to the soil, such as phosphate availability (Purnomo et al. 2021). Nurfitriani (2018) isolated four bacterial species from small-scale gold mining in Sekotong, which were able to grow on 5 ppm mercury media, namely *Brevundimonas vesicularis, Nitrococcus mobilis, Fusobacterium necrogenes*, and *Fusobacterium aquatica*. *Brevundimonas vesicularis* bacteria can accumulate Hg up to 2.17 ppm Hg, and its resistance and rapid response to heavy metals are attributed to a resistance mechanism. In high-concentration mercury media (above 25 ppm), indigenous microbial consortia can be obtained as bioremediation agents (Nurfitriani 2018). This study aims to identify types of indigenous microbes that have the potential as bioremediation agents, determine the ability of these microbial species to influence chemical properties, and evaluate their ability to reduce mercury content.

## MATERIALS AND METHODS

### Location and time

The research was conducted between July and September 2022 in several locations. Microbiology tests for isolating the bacteria were carried out at the Microbiology Laboratory of the Faculty of Mathematics and Natural Sciences, University of North Sumatra. Soil chemical properties were

tested at the Soil Fertility Laboratory, Faculty of Agriculture, University of North Sumatra. The Mercury Reduction Power Test was conducted using AAS at the General and Food Analysis Laboratory of PT Mutu Agung Lestari in Medan Selayang. The soil samples used in the research were taken from gold mining sites in the village of Penyabungan Jae, Mandailing Natal Regency (GPS 0.8513085, 99.5360124).
The findings from this research could serve as reference material for land conservation efforts, specifically for reclaiming land contaminated with heavy metals for agricultural or other purposes.

**Methods**

The research method consisted of five stages. Firstly, the isolation of indigenous bacteria was performed using selective media (NA+ HgCl) with concentrations of 25 ppm, 50 ppm, and 75 ppm. Secondly, the potential of plant growth promoting microorganisms (PGPM) was tested, which included nitrogen fixation, phosphate dissolving ability, and IAA hormone production. Thirdly, mercury reduction testing was conducted using NA isolates. Fourthly, soil tests were identified using Bergey's Manual of Determinative Bacteriology. Finally, the data obtained were analyzed statistically using ANOVA and Duncan's Multiple Range Test.

**Bacterial isolation**

Samples collected from the mining waste area were isolated using serial dilution. The water sample (1 mL) was diluted with 9 mL of distilled water and made into dilutions of $10^1$ to $10^8$. Dilutions of $10^4$, $10^6$, and $10^8$ were taken (1 mL) and poured into Petri dishes containing nutrient agar medium supplemented with $HgCl_2$ solution. The pour plate technique was used for the isolation process, followed by incubating the Petri dishes at 37 ºC for 2 × 24 hours to obtain mercury-resistant bacterial isolates (Amelia 2016; Hasibuan et al. 2017). Bacteria that can grow at the highest concentration of mercury ($HgCl_2$) were selected from the water sample and then inoculated on nutrient agar media (containing yeast extract 2g $L^{-1}$, bactopeptone 5g $L^{-1}$, NaCl 5g $L^{-1}$, agar, and water) and purified using methods described by Blake et al. (1993) and Pratiwi (2012).

**Plant growth promoting microbial (PGPM) characterization test**

**Nitrogen fixation**

The nitrogen fixation activity test was conducted using a nitrogen-free mineral medium (NFMM) containing 0.7% glucose and 2% bromothymol sulfonphthalein, which is commonly used for isolating nitrogen-fixing bacteria or fungi. The composition of the medium consisted of (g $L^{-1}$): $K_2HPO_4$ (1.0), $CaCl_2$ (1.0), NaCl (0.5), $MgSO_4 \cdot 7H_2O$ (0.25), $FeSO_4 \cdot 7H_2O$ (0.01), $Na_2MoO_4 \cdot 2H_2O$ (0.01), MnSO4·5H2O (0.01), with glucose as the carbon source (20g $L^{-1}$). In the case of solid medium, 2% agar was added.

**Phosphate (P) dissolving ability test**

For testing the ability of phosphate solubility, the Pikovskaya selective media was used with the addition of tricalcium phosphate (TCP) as a source of phosphate. The media was sterilized and then poured into a petri dish and allowed to solidify. After that, bacterial and fungal isolates were taken from the frozen media using an inoculation needle and scratched or planted onto the surface of the media in a zig-zag pattern. The plates were then incubated for 48 hours at 30 ºC. Bacterial isolates capable of dissolving phosphate were characterized by forming clear zones (halos) around the bacterial colonies (Purwaningsih. 2003). The ability of phosphate solubility (E) was measured based on the formula proposed by Oedjijono et al. (2014).

$$E = \frac{Phosphate\ dissolution\ diameter(s)}{Colony\ growth\ diameter(G)} \text{x } 100$$

**Indole acetic acid (IAA) hormone-producing test**

To test the ability of bacteria and fungi to produce IAA, nutrient broth (NB) or potato dextrose broth (PDB) and Salkowski reagent were used. The isolates were cultured on NB or PDB media supplemented with 0.1 g $L^{-1}$ tryptophan and incubated in the dark at room temperature for 48 hours. After centrifugation, the supernatant was mixed with Salkowski reagent and incubated for 24 hours in the dark at room temperature. The intensity was measured at 535 nm using a UV spectrophotometer. The

concentration of IAA was determined using a standard curve ranging from 0 to 50 ppm. This method was based on the study conducted by Kesaulya in 2015.

**Mercury reduction test**

To test the reducing power of mercury-resistant bacterial isolates, stocks already present in slanted natrium agar media were used. One dose of the isolate was taken from the Sodium agar stock and inoculated into natrium broth liquid containing $HgCl_2$. The mixture was then incubated for 24 hours at room temperature, at a speed of 100 rpm on an Incubator- shaker. The resulting culture was centrifuged at 5000 rpm for 5 minutes to separate the bacteria from NB media, and the supernatant was analyzed using an atomic absorption spectrophotometer (AAS) to determine metal concentrations This mercury analysis followed the Indonesian National Standard (SNI) mercury testing method 06-2462-1991 (Dirayah et al 2005; Amelia et al. 2016).

The gram staining method was employed to differentiate between gram-positive and gram-negative bacterial species. Bacterial culture was taken from the NA medium, and one colony was smeared onto a slide, then fixed above a Bunsen burner at a distance of 20 cm. Two to three drops of Gram A (crystal violet) were added to the bacterial smear and left to stand for a minute. The smear was then rinsed with running water and left to dry. Next, Gram B solution (iodine solution) was added and left to sit for a minute before being rinsed under running water and dried. The smear was washed with solution C (96% alcohol) for 30 seconds, rinsed with water, and dried. Finally, solution D (Safranin) was added and left for 30 seconds, after which the smear was rinsed with running water and dried using blotting paper.

**Soil test**

The bioremediation agent microbial testing was conducted on mercury-contaminated soil. Prior to testing the microbes on the soil, microbial inoculum was prepared (Rahayu 2022). The preparation of the microbial inoculum involved using microbes from the results of the Mercury Reduction Power Test (lab scale) and PGPM with the most potential. The best microbes (fungus/bacteria) were cultured on peptone glucose extract (PGE) medium and activated three times using the same medium for 24 hours at 37 ºC. Activated culture was then incubated at 150 ºC for 24 hours. Each inoculum was then inoculated into a PGE agar medium to observe its activity results. The inoculums on PGE agar media were incubated for 24 hours, and the number of colonies was counted. Every single inoculant was then inoculated into 2 litres of PGE medium, reaching a minimum number of cells: $10^9$ cells $mL^{-1}$. The final result was an inoculum ready for bioremediation testing on mercury-contaminated soil.

The chemical properties of the soil were also measured, including pH ($H_2O$), N-total (%), and P-available (ppm). The microbial testing of bioremediation agents was then carried out on soil contaminated with mercury. One kilogram of air-dried soil was weighed and placed into a bioremediation reactor that was aerated with air, then inoculated with microbial suspension and stirred evenly.

**Identification of bacteria**

The characterization of bacteria was conducted through several stages, including the determination of colony morphology, gram staining, gelatine hydrolysis test, citrate test, TSIA test, and SIM test. Once the bacterial characteristics were determined, the identification process was carried out based on Bergey's Manual of Determinative Bacteriology.

## RESULTS

**Bacterial isolation**

From the NA media, 9 bacterial isolates were obtained, with 3 isolates each growing at concentrations of 25 ppm, 50 ppm, and 75 ppm of mercury. Based on the colony morphology, 3 isolates with the highest number of colonies were selected: NA 1 (50 ppm), NA 2 (25 ppm), and NA 3 (75 ppm). The three isolates were then used for further testing (Figure 1).

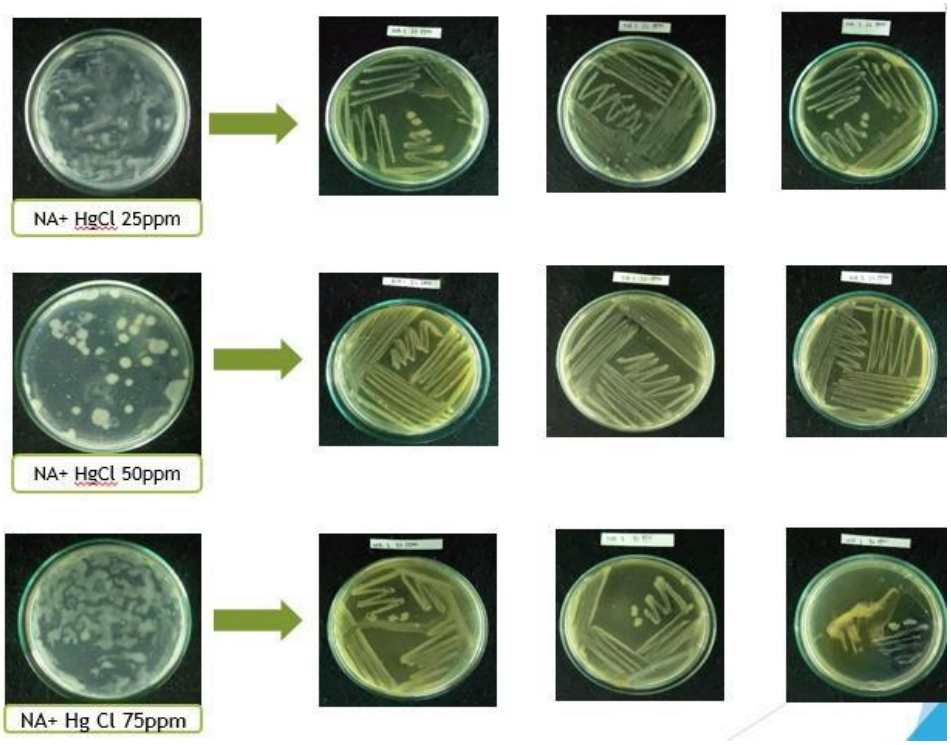


**Figure 1.** Results of isolation and purification of indigenous bacteria

## Characterization of plant growth promoting microbes (PGPM)

### Phosphate solvent

From the 9 isolated samples, only 3 isolates showed the ability to dissolve phosphate in the phosphate solvent test. These 3 isolates, namely NA 1 (50 ppm), NA 2 (25 ppm), and NA 3 (75 ppm), were then inoculated on Pikovskaya media to determine their ability as phosphate solubilizers. The formation of a clear zone around the colony indicated the ability of the bacteria to dissolve phosphate (as shown in Figure 2).

### IAA hormone production

The qualitative results in Figure 3 revealed a color difference among the 3 isolates after the addition of Salkowski reagent, with isolates a and c showing a more distinct pink color. This qualitative observation was further supported by the quantitative data on the absorbance values, which were as follows: a). NA 1 (50 ppm): 1.042, b). NA 2 (25 ppm): 0.712, and c). NA 3 (75 ppm): 0.921.

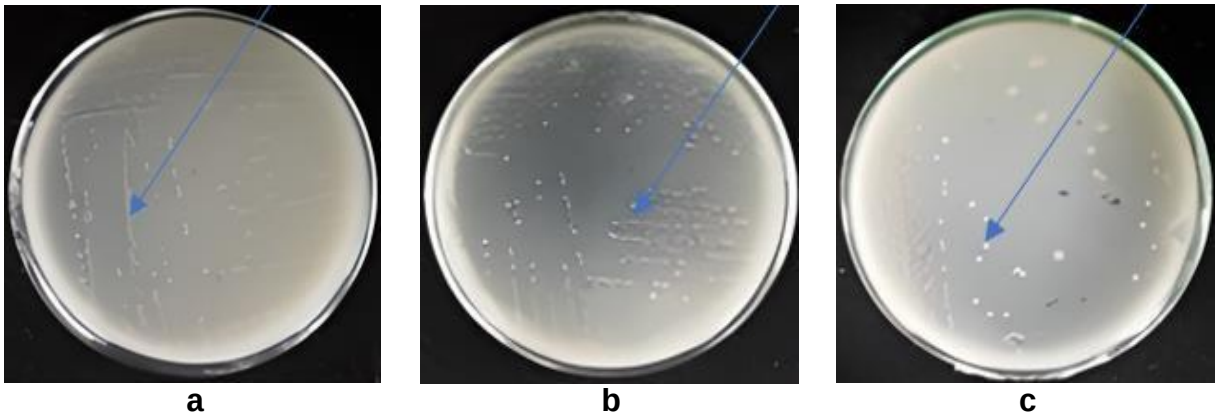


**Figure 2.** Phosphate solvent isolate: a). NA 1 (50 ppm), b). NA 2 (25 ppm), and c). NA 3 (75 ppm)

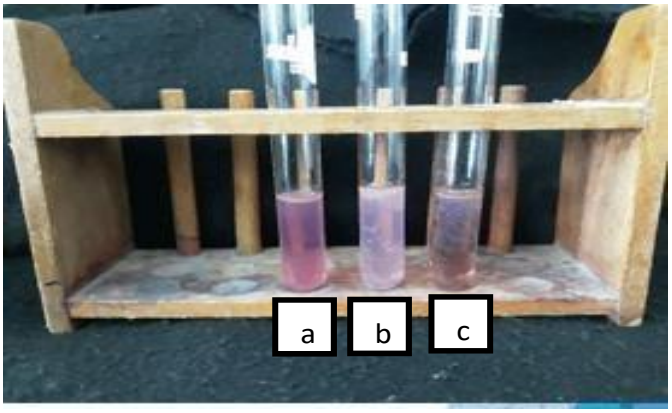


**Figure 3.** IAA hormone-producing isolates: a). NA 1 (50 ppm), b). NA 2 (25 ppm), and c). NA 3 (75 ppm)

**Nitrogen fixation**

Nitrogen fixation tests were carried out on all 9 isolates, but none contained nitrogen. This is likely due to the specific conditions required for nitrogen fixation to occur, including the presence of nitrogenase enzymes, a reaction and appearance of Gram-positive (+) bacteria, an anaerobic atmosphere, the presence of a reductant (electron source), the presence of ATP, and the absence of inhibitors.

**SOIL TEST**

**Soil chemical properties**

The chemical properties of soil were analyzed in the treatment group, where PGP bacteria were incubated for 14 days with three isolates, namely NA1 (50 ppm), NA 2 (25 ppm), and NA 3 (75 ppm). The parameters observed were pH ($H_2O$), N-total (%), and P-available (ppm), and the results are presented in Table 1. The samples used included initial mercury-contaminated soil (control) and mercury-contaminated soil with the addition of each of the three isolates. The pH of each sample was neutral with an average value of 6. The N-total average was low at 0.20%. However, P-available for control and NA 3 (75 ppm) had an average of 10 ppm, which is considered low, while for NA 2 (25 ppm) and NA 1 (50 ppm), it was 14.08 ppm and 12.03 ppm, respectively, which is considered moderate according to PPT (1983) regarding the standard value of soil chemical properties.

**Level of mercury in soil**

Table 2 shows the results of the observations, which include samples of initial mercury-contaminated soil (control) and mercury-contaminated soil treated with each of the three isolates. Although the mercury content is still very high based on the criteria for mercury content in soil, there is a decrease in the amount of mercury after the soil is incubated with PGP.

**Table 1**. Soil chemical parameters on land contaminated with gold mine waste with and without bacterial inoculation

| No. | Parameters | | | |
|---|---|---|---|---|
| | Sample | pH ($H_2O$) | N-total (%) | Available-P (ppm) |
| 1 | Control | 6.31 | 0,24 | 10.27 |
| 2 | Isolate NA 1;50 ppm | 6.70 | 0,19 | 12.03 |
| 3 | Isolated NA 2; 25 ppm | 6.12 | 0,21 | 14.08 |
| 4 | Isolated NA 3;75 ppm | 6.27 | 0,18 | 10.37 |

The results presented in Table 2 demonstrate that the incubation of soil with Isolate NA1 (25 ppm) resulted in the highest reduction of mercury content compared to the incubation with isolates NA2 (50 ppm) and NA3 (75 ppm). Specifically, the initial mercury content of 36.3 ppm was reduced to 19.8 ppm, indicating a decrease of 16.5 ppm. These findings suggest that the PGP bacterial isolates have a potential impact on reducing the mercury content in the gold mining area located in the Penyabungan Jae Village of Mandailing Natal Regency.

**Table 2.** Soil Mercury content before and after bacterial incubation treatment

| Item | Mercury Content (ppm) |
|---|---|
| Early Land | 36,3 |
| Isolate NA 1 (25 ppm) | 19,8 |
| Isolate NA1 (50 ppm) | 29,3 |
| Isolate NA 1 (75 ppm) | 31,7 |

### Identification of bacteria

Identification results based on morphological and biochemical tests can be seen in Table 3 based on colony shape, margin, elevation, color, cell shape, gram staining, gelatin test, citrate test, TSIA test, and SIM test (Krieg et al. 2010).

## DISCUSSION

Bacteria have the ability to adapt to heavy metal-polluted environments, which makes them more resistant to pollutants (Mathivanan et al. 2021). Generally, the resistance properties of bacteria are due to mechanisms such as biotransformation (oxidation-reduction), bioprecipitation (deposition of metal ions on the cell surface through cation elimination), and biosorption (use of natural or recombinant bacterial biomass to absorb metal ions) (Osman et al. 2019). The results of the study showed that only 9 bacterial isolates could be obtained using media with the addition of $HgCl_2$, which were classified as having a relatively low capacity for mercury reduction, but only 3 isolates had PGP properties (Cilliers et al. 2022).

The study of PGP potential in the three selected Indigenous bacteria (BRM) is crucial for their utilization as agents for land reclamation in areas polluted by gold mining waste. This potential is expressed through nitrogen fixation, phosphate solubilization, and IAA hormone production (Suharjono and Yuliatin 2019). However, only nine isolates of mercury-reducing bacteria were obtained in small amounts, which can be attributed to the high level of mercury in the soil (36.3 ppm/250 grams), far exceeding the standard concentration of 0.174 according to the US Department of Commerce's National Oceanic and Atmospheric Administration (NOAA). The chemical properties of the soil are directly

**Table 3**. Microscopic identification by morphological and biochemical tests

| No. | Code Isolation | Colony Form | Line | Height | Color | Cell Shape | Gram | Jelly | Citrid Acid | NO | Sim | Genus Description |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Na 1;50 ppm | Irregular | Wavy | Flat | White milk | Monobas-ilus | Negative | - | - | A A | + | *Pseudomonas* |
| 2 | Na 2; 25 ppm | Round | Wavy | Flat | White milk | Streptoc-ocus | Negative | - | - | K | + | New series |
| 3 | At 3;75ppm | Irregular | Round | Flat | White milk | Diplobacilli | Negative | - | + | A A | + | *Klebsiella* |

affected by the concentration of soil mercury, as the pH before and after bacterial inoculation showed no significant difference, and the total N and available P levels remained low and relatively unchanged compared to the initial soil.

According to Rahayu's research (2019), three bacterial isolates with PGP abilities have been isolated and identified from soil contaminated with gold mine waste. These bacteria belong to the genera *Pseudomonas, Neisseria*, and *Enterobacteriaceae*. Bacterial membranes can absorb contaminated organic components and affect their physiological functions. Bacteria that survive in toxic environments can be used as contaminant degrading agents through bioremediation technology. *Pseudomonas fluorescens*, an indigenous bacterial species with strong adaptive properties, is reported to be able to adapt to soil contaminated with oil waste in Bojonegoro.

Plant growth-promoting bacteria (PGPB) are microorganisms with great potential as biological agents to increase agricultural productivity. These microbes are important in improving soil texture and fertility, regulating nutrition and hormone balance, secreting extracellular molecules and bio stimulants, inducing resistance to pathogens, and modulating plant stress responses. Among the categories of PGPB, phosphate-solubilizing bacteria have been extensively studied. These bacteria have the ability to solubilize insoluble phosphates in the soil, making them available for plant uptake. Phosphorus is one of the essential macronutrients required for plant growth (Meng et al. 2021). One of the hormone-producing bacteria is *Pseudomonas vulgaris*, which produces hormones such as IAA, gibberellins, cytokinins, and abscisic acid (Widyati 2016).

The bacterial isolate identified using Bergey's Manual of Determinative Bacteriology belongs to *Pseudomonas*, with the isolated code NA1; 50 ppm. *Pseudomonas* is known for synthesizing metabolites that promote plant growth in soil (Oedjijono et al. 2022). It is a gram-negative, aerobic bacilli with irregular colony shapes, small to medium sizes, smooth or wavy edges, and flat elevation. Moreover, *Pseudomonas* is also recognized for its ability to degrade heavy metals, which makes it a useful bioremediation agent for environments polluted with toxic compounds. The colonial morphological characteristics of this bacterium include coccus-shaped and gram-negative cells.

The bacterial isolate with the code NA2 (25 ppm), belonging to the genus *Neisseria*, has been found to have the potential to promote plant growth. *Neisseria* bacteria are known for their ability to solubilize phosphate and inhibit the growth of plant pathogens (Safriani RS et al. 2020). The *Neisseria* genus includes species such as *Neisseria gonorrhoeae*, *N. bacilliformis, N. cinerea, N. elongata, N. lactamica, N. mucosa, N. flava*, and many others (Reiner 2017).

The third isolate, with the isolated code NA3 (75 ppm), belongs to the genus *Klebsiella*, which is a gram-negative bacterium from the *Enterobacteriaceae* family. It shows an acidic/acidic (A/A) reaction in TSIA and other characteristics such as positive gas detection and citrate test. *Klebsiella* bacteria are also known as part of the PGPR group of bacteria, acting as biostimulators or hormone producers (Prasad et al. 2019). Several studies have reported the potential of mercury-reducing and plant growth-promoting (PGP) bacteria, including *Pseudomonas*, *Azotobacter, Arthrobacter, Aeromonas, Acinetobacter, Bradyrhizobium, Acinetobacter, Allorhizobium, Agrobacterium, Azoarcus, Azorhizobium, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Enterobacter, Frankia, Flavobacterium, Klebsiella, Micrococcus, Streptomyces, Serratia*, and *Thiobacillus*. PGPR bacteria have various benefits, such as biofertilization, photostimulation, biopesticides, and bioremediation, and can increase crop yield in agriculture (Sing et al. 2016, Sing et al. 2017, Sing et al. 2020).

## CONCLUSION

The potential bioremediation agents obtained from gold mining areas in North Sumatra consist of three indigenous microbial types, namely *Pseudomonas*, *Neisseria, and Klebsiella* bacteria. These microbes have shown the ability to reduce mercury levels in contaminated soil. Although the bacterial isolates can increase soil available P and maintain soil pH, they cannot increase soil in

land contaminated with gold mine waste. The mercury content in the soil before reduction was 36.3 ppm, but it decreased to 19.8 ppm after treatment with NA2 isolate (25 ppm) (*Pseudomonas* bacteria), to 29.3 ppm after treatment with NA1 isolate (50 ppm) (*Neisseria* bacteria), and to 31.7 ppm after treatment with NA3 isolate (75 ppm) (*Klebsiella* bacteria).

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## REFERENCES

Alori ET, Gabasawa AI, Elenwo CE, Agbeyegbe OO (2022) Bioremediation techniques affected by limiting factors in the soil environment. Frontiers in Earth Science 47. doi: 10.3389/fsoil.2022.937186

Amelia TF, Ace B, Herpandi (2016) Aktivitas reduksi merkuri pada bakteri yang diisolasi dari air dan sedimen di sungai musi. Jurnal Teknologi Perikanan 5: 1. doi: 10.36706/fishtech.v5i1.3522

Anggriany PS (2018) Utilization of indigenous bacteria in reducing cu heavy metal in etching printed circuit board (PCB) liquid waste. (Doctoral dissertation, UAJY) 3: 2.

Blake (1993) Chemical transformation of toxic metals by *Pseudomonas* strain from a toxic waste site. Environmenlal Toxicology and Chemistry.

Dirayah, Husain, Muchtar (2005) Pengkompleks logam pb dan cd dari limbah cair PT kawasan industri makassar. Jurnal Marina Chimica Acta 6(1).

Cilliers C, Neveling O, Tichapondwa SM, Chirwa EM, Brink HG (2022) Microbial Pb(II)-bioprecipitation:characterizing responsible biotransformation mechanisms. Journal of Net Production 374: 133973. doi: 10.1016/j.jclepro.2022.133973

Demmmallino EB, Ibrahim T, Karim A (2018) Farmers in the middle of a mine: phenomenological study of the effects of policy implementation on the lives of farmers in morowali. Journal of social economics of agriculture 14: 2.

Fauziah N, M. Idris (2022) The effect of liquid tofu waste and growing media on the growth and yield of long beans (*Vigna sinensis* L.). Journal of Biotechnology & amp; Indonesian Biosciences (JBBI) 9(2): 217–226. doi: 10.29122/jbbi.v9i2.5492

Hasibuan et al (2017) Identifikasi bakteri berasal dari sungai batang bungo di desa tanjung gendang kabupaten bungo propinsi jambi sebagai bahan pengayaan praktikum mikrobiologi. Pendidikan Biologi FKIB Universitas Jambi.

Kesaulya H, Zakaria B, Syaiful SA (2015) Isolation and physiological characterization of pgpr from the potato rhiosphere in medium soil on buru island. Italian Oral Surgery 3:190–199. doi:10.1016/j.profoo.2015.01.021

Krieg NR et al (2010) A consistent and accurate ab initio parametrization of density functional dispersion correction (DFT-D) for the 94 elements H-Pu. The Journal of chemical physics 132(15): 154104. doi: 10.1063/1.3382344

Mathivanan K, Chandirika JU, Vinothkanna A, Yin H, Liu X, Meng D (2021) Bacterial adaptive strategies to overcome metal toxicity in contaminated environments–an overview. Ecotoxicology and Environmental Safety 226:112863. doi: 10.1016/j. ecoenv.2021.112863

Meng X, Chen WW, Wang YY, Huang ZR, Ye X, Chen LS, Yang LT (2021) Effects of phosphorus deficiency on the absorption of mineral nutrients, photosynthetic system performance and antioxidant metabolism in citrus

grandis. Plos One 16(2): e0246944. doi:10.1371/journal.pone.0246944

Nurfitriani S, Arisoesilaningsih E, Nuraini Y, Handayanto E (2022) Formation of biofilms by mercury resistant bacteria from soil contaminated by small-scale gold mine waste. Biodiversity Journal of Biodiversity 23(2). doi: 10.13057/biodiv/d230242

Nurhayati A, Ummah ZI, Shobron S (2018) Environmental damage in Al-Quran suhuf. 30(2): 194-220.

Nuryana D (2017) Bioremediation of petroleum pollution. Journal of Earth Energy Engineering, 6(2): 9-13.

Oedjijono, Soetarto ES, Moeljopawiro S, Djatmiko HA (2014) Promising plant growth promoting rhizobacteria of *Azospirillum* spp. isolated from iron sand soils. Purworejo coast central Java, Indonesia 5(3): 302–308.

Oedjijono O, Lestari S, Samsudin LS, Hermilia H (2022) Bioremediation of batik wastewater by rhizobacteria isolated from iron sand soil tolerant of pb and zn: rhizobacteria tolerant of Pb and Cd. Biodiversity Journal of Biodiversity 23(1). doi: 10.13057/biodiv/d230136

Osman GE, Abulreesh HH, Elbanna K., Shaaban MR (2019) Recent advances in metal-microbe interactions: prospects in bioremediation. J Pure Appl Microbiol 13(1):13-26. doi:10.22207/JPAM.13.1.02

PPT (1983) Term of reference tipe a, jenis dan macam tanah di indonesia untuk keperluan survey dan pemetaan tanah daerah transmigrasi. Pusat Penelitian Tanah.

Prasad M, Srinivasan R, Berde CV, Chaudhary M, Jat LK (2019) Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges of pgpr amelioration in sustainable agriculture. Elsevier 315-344.

Pratiwi (2012) Penapisan bakteri resisten terhadap merkuri sebagai alternatif agen bioremediasi pada pencemaran tanah pertambangan. Institut Pertanian Bogor.

Purnomo B, Rahmawati NS, Nuraini Y (2021) Utilization of phosphate solubilizing bacteria in soil to optimize the use of coal fly ash to increase available P in Ultisol soil. Journal of Mining and Degraded Land Management 8(4): 2937-2946. doi: 10.15243/jdmlm.2021.084.2937

Purwaningsih (2003) Isolasi, populasi dan karakterisasi bakteri pelarut fosfat pada tanah dari taman nasional bogani nani wartabone, sulawesi utara. Biologi 3(1).

Putri WA, Rahayu HM, Khasanah AU, Sembiring L, Kawaichi M, Purwestri YES (2021) Identification of mercury resistant streptomyces isolated from *cyperus rotundus* l. rhizosphere and molecular cloning of mercury(ii) reductase genes . Indonesian Journal of Biotechnology 26(4): 206-213. doi: 10.22146/ijbiotech.65989

Rahadi et al (2020) An investigation of pro-environmental behaviour and sustainable development in Malaysia. Sustainability 12(17): 7083.

Rahayu DR, Mangkoedihardjo S (2022) Bioaugmentation study to reduce heavy metal concentrations in water areas using bacteria (case study: mercury pollution in the krueng sabee river, aceh jaya). ITS Engineering Journal 11(1): 15-22. doi:10.12962/j23373539.v11i1.82791

Rahayu YS, Yuliani, Trimulyono G (2019) Isolation and identification of hydrocarbon degrading bacteria and phosphate dissolving bacteria in oil contaminated soil in bojonegoro, east java, indonesia. Indonesian Journal of Science & Technology. doi: 10.17509/ijost.v4i1.14923

Reiner (2017) Lipid-lowering nutraceuticals in clinical practice: position paper from an

international lipid expert panel. Nutrition reviews 75(9): 731-767. doi: 10.1093/nutrit/nux047

Safriani RS, Fitri L, Ismail SY (2020) Isolation of potential plant growth promoting *Rhizobacteria* (PGPR) from Cassava (*Manihot Esculenta*) rhizosphere soil. Journal of Biology & Biology Education: Biosaintifika. doi: 10.15294/biosaintifika.v12i3.25905.

S Nurfitriana, U Chasanah, Y Nuraini, A Fiqri, E Handayanto (2018) Ability of bacterial mercury accumulation isolated from small-scale gold mine tailings. Proceedings of the National Seminar on Suboptimal Land.

Singh JS, Kumar A, Rai AN, Singh DP (2016) Cyanobacteria: a valuable biological resource in agriculture, ecosystems and environmental sustainability. Frontiers in microbiology 7:529.

Singh MP, Singh P, Singh RK, Solanki MK, Bazzer SK (2020) Plant microbiomes: understanding benefits above ground phytobiomes: current insights and future views. Springer 51-80.

Singh R, Parihar P, Singh M, Bajjuz A, Kumar J, Singh S, VP Singh, Prasad SM (2017) Uncovering the potential applications of cyanobacteria and algal metabolites in biology, agriculture, and medicine: current status and future prospects. Frontiers in microbiology 8: 515.

Suharjono S, Yuliatin E (2022) Coffee plant rhizospheric bacterial communities and their potential as plant growth promoters. Journal of Biodiversity 23(11). doi: 10.13057/biodiv/d231136

Widyati E (2016) The role of phytohormones in plant growth and their implications for forest management. Galam BPPLH 2(1): 11-22.