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Identification and Mercury Sensitivity Test of Rhizosphere Bacteria From *Ageratum conyzoides* Plants Growing In Ratatotok Gold Mine, North Sulawesi

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ABSTRACT

Indonesia is known for its abundant natural resources, such as gold. However, the mercury-based amalgamation method in illegal gold mining causes environmental pollution. This research explores the potential of utilizing mercury-resistant rhizosphere bacteria from *Ageratum conyzoides*, a gold hyperaccumulator plant, to develop an effective bioremediation strategy for contaminated gold mining areas. This descriptive-exploratory study aims to identify and determine the sensitivity of mercury in five rhizosphere bacteria isolates from previous research. Bacteria were isolated from the roots of *Ageratum conyzoides* growing in the Ratatotok gold mining area of North Sulawesi. Bacteria identification was determined using profile-matching methods based on microscopic, macroscopic, physiological, and biochemical features. Similarity Indexes were determined using dendrogram visualization with MVSP software. Mercury sensitivity was analyzed using the cup-plate technique, varying mercury concentrations from 0 ppm to concentrations where clear zones appeared, indicating bacterial growth inhibition. The result indicated that the five isolates were bacteria from the genera Alcaligenes, Pseudomonas, Enterococcus, Streptococcus, and Micrococcus. Notably, Pseudomonas showed the highest potential for mercury resistance, being mercury sensitive at a concentration of 470 ppm, followed by Alcaligenes (110 ppm), Enterococcus (30 ppm), and both Streptococcus and Micrococcus (20 ppm).

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1. INTRODUCTION

Indonesia possesses a variety of natural resources, including gold. One of the areas that has the potential for natural resources in the form of gold is North Sulawesi Province. Based on data from the Mining and Energy and Mineral Resources Service of North Sulawesi Province in 2019, in Minahasa Tenggara Regency with a gold potential of 22,250,096 tons, there are only 5 gold mining industries with IUPs, so there are still several illegal gold mining activities (PETI), such as in Ratatotok Selatan Village, Ratatotok District (Gundo *et al.*, 2020). The existence of illegal gold mining (PETI) in Ratatotok District, Southeast Minahasa Regency, can cause environmental pollution due to mining activities conducted without proper authorization, and has the potential to be

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carried out uncontrollably. The illegal gold mining process in Ratatotok is known to still be carried out with simple equipment and methods, namely using the amalgamation method or with the help of mercury compounds to form an amalgam. This process will produce liquid waste containing mercury and has the potential to pollute the environment around the mining site and also mine workers (Sumendap *et al.*, 2023; Tongkotow *et al.*, 2023). Mercury pollution in the environment was discovered in Rachmansyah *et al.* (2017), where the Hg content in the waters of Ratatotok Bay in all samples exceeded the threshold value. In addition, Hg contamination was found in residents' wells that were <50 m from the PETI waste disposal pond.

Phytomining is an alternative method that uses hyperaccumulator plants to extract valuable metals such as gold, this technique can maximize recovery from low-grade or abandoned mine sites while also reducing environmental risks through land remediation (Kusuma & Haeruddin, 2022). The success of phytomining is influenced by soil properties, including pH, texture, salinity, and contamination levels, which affect plant growth and metal availability. The rhizosphere, the soil zone around roots, hosts microbial communities that play a key role in contaminated soils (Wang *et al.*, 2020). Some rhizosphere bacteria are resistant to mercury, such as three Azotobacter isolates from the ITS Eco Urban farming land that survived at Hg concentrations up to 20 mg/L (Khotimah & Zulaika, 2014). Although resistance does not necessarily mean degradation, it allows bacteria to survive under toxic conditions, maintain interactions with plants, and support growth. These interactions may enhance the performance of hyperaccumulator plants in Hg-rich soils by improving tolerance and metal bioavailability, thereby contributing to both phytomining and bioremediation.

According to Aminatun *et al.* (2024), exploration of plants growing in former gold mining areas of Ratatotok identified *Ageratum conyzoides* (bandotan) as the plant with the highest gold content. As a natural hyperaccumulator, its ability to thrive may depend on associations with rhizosphere bacteria that tolerate mercury. Investigating these bacterial communities is crucial, since high Hg levels could limit plant establishment and reduce phytomining potential. Therefore, this study aims to identify rhizosphere bacteria from *Ageratum conyzoides* growing in the Ratatotok gold mine site, North Sulawesi, and to analyze their mercury resistance through sensitivity tests.

2. RESEARCH METHOD

This research used five isolates of rhizosphere bacteria from *Ageratum conyzoides* plants growing at the Ratatotok gold mine site, North Sulawesi with isolate codes BDT1, BDT2, BDT3, BDT4, and BDT5. The five isolates used in this study were bacterial isolates that had been isolated in previous studies as a continuation of the vegetation exploration process at the Ratatotok gold mine site, North Sulawesi (T. Aminatun, personal communication, June 3, 2023). This research was conducted from October 2023 to February 2024 at the Microbiology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta, Special Region of Yogyakarta, Indonesia.

2.1. Research Tools and Materials

The tools used in this study were: petri dishes, test tubes, durham tubes, blue tips, pipettes, object glasses, cover glasses, a microscope, 10mL measuring cylinder, erlenmeyer flasks, an analytical scale, spatula spoons, Laminar Air Flow (LAF), UV-VIS Spectro, incubators, refrigerators, autoclaves, inoculation needles, bunsen burners, gas lighters, beakers, 100uL-1000uL micropipettes, 1mL-10mL micropipettes, hot plates, magnetic stirrers, vortexes, and universal pH indicator charts.

The materials used in the study were rhizosphere bacterial isolates, distilled water, cotton plugs, sterile cotton swabs, filter paper, Nutrient Agar (NA) media, Nutrient Broth (NB) media, Urea base Agar media, Simmon's Citrate (SC) media, Sulphite Indole Motility (SIM) media, Lysine Iron Agar (LIA) media, Methyl red Voges Proskauer (MRVP) media, Mueller's Hinton Agar (MHA) media, glucose, mannitol, fructose, galactose, maltose, sucrose, lactose, starch, tryptone, KOH 40%, alpha naphthol, Methyl red, Ethanol 95%, methylene green, crystal violet, lugol, acetone-alcohol, safranin, Phenol red, H_2O_2 30%, NaOH 0.1N, HCl 0.5N, NaCl, $HgCl_2$, and universal pH indicator strips.

2.2. Bacterial Characterization

Bacterial isolates were characterized using phenotypic characters. Physiological characters are carried out by conducting growth tests using different pH, salinity, and temperature variations with modified nutrient broth media. Macroscopic characters are obtained by observing colony shape, colony color, colony margin, and colony elevation in bacterial colonies inoculated on Nutrient Agar plate media (Pulungan & Tumangger, 2018).

Microscopic characters are obtained by performing gram staining and endospore staining. Gram staining was performed by aseptically spreading bacterial isolates onto a glass slide. The smear was fixed by passing the slide through the flame of a Bunsen burner. The smear was subsequently flooded with four reagents in the following sequence: the first reagent, Gram A (crystal violet), was applied for 1 minute; this was followed by Gram B (Lugol's iodine), which was allowed to act for another minute. The slide was then treated with Gram C (acetone-alcohol) for 10 to 20 seconds to decolorize the smear, after which it was rinsed gently with running water. Finally, the smear was stained with Gram D (safranin) for 1 minute before being rinsed again with running water and allowed to air dry. The morphological characteristics of the bacterial cells and their coloration (indicative of Gram type) were observed under a microscope (Sine *et al.*, 2017).

Endospore staining was conducted by aseptically smearing bacterial isolates onto a glass slide, then fixed by passing it through the Bunsen flame. The slide was placed over boiling water on a beaker glass and flooded with malachite green dye. The smear was allowed to sit over the boiling water for 5 minutes, with malachite green gradually applied to prevent drying. Following this incubation, the smear was allowed to cool and rinsed with running water. Once dry, the smear was flooded with safranin dye for 1 minute, followed by another rinse with running water. Endospores were observed as green, while vegetative cells appeared red (Fauzaan *et al.*, 2022).

Biochemical characters are obtained by conducting several tests, such as carbohydrate fermentation, oxygen requirement, starch hydrolysis, gelatin hydrolysis, catalase, urease, Lysine decarbooxylase, and IMViC. The carbohydrate fermentation test was performed on Phenol red broth base media modified with 0.5% of several types of sugars (glucose, galactose, sucrose, maltose, lactose, fructose, and mannitol) and then incubated for 24-48 hours at 37˚C. Positive results were shown by a color change of the media from red to yellow and gas formation in the Durham tube (Cappuccino & Welsh, 2017).

The oxygen requirement test was conducted on nutrient broth media and incubated for 24 hours at 37˚C. Aerobic bacteria will grow on the surface of the medium, anaerobic bacteria will grow at the base of the medium, microaerophilic bacteria will grow in clusters slightly below the surface of the medium, and facultatively anaerobic bacteria will grow spread throughout the medium (Cappuccino & Welsh, 2017).

The starch hydrolysis test was conducted using starch agar media. Iodine/lugol solution was dripped around the bacterial colony on starch agar and left for several minutes. Positive result was shown by the presence of a clear zone around the bacterial colony (Cappuccino & Welsh, 2017).

The gelatin hydrolysis test was carried out using nutrient gelatin media and incubated for 24 hours at 37˚C. After 24 hours, the media was stored in an incubator at 4˚C for 30 minutes; positive result was indicated by the media remaining in liquid form (Cappuccino & Welsh, 2017).

The catalase test was carried out by aseptically smearing bacterial isolates onto a glass slide, then dripped with 3% H_2O_2. Positive results are indicated by bubbles formation (Cappuccino & Welsh, 2017).

The urease test was carried out by aseptically taking one loop of bacterial isolate and then streaking it on a slanted Urea Base Agar medium. The media was incubated at 37°C for 24 hours. Positive results were indicated by a color change from yellow to purplish red (Ulfa *et al.*, 2016).

The lysine decarboxylase test was carried out by inoculating bacterial isolates on Lysine Iron Agar (LIA) media. Furthermore, the media was incubated for 24 hours at 37˚C, and the color change was observed. Positive results were indicated by a purple color at the bottom of the media (Wulandari & Purwaningsih, 2019).

The sulfite, indole, and motility (SIM) test was carried out by inoculating bacteria on SIM media. The isolates were incubated for 24 hours at 37 °C. Observations were made by looking for

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black precipitate in the media for H_2S formation; bacterial growth was seen spreading from inoculation line for motile bacteria; and when dripped with KOVACS reagent, a purplish red ring was formed for indole production (Yulvizar, 2013 in Nuryanti *et al.*, 2021).

The citrate test was carried out by inoculating one loop of bacterial isolate on Simmon's Citrate (SC) slant agar media. The media was incubated for 24-48 hours, and observations were made by looking for a change in the green color of the media to blue (Cappuccino & Welsh, 2017).

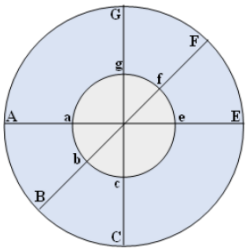
The MRVP test was carried out by inoculating bacterial isolates on Methyl red Voges Proskauer broth (MRVP broth) media, and then the media was incubated at 37°C for 3 days. Next for methyl red test, the media was added with two drops of methyl red indicator, and observations were made by looking at the change in red color on the media. And for the Voges Proskauer test, the media was dripped with five drops of 40% KOH and ten drops of 5% α-naphthol. A positive result was shown by a red (pinkish red) color formation on the media (Cappuccino & Welsh, 2017).

2.3. Bacteria Identification

Bacteria were identified using profile-matching method based on macroscopic, microscopic, physiological, and biochemical characters to obtain reference genera with Bergey's Manual of Systematic Bacteriology as a reference. Furthermore, the profile-matching results were further analyzed using the MVSP software to analyze similarity indexes through dendogram visualization (Yulianti & Rakhmawati, 2017).

2.4. Mercury Sensitivity Test

The Mercury sensitivity test was conducted using the cup-plate technique. Bacterial isolates were suspended in 0.85% NaCl, and the turbidity was adjusted to equivalent to McFarland 0.5 by adding sterile distilled water. Then, bacterial suspension was inoculated into Mueller Hinton Agar media using a sterile cotton bud. Five wells on each plate were made using sterile pipettes, each of which was filled with sterile distilled water as a control, as well as with $HgCl_2$ at Hg^{2+} concentrations of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and so on until a clear zone of inhibition was observed. Clear zones were observed and measured using a digital caliper as shown in Figure 1.



Description :
AE, BF, and CG = total diameter
ae, bf, and cg = well diameter
Measurement 1 = AE-ae
Measurement 2 = BF-bf
Measurement 3 = CG-cg

Clear Zone Diameter :

$$\frac{\textbf{Measurement 1} + \textbf{Measurement 2} + \textbf{Measurement 3}}{3}$$

Figure 1. Clear Zone Measurement

3. RESULTS AND ANALYSIS

3.1. Bacterial Characterizations

Characterization of the five bacterial isolates showed significant variations in morphological and biochemical characters. Microscopic examination showed that most isolates were spherical (cocci), and only one isolate was rod-shaped (bacilli). Biochemical tests showed diverse metabolic abilities, including differences in fermentation patterns and enzyme production. These results are shown in Table 1.

Table 1. Characterization Results

Characters	Isolat Codes				
	BDT1	BDT2	BDT3	BDT4	BDT5
		Colony Morphology			
Colony color	Yellowish white	Greyish white, medium turn green	White	White	Yellow
Colony shape	Round	Irregular	Round	Round	Round

Margin	Entire	Curled	Entire	Entire	Entire
Elevation	Convex	Raised, spreading edge	Raised	Flat	Flat
Oxygen requirements	Facultative anaerob	Aerob	Facultative anaerob	Facultative anaerob	Aerob
		NB (Broth)			
Growth type	Uniform turbidity	Pellicle	Flocculent	Uniform turbidity	Pellicle
		NA Slant			
Growth type	Beaded	Filiform	Beaded	Filiform	Beaded
		Microscopic			
Gram	-	-	+	+	-
Endospora	-	-	-	-	-
Cell shape	Coccus	Bacil	Coccus	Coccus	Coccus
Configuration	Mono	Mono	Strepto	Strepto	Mono
		Temperature			
27°C	+	+	+	+	+
-4°C	+	+	-	+	-
50°C	-	-	-	-	-
		pH			
pH 5	-	+	+	-	+
pH 7	+	+	+	+	+
pH 9	+	+	+	+	+
		Salinity			
1%	+	+	+	+	+
5%	+	+	+	+	+
10%	+	+	-	-	+
		Carbohydrate Fermentation			
Sucrose	+A/-G	-	+A/-G	-	+A/-G
Galactose	-	-	+A/-G	+A/-G	+A/-G
Maltose	-	-	+A/-G	+A/-G	+A/-G
Glucose	-	-	+A/-G	+A/-G	+A/-G
Mannitol	-	-	-	+A/-G	-
Lactose	-	-	+A/-G	-	-
Fructose	-	-	+A/-G	+A/-G	+A/-G
		Enzyme			
Gelatinase	-	-	-	-	-
Urease	-	-	-	+	+
Amilase	+	-	-	-	-
Katalase	+	+	-	+	+
Lysine	+	+	-	+	-
		IMViC Test			
Citrate	+	+	-	-	-
MR	-	-	+	+	-
VP	-	-	-	-	-
H_2S	-	-	-	-	-
Indol	-	-	-	-	-
Motility	-	+	-	-	-

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Based on the characterization results (Table 1), BDT1 is a facultative anaerobic, Gram-negative coccus that does not form endospores. It forms round colonies with entire margins, convex elevation, and a yellowish-white color. Biochemically, it is positive for catalase, amylase, and lysine decarboxylase, but negative for gelatinase and urease. It grows at pH 7 and 9, at temperatures of 27 °C and 4 °C, and in 1%, 5%, and 10% NaCl. It ferments sucrose and utilizes citrate.

BDT2 is an aerobic, Gram-negative bacillus that does not form endospores. Colonies are irregular with curled margins, raised elevation with spreading edges, and a grayish-white color; the medium turns green. It is catalase- and lysine decarboxylase-positive, but negative for amylase, gelatinase, and urease. It grows at pH 5, 7, and 9, at 27 °C and 4 °C, and in 1%, 5%, and 10% NaCl. All carbohydrate fermentation tests were negative. It utilizes citrate and is motile.

BDT3 is a facultative anaerobic, Gram-positive coccus that does not form endospores. Colonies are round with entire margins, raised elevation, and a white color. It is catalase-, urease-, lysine decarboxylase-, gelatinase-, and amylase-negative. It grows at pH 5, 7, and 9, at 27 °C, and in 1% and 5% NaCl. It is methyl red-positive and ferments fructose, galactose, maltose, glucose, lactose, and sucrose.

BDT4 is a facultative anaerobic, Gram-positive coccus that does not form endospores. Colonies are round with entire margins, flat elevation, and a white color. It is positive for catalase, urease, lysine decarboxylase, and methyl red, but negative for gelatinase and amylase. It grows at pH 7 and 9, at 27 °C and 4 °C, and in 1% and 5% NaCl. It ferments fructose, galactose, maltose, glucose, and mannitol.

BDT5 is an aerobic, Gram-negative coccus that does not form endospores. Colonies are round with entire margins, flat elevation, and a yellow color. It is positive for catalase and urease, but negative for lysine decarboxylase, gelatinase, and amylase. It grows at pH 5, 7, and 9, at 27 °C, and in 1%, 5%, and 10% NaCl. It ferments sucrose, fructose, galactose, maltose, and glucose.

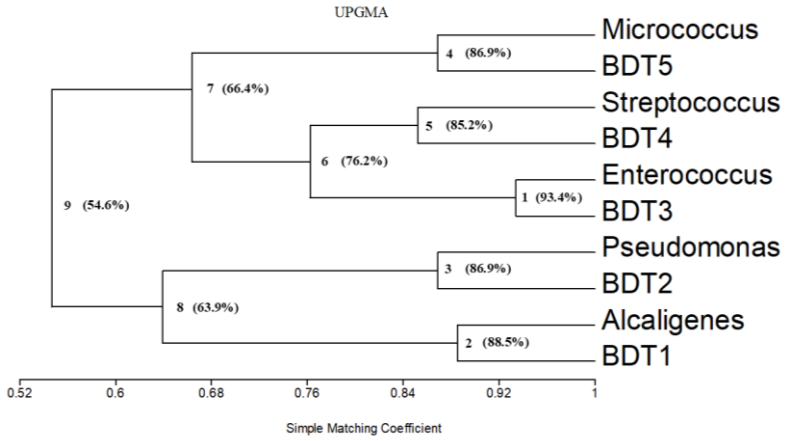

Figure 2. Relationships Dendogram

Based on the characterization results, profile matching analysis was carried out and several reference genera were obtained, which is Alcaligenes, Pseudomonas, Enterobacter, Streptococcus, and Micrococcus. Furthermore, all characters from the five isolates and the reference genera were analyzed using Microsoft Excel and MVSP software to obtain a dendogram graph. The dendrogram graph constructed using the UPGMA method and Simple Matching Coefficient illustrates the phenotypic similarity among the five rhizosphere bacterial isolates (BDT1, BDT2, BDT3, BDT4, and BDT5) compared with reference genera (Figure 2). The isolates were grouped into two major clusters at 54.6% similarity.The first cluster (63.9% similarity) consisted of BDT1 and BDT2, which were closely related to the genera Alcaligenes (88.5% similarity with BDT1) and Pseudomonas (86.9% similarity with BDT2). The second major cluster (66.4% similarity) included BDT3, BDT4, and BDT5. Within this cluster, BDT3 showed the highest similarity (93.4%) with Enterococcus,

BDT4 was grouped with Streptococcus (85.2% similarity), and BDT5 was associated with Micrococcus (86.9% similarity).

These results indicate that the rhizosphere bacterial isolates from Ageratum conyzoides are phenotypically diverse, spanning both Gram-negative genera (Alcaligenes, Pseudomonas) and Gram-positive genera (Enterococcus, Streptococcus, Micrococcus). The high similarity coefficients (>85%) between the isolates and their respective reference taxa suggest that the phenotypic identification was reliable. Further explained by Goodfellow & O´Donnell (1993 in Salaki *et al.*, 2010), based on the taxospecies concept, if the cluster similarity index is ≥70%, it can be concluded that the members of the cluster are members of the same species because they have a high similarity index. Based on the dendogram results, 9 clusters were obtained with varying similarity index.

The characteristics of the BDT1 isolate have high similarity with the genus Alcaligenes, according to Bergey (1994), which is a gram-negative bacteria that is round, motile, and is found in soil and water. Research by Pineda *et al.* (2023) states that the bacteria Alcaligenes faecalis isolated from gold mining wastewater in Picacho, Winterhaven, Imperial County, California, are able to help the gold-washing process.

The characteristics of the BDT2 isolate have high similarity with the genus Pseudomonas, according to Brenner *et al.* (2007), which is a gram-negative bacteria in picthe form of bacilli or short bacilli, aerobic, catalase-positive, motile, and produces yellow to green pigments. In addition, several species of bacteria, such as Pseudomonas putida and Pseudomonas fluorescens, can be isolated from the soil. It is further explained that this pseudomonas genus is often found in soil or water contaminated with heavy metals such as mercury, lead, arsenic, and others. Research by Peix *et al.* (2018, in Christita *et al.*, 2018) found that Pseudomonas aeruginosa bacteria isolated from wastewater can survive or tolerate various types of contaminants, one of which is reducing heavy metal contaminants in mining areas.

The characteristics of the BDT3 isolate have high similarity to the Enterococcus genus. According to Vos *et al.* (2011), enterococcus genus bacteria are gram-negative bacteria, arranged in a short chain configuration, do not produce endospores, have yellowish-white pigments, have negative catalase, and have a dominant end product of lactic acid from glucose fermentation. In addition, the Enterococcus genus is known to be isolated from various habitats such as food, plants, soil, and water. This is in line with the research of Christita *et al.* (2018), who found isolates of enterococcus genus bacteria from wastewater from nickel mines in East Halmahera.

The characteristics of the BDT4 isolate have high similarity to the genus Streptococcus. According to Vos *et al.* (2011), Streptococcus genus are gram-positive bacteria, round in shape, have a chain or pair configuration, are nonmotile, do not produce endospores, are facultative anaerobes, and ferment carbohydrates with lactic acid production without gas production. Based on research by Christita *et al.* (2018), 39 isolates of Streptococcus genus bacteria were found in nickel mine wastewater in East Halmahera. In addition, in the research of El-Imam *et al.* (2023), isolates of Streptococcus genus bacteria were also found from Jebba gold mining tailings, Nigeria.

The characteristics of the BDT5 isolate have high similarity to the genus Micrococcus; according to Parte *et al.* (2012), Micrococcus is a gram-positive bacteria in the form of a sphere, nonmotile, aerobic, catalase-positive, does not produce endospores, and has yellow or slightly orange colonies. In the study of Daibova *et al.* (2019, in Jorjani *et al.*, 2022), it was found that natural microbes such as Pseudomonas sp. and Micrococcus sp. are known to be able to help the gold washing process by 87.8% to 92.2%.

3.2. Mercury Sensitivity Test

The cup-plate method, involving the creation of wells on agar plates, was used in this study to determine the sensitivity of isolates to certain mercury concentrations, based on the presence or absence of an inhibition (clear) zone. Mercury(II) chloride ($HgCl_2$) was used as the mercury source in this assay due to its good solubility in water (Ulfa *et al.*, 2016). The result are shown in Table 2.

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Table 2. Mercury Sensitivity Test Result

Isolate Codes	Lowest Mercury Concentration with Clear Zone (ppm)	Clear Zone Diameter (mm)
BDT1	110 ppm	2.12
BDT2	470 ppm	1.80
BDT3	30 ppm	4.52
BDT4	20 ppm	3.62
BDT5	20 ppm	2.85

The isolate that formed a clear zone at the highest mercury concentration was BDT2, suspected to belong to the genus Pseudomonas, showing sensitivity at 470 ppm. Isolate BDT1 (Alcaligenes) exhibited sensitivity at 110 ppm, while BDT3 (Enterococcus) demonstrated sensitivity at 30 ppm. Isolates BDT4 (Streptococcus) and BDT5 (Micrococcus) both showed sensitivity at 20 ppm. According to this results, Pseudomonas bacteria are known to have several species that are resistant to mercury. Based on research by Sutanto *et al.* (2018), it was found that Pseudomonas bacteria were able to grow in media with added mercury up to a concentration of 40 ppm. Furthermore, in the latest research by Robas Mora *et al.* (2022), a strain of *Pseudomonas mercurytolerans* was found in ex-mining soil with a mercury concentration of 1,710 ppm.

The results of the mercury sensitivity test on the BDT1 isolate or Alcaligenes bacteria are supported by the results of research conducted by De & Ramaiah (2007) that several species of bacteria of the genus Alcaligenes are known to be able to grow in media with the addition of mercury up to 75 ppm. Furthermore, in the research of Mondragón *et al.* (2011), it is known that Enterococcus bacterial isolates can grow on media with the addition of $HgCl_2$ 200, 250, 300, and 350 µM, but the mercury concentration in ppm is unknown. In addition, Giri's (2011) study also found bacterial isolates from the Enterococcus genus that were able to grow at mercury concentrations reaching 100 ppm and bacteria from the Streptococcus genus that were able to grow at mercury concentrations reaching 50 ppm. Meanwhile, in the micrococcus genus, the presence of the merA gene was found, which is known to play an important role in the mercury resistance process, namely by encoding the mercury reductase enzyme, which helps the process of reducing Hg^{2+} to Hg^0. Wijaya *et al.* (2021).

4. CONCLUSION

Based on the identification results, the five isolates of rhizosphere bacteria from *Ageratum conyzoides* plants growing at the Ratatotok Gold Mine site, North Sulawesi, came from the genus Alcaligenes Pseudomonas, Enterococcus, Streptococcus, and Micrococcus. The isolates with the highest mercury resistance potential were Pseudomonas bacteria (BDT2) which were sensitive to a mercury concentration of 470 ppm, then Alcaligenes bacteria (BDT1) at a mercury concentration of 110 ppm, Enterococcus bacteria (BDT3) at a mercury concentration of 30 ppm, and Streptococcus and Micrococcus bacteria (BDT4 and BDT5) at a mercury concentration of 20 ppm.

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