Identification of Mercury and Cyanide Degrading Bacteria in Sekotong People's Mining

Suhadi(✉), S. Zubaidah, Sueb, R. Farahilda, M. Syamsussabri, and A. N. Rochmah

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Malang, Indonesia
suhadi@um.ac.id

Abstract. The people's gold mining in West Lombok district, especially in the Sekotong sub-district, reached 1497 glondong units and 570 (38%) was disposed into the river. If the situation continued, problems would arise in the environment. This study aimed to determine the microorganisms found in gold mining waters. Purposive sampling was used to collect the samples, taking into account the location of the gold processing operations in the Pelangan village, Sekotong sub-district, and West Lombok district. The procedure consisted of sampling, isolation and identification of bacteria. The results of found that based on observational data on macroscopic and biochemical characteristics, the 1st bacterial isolate was identified as Moraxella sp. 64.70%, and the second isolate of bacteria was identified as Pediococcus spp. 81.25% and the 3rd bacterial isolate was identified as Moraxella sp. 92.13%. It concluded that bacteria was found in the gold mining location of Pelangan village, Sekotong sub-district, West Lombok district were Moraxella sp., and Pediococcus spp.

Keywords: People's Mining · Bioremidiation · Cyanide · Mercury

1 Introduction

Gold resources are very abundant in Indonesia [1]. Even though there are 90 countries that maintain gold mines, only 20 of them actually supply any of the world's gold. With a gold production of 73.1 tons and gold reserves of almost 3000 tons, Indonesia is ranked eighth in the global [2]. The island of Lombok, which is located in West Nusa Tenggara, is very popular with the public for gold mining [3]. Sekotong is one of the sub-districts in West Lombok which has an area of 330.45 km^2 [4].

Small-scale miningior known in Indonesia as community gold mining, community mining or small-scale many have not applied the principles of "good mining practices" in terms of health, safety and the environment in carrying out their mininghactivities. For example, the widespread use of mercury (mercury) in gold extraction activities by small-scale artisanal mining [5]. According to Syamsussabri's observations, [6] the Sekotong people manage mining gold using basic, minimal procedures without considering the environmental safety concerns due to the waste pollution they produce. Techniques such amalgamation and cyanidation are used in the gold processing process. Heavy metals

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and potentially hazardous chemicals, such as CN and Hg, are known to be used in the community's mining activities. [6].

One of the environmental pollutants is heavy metals, so waste containing heavy metals must be managed as well as possible before being disposed of in the environment [7]. Bioremediation is a biological environmental technique that is able to reduce environmental pollution caused by heavy metals, with the help of microbes [7]. Enzymes made by microorganisms modify hazardous pollutants' chemical structures, which is a process known as biotransformation. Biotransformation eventually progresses to biodegradation, wherein hazardous pollutants lose a few of their toxic properties and eventually break down into harmless metabolites [8]. Microorganisms can be utilized and used to reduce environmental damage. [9].

The release of waste from the gold processing industry into the environment is also what produces Hg and CN pollution. Additional symptoms includes decreasing soil and water quality, which will possibly influence other organisms and cause disease. [6]. This research needs to be done because mercury (Hg) and cyanide (CN) pollution in the Sekotong people's gold mine has passed the threshold set by WHO, this pollution is known to have entered aquatic organisms that have economic value and consumption by the community [12]. In Sekotong, community mining is still going on, and no safety precautions have been implemented. If this problem is not handled wisely, of course it can endanger the environment and human life in the future.

First observations made by researchers taking water samples at the Sekotong people's gold mine, observations and analysis were carried out at the Airlangga University Laboratory, Surabaya. These circumstances make it necessary to conduct research related to microorganisms that are able to degrade Hg and CN pollutants due to community gold mining activities in Sekotong. The purpose of this study was to determine the microorganisms capable of degrading Hg and CN pollutants due to community gold mining activities in Sekotong, West Lombok, NTB Province.

2 Methods

The aim of this quantitative descriptive study is to discover more about the bacteria that can degrade Hg and CN pollutants in the gold mine of the Sekotong people. At Airlangga University's Biology Laboratory, observations and analysis were carried out.

The research population for water sampling is three to four locations, including: Middle Sekotong Village (Telaga Lebur Hamlet), Cendi Manik Village (Sayong Daye Hamlet), West Sekotong Village (Lendang Re 1) Village, West Sekotong Village (Lendang Re 2 Hamlet). Water samples were taken by purposive sampling with consideration of the location of gold processing activities. The number of samples was taken at several points around the Sekotong gold mine. The tools and materials used in the study included 100 ml sample bottles, funnels, measuring cups, tweezers, and stationery, and the Global Positioning System (GPS). Water sample, tissue, label paper (Fig. 1).

Research procedures consist of, sampling, isolation of bacteria, and identification of bacteria. The stages of taking water samples are as follows: (1) Water samples are taken in the holding pond at a depth of 0.8 d (times depth) with the provision that the sample point is taken in the middle area of the reservoir with the aim of knowing the average

Fig. 1. Sampling research map

level of pollution; (2) a water sample is taken and put in a 100 ml sample bottle; (3) water samples were stored in an ice box at a temperature of 00 °C 60 °C [10, 11].

The stages of bacterial isolation are as follows: (1) performing a gradual dilution of the sample using a physiological saline solution of 0.85% NaCl up to a dilution of 10–4; (2) pour plate from 10–2, 10–3, and 10–4 in duplicate using nutrient agar (NA) media then incubated at room temperature for 48 h to get the bacteria in the sample; (3) counting and observing the morphology of the growing bacterial colonies; (4) colonies with different morphology were declared as different bacterial isolates; (5) refining the three isolates that grew the most for further identification.

The stages of bacterial identification are as follows: (1) Rejuvenating pure cultures of bacteria to be identified with an age range of 18–24 h on slanted NA; (2) Gram staining to determine the type of Gram and the shape of the bacteria to be identified; (3) Conducting oxidase test of slanted NA bacterial samples to determine the type of kit to be used. Positive oxidase is indicated by the reaction and the formation of purple color on the oxidase strip paper, while negative oxidase does not form purple color; (4) Make a suspension of bacteria in a 10 mL physiological saline solution of 0.85% NaCl; (5) Adding 200 L of bacterial suspension into each well; (6) Cover the part of the well circled in black with immersion oil if the oxidase test is positive and the part circled in black and black dotted for the oxidase test is negative; (7) After all suspensions have entered, close the well seal again and incubate in an incubator at 300 °C for 24 h (8) Add reagents for wells that require further detection after incubation (9) Physiological observation data for Gram-negative bacteria are read (presented in biochemical table) is entered in the Microbact TM Gram Negative Identification System program for identification (10) Data from macroscopic, microscopic, and biochemical observations of Gram positive bacteria are used to identify isolates by calculating the Jaccard index (Real and Vargas, 1996) which includes positive and negative similarities. of the character of each species [12] (Tables 1 and 2).

Table 1. Results of Macroscopic and Microscopic Characteristics

No.	Characteristics	Observation result		
		Isolate to 1	**Isolate to 2**	**Isolate to 3**
1.	Colony shape	*Circular*	*Circular*	*Circular*
2.	Colony edge	*Entire*	*Entire*	*Entire*
3.	Colony elevation	*Flat*	*Flat*	*Flat*
4.	Colony optics	*Translucent*	*Opaque*	*Transparent*
5.	Colony color	*Light Yellow*	*White*	*White*
6.	Cell shape	*Bacil*	*Coccus*	*Bacil*
7.	Gram stain	*Negative*	*Negative*	*Negative*
8.	Endospore	-	-	-

3 Result

3.1 Bacterial Identification Results

Based on the observational data on macroscopic, microscopic, and biochemical characteristics, the 1st bacterial isolate was identified as *Moraxella* sp. 64.70%, the second bacterial isolate was identified as *Pediococcus* spp. 81.25%, and the 3rd bacterial isolate was identified as *Moraxella* sp. 92.13% (Figs. 2, 3, 4 and 5).

The types of bacteria found in the Sekotong people's gold mine are presented in the following figure: presented in the following figure:

4 Discussion

Sekotong District, West Nusa Tenggara Province (NTB) is the location of smallholder gold mining. Has been going on since 2008 until now. The gold processing process still uses a simple technique, namely amalgamation and cyanidation [6]. The latest data shows that mercury (Hg) and cyanide (CN) contamination in the Sekotong people's gold mine has passed the threshold set by WHO, which is 0.001 ppm for Hg and 0.0035 for CN. In water, it is known that Hg contamination ranges from 0.4 to 3.35 ppm, while CN pollution ranges from 5.31 to 13.27 ppm [12]. This pollution is known to have entered aquatic organisms that have economic value and consumption by the community [13].

Hg contamination is known to be present in some aquatic organisms with levels of 1.36 to d. 23.43 ppm as well as CN pollution with levels of 18.21 to d. 90.22 ppm. Hg and CN pollution have even accumulated to humans, it is known that Hg levels in human hair reach a value of 36.34 ppm while HCN levels in human hair are 23.06 ppm [13]. This is a very large number and exceeds the threshold that has been set. We know for ourselves that Hg and CN are toxic substances that are very dangerous for human health [7]. If this problem is not handled wisely, of course it can endanger the environment and human life in the future. Researchers have conducted research related to pollution problems found

Table 2. Results of Biochemical

No.	Characteristics	Observation result		
		Isolate to 1	**Isolate to 2**	**Isolate to 3**
1	Oxidase	+	−	+
2	Motilitas	−	−	−
3	Nitrate	−	−	−
4	Lysine	+	+	+
5	Ornithine	−	−	−
6	H_2S	−	−	−
7	Glucose	−	+	−
8	Mannitol	−	−	−
9	Xylose	−	+	−
10	ONPG	+	−	+
11	Indole	−	−	−
12	Urease	−	−	−
13	VP	−	−	−
14	Citrate	+	−	−
15	TDA	−	−	−
16	Gelatin	+	−	+
17	Malonate	+	−	−
18	Inositol	−	−	−
19	Sorbitol	−	−	−
20	Rhamnose	−	−	−
21	Sucrose	−	−	−
22	Lactose	−	+	−
23	Arabinose	−	+	−
24	Adonitol	−	−	−
25	Raffinose	−	−	−
26	Salicin	−	−	−
27	Arginine	−	−	−
28	Katalase	+	+	+

in the waters oftthe people gold mining in Sekotong, related to microorganisms that are able to degrade Hg and CN pollutants. The microorganism is the bacteria *Moraxella* sp. And *Pediococcus* sp.

Bacteria *Moraxella* sp is a gram-negative bacterium, gram-negative bacteria are able to absorb red color (absorb safranin dye), when observed under a microscope, in the gram

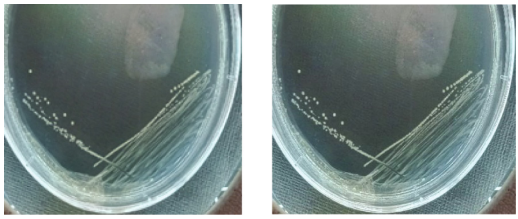

Fig. 2. Macroscopic results of the 1st and 3rd isolates identified as *Moraxella* sp.

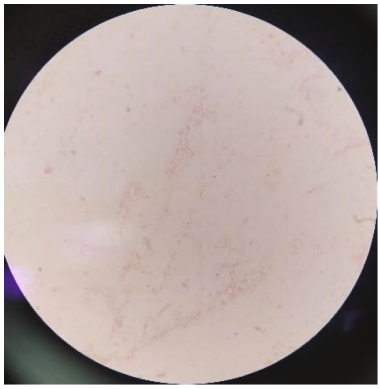

Fig. 3. Microscopic results of the 1st and 3rd isolates identified as *Moraxella* sp

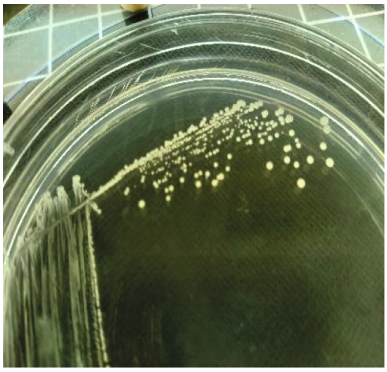

Fig. 4. Macroscopic results of the 2nd isolate identified as *Pediococcus* sp.

staining process (14). *Moraxella* sp bacteria are rods or short rods, these bacteria can be found in aquatic environments, and soil [15]. *Moraxella* sp bacteria are able to degrade pollutants in the form of Hg and CN, where in the Sekotong people's gold mining it is known that there is mercury (Hg) and cyanide (CN) pollution, which is a result of mining activities carried out by local people. *Moraxella* sp bacteria are able to degrade cyanide (CN) by utilizing cyandia as a carbon and nitrogen source [16]. *Moraxella* sp

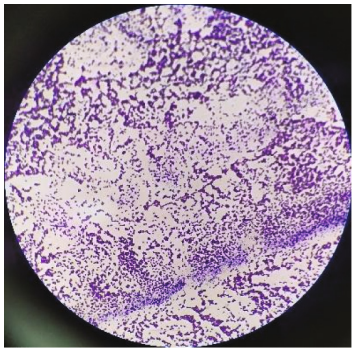

Fig. 5. Microscopic results of the 2nd isolate identified as *Pediococcus* sp.

bacteria are able to reduce the concentration of cyanide (CN) pollutants, by degrading CN into ammonia and converting it into nitrate as a final byproduct [16].

Pediococcus sp bacteria are classified as gram-positive bacteria, gram-positive bacteria are able to show a blue-purple color (absorb crystal violet dye), when observed under a microscope, in the gram staining process [15]. *Pediococcus* sp bacteria are able to degrade pollutants in the form of mercury (Hg) and cyanide (CN). Several studies have shown that *Pediococcus* sp bacteria are able to degrade pollutants in the form of mercury (Hg). The use of lactic acid bacteria in the remediation process has proven effective, lactic acid bacteria (*Pediococcus* sp) have good ability to remediate water with heavy metal contamination [17]. Furthermore, the unique cell wall structure of these bacteria enables them to bind heavy metals. Peptidoglycan, teichoic, and lipoteichoic acid components compose the cell wall. The structure of polysaccharides and exopolysaccharides allows the presence of adhesion forces on macromolecules [17]. The results of the study, based on observational data on macroscopic, microscopic, and biochemical characteristics, the 1st bacterial isolate was identified as *Moraxella* sp. 64.70%, the second isolate bacteria identified as *Pediococcus* sp. 81.25%, and the 3rd bacterial isolate was identified as *Moraxella* sp. 92.13% [12], these bacteria are able to degrade Hg and CN pollutants found in the Sekotong people's gold mine.

5 Conclusion

The people's gold mining in Sekotong is polluted with Hg and CN pollutants which have exceeded the predetermined threshold. From the results of research that has been carried out to reduce these pollutants, there are microorganisms that are able to degrade these pollutants, namely *Moraxella* sp bacteria and *Pediococcus* sp bacteria.

References

1. W. H. Utomo, R. Suntari, N. Arfarita, Suhartini, & E. Handayanto, Rehabilitation of Artisanal Small-scale Gold Mining Land in West Lombok, Indonesia: 3, Exploration of Indigenous Plant Species and The Associated Mycorrhiza for Phytomycoremediation of Mercury Contaminated Soils, *American-Eurasian Journal of Sustainable Agriculture*, 8(1), 2014, 34–41.

2. A. Lamare, *Top 10 Gold Producing Countries in the World. Celebrity Networth.* (Online), 2013, (https://www.celebritynetworth.com/articles/entertainment-articles/top-10-gold-producing-countries-world/).
3. L.G.S Astiti, & T. Sugianti, Dampak Penambangan Emas Tradisional pada Lingkungan dan Pakan Ternak di Pulau Lombok, *Sains Peternakan Vol. 12 (2)*, September 2014: 101-106.
4. Badan Pusat Statistik (BPS) Lombok Barat, *Kabupaten Lombok Barat Dalam Angka*, BPS-Statistics of Lombok Barat Regency, 2018
5. H. Nugroho, Tinjau Ulang Kebijakan Mengenai PETI (Pertambangan Tanpa Izin) di Indonesia, *The Indonesian Journal of Development Planning*. 4 (2), 2020
6. M. Syamsussabri, Suhadi & Sueb, Kelayakan Modul Pencemaran Lingkungan Berbasis Environmental Worldview dan Environmental Attitudes, *Jurnal Pendidikan: Teori, Penelitian, dan Pengembangan*, *4*(9), 2019, 1207–1212. http://journal.um.ac.id/index.php/jptpp
7. M. Alfian, Supartono, & S. Mursiti. Bioremediasi Logam Pb pada Limbah Tekstil dengan Staphylococcus aureus dan Bacillus subtilis, *Indo. J. Chem. Sci,* 6 (3), 2017
8. S. Yani, Bioremediasi Limbah Merkuri Dengan Menggunakan Mikroba Pada Lingkungan Yang Tercemar, Edisi Juni 2011 Volume V No. 1 - 2 ISSN 1979–8911, 2011
9. J.H., D. Lamb, P. Paneerselvam, G. Choppala, J.W. Chung, & N. Bolan, Role of Organic Amendments on Enhanced Bioremediation of Heavy Metal (Loid) Contaminated Soils, *Journal of Hazardous Materials*, 1(85), 2011, 549–574.
10. R.B. Baird, A.D. Eaton, & E.W. Rice, *Standard Methods for the Examination of Water and Wastewater, 23rd Edition. Washington*, DC: American Public Health Association, American Water Works Association, Water Environment Federation, 2017
11. A. Hadi., Pengambilan Sampel Lingkungan. Jakarta, 2015
12. Laporan Unit Layanan Biologi Fakultas Sains dan Teknologi Universitas Airlangga
13. Suhadi., Sueb., & Syamsussabri, Mercury and Cyanide Pollution on the Aquatic Organism in Sekotong People and Gold Mining, *Journal of Physics: Conference Series*, 2019, doi:https://doi.org/10.1088/1742-6596/1417/1/012032
14. D. P. Sari, A. Hermansyah., & R. Elvia, Isolasi Bakteri Dari Tanah Tempat Pembungan Akhir (TPA) Air Sebakul Sebagai Agen Biodegradasi Limbah Plastik Polyethylene, *Jurnal Pendidikan dan Ilmu Kimia*, 4(2): 98–106 (2020).
15. N., H. Mukti, R. Laras, & Romadhon. Aktivitas Antibakteri Isolat Bakteri Asam Laktat Dari Peda Dengan Jenis Ikan Berbeda Terhadap *E. Coli* Dan *S. aureus*. *Jurnal Ilmu dan Teknologi Perikanan* Volume 1 No 2 (2019).
16. S. Potivichayanon, & Kitleartpornpairoat, Biodegradation of Cyanide by a Novel Cyanide-grading Bacterium, *Journal of World Academy, Engineering and Technology*, 2010, 1362–1365.
17. L, A. Huet, & D. Puchooa, Bioremadiation of heavy metals from aquatic environment through microbial processes: A potential role for probiotics?, *Journal of Applied Biology & Biotechnology* Vol. 5 (6). 2017