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Potential of Chemolitotrophic Bacteria from Gold Mining Area in Sulfur Oxidation Process

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Abstract

Gold in nature is covered by rocks which contain sulfide minerals such as pyrite, chalcopyrite, arsenopyrite, and others sulfide minerals. Chemolithotrophic bacteria have the ability to oxidize the sulfur compounds and can be used in the process of releasing gold from carrier rocks which contain sulfide minerals. This research aimed to explore and identify the chemolithotrophic bacteria from gold mining areas as well as determine their potential for sulfur oxidation. The methods used in this study were exploring the potential of bacteria in sulfur oxidation and describing the variety of bacteria that were isolated from gold mining areas by 16s rRNA identification. The results showed that there were six isolates from isolation with Starkey solid medium, i.e. Bl-1, B1-2, B1-3, B1-4, B1-5 and B1-6 that were similar to *Paenibacillus* sp., *Enterobacter ludwigiis* train E8-13, *uncultured Burkholderia* sp., *Uncultured bacterium* clone N4.5, *Bacillus subtilis* strain CICC 10023, and *Bacterium enrichment* culture clone 02 respectively. The B1-3 isolate showed the highest increase of sulfate compound in the medium (8.04 % at 649.55 ppm). This indigenous bacteria will be able to be used to release gold from rock which contains sulfide minerals and reduce the use of hazardous chemicals commonly used in gold mining.

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INTRODUCTION

Characteristics of the gold deposit in nature can be identified by the type of minerals ore in the mining area. The several types of minerals orein the gold mining area are quartz, pyrite, carbonate, barite, fluorite, monazite, ilmenite, silicate, and some other sulfide minerals (Eugene and Mujumdar, 2009). Purawiardi and Ginting (2008) reported some of minerals type in the mining area as acarrier rock of gold, like quartz, goethite, argentite, electrum, galenaand pyrite which have a bright yellow coloror pink sometimes.

Conventional gold mining use toxic chemicals like cyanide to separate the gold from ore that contains sulfide minerals. Excessive use of cyanide for the dissolution of gold is associated with environmental risks. This process can be replaced with abiological method with bacteria as environmentally friendly dissolution agent (Olson, 1991).

Sulfur-oxidizing bacteria are part of chemolithotrophic bacteria. These bacteria have the ability to utilize inorganic sulfur compound and degrade insoluble compound to soluble compound (Sabra *et al*. 2011). This capability can be applied to remove interfering metal sulfides from ore-bearing precious metals before cyanidation treatment (Rohwerder *et al*., 2003). Bacterial used in gold recovery process can increase the result up to 95 % (Bosecker, 1997).

The previous study reported that some of sulfur-oxidizing bacteria belong to thegeneraof *Leptospirilum*, *Thiobacillus*, *Bacillus*, *Beggiota, Thiothrix, Thermothrix, Thiovolum, Acidianus, Sulfolobus, Thioalcalimicrobium* and *Thioalkallividrio* (Rojas-Avelizapa *et al*., 2013), *Thiomonas* (Han *et al*., 2013), *Sulfobacillus* (Okibe *et al*., 2003). Donati and Sand (2007) reported several genera of bacteria which play a role in the bioleaching process i.e. *Alicyclobacillus, Caldibacillus, Ferrimicrobium, Ferroplasma, Metallosphaera,* and *Sulfurococcus.*

Some countries such as Australia, Brazil, Ghana, Peru and South Africa have used the process of oxidation of sulfide minerals in the gold mining (Gonzales *et al*., 2004). This technique uses microbes in the oxidation of sulfur as the main compound of minerals ore in the rock of gold. This technique is known as bioleaching. Based on the above case, this study was conducted to explore the spesies and potential of indigenous khemolitotrophik microbial from gold mining area in doing sulfur oxidation as one of process in gold mining.The chemolithoauotrophic bacteria found from gold mining in this study were identified with 16s rRNA and were monitored for their potential in bioleaching experiment to oxidize gold ores. The discovery of these bacteria can provide benefits in the process of safely releasing gold by reducing the use of cyanide as danger chemicals, especially in gold mining conducted by individuals.

METHODS

Microorganism and culture

Bacteria were isolated from the gold ore of a gold mine, through isolation, purification and colony morphological identification processes. The medium used was Starkey solid medium which contained: KH_2PO_4 3 g/L, $MgSO_4$ $7H_2O$ 0,2 g/L, $CaCl_2$ $2H_2O$ 0,2 g/L, $(NH_4)_2SO_4$ 0,5 g/L, trace of $FeSO_4$. Cultivation was maintained at 30 ˚C and pH 8 (Vidyalakshmi and Sridar, 2006).

Ore samples

Gold ore was obtained from traditional mining in Banyumas which was crushed to 200 mess size. Element analysis was carried out at Analytical Chemistry Laboratory of Gadjah Mada University with X-ray Diffraction (XRD) and Atomic Absorption Spectroscopy (AAS).

Screening of sulfur-oxidizing bacteria

Isolates were grown on Starkey liquid medium during incubation for twoweeks and then were titrated using five mM solution of iodine with 0.5 N starch as indicator. This method was used to measure the thiosulfate ions consumption as the one of theintermediate sulfur compound produced during sulfur oxidation (Ohba and Owa, 2005).

DNA extraction and PCR amplification of bacterial 16S rRNA genes

The sample for 1.5 mL liquid isolates were centrifugated 10000 g for 15 minutes. Materials for extraction used 50 µL tenderizer 35%, 50 µL Sodium Dodecyl Sulfate 20% alcohol absolute, ethanol 70% and TE buffer for storage at -20 ˚C. Polymerase chain reaction (PCR) amplification of 16s rRNA of isolate sample was performed in atotal volume of 30 µL using primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1540R (5'-AAGGAGGTGATCCAACCGCA-3'). PCR reaction program was carried out and followed by Wei-min *et al*. 2009. PCR products were detected by 1.5 % agarose gel electrophoresis.

Sequencing and identification

DNAproductfrom amplification was sent to 1st BASE Singapore for nucleotide base sequencing. Nucleotide chain from sequencer was analyzed by Basic Local Alignment Search Tool (BLAST) from National Center for Biotechnology Information (NCBI).

Bioleaching experiment

Bioleaching experiment was performed with Yuan *et al.* (2013) modified at medium and incubator-type. This test was conducted at 250 mL flask containing 100 mL Starkey medium and 10% (v/v) isolate (10^6cell/mL) then 5% (w/v) gold ore size was less than 74 µm in diameter was added. This gold ore suspension was incubated at 30 ˚C for 30 days in R-L incubator. Sulfate in medium was measured by UV-Vis at the beginning and in the end of the experiment. During the leaching process, total bacteria were measured with Total Plate Count (TPC) method using spread plate technique and pH of the solution was measured with pH meters every seven days.

Data Analysis

Data of the isolates were analyzed by descriptive analytic to refer their characters and their potential in sulfur oxidation with gold ore.

RESULTS AND DISCUSSION

Ore samples from gold mining were smoothed up to 20 mess to know the dominant of minerals content.Ore analysis with XRD indicated the sample was dominated by quartz as the three highest peak d= 3.34 Å, d= 3.24 Å dan d= 3.31 Å (Fig. 1). Based on the results, the most common type of minerals found in gold ore taken from the gold mine was the quartz mineral (SiO_2) type. From the sample it was also detected the presence of sulfide minerals content and low grade gold content (25 ppm) by AAS. The quartz mineral (SiO_2) is a type of mineral with hollow rock characteristics. Cavities in quartz minerals is filled with gold and the number of fine veins contained in quartz minerals becomes the parameter of the gold content contained in the rock (Purawiardi & Ginting, 2008).

XRD analysis showed there were three top peaks which were analyzed as quartz. Besides that, sulfide minerals such as augite, hematite, goethite and pyrite were also detected in the sample even though in low intensity. AAS analysis also showed gold content in sample. Purawiardi and Ginting (2008) recognized the dominaton of quartz in gold mine area at peak d=3,34 Å. Hutamadi and Mulyana (2006) also detected dominate of quartz at the potential area for gold mining in Banyumas.

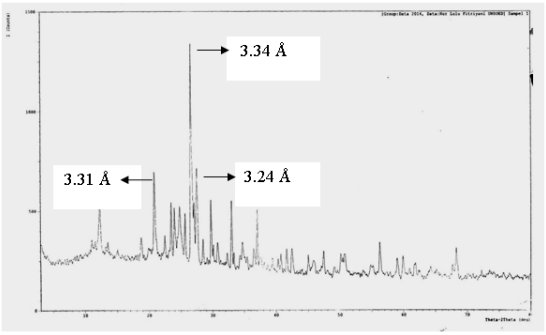

Figure 1. Result of XRD analysis from gold ore

The Isolation of Chemolithotrophic Bacteria from Gold Mining

Isolation with Starkey medium fromgold ore resulted in six isolates. Starkey media contains only minerals without the addition of sources of sugar and carbon sources, so the bacteria thatcan grow is a type of chemolithotrophic bacteria. Chemolithotrophic bacteria is a type of bacteria that is capable of using inorganic compounds as acarbon source in its growth through oxidation or reduction process.All isolate bacteria from isolation process were screened by Starkey liquid medium to known their potential capability in sulfur oxidation. The result showed that all of theisolates from gold mining area have thepotential in sulfur oxidation. The bacteria in cultivation using Starkey liquid media changed their color to violet after the titration with 0.8 mL iodine which indicated the occurrence of sulfur oxidation. The positive result of thiosulfate (intermediate compounds of the sulfur oxidation process) formation was indicated by the change of color of the medium to blue as a result of reaction between thiosulfate and iodine solution with starch solution (Ohba and Owa, 2005). Rawlings (2005) added that the process of sulfur oxidation through the thiosulfate pathway will produce thiosulfate as the main intermediate and sulfate compounds as the end product. Isolates were detected as chemolithotrophic bacteria through the identification process of DNA isolation continued with amplification of DNA with PCR and sequencing.

BLAST analysis showed similar results between Bl-1 isolate and Paenibacillus sp. G1, Bl-2 isolate with Enterobacter ludwigiistrain E8-13, Bl-3 isolate with uncultured Burkholderia sp., Bl-4 isolate with uncultured bacterium clo-

neN4.5, Bl-5 isolate with Bacillus subtilis strain CICC 10023 and Bl-6 with Bacterium enrichment culture clone 02. From BLAST analysis, it was known thatthere weretwo isolate as uncultureable bacteria while the isolation process using the agar plate produced six isolates capable of growing on Starkey media. Williams *et al.* (2007) suggested that some of uncultured bacteria have the ability to grow in the isolation process using agar medium because the inter-species symbiosis with nutrient exchange. Other that the presence of quorum-sensing capable of inducing the synthesis of a protein used for intercultural communication. D'Onofrio *et al.* (2010) reported the presence of acyl-desferrioxamine siderophores is capable of assisting uncultured bacterial growth in the cup medium. The existence of other organisms greatly affect the growth of an individual on a medium (Bintari et.al., 2008)

BLAST analysis showed that there was a similarity between BL-1 isolate with Paenibacillus sp. G1. This bacterium is a type of bacteria that has a wide enough habitat and is often found in the soil, Gram-positive bacteria with stem-shaped cells and are chemoorganotrophic bacteria (Mead *et al.*, 2012). Experiment with Bl-1 isolate detected decreasing concentration of sulfate compound in a medium, apparently, this bacterium was sulfate-reducing bacterium. Gray *et al.* (2003) reported Paenibacillus sp. was potential to desulfurization. Hollibaugh *et al.* (2007) also studied the role of Paaenibacillus lentimorbus in sulfide oxidation and reduction of arsenic.

Bl-2 isolate has similarity with *Enterobacter ludwigii* which has the form of rod-shaped cell and Gram-negative bacteria. *E. Ludwigii*has the ability in bioremediation process to reduce Cd levels in plants (Pau-Roblot *et al.*, 2012) and has the ability to degradate hydrocarbon compounds (Yousaf *et al.*, 2011).

Bl-3 colonies have transparent, flat, stable colonies variant characteristics and has a colony diameter of 2-3 mm (Jewell, 2000). Bl-3 has similarity with *uncultured Burkholderia* sp (BLAST analysis). *Burkholderia* sp. is a rod-shaped cell, chemolithotrophic bacteria and grows in the pH of 3. This bacteria reported of capable in oxidizing thiosulfate and has the *soxB* gene. *SoxB* gene is a crucial gene to sulfur oxidation found in the sulfur-oxidizing bacteria group as mentioned by Bhowal and Chakraborty (2011).

Sulfur Oxidation Activity of Six Isolate Bacteria Bl

There were increases of sulfate compound in the medium after the inoculation of substrate with Bl-3 isolate by 8.04% at 649.55 ppm and 2.67 % with mix cultures. Experiment with Bl-3 isolates shown the increasing concentration of sulfate compound, it predicted that this isolate was potential to separate gold from sulfide minerals at ore. From Table 1 we know that bioleaching experiment with five isolates except Bl-3 isolate can decrease the sulfate compound. The decreasing sulfate content in experiments by using Bl-5 isolate is thought to be due to the Bl-5 which is similar to *Bacillus subtilis*has the ability to reduce sulfate to sulfide (Ehrlich and Newman, 2009) because it has *sulfonucleotide reductase* (SNR) enzymes (Berndt *et al.*, 2004).

Table 1. Table of measured sulfate compound in medium after bioleaching experiment at last incubation

Sample	Sulfate content (ppm)	Percentage of increase or reduce sulfate compound
Control	601.22	0
Bl1	584.49	- 2.78
Bl2	543.14	- 9.66
Bl3	649.55	+8.04
Bl4	522.56	-13.08
Bl5	586.46	- 2.46
Bl6	514.88	-14.36
Mix cultures	617.25	+2.67

Note: (+) there is an increase of sulfate compound in medium, (-) there is sulfate reduction in medium

Five of experiments except the one with Bl-3 isolate decreased the sulfate content in the media (Table. 1). The results of sulfate compound analysis showed that there were only two experiments with the sulfur oxidation of gold ore. Increased sulfate compound as a result of the activity of Bl-3 isolate that are capable of oxidizing gold ore. These results indicate the potential of Bl-3 isolate to gold leaching in gold minning. During bioleaching experiment, the population of Bl-3 isolate indicated the best growth up to 5.8 x 10^6 CFU/mL at the final time of incubation. We discovered that population of each isolate was varied from 10^2 to 10^6 CFU/mL (Fig.2) during bioleaching experiment. Meanwhile, pH value during bioleaching process was not decreased significantly, i.e. 5 to 4.8.

Population of each isolate during bioleaching experiment is shown in Figure 2. It was found that growth of Bl-3 was the highest among all iso-

lates. This result is obtained from the calculation of total plate count in incubation of the first week of $4.2x10^5$ CFU/mL and peaked at fourth week incubation by $5.8x10^6$ CFU/mL. The high number of bacteria isolate of Bl-3 at the fourth week is directly proporsional to the increase of sulfate content in media of 48.33 ppm. The growth of the isolates is due to their capability to utilize inorganic sulfur material in the medium as their energy source even though their strength of capability was various. Chemolithotrophic bacteria have the ability toutilize inorganic compounds (Vandanyan and Vandanyan, 2014).

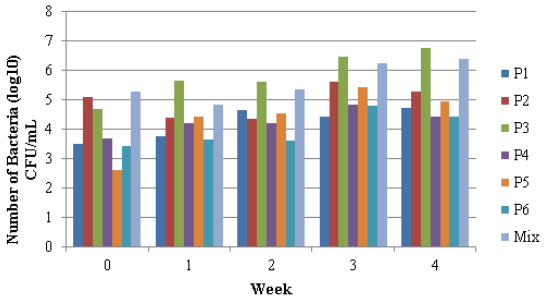

Figure 2. Graphic of population of each isolate every week during bioleaching experiment

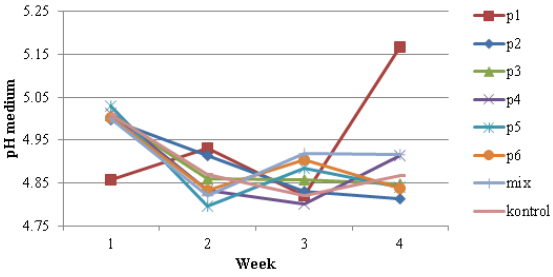

Figure 3. Graphic of pH condition every week during bioleaching experiment

From graphic of pH condition (Figure 3), the decreasing sulfate compound from medium are indicated by the increase of pH values. It was caused by releasing ion OH^- from the reduction of sulfate reaction (Widyati 2007; Yuliana 2012). Experiment with Bl-3 isolate shown stable pH value after seven days of incubation. This condition can be due to the low sulfur oxidation. Okibe *et al*. (2003); Wei-min *et al.* (2009); Rojas-Avelizapa *et al*. (2013); Han *et al*. (2013) and Vandanyan & Vandanyan (2014) reported that oxidation of sulfur decreasing pH value up to 3. Sulfur oxidation processes occurred during bioleaching experiments with the BL-3 isolate may be developed to assist in the release of gold from the sulfide minerals that mask it. Utilization of sulfur oxidizing bacteria can also reduce the use of harmful chemical compounds such as cyanide which is widely used in gold mining and can optimize gold recovery from gold ore.

CONCLUSION

Based on the result and discussion, it is known that indigenous bacteria from gold mine have potential in sulfur oxidation process. It is indicated by the increase of sulfate amount in experimental media containing isolate Bl-3 (*uncultured Burkholderia* sp.) by 8.04%. The result of bacteria identification obtained 6 isolates of chemolitotrop bacteria which are known to have similarity with the following type of bacteria: *Paenibacillus* sp. G1, *Enterobacter ludwigii* strains E8-13, *uncultured Burkholderia* sp., Uncultured bacterium clone N4.5, *Bacillus subtilis* strain CICC 10023 and *Bacterium enrichment* culture clone 02.

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