

Chromium Precipitation Activity and Molecular Characterization of Sulfate-Reducing Bacteria

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ABSTRACT. Chromium is one of the metals used in many areas of industry., However, chromium is toxic to organisms when present in large quantities in the environment. One of the method for treatment of hazardous waste containing chromium in the aquatic environment can be removed by bioremediation using sulfate-reducing bacteria (SRB). Therefore, the purpose of this research were to analyze the chromium precipitation activity of sulfate-reducing bacteria isolated from sulfate reducing bioreactor and its molecular identification using 16S rRNA gene sequences. The result observed that the isolate of sulfate-reducing bacteria (KGP1 strain) has chromium tolerancy ability up to 5 ppm. It also showed that the strain KGP1 could precipitate chromium up to 0.141 ppm (79 %) on 5 days incubation. Based on 16S rRNA gene sequences, this strain identified as *Desulfovibrio aerotolerans*.

Keywords: Sulfate-reducing bacteria · 16S rRNA · Chromium.

1 INTRODUCTION

Chromium is one of the metals used in many areas of industry such as metallic coating, textile, leather tanning, dye production and wood preservation. However, chromium may be toxic to organisms when present in large quantities in the environment. In humans, hexavalent chromium (Cr⁶⁺) may cause various health problems such as dermatitis, respiratory disorders, even cancer because it is carcinogenic (Jobby *et al.*, 2018).

Several treatment technologies have been developed to remove chromium from water. Those technologies most often used physico-chemical techniques, chemical oxidation, ion exchange, adsorption via activated carbon and membrane separation (Jafari *et al.*, 2017; Makrianni *et al.*, 2017; Vi *et al.*, 2018). All of these

methods have several disadvantages such as high operational cost, production of chemical sludge and sludge disposal problems. However the application of the sulfate reducing bacteria has been extensively reported in the literature for the treatment of liquid wastes containing chromium (Pagnanelli *et al.*, 2012; Márquezreyes *et al.*, 2013; Gong *et al.*, 2018; Gan *et al.*, 2019; He *et al.*, 2019). The SRB can provide the counter reactant (hydrogen sulfide) for the process of precipitation of metals ions in their form respective sulfides by sulfate reduction process (Hussain *et al.*, 2016). According at Joutey *et al.* (2015), this bacterium is able to remove chromium 100 times faster than the chromium reducing bacterium.

In a previous study, the sulfate-reducing bacteria strain (KGP1) has been isolated from Sulphate reducing bioreactor. This bacteria was investigated simultaneously reduce sulphate and precipitate manganese (Retnaningrum and Wilopo, 2017). However, the ability of that bacteria in precipitating of others heavy

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metal especially chromium which released from leather tanning home industries at Kota Gede, Yogyakarta, Indonesia is still unknown. This bacteria then necessary to be identified molecularly for further application. Therefore this research was conducted to determine the ability of bacteria strain in chromium precipitation and identify bacterial isolate based on the 16S rDNA gene sequences.

2 MATERIALS AND METHODS

2.1 Strain preparation

The KGP1 strain was grown in a glass bottle on a modified Postgate B medium. This modified medium containing (g/L): MgSO₄·7H₂O 0.5, NH₄Cl 1.0, K₂HPO₄ 0.5, sodium lactate 50 % 14 mL, CaCl₂ 1.0, yeast extract 1.0, FeSO₄·7H₂O 0.5 and ascorbic acid 0.1 (Postgate, 1984). Bacteria strain was then cultured and incubated at 37°C for 72 hours in the anaerobic chamber repeatedly 3 times. The final culture was then transferred into the 100 ml bottle containing 50 ml modified Postgate B medium with addition of chrome concentration variation for the metal resistance test.

2.2 Determination chromium tolerance and precipitation potential

The influence of chromium on the bacterial growth was investigated by exposing strain KGP1 with different concentrations of chromium (1, 3, 5 and 7 ppm). The culture then was incubated at 37°C for 5 days. At 0, 3 and 5 days of incubation, the bacterial growth was measured based on its turbidity using spectrophotometer λ 600 nm. The chromium tolerance of strain was evaluated by the MTC (maximum tolerated concentration), which is defined as the maximum metal concentration above which no bacterial growth is observed. Whereas, the chromium concentration which the bacterial growth measured was selected for further precipitation potential analysis. At the same interval incubation, chromium concentration of culture was measured using Atomic absorbance spectrophotometry (AAS).

2.3 Sequencing 16S rRNA gene analysis

Total genomic DNA was extracted from cell cultures grown on modified Postgate B media. The cells were harvested from 20 mL of cell cul-

ture by centrifugation at 4000 rpm for 10 min and twice washed with chilled deionised water. DNA extraction was carried out by modified extraction from cell lysis solution method and continued by precipitation solution. DNA extract was then amplified by the PCR method (Polymerase Chain Reaction) for identification. Two universal bacterial 16S rRNA primers (27F; 5' AGAGTTTGATCMTGGCTCAG 3', and 1429R; 5' TACGGYTACCTTGTTACGACTT 3') and also Mix PCR were used (Ahn *et al.*, 2017; Nurhikmayani *et al.*, 2019). Products of the PCR process were checked by gel electrophoresis on a 1% agarose gels and stained by SYBR® green. Sequencing was carried out by bidirectional sequencing method. The result of sequencing was compared with known sequences in the Gen Bank database use BLAST at www.ncbi.nlm.nih.gov to identify the most similar sequence alignment.

3 RESULTS AND DISCUSSION

3.1 The chromium tolerance

The growth of the strain in modified Postgate B medium was shown by blackened medium. This change was caused by the growth of sulfate-reducing bacteria and the occurrence of sulfate reduction (Postgate, 1984). On sulfate reducing process, SRB will produce hydrogen sulfide which effective as indirect chemical Cr(VI) reductans under anoxic environmental condition (Cheung and Gu, 2007; Joutey *et al.*, 2015). The color change in the medium occurs due to the interaction between hydrogen sulfide and chromium metal which causes Cr(VI) reduction to Cr(III), the form of precipitate chromium. The reaction process will be caused the formation of black color and the increasing amount of chromium precipitation. Based on Gong, *et al.* (2018) research, production of hydrogen sulfide by sulfate-reducing bacteria occurs during the cell growth process, hydrogen sulfide able to increase Cr(III) deposition. Therefore the color of the media can be one of the indicators of chromium deposition on the media, the more concentrated the color of the media to eat more and more chromium metal that precipitates.

According to the OD (Optical Density) measurement results shown in Figure 1, different values of ODs occur between Strain KGP1 and

incubation times at different chromium concentrations. The result shown that the OD value of Strain KGP1 increasing from 0 days to 5 days. Figure 1 shown that Strain KGP1 was having capability resistance of chromium up to 5 ppm, but the isolate growth was depressed by chromium started on 3 ppm concentration of chromium. The highest OD values on the fifth day was 1.503 on the medium containing 1 ppm chromium concentration.

3.2 The chromium precipitation potency

Based on the test results of Figure 2, on day 0 when bacteria were inoculated on a medium containing chromium metal, there was a decrease of chromium concentration up to 0.519 ppm (37.75 %) in strain KGP1. The process of decreasing the chromium concentration is caused by the efforts of sulfate-reducing bacteria in defending themselves from the stressors present in the environment ie heavy metal chromium. According to Cardin *et al.* (2002), the presence of Cr(VI) in the media will lead to changes in the metabolism of lactate in the sulfate-reducing bacteria *Desulfovibrio vulgaris*. This will lead to inhibition of anabolic processes and growth with concomitant energy production. When bacterial cells are exposed to Cr(VI) on the media, the first thing that happens was the bacterial cell will lower the redox potential of the culture medium by catabolism lactate as an energy source to maintain conditions for survival.

The Strain KGP1 (Figure 2) showed that isolate was able to decrease the chromium concentration in the medium at days 5 up to 0.141 ppm (79 %) in Strain KGP1 compared by control. This is also followed by changes in media color and turbidity. The color change in the media occurs due to the interaction between Hydrogen sulfide and chromium metal which causes Cr(VI) to be Cr(III) reduction in the form of a precipitate. The reaction process will cause the formation of black color and the increasing amount of sediment. According to Gong *et al.* (2018) production of hydrogen sulfide in sulfate-reducing bacteria occurs during the cell growth process, hydrogen sulfide able to increase Cr(III) precipitation.

3.3 Sequencing 16S rRNA gene analysis

The Strain KGP1 has been identified using 16s rRNA gene analysis. Based Table 1, The Strain KGP1 was indentified as *Desulfovibrio aerotolerans* with percent identity 99 %. According to proposed guidelines for bacterial classification, strains with less than 97 % similarity in 16S rRNA gene sequence represent different bacterial species, but if it was above 97 % the organism can be considered the same species (Wang *et al.*, 2015).

4 CONCLUSION

Isolated SSK02 with a precipitation ability of chromium up to 79 % on the fifth day. Based on molecular invention using 16S rRNA, Strain KGP1 was indentified *Desulfovibrio aerotolerans* with percent identity 99 %.

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REFERENCES

- Ahn, H., Kim, J., Kim, W. J. (2017) Isolation and Characterization of Bacteriocin-producing *Pediococcus acidilactici* HW01 from Malt and Its Potential to Control Beer Spoilage Lactic Acid Bacteria. Food. Contr. 80: 59-66.
- Chardin, B., Dolla, A., Chaspoul, F., Fardeau, M. L., Gallice, P., and Bruschi, M. (2002) Bioremediation of Chromate: Thermodynamic Analysis of the Effect of Cr(VI) on Sulfate-Reducing Bacteria. J. Appl. Microbiol. Biotechnol. 60: 352-360.
- Cheung, K. H., and Gu, J. D. (2007) Mechanism of hexavalent chromium detoxification by microorganisms and bioremediation application potential: a review. Int. Biodeterior. Biodegradation. 59:8-15.
- Gan, M., Gu, C., Ding, J., Zhu, J., Liu, X., and Qiu, G. (2019) Ecotoxicology and Environmental Safety Hexavalent chromium remediation based on the synergistic effect between chemoautotrophic bacteria and sulfide minerals. Ecotoxicol. Environ. Saf. 173: 118-130.
- Gong, Y., Werth, C. J., He, Y., Su, Y., Zhang, Y., and Zhou, X. (2018) Intracellular versus extracellular accumulation of Hexavalent chromium reduction

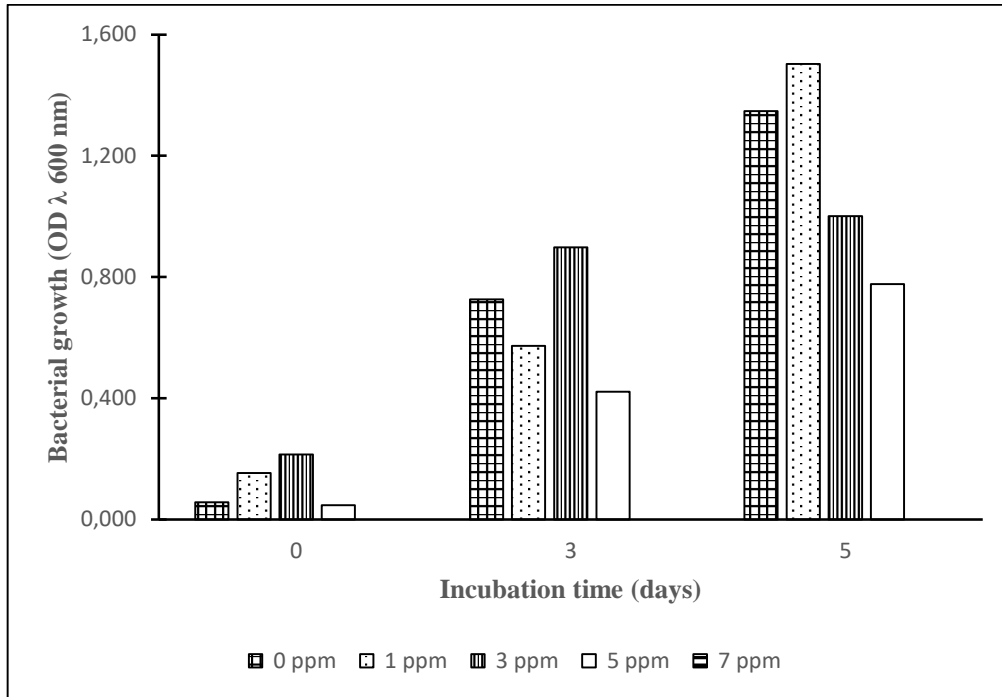


Figure 1: Growth strain KGP1 on medium postgate B containing different concentration of chromium on 5 days incubation.

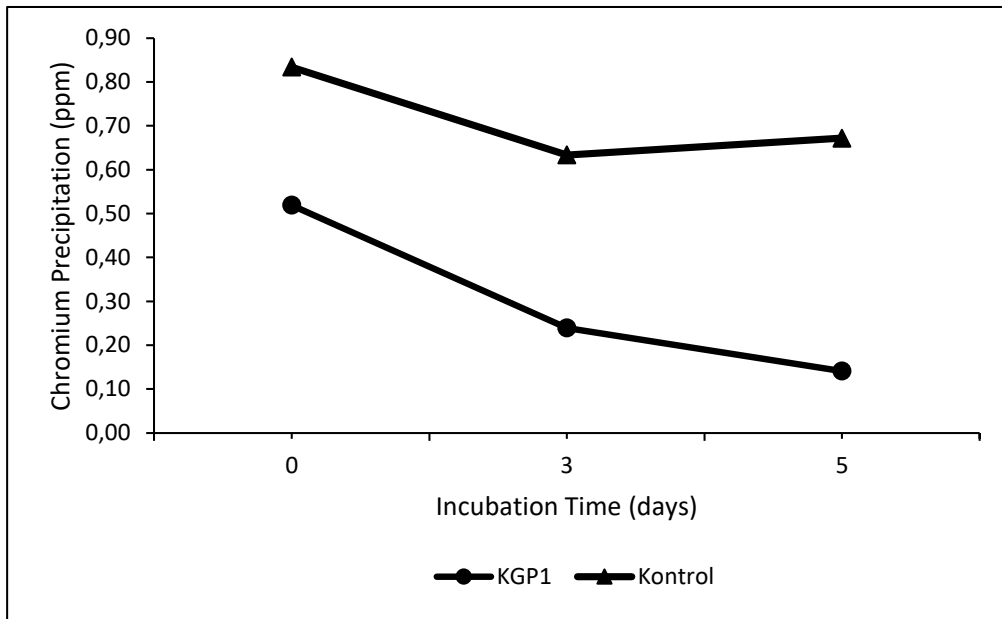


Figure 2: The chromium precipitation of strain KGP1.

Table 1: Result of sequencing 16S rRNA gene Analysis.

Strain	Homolog Species	Percent Identity	Accession
KGP1	<i>Desulfovibrio aerotolerans</i> strain MB28	99 %	MF098559.1
	<i>Desulfohalobium retbaense</i> strain MM25	94 %	MG907121.1
	<i>Desulfotalea artica</i> strain MM32	9 %	MG928386.1

- products by *Geobacter sulfurreducens* PCA. *Environ. Pollut.* 240: 85-492.
- He, Y., Gong, Y., Su, Y., Zhang, Y., and Zhou, X. (2019) Chemosphere Bioremediation of Cr(VI) contaminated groundwater by *Geobacter sulfurreducens*: Environmental factors and electron transfer flow studies. *Chemosphere.* 221: 793-801.
- Hussain, Ali, Hasan, A., Javid, A., and Qazi, J. I. (2016) Exploited application of sulfate-reducing bacteria for concomitant treatment of metallic and non-metallic wastes: a mini review. *Review article 3. Biotech.* 6: 19.
- Jafari, M. R., Hosseini, S. M., Musavi Andani, S. M. J., Hamidi, A. R., and Madaeni, S. S. (2017) Ionic behavior modification of cation exchange ED membranes by using CMC-co-Fe₃O₄ nanoparticles for heavy metals removal from water. *J. Iran. Chem. Soc.* 14: 1011-1021.
- Jobby, R., Jha, P., Yadav, A. K., and Desai, N. (2018) Biosorption and biotransformation of hexavalent chromium [Cr (VI)]: A comprehensive review. *Chemosphere.* 207: 255-266.
- Joutey, Nezha, T., Sayel, H., Bahafid, W., and El Ghachtouli, N. (2015) Mechanisms of Hexavalent Chromium Resistance and Removal by Microorganisms. Whitacre, D. M. (ed.) in *Rev. Environ. Contam. Toxicol.* 233: 45-63.
- Márquez-reyes, J. M., López-chuken, U. J., Valdegonzález, A., and Luna-olvera, H. A. (2013) Removal of chromium and lead by a sulfate-reducing consortium using peat moss as carbon source. *Bioresour. Technol.* 144: 128-134.
- Nurhikmayani, R., Daryono, B. S., and Retnaningrum, E. (2019) Isolation and molecular identification of antimicrobial-producing Lactic Acid Bacteria from *chao*, South Sulawesi (Indonesia) fermented fish product. *Biodiverditas.* 20: 1063-1068.
- Pagnanelli, F., Viggi, C. C., Cibati, A., Uccelletti, D., Toro, L., and Palleschi, C. (2012). Biotreatment of Cr (VI) contaminated waters by sulphate reducing bacteria fed with ethanol. *J. Hazard. Mater.* 199-200: 186-192.
- Postgate, J.R., (1984) *The Sulphate Reducing Bacteria*, 2nd Ed., University Press, Cambridge, UK, 20-30.
- Retnaningrum, E., and Wilopo, W. (2017) Removal of Sulphate and Manganese on Synthetic Wastewater in Sulphate Reducing Bioreactor Using Indonesian Natural Zeolite. *Indones. J. Chem.* 7: 203-210.
- Vi, S. C., Fern, P. M., and Figueroa, L. I. C. (2018) Bioremediation strategies for chromium removal: Current research, scale-up approach and future perspectives, *Chemosphere.* 208.
- Wang, X., King, J., and Mayer, L. W. (2015) A phylogenetic Perspective on Molecular Epidemiology. In *Molecular Medical Microbiology*. 2nd Edition. Volume 1. Academic Press.