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In-Vitro Chromium Bioremediation Potential Using Bacteria Consortium from Chromium Polluted Soil

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ABSTRAK

Penelitian ini ditujukan untuk mendapatkan formulasi konsorsium bakteri yang memadai untuk penanggulangan limbah krom pada lingkungan, dimana bakteri Bacillus sp., Bacillus licheniformis dan Bacillus anthracis yang diambil dari lingkungan tercemar krom memiliki potensi sebagai agen bioremediasi limbah krom. Penggunaan bioremediasi yang dinilai efektif dan efisien dalam penanggulangan limbah dan telah menjadi solusi untuk membersihkan lingkungan dari pencemaran tanpa harus menggunakan bahan kimia berlebihan. Metode dalam penelitian ini menggunakan metode kuantitatif dengan pendekatan eksperimental. Sampel diisolasi dari tanah kemudian diseleksi menurut resistensinya terhadap kandungan krom, lalu diidentifikasi secara biokimia dan molekuler, setelah itu sampel diuji kandungan krom pada awal pengamatan hingga akhir pengamatan pada jam ke 12. Temuan penelitian ini menunjukkan konsorsium bakteri Bacillus sp. dan Bacillus anthracis memiliki sinergitas yang paling baik yang ditandai dari terlihatnya pertumbuhan koloni streak. Pada uji reduksi kandungan krom, konsorsium Bacillus sp. dan Bacillus anthracis memiliki persentase reduksi logam krom yang paling tinggi, mencapai 26% sedangkan persentase dari konsorsium Bacillus licheniformis dan Bacillus anthracis memiliki persentase 9.4% menjadi yang paling rendah dari seluruh konsorsium yang diuji. Dari penelitian ini disimpulkan konsorsium Bacillus sp. dan Bacillus anthracis merupakan formulasi konsorsium terbaik dalam mereduksi kandungan krom dari seluruh konsorsium yang diuji dalam penelitian ini, menjadikan konsorsium Bacillus sp. dan Bacillus anthracis sebagai konsorsium potensial untuk diaplikasikan dalam sistem bioremediasi limbah krom.

Kata kunci: Limbah krom, Konsorsium bakteri, Reduksi Krom, Bakteri resisten krom, Bioremediasi limbah krom

ABSTRACT

This research aims to obtain an adequate formulation of bacterial consortium for chromium waste management, where the bacteria *Bacillus* sp*., Bacillus licheniformis* and *Bacillus anthracis* obtained from the chromium polluted environment have the potential as bioremediation agent of chromium waste. The use of bioremediation is considered to be effective and efficient in waste management and has become the solution to cleanse the environment from pollution without excessive use of chemical substances. The methods used in this research are quantitative methods with an experimental approach. Sample isolated from soils and then selected based on their resistance toward chromium stress, the sample then identified using biochemical and molecular method, after that the sample chromium content is tested in the beginning of the observation until the end of observation at the 12th hour. This research shows that the bacterial consortia of *Bacillus* sp*.* and *Bacillus anthracis* have the best synergy that is marked by the growth of streak colonies that are clearly seen. In the chromium reduction test, the *Bacillus* sp*.* and *Bacillus anthracis* consortia have the highest chromium reduction percentage, reaching 26% meanwhile the percentage of *Bacillus licheniformis* and *Bacillus anthracis* consortia have the reduction percentage of 9.4% marked as the lowest out of all the tested consortia. The conclusion of this research is the consortia of *Bacillus sp.* and *Bacillus anthracis* is the best consortia in chromium reduction property out of all consortia that is tested in this research, this shows that the *Bacillus* sp*.* and *Bacillus anthracis* consortia as the highest potential consortia to be applied in chromium waste bioremediation systems.

Keyword: Chrome waste, Bacterial consortium, Chrome reduction, Chrome resistant bacteria, Chrome waste bioremediation

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1. INTRODUCTION

Chrome metal is one of the heavy metals commonly found in nature and one of the abundant natural heavy metals of earth. The oxidation phase of the chrome metals varies, with the most stabilized valencies and commonly found in nature as macronutrient is (Cr (III)) (Reif & Murray, 2024). The derivative of trivalent chromium is hexavalent chromium (Cr (VI)) is the result of trivalent chromium oxidation. This hexavalent chromium is considered toxic and carcinogenic, the soluble ions are capable of penetrating cell wall and dangerous for any organism (Coetzee *et al*., 2020).

In nature, hexavalent chromium formed due to the oxidation of trivalent chromium with other substances that naturally exist or due to the interaction with another polluting agent (Mandiwana *et al*., 2007). Chromium pollutions are gradually increasing due to the growth and expansion of industrial sector (Sun *et al*., 2022). The usage of chromium on the industries sector such as tanneries, metallurgies and textiles which mostly using hexavalent chromium on their production processes, resulting the increase of chromium pollution (Iskra & Fedoruk, 2022). Unregulated chrome waste removal from the industries that uses chrome in their production processes are also the reason of the increasing in chromium pollution (Titah *et al*., 2021).

Chromium waste management through bioremediation is seen to give effective result in waste removal and nature preservation (Coetzee *et al*., 2020). Bacteria used in bioremediation due to their ability to adapt in polluted environment (Dell'Anno *et al*., 2021). The bacteria that isolated from hexavalent chromium polluted soil tends to be resistant to chromium pollution and have the ability to degrade chromium (Adamu & Adamu, 2022).

The usage of bacterial consortia in bioremediation is seen as the more effective method due to the difference in ability of each bacteria to degrade the polluting agent (Paneerselvam *et al*., 2013). There are 20% increase in bioremediation time, making the duration for bioremediation shorter with the same amount of degraded chromium pollutant (Asri *et al*., 2023). The efficiencies of each bacterial consortia are different due to their difference in characteristics, potential and compatibility of each consortium (Adamu & Adamu, 2022).

This research aims to identify the isolated bacteria from chrome polluted soil and get the combination of bacteria species that have potential to degrade the chromium content to be used in chrome waste management.

2. METHODOLOGY

2.1. Research Location

This research takes place in Environmental Research Laboratory of Biology at Fakultas Pendidikan Matematika dan Ilmu Pengetahuan Alam (FPMIPA) Universitas Pendidikan Indonesia.

2.2. Materials and Instrument

The instrument used in this research are petri dish, test tube, erlenmeyer flask, sample bottle, disposable cuvette, measuring glass, beaker glass, inoculating loop, spiritus burner, object glass, microplate 96 well, digital scale, magnetic stirrer hotplate, autoclave, shaker, incubator, micropipette, micropipette *tips,* microscope, spectrophotometer UV-vis, *microplate reader,* PCR Veriti 96 well fast thermal cycler (applied biosystems), UV transilluminator, electrophoresis *kit*

The materials used are nutrient agar (NA), nutrient broth (NB), $K_2Cr_2O_7$, crystal violet, safranin, spiritus, malachite green, alcohol 96%, distilled water, 1,5-diphenylcarbazide (DPC), H_2SO_4, buffer phosphate, buffer tis-EDTA (TE) 1X, agarose powder, loading dye, DNA ladder (marker), goTaq green PCR master mix 2X, nuclease-free water, primer forward 27F & reverse 1492 R, GelRed nucleic acid gel stain.

2.3. Working Procedure

a. Bacteria isolation

Bacteria sampled from chrome polluted soil then isolated by dissolving and diluting the soil sample with the ratio of 1mg soil / 10 ml distilled water eight times from 10^{-1} to 10^{-8}. The chosen isolated sample taken from the last three dilution from 10^{-6} to 10^{-8} and inoculated in NA media then incubated at 37°C for 24 hours. The grown bacteria only the ones with chromium resistant characteristics.

b. 16s rRNA Species Identification

The species of the bacteria is identified by extracting and isolating the bacteria DNA then the DNA sample is amplified using PCR (Polymerase Chain Reaction) to amplify the 16s region. The amplified sample then visualized using electrophoresis method to see the amplification quality. The sample then sequenced to get the DNA nitrogen base sequence then the base sequence analysed using MEGA 12 software to get the data in the FASTA format. The FASTA sequence then analysed using BLAST (Basic Local Alignment Search Tool) to see which species that have similar nitrogen base sequence.

c. Bacteria Consortium Compatibility Test

The bacteria compatibility in each consortium is tested by seeing each interaction of the bacteria using the cross-streak method to see the lysis area marked by Inhibition zone, marked by a clear area around the intersection, the existence of the inhibition zone implying the bacteria are not compatible (Denaya *et al*., 2021)

d. Chromium Reduction by Consortium

Chromium reduction test measured by using DPC method to detect the hexavalent chromium content in the medium

3. RESULT AND DISCUSSION

3.1. Bacteria Isolation

The isolated bacteria showing 3 different colonies with a distinct characteristic in each colony as shown at Figure 1.

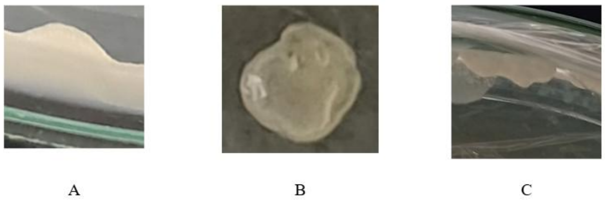

Figure 1. Bacteria Colony Morphology

Bacteria A having irregular shaped colony with lobate type margin, no elevation (flat) and milky white colour. Bacteria B have circular shaped colony, clear white colour with entire type margin dan and have a raised colony. Bacteria C having irregular shaped colony with milky white colour and undulate type margin, the colony also shows some raised elevation. The difference in colony morphology caused by varies gene expression and different metabolism of each bacteria (Maeda *et al*., 2018).

3.2. 16s rRNA Species Identification

16s rRNA analysed using BLAST and shows that all of the sample belong in the same genus taxonomy level with the genus *Bacillus.* The detail of every species of the sample shown in the Table 1.

BLAST result shows the sample of B and C are identified to the species level, but the sample A only identified to genus level. Non-specific identification happens due to bad PCR quality where only partial of the reverse or forward sample, such as in sample A where only the forward primer that partially amplified. Watanabe et al (2021) says that unspecified identification mostly caused by bad PCR result.

Table 1. BLAST Analysis Result

Sample	Closest Relatives	Similarity	Notes
A	*Bacillus pacificus* strain MCCC 1A06182 (NR_157733.1)/ *Bacillus paramycoides* strain MCCC 1A04098 (NR_157734.1)/ *Bacillus paranthracis* strain MCCC 1A00395 (NR_157728.1)/ *Bacillus albus* strain MCCC 1A02146T (NR_157729.1)/ *Bacillus cereus* ATCC 14579 (NR_074540.1)	99,84%	639 bp (partial 27 f) (genus level, species unidentified, written as *Bacillus* sp.)
B	*Bacillus licheniformis* strain DSM 13 (NR_118996.1)	99,86%	1402 bp
C	*Bacillus anthracis* strain ATCC 14578 (NR_041248.1)	99,60%	519 bp (partial)

Phylogeny tree shown in Figure 2 shows that Sample A bacteria *Bacillus* sp. is closely related to sample C *Bacillus anthracis*. Indicating that bacteria with similarity in nitrogen base sequence with sample A (*Bacillus pacificus, Bacillus paramycoides, Bacillus paranthracis, Bacillus albus, Bacillus cereus*) are closely related to sample C bacteria (*Bacillus anthracis*). The bootstrap of the two sample shows the value as 100, indicating the confidence level of the two bacteria relation are very strong. Both of the bacteria sample still have relation with bacteria sample B (*Bacillus licheniformis*) but does not seem to be closely related.

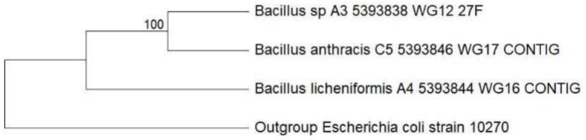

Figure 2. Interspecies Phylogeny Relation Tree

Bacteria species of sample A (*Bacillus pacificus, Bacillus paramycoides, Bacillus paranthracis, Bacillus albus, Bacillus cereus*) included in *Bacillus cereus* group, same as the sample C bacteria (*Bacillus anthracis*) that also included in the *Bacillus cereus* group (Liu *et al.,* 2017). Sample B bacteria (*Bacillus licheniformis*) is a part of *Bacillus subtilis* group which considered as the distant relatives of *Bacillus cereus* group (Iqbal *et al*., 2022).

3.3. Bacteria Consortium Compatibility Test

There is total 4 consortium used in this research, Formula A (*Bacillus* sp. – *Bacillus licheniformis*), Formula B (*Bacillus* sp. - *Bacillus anthracis*), Formula C (*licheniformis* – *Bacillus anthracis*) and Formula D which is the combination of all the bacteria used in this research (*Bacillus* sp. – *Bacillus licheniformis* – *Bacillus anthracis*). But for the compatibility test only testing the compatibility of Formula A, Formula B and Formula C, while for Formula D, the compatibility is concluded from the result of each species interaction.

The combination of bacteria in consortium is tested for their compatibility to see how the bacteria interact in the consortium as shown in Figure 3.

Bacillus sp. - *Bacillus licheniformis* *Bacillus* sp. - *Bacillus anthracis* *Bacillus licheniformis* - *Bacillus anthracis*

Figure 3. Consortium Compatibility

The most compatible consortium shown by Formula B where no inhibition zone is seen on the intersection area. As for the other consortium, there is no growth shown in Formula A medium and there is clear area around the intersection of Formula C bacteria medium, implying there is inhibition zone. This result shows that bacteria combination of Formula A and Formula C are not synergic with each other.

From the test we could also conclude on how the three bacteria would interact in Formula D, where only two of the bacteria that will show synergy, indicating that the three bacteria might not synergise if combined.

3.4. Chromium Reduction by Consortium

The result of chrome reduction by consortium in Figure 4 shows that bioremediation by Formula B consortium have a better chromium reduction quantity and quality compared to the other consortium.

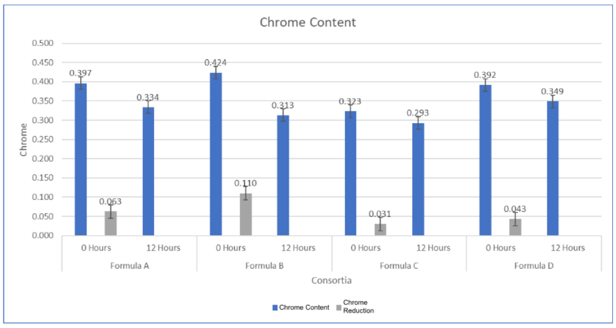

Figure 4. Chrome Reduction by Each Consortia

From the four consortia used in the research, Formula B consortium shows higher potential in chromium reduction and higher chromium reduction percentage compared to the other consortium. Figure 5 shows the percentage of each consortium in chromium bioremediation, where the Formula B consortium shows the highest percentage of all reaching 26% of chrome removal.

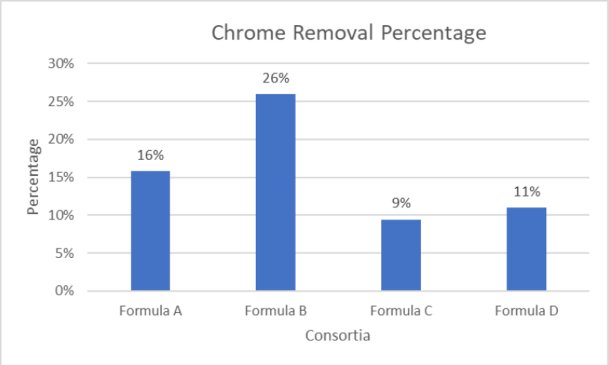

Figure 5. Consortia Chrome Reduction Percentage

The other consortium shows very low percentage with Formula C consortium showing the removal percentage of 9,4% not even reaching 10% of chromium removal. The other consortia reaching above 10% of chromium removal but none of the consortium reaching 20% chromium removal, only Formula A shows 15,8% of chrome removal, making it the second highest and Formula C shows 11% making it the second lowest potential.

The removal percentage could be affected by compatibility of bacteria in consortium, where the Formula B bacteria are close relatives, making it more compatible than the other three consortium. Lin *et al* (2023) on their research mentioned that the compatibility of bacteria in consortium could boost the metabolism activity that reduces chromium. Dwisandi *et al* (2021) also mentioned that a good consortium synergy would lead to a more effective heavy metals degradation, because the interaction of each bacteria could affect their enzyme activities that the bacteria with low synergy could inhibit each other growth and metabolism, making the heavy metals degradation less stable.

4. CONCLUSION

Consortium formula with the highest potential in this research is shown by Formula B (*Bacillus* sp. – *Bacillus anthracis*). The good synergy of the two bacteria made the two bacteria more active in reducing chromium content in the medium. This consortium formula could be developed to be used in chromium waste management to be applied to waste management of the industries that uses chromium in their production. In the future, improvement such as the development of standard curve to see the exact viable bacteria cell in every growth phase and chromium content measurement using AAS (Atomic Absorption Spectroscopy) to see the impact of chromium exposure toward bacteria cell structure.

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