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# Isolation and Selection of Indigenous Bacteria Resistant Nickel and Chromium from Nickel Post Mining Land in Pomalaa, Indonesia

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**Abstract:-Nickel is used for various commercial and industrial needs. The main problem currently arising from the nickel post mining land in Pomalaa is the high level of heavy metals Ni and Cr in soil. The use of microorganisms to reduce the toxic metals on contaminated soils is environmentally friendly. This research was conducted to obtain indigenous bacteria that were resistant to the content of Ni and Cr at a concentration of 10 ppm. The research methods carried out to achieve the research objectives are isolation and selection. The selection results from 96 indigenous single bacteria that were regenerated on solid NB media obtained 41 bacterial isolates which had density and the ability to grow very fast (+++) i.e. bacteria that could grow at <2 days, 17 bacterial isolates rather quickly (++) namely bacteria that are able to grow within 2-3 days, 22 bacterial isolates are rather slow (+) i.e. bacteria that grow in more than 3 days and 16 bacterial isolates do not grow. The selected indigenous bacteria, further can be used as biological agents in reducing the toxic effects of metals on contaminated soil.**

***Keywords:-*** *indigenous bacteria; chromium; nickel; mining.*

## I. INTRODUCTION

Nickel mines in Indonesia are in West Kalimantan, Maluku, Papua, South Sulawesi, Central Sulawesi and Southeast Sulawesi. Nickel is used for various commercial and industrial needs. In recent years, along with the increasing number of mining activities in various regions in Indonesia, mining activities have begun to face problems, one of which is environmental pollution. This condition is faced by nickel post mining land in pomalaa, Southeast Sulawesi.

The main problem currently faced by nickel post mining land in pomalaa is the toxicity of heavy metals nickel (Ni) and chromium (Cr) in soil. This research was based on preliminary research [1], from the results of the study it was known that occurred toxicity of heavy metals Ni and Cr on nickel post mining land in Pomalaa. High Ni and Cr concentrations in the soil can damage the environment both soil and water and inhibit plant growth because it is Toxic. The toxicity limit for plants for Ni is 100 ppm and Cr is 20 ppm [2].The handling of waste disposal at the nickel post mining land in pomalaa is revegetation, but the revegetation effort has not been able to restore soil fertility. The results of plant nutrient analysis on revegetated land showed deficiencies of K, Ca, Fe, Cu, and Mn [1]. Nutrient deficiency is one indicator of low soil fertility. Therefore improvements in rehabilitation and reclamation are absolutely necessary. One of the ways is to use microorganisms as reducer agents.

The use of microorganisms to reduce metal toxic effects on contaminated soil has been a concern of researchers because it is more environmentally friendly [3]. The bacterial combination from P.MA with a six-day incubation period reduced the Cr (IV) content by 61.59% [4]. Microbial consortium PG 65-06 (A): PG 97-02 (B): MR 1.12-05 (C): A1 (D) ratio of 1: 1: 1: 1 resulted in a decrease in the distribution coefficient of the Pb exchange rate of 21% originally 19.3 mg / kg to 15.91 mg / kg and an increase in residual phase of Pb of 146% which was originally 7.77 mg / kg to 17.00 mg / kg [5]. Chlorella vulgaris has the ability as bioremediator of Cr, Chlorella vulgaris has polyamine which acts to protect or as a protection in a contaminated environment [6]. This research was conducted to obtain indigenous bacteria that were resistant to the content of Ni and Cr at a concentration of 10 ppm. The selected indigenous bacteria, further can be used to reduce the toxic effects of metals on contaminated soil.

## II. METHODS

Indigenous bacteria obtained from soil at nickel post mining land in pomalaa. Soil sample were taken from three locations, namely the southern mine (TS), the middle mine (TT) and the northern mine (TU) at depths of 0-30cm, 30-60cm and 60-90cm using soil drills. Soil samples are then put into paper bags, composited and stored at room temperature. Subsequent research was carried out at the Research Center for Biotechnology at Hasanuddin University, Makassar. The Isolation begins by weighed 10 grams of soil, then the soil is diluted serially in $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ with sterile aquadest. Taken 50 ml of the suspension solution at $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilutions which then inoculated on a petri dish containing solid NB media. This stage is done to see the ability of bacterial adaptation on NB media as the isolation media. Furthermore, bacteria which had density and the ability growing faster than others, regenerated on selective media namely solid NB media containing 10 ppm of $NiCl_2$ and $CrCl_2$ solution. This stage was carried out to obtain adaptive bacteria with nickel and chromium, and then the culture of bacteria was incubated for 2-3 days at $30^0$C. After visible growth of bacteria on selective media the research continued with the purification. the Purification was carried out on solid NB

media to obtain single bacterial colonies. The single bacterial colonies selected based on growth speed and growing density.

## III. RESULT AND DISCUSSION

Soil samples from southern mine (TS) divided into five locations namely G, R, S, Q and RST, middle mine (TT) divided into four locations namely TLB, TTC, TLE, TT virgin and northern mine (TU) divided into three locations namely I, II and III. The dilution stage is carried out to reduce bacterial density at the isolation stage. Isolation bacteria from nickel post mining land was taken from $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilutions. At this stage all indigenous bacteria from nickel post mining land can adapted and grown on solid NB media. Furthermore, isolation was carried out on selective NB media containing 10 ppm $NiCl_2$ and $CrCl_2$. Indigenous bacterial isolation on selective NB media is presented in Table 1.

| Soil samples | Dilution $10^{-3}$ | | Dilution $10^{-4}$ | | Dilution $10^{-5}$ | |
|---|---|---|---|---|---|---|
| | Media $NiCl_2$ | Media $CrCl_2$ | Media $NiCl_2$ | Media $CrCl_2$ | Media $NiCl_2$ | Media $CrCl_2$ |
| G | + | + | + | + | + | + |
| R | + | + | + | - | + | - |
| S | + | + | - | + | + | - |
| Q | + | - | + | - | + | + |
| RST | + | + | + | - | - | - |
| TLB | + | + | + | + | - | + |
| TTC | + | + | - | + | + | + |
| TLE | + | + | + | + | + | + |
| TT Virgin | + | + | + | + | + | + |
| I | + | + | + | + | - | - |
| II | + | + | - | + | + | + |
| III | + | + | + | + | - | + |

Table 1. Indigenous bacterial isolation on selective NB media

Isolation bacteria on selective NB media in the $10^{-3}$, $10^{-4}$ and $10^{-5}$ dilution series showed that bacterial colonies that was able to adapt and grow from all dilution series containing 10 ppm $NiCl_2$ and $CrCl_2$ solutions in the media were bacteria from samples G, TLE and virgin TT. It was found that not all bacterial samples were able to grow on media containing nickel and chromium. The results of this reaserch prove that the level of bacterial tolerance to heavy metals varies. This difference can be caused by the type of bacteria itself. Then all bacteria that grew on selective NB media containing 10 ppm nickel and chromium were regenerated on NB media to obtain single bacterial colonies. The single bacterial colonies selected based on growth speed and growing density. The growth speed of isolates was divided into three groups, very fast (<2 days), fast (2-3 days), slow (> 3 days) and not growing. The growth speed of single indigenous bacteria on NB media is presented in Table 2.

| Soil Samples | Isolate | Growing Speed | | | |
|---|---|---|---|---|---|
| | | < 2 Day | 2-3 Day | >3 Day | Not growing |
| G | Gni-31 | +++ | | | |
| | Gni-32 | +++ | | | |
| | Gcr-3 | | | + | |
| | Gni-41 | +++ | | | |
| | Gni-42 | +++ | | | |
| | Gcr-4 | | ++ | | |
| | Gni-51 | +++ | | | |
| | Gni-52 | +++ | | | |
| | Gcr-5 | +++ | | | |
| R | Rni-31 | +++ | | | |
| | Rni-32 | +++ | | | |
| | Rni-33 | +++ | | | |
| | Rni-35 | +++ | | | |
| | Rcr-3 | +++ | | | |
| | Rni-41 | +++ | | | |
| | Rni-42 | +++ | | | |
| | Rcr-4 | | | | - |
| | Rni-5 | | ++ | | |
| | Rcr-5 | | | | - |
| S | Sni-31 | +++ | | | |
| | Sni-32 | +++ | | | |
| | Sni-34 | +++ | | | |
| | Scr-3 | +++ | | | |
| | Sni-4 | | | | - |
| | Scr-4 | +++ | | | |
| | Sni-5 | +++ | | | |
| | Scr-5 | | | | - |
| Q | Qni-31 | +++ | | | |
| | Qni-32 | +++ | | | |
| | Qcr-3 | | | | - |
| | Qni-41 | +++ | | | |
| | Qni-42 | +++ | | | |
| | Qcr-4 | | | | - |
| | Qni-5 | +++ | | | |
| | Qcr-5 | +++ | | | |
| RST | RSTni-31 | | ++ | | |
| | RSTni-32 | | ++ | | |
| | RSTcr-3 | | | + | |
| | RSTni-4 | | | + | |
| | RSTcr-4 | | | | - |
| | RSTni-5 | | | | - |
| | RSTcr-5 | | | | - |
| TLB | TLBni-31 | | ++ | | |
| | TLBni-32 | +++ | | | |
| | TLBni-33 | +++ | | | |
| | TLBni-34 | | ++ | | |
| | TLBcr-31 | | ++ | | |
| | TLBcr-32 | | ++ | | |
| | TLBcr-33 | | ++ | | |
| | TLBni-4 | +++ | | | |
| | TLBcr-4 | | ++ | | |
| | TLBni-5 | | | | - |
| | TLBcr-5 | | | + | |

| | | | | | |
|---|---|---|---|---|---|
| TTC | TTCni-3 | | | + | |
| | TTCcr-31 | | ++ | | |
| | TTCcr-32 | | ++ | | |
| | TTCcr-33 | +++ | | | |
| | TTCcr-34 | +++ | | | |
| | TTCcr-35 | +++ | | | |
| | TTCcr-36 | +++ | | | |
| | TTCni-4 | | | | - |
| | TTCcr-4 | | | | - |
| | TTCni-5 | | | + | |
| | TTCcr-5 | +++ | | | |
| TLE | TLEni-3 | | ++ | | |
| | TLEcr-3 | | | + | |
| | TLEni-4 | | | + | |
| | TLEcr-4 | | | + | |
| | TLEni-5 | | | + | |
| | TLEcr-51 | +++ | | | |
| | TLEcr-52 | +++ | | | |
| TT *Virgin* | *virgin* ni-3 | | | + | |
| | *virgin* cr-31 | +++ | | | |
| | *virgin* cr-32 | +++ | | | |
| | *virgin* ni-4 | | | + | |
| | *virgin* cr-4 | | | + | |
| | *virgin* ni-5 | | | + | |
| | *virgin* cr-5 | | ++ | | |
| I | Ini-3 | | | + | |
| | Icr-3 | | | + | |
| | Ini-4 | | | + | |
| | Icr-4 | | ++ | | |
| | Ini-5 | | | | - |
| | Icr-5 | | | | - |
| II | IIni-3 | | ++ | | |
| | IIcr-3 | +++ | | | |
| | IIni-4 | | | | - |
| | IIcr-4 | +++ | | | |
| | IIni-5 | | ++ | | |
| | IIcr-5 | | | + | |
| III | IIIni-3 | | | + | |
| | IIIcr-3 | | | + | |
| | IIIni-4 | +++ | | | |
| | IIIcr-4 | | | + | |
| | IIIni-5 | | | | - |
| | IIIcr-5 | | | + | |

Table 2. The growth speed of single indigenous bacteria on NB media

The selection results from 96 indigenous single bacteria that were regenerated on solid NB media obtained 41 bacterial isolates which had density and the ability to grow very fast (+++) i.e. bacteria that could grow at <2 days, 17 bacterial isolates rather quickly (++) namely bacteria that are able to grow within 2-3 days, 22 bacterial isolates are rather slow (+) i.e. bacteria that grow in more than 3 days and 16 bacterial isolates do not grow. Based on the results of the above research, indigenous bacteria originating from mined land are very suitable to be used as biological agents to reduce the toxic effects of metals because they have high growth speeds and adaptability to heavy metal contaminated soils, especially the toxic effects of Ni and Cr metals. The mechanism of microbial tolerance to metals is by means of metal efflux outside the cell [7]. The ability of microorganisms to adapt to contaminated environmental conditions has been widely investigated [8]. Diagram of indigenous single bacterial growth on solid NB media is presented in Figure 2.

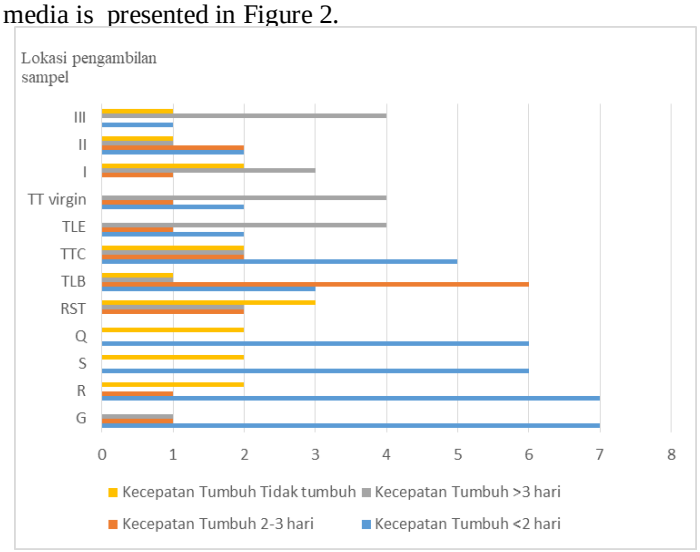


Figure 2. Diagram of indigenous single bacterial growth on solid NB media

The growth diagram in Figure 2 shows that most sampling locations have indigenous bacteria that have the potential to be further tested as biological agents for reducing heavy metals. The use of bacteria as a heavy metal reducing agent is an effective, efficient, easy and inexpensive technique for cleaning soil and water contaminated by toxic compoundsunds [9] [10].

## IV. CONCLUSION

The selection results from 96 indigenous single bacteria that were regenerated on solid NB media obtained 41 bacterial isolates which had density and the ability to grow very fast (+++) i.e. bacteria that could grow at <2 days, 17 bacterial isolates rather quickly (++) namely bacteria that are able to grow within 2-3 days, 22 bacterial isolates are rather slow (+) i.e. bacteria that grow in more than 3 days and 16 bacterial isolates do not grow.

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