Bioremediation and Identification of Potential Indigenous Bacteria from Field of Iron Mining and Pesticide Application

Sunil Kumar Jha

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 12
Issue: 04

Res Jr of Agril Sci (2021) 12: 1372–1376

CARAS

Res. Jr. of Agril. Sci. (July-Aug 2021) 12(4): 1372–1376
www.rjas.org

Full Length Research Article

Bioremediation and Identification of Potential Indigenous Bacteria from Field of Iron Mining and Pesticide Application

Sunil Kumar Jha*[1]

Received: 09 May 2021 | Revised accepted: 13 Jul 2021 | Published online: 04 Aug 2021
© CARAS (Centre for Advanced Research in Agricultural Sciences) 2021

ABSTRACT

The study was carried out to isolate bacteria from a field having history of mining and for their identification biochemical test were performed. The mining land was later used for agriculture. History with mining led to degradation of soil fertility. Bacteria are biological agents that are environmental friendly, which can be used to reduce heavy metal toxicity and increase soil fertility on post-mining land. On the basis of biochemical analysis ten indigenous bacterial colony were isolated which were resistant to pesticide and iron toxicity were found to belong to the genus *Bacillus sp*. is gram-positive bacteria that do not have the catalase enzyme, do not have the ability to use carbohydrates and produce endospores in their life cycle while indigenous bacteria from the genus *Clostridium sp., Streptomyces sp.* is a gram-positive bacteria which in part has the enzyme catalase, all of which have the ability to use carbohydrates and only some of them produce endospores.

***Key words*:** Agriculture, Bioremediation, *Bacillus*, Catalase, Endospore

Joda is a iron producing sub-district in Odisha, India. The management of iron post-mining land in Joda is re-vegetation, various types of plants are planted in post-mining land, but the re-vegetation effort is not enough to restore soil fertility. The results of the nutrient analysis of plants on re-vegetated land showed deficiencies of K, Ca, Fe, Cu, Mn. The results of the analysis of heavy metals are known to occur in the toxicity of nickel (Ni) and Chromium (Cr) [1]. The toxicity limit for plants for iron is 125 ppm, nickel is 100 ppm and chromium is 20 ppm [2]. The limit of iron concentration seldom found at concentration greater than 10ppm, nickel metal content in the soil, water, and the human body is 4-80 ppm [3]. Iron, Nickel and chromium are included in heavy metals whose availability in high concentrations in soil can be toxic so they degrade soil fertility and inhibit plant growth. High concentrations of heavy metals will inhibit growth, change morphology, and interfere with the metabolism of organisms in vitro [4].

Indigenous bacteria may be an indicator of heavy metal presence and bacteria isolated from their habitat and then cultured in vitro. One effort to reduce heavy metal toxicity and increase soil fertility on post-mining land is by utilizing indigenous bacteria in the mine. According to EPA 2010 parameterization is defined as a spontaneous process in which the microbial process is used to degrade, break down, or transform hazardous contaminants into less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from environmental media [5]. Bacteria in site habitats contaminated with heavy metals develop several mechanisms of tolerance to heavy metals, most of the mechanisms of microbial tolerance to metals are by metal efflux outside the cell [6]. Bacteria isolated from an environment contaminated with heavy metals have resistance to heavy metals around them [7]. The use of bacteria to degrade polluted soil has been widely studied. Flavobacterium, Pseudomonas carry out bioremediation of total petroleum hydrocarbons [8]. Bacillus sp. is capable of degrading toluene-contaminated soil [9]. Bacillus bacteria have a good ability to degrade heavy metals such as Fe, Zn, Ni, Cu, and Pb [10]. Biochemical tests and identification were carried out to characterize pure culture from isolation through its morphological and physiological properties.

The results of biochemical tests can be a recommendation in future research to obtain potential bacteria as biological agents that can be used to reduce metal toxicity on post-mining land and increase soil fertility. This research is expected to add the treasure of science, especially in the field of environmental biotechnology, where the use of indigenous bacteria can be one of the technological choices that can restore contaminated soil conditions through biological processes that are economically and functionally able to compete with other remediation technologies.

MATERIALS AND METHODS

* Sunil Kumar Jha
✉ skjhaenv@gmail.com

[1] Laboratory of Environmental Biotechnology, University Department of Botany, Ranchi University, Ranchi, Jharkhand, India

Soil sample were collected from field having history of iron mining and pesticide application from Joda, Odisha. This study began with an analysis of soil samples where indigenous bacteria were isolated. Furthermore, the research is divided into two stages. The first stage is a gram test (3% KOH), and a biochemical characteristic test. Biochemical characteristic tests include catalase (3% H_2O_2), Oxidation-Fermentation (O-F) test, and endospores test. The second stage is morphological observation and identification.

Isolation of Bacteria from soil at the sampling location. The bacterial isolate were characterized based on morphological and biochemical characteristic [11] was done in environmental biotechnology lab of University department of Botany, Ranchi University, Ranchi, Jharkhand. All together 10 isolates were isolated in pure form. The colony characteristics of the selected bacterial isolate size, shape, color, margin, elevation, consistency and opacity were observed and recorded. The selected bacterial isolate was gram stained and observed under the light microscope at 100X magnification to study the cell morphology. The isolates were maintained in MSGA slants.

Gram staining

Gram staining was performed for all isolated colonies according to the standard procedure. A smear of bacterial cells was prepared on a clean glass slide by a gentle heat fixation. The heat fixed smear was flooded with crystal violet solution for one minute. Smear was washed with water followed by adding mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and rinsed with water. Finally, safranin was used as counter stains for 60-80 sec and washed with water. Cells were then examined under microscope.

Gram test (KOH 3%)

Gram test method with 3% KOH [12]. The gram test is carried out by taking one bacterial isolate and then mixing it with two drops of 3% KOH solution on top of the object glass, stirring circularly for 5-10 seconds with one needle, observed the formation of mucus. If the mucus is formed on top of the glass, the object indicates the isolate is a gram-negative bacterium, if it is not slimy it indicates gram-positive bacteria [13].

Catalase test (H_2O_2 3%)

Hydrogen peroxide (3 percent) was added to 24-48 hours bacterial culture growing on slants. Formation of bubbles indicated positive reaction. If there are bubbles indicating positive catalase bacteria if there are no bubbles, including negative catalase bacteria [14].

Oxidation - Fermentation test (O-F) [15]

The oxidase test is used to identify bacteria that produces cytochrome c oxidase, an enzyme of the bacterial ETC. All bacteria that are oxidase positive are aerobic and can use oxygen as a terminal electron acceptor in respiration. Oxidase test was performed with 1% solution of N, N, N^1N^1-Tetra methyl-p- phenyldiamine-dihydrochloride which was socked in a piece of filter paper. A portion of the colony of the test organism was picked up with a sterile tooth pick and touched on to the paper with impregnated reagent. A dark purple color development within 5- 10 second was considered positive and no change of color was interpreted as a negative result for oxidase. Thick suspension of bacteria were made in sterilized water in a sterile Petri plates, oxidase discs procured from Hi-Media chemicals, Bombay were placed in these suspensions. Formation of color was indicative of oxidase positive reaction.

RESULTS AND DISCUSSIONS

Gram test (KOH 3%) and Catalase test (H_2O_2 3%)

Gram tests are used to distinguish gram-positive and gram-negative bacteria. The ten indigenous bacterial isolates that were tested, all of them were gram-positive (+) bacteria which were characterized by the absence of mucus formed on the object of observation. Catalase test using 3% hydrogen peroxide (H_2O_2) was carried out to determine the presence or absence of the Catalase enzyme in bacteria. Catalase is an enzyme produced by living organisms, including bacteria to catalyze H_2O_2 into H_2O and O_2. Hydrogen peroxide is formed during aerobic metabolism, so microorganisms that grow in an aerobic environment can emit these toxic substances. The catalytic test uses 3% H_2O_2 because H_2O_2 is one of the components produced by bacteria during the aerobic respiration process. Gram test results and Catalase tests on ten indigenous bacterial isolates varied results [16]. Gram test and Catalase test on ten indigenous bacterial isolates are presented in (Table 1).

Table 1 Gram test and Catalase test on ten indigenous bacterial isolates

Isolates	Gram Test (KOH 3%)		Catalase Test (H_2O_2 3%)	
	Observation	Results	Observation	Results
S_1	Mucus is not formed	+	There are bubbles	+
S_2	Mucus is not formed	+	There are no bubbles	-
S_3	Mucus is not formed	+	There are no bubbles	-
S_4	Mucus is not formed	+	There are no bubbles	-
S_5	Mucus is not formed	+	There are bubbles	+
S_6	Mucus is not formed	+	There are bubbles	+
S_7	Mucus is not formed	+	There are no bubbles	-
S_8	Mucus is not formed	+	There are no bubbles	-
S_9	Mucus is not formed	+	There are bubbles	+
S_{10}	Mucus is not formed	+	There are bubbles	+

Oxidation - Fermentation (O-F) test and endospores test [15]

Oxidation-Fermentation (O-F) are two important processes in the metabolism of microorganisms. O-F test is carried out to determine the level of ability of microorganisms to use carbohydrates while endospores are carried out to distinguish bacterial spores from vegetative cells. *Clostridium*, *Streptomyces*, *Desulfotomaculum*, and *Bacillus* are bacteria that produce endospores in their life cycle. Endospore is a dormant form of vegetative cells, so its

metabolism is inactive and is able to withstand environmental stresses such as heat, dryness, cold, radiation and pollutants. The results of O-F and endospores tests on ten indigenous bacterial isolates showed mixed results. The O-F and endospores tests on ten indigenous bacterial isolates are presented in (Table 2).

Table 2 Oxidation – fermentation (O-F) test and endospores test on ten indigenous bacterial isolates

Isolates	O-F test		Endospores test
	Hasil		Results
	Oxidation	Fermentation	
S_1	+	+	-
S_2	+	+	+
S_3	-	-	+
S_4	+	+	+
S_5	+	+	-
S_6	+	+	+
S_7	+	+	+
S_8	+	+	+
S_9	+	+	+
S_{10}	+	+	-

Identification

Identification was carried out to determine the genus of indigenous bacterial isolates tested. Based on the results of identification of ten indigenous bacterial isolates which tested were obtained three genera of bacteria, namely *Bacillus sp.*, *Clostridium sp*, and *Streptomyces sp*. [17]. The identification results of the ten indigenous bacterial isolates are presented in (Table 3).

Table 3 The identification results of ten indigenous bacterial isolates

Isolates	Pigmentation	Colony form	Colony margin	Elevation	Identification
S_1	Brownish yellow	Irregular	Undulate	Raised	*Clostridium sp*
S_2	White cloudy	Irregular	Undulate	Raised	*Clostridium sp*
S_3	White	Irregular	Undulate	Raised	*Bacillus sp*
S_4	brownish white	Irregular	Undulate	Raised	*Clostridium sp*
S_5	White cloudy	Irregular	Undulate	Raised	*Clostridium sp*
S_6	White	Rhizoid	Filamentous	Raised	*Clostridium sp*
S_7	Brownish white	Irregular	Undulate	Raised	*Clostridium sp*
S_8	White cloudy	Irregular	Entire	Raised	*Clostridium sp*
S_9	White cloudy	Irregular	Undulate	Raised	*Clostridium sp*
S_{10}	Dark brown	Irregular	Filamentous	Raised	*Streptomyces sp*

S. No.	Characteristics	*Bacillus sp.*	*Clostridium sp.*	*Streptomyces*
A.	Glucose	+	+	+
B.	Lactose	-	-	-
C.	Sucrose	A	-	-
A.	Citrate utilization	+	-	-
B.	Starch hydrolysis	+	-	-
C.	Gelatin hydrolysis	+	+	+
D.	Litmus milk reaction	-	-	-
E.	Nitrate reduction	+	+	+
F.	Nitrite reduction	+	-	-
G.	H2S production	+	+	+
H.	Urease	-	+	+
I.	Arginine dihydrolase	+	-	-
J.	HCN production	-	-	-
K.	Methyl red	-	-	-
L.	ONPG	-	-	-
M.	Oxidase	+	-	-
N.	Catalase	+	-	-
O.	Ammonia production	+	-	-
P.	IAA production	+	-	-
Q.	Siderophore production	-	-	-
	Utilization of sugars and other carbon sources			
A.	Glucose	+	-	-
B.	Lactose	+	-	-

C.	Galactose			
D.	Mannose	+	-	-
E.	Arabinose	+	-	-
F.	Xylose	+	-	-
G.	Raffinose	+	-	-
H.	Cellulose	+	+	+
I.	Starch	-	+	+
J.	Mannitol	-	-	-
K.	Glycerol	-	-	-
L.	Dulicitol	+	-	-

The ten isolates of selected bacteria identified, eight bacterial isolates were identified as *Clostridium sp.*, one as *Streptomyces sp.* and one bacterial isolate as *Bacillus sp*. Microbes that are tolerant of the environment contaminated with heavy metals potentially become biological agents for heavy metal accumulation. This bacterium can be used to overcome environmental pollution caused by heavy metals. Bacteria, molds, algae, and yeast can accumulate heavy metals Au, Ag, Cu, Cd, Fe, Zn and Ni [18]. *Pseudomonas, Thiobacillus, Bacillus,* and N_2 fixing bacteria are reported to be able to accumulate heavy metals [19]. Also, bacteria are soil microorganisms that play an important part in the process of reforming organic matter and procuring minerals for high-level plant growth processes [20]. Bacteria on the soil also play a role as producers of additives. Soil microbes are capable of producing additives such as antibiotics, biopesticides, microbial toxins, growth regulators (ZPT), and enzymes [21].

A *Bacillus* is a group of bacteria that can act as phosphate solubilizers in the soil. *Clostridium* is a group of bacteria that can bind free nitrogen from the air, in bioremediation *Streptomyces* make themselves resistant to heavy metal. Based on the results of the research known indigenous bacteria from the genus *Bacillus sp.* is gram-positive (+) bacteria that do not have the catalase enzyme, do not have the ability to use carbohydrates and produce endospores in their life cycle while indigenous bacteria from the genus, *Streptomyces sp., Clostridium sp*. is a gram-positive (+) bacteria which in part has the enzyme catalase, all of which have the ability to use carbohydrates and only some of them produce endospores. This *Bacillus sp.* and *C. sp*. will be further tested for its capability to reduce Iron and chromium metals with spectrophotometric analysis method and their ability to produce auxin and gibberellin hormones, phosphate dissolution, and nitrogen fixation. Field studies and the making of bioremediator formulations are research sustainability plans so that these indigenous bacteria can be used as biological agents to reduce metal toxicity on post-mining land and increase soil fertility.

CONCLUSION

The results of this study obtained three types of indigenous bacteria from the genus *Bacillus sp., Clostridium sp. and* Streptomyces sp which have varied characterizations of biochemical tests. These bacteria are potential agent in bioremediation to be further tested so this indigenous bacteria can be used as biological agents to reduce metal toxicity on post-mining land and to increase soil fertility so that better crops.

LITERATURE CITED

1. Widiatmaka S, Kusmaryanti N. 2010. Karakteristik Pedologi dan Pengelolaan Revegetasi Lahan Bekas Tambang Nickel; Studi Kasus Lahan Bekas Tambang Nikel Pomalaa, Sulawesi Tenggara. *Journal Tanah Link* 12(2): 1-10
2. Charman PEV, Murphy BW. 1991. *Soils, their Properties and Management*. A Soil Conservation Handbook for New South Wales. University Press, Sidney
3. U.S. Department of Health and Human Services. 2005. Toxicological Profile for Nickle, Public Health Service, Agency for Toxic Substances and Disease Registry. Atlanta. Available at www.atsdr.cdc.gov/toxprofiles/tp15.pdf
4. Blaudez D, Botton B, Chalot M. 2000. Effects of heavy metals on nitrogen uptake by mycorrhizal birch seedlings. *FEMS Microbiol. Ecology* 33: 61-67.
5. Tarekegn MM, Fikirte Zewdu S, Ishetu AI, Yildiz F. 2020. Microbes used as a tool for bioremediation of heavy metal from the environment. *Cogent Food and Agriculture* 6(1). DOI:10.1080/23311932.2020.1783174.
6. Spain A. 2003. Implication of microbial heavy metal tolerance in the environment. *Review in Undergraduate Research* 2: 1-6.
7. Chojnacka K. 2010. Biosorption dan Bioaccumulation, the prospect of partial application. *Environment International* 36: 299-307.
8. Kitts LC, Kaplan WC. 2004. Bacterial succession in a petroleum land treatment. *Appl. Environ. Microbiology* 70(3): 1777-1786.
9. Komar MS, Irianto. 2000. Pengaruh Penambahan Kultur Bacillus UK 41 dan UK 44 Terhadap. *Journal Sains dan Matematika* 8(1): 1-5.
10. Rahayu SP, Sumingkrat Siti NT, Siti A, Trinity A, Rofienda, Deni. 2006. Penelitian bioremediation (Ex-situ) Tanah Terkontaminasi Limbah B3 yang mengandung Logam Berat. *Bulletin Penelitian* 28(1): 8-17.
11. Barrow, Feltham. 1993. Cowan and steel's manual for the identification of medical bacteria. Camb. Univ. Press, New York, USA
12. Abegaz K. 2007. Isolation, characterization, and identification of lactic acid bacteria involved in traditional fermentation of border Ethiopian cereal beverages. *African Journal of Biotechnology* 6(12): 1469-1478
13. Jay JM. 1992. *Modern Food Microbiology*. 4th Edition. New York: Chapman and Hall.
14. Hadioetomo. 1990. Mikrobiologi Dasar Dalam Praktek. PT. Gramedia. Jakarta.

15. MacFaddin JF. 1985. *Media for Isolation Cultivation-Identification Maintenance of Medical Bacteria*. Williams and Wilkins, Baltimore.
16. Winn WC, Koneman EW. 2006. *Koneman's Color Atlas and Textbook of Diagnostic Microbiology*. Lippincott Williams and Wilkins, Philadelphia, United States.
17. Harley JP. 2005. *Laboratory Exercises in Microbiology*. Sixth Edition. New York: The McGraw-Hill Companies, Inc.
18. Gadd GM, White C. 1993. Microbial treatment of metal pollution working biotechnology. *Trends in Biotechnology* 3(2): 353-359.
19. Mullen MD, Wolf DC, Ferris FG, Beveridge J, Fleming CA, Bailey GW. 1989. Bacterial sorption of heavy metal. *April. Environ. Microbial*. 55: 3143-3149.
20. Abercrombie M, Hickman M, Jhonson ML, Thain M. 1993. Kamus lengkap Biologi. Penerjemah: T. Siti Sutarmi dan Nawangsari Sugiri. Jakarta. Erlangga Hanafiah, A.K. 2010. Dasar-Dasar Ilmu Tanah. PT.
21. Bodenhausen N, Bortfeld-Miller M, Ackermann M, Vorholt JA. 2014. A synthetic community approach reveals plant genotypes affecting the phyllosphere microbiota. *PLOS Genetics* 10: e1004283.