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Identification and Characterization of Bacteria from Uranium Contaminated Soil

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***Abstract*— *This study was performed to identify and characterization of microbes from uranium contaminated soil. The isolates was identified via gram's staining and characterized by biochemical tests according to Bergey's Manual. Three isolates were identified and purified for further analysis. C1 (Corynebacterium xerosis), C2 (Bacillus pumilus) and (C3) Deinococcus grandis have been identified. All isolates were showed positive result for alkaline phosphate production. Further study to be needed to examine remove heavy metals from soil and water.*

***Keywords*— *Uranium, Characterization, Contaminated, Alkaline Phosphate, Biochemical.*

I. INTRODUCTION

Uranium in the surroundings refers to the science sources and effects in humans and animals. Uranium is faintly radioactive and leftovers so for the reason that of its long physical half-life. The biological half-life of uranium is about 15 days. Uranium exposure normally functioning organs like kidney, brain, liver, heart and several other systems can be affected because uranium is a toxic metal. The uranium solubility in soil is dependent upon numerous factors like soil texture, pH, redox potential, microbial activity, temperature, organic and inorganic compounds, and moisture (C.K. Gupta, 2003). Soluble forms of uranium can move around with soil water and be uptake by aquatic organisms and plants or volatilized (J.C. Igwe et al., 2005).

Present is a rising trend of uranium accumulating in soils because of numerous purpose or mistaken practices. Political and Public pressure to explain a problem condition of this nature occurs while serious toxic levels are reached. As a outcome, there would be many kind of risk for agro-systems, ecosystems and health. It is recommended that knowledge of the mechanisms of contaminants that manage the actions of such heavy metals and proposition of remediation treatments (Berthelin and Leyval., 2000). The improvement of nuclear fuel essentials, includes uranium, from solid systems is of broad curiosity for exploiting emergent energy assets. The elimination of toxic heavy metals and radioactive elements from contaminated sources is a valuable topic to explore in terms of environmental control. Researchers have been especially paying attention on studying microorganisms that eliminate uranium including bacteria (A. M. Marques et al., 1991, G. W. Strandberg et al., 1981, M. Z.-C. Hu et al., 1996, Y. Andres et al., 1993), actinomycetes (J. J. Byerley et al., 1987; N. Friiss et al., 1986; Z. Golab et al., 1991) fungi (M. Galun et al., 1983a, 1983b; M. Tsezos et al., 1981; C. White et al., 1990) and yeasts.(S. E. Shumate et al., 1978)

In uranium deposits, we could suppose that some microorganisms having a high ability to adsorbing uranium and further species of microorganisms may present in mine soil systems that can filter uranium from ore. Therefore, it's useful to find additional microorganisms having ability to eliminate uranium from uranium deposits.

We have examined identification of bacteria uranium soil sample. We identified some strains of bacteria which has alkaline phosphate producing capability. The microbes may be useful as an adsorbing agent for elimination of heavy metals present in seawater, effluents and other sources.

II. MATERIALS AND METHODS

A. *Sample Collection*

The soil sample was collected the soil surface (0-6 cm) and at a depth of approximately 30 cm from uranium mining sites and placed in sterilized polyethylene bags using a sterilized spatula. Sample was stored at 4 ºC until analysis. All reagents were purchased from hi-media and Sigma Aldrich.

B. *Isolation of Bacteria*

Soil sample was sieved (2 mm) to remove large size particles. The bacteria were initially isolated by spread plate method. Plating dilutions (10^{-7}) of soils in saline solution (0.9% NaCl) on nutrient agar media and then incubated at 37 °C for 24 hours. Individual colonies of bacteria that different in shape and color were selected and pure culture was done by streaking on nutrient agar media. The bacterial isolates were maintained on nutrient agar at 4°C.

C. *Gram Staining*

Gram staining was performed for all isolated colonies according Aneja, K.R (2003). A smear of bacterial cells was prepared in glass slide. The smear was added 2-3 drops crystal violet solution for one minute then smear was washed with distilled water followed by adding 4-5 drops mordant Gram's iodine. After than smear was decolorized by adding 95% ethyl alcohol and washed with distilled water. Finally safranin was added as a counter stains for 60-80 sec. and washed with distilled water. Cells were examined under microscope.

D. *Biochemical Tests of Pure Cultures*

Identification of the pure cultures was identified according to Bergey's manual. Different biochemical tests were performed namely Endospore staining, Catalase test, Starch hydrolysis test, Indole test, Indole production test, MR-VP test, Citrate Utilization Test, Nitrate reduction test, Mannitol fermentation, Glucose fermentation, fructose fermentation,

Lactose fermentation, 6.5% Nacl, H_2S production, Casein Hydrolysis test, Motility test and Gelatin hydrolysis test.

E. Starch Hydrolysis Test

Starch agar media was used for this test. Pure culture was streaked on media and kept in incubation at 37°C for 24 hours. After incubation media was flooded with iodine solution. Clear zone around the colony was shown positive result.

F. Indole Production Test

Tryptone broth was used for indole production test. Bacterial colony was inoculated on media and kept for incubation at 37° C for 24 hours. After incubation 0.2 ml kovac's reagent was added and after 5 min. cherry red color was indicates positive result.

G. Citrate Utilization Test

Simmons Citrate agar media prepared for test. Bacterial colony was streaked on media and kept for incubation at 37°C for 36 hours. After incubation, Positive result the color of media changes from green to blue.

H. Nitrate Reduction Test

Nitrate broth was used for test. Bacterial colony was inoculated on media and kept for incubation at 37°C for 24 hours. After incubation 5 drops of Sulfanilic Acid and 5 drops of Alpha-naphthylamine reagent was added. Positive result interpreted by the formation of cherry red to pink colouration.

I. Carbohydrates Fermentation Test

Phenol Red Carbohydrate Fermentation broth was prepared and bacterial colony was inoculated on media. Media was kept for incubation at 37°C for 36 hours. Positive result interpreted by the change in color of Phenol red from Red to yellow/orange color.

J. Screening of Bacteria for Alkaline Phosphate Production

Phosphate agar plates were prepared and streaked colonies over the media. Appearance of yellow colour streaked indicates the positive reaction for alkaline phosphate production.

III. RESULT AND DISCUSSION

A. Isolation of Bacteria from Soil Sample

Bacterial colonies were isolated from soil samples and isolated bacterial colonies were shows below figure 1. Three colonies were selected and pure culture of isolated colony were presented in fig. 2

B. Staining and Biochemical Tests

Gram's staining and various biochemical test of three purified culture results were presented in Table 1. *Corynebacterium xerosis* (C1) (Guido Funke et al., 1996), *Bacillus pumilus* (C2) (Ammini Parvathi et al., 2009) *and Deinococcus grandis* (C3) (Shibai, et al., 2019) were identified through various biochemical tests presented below.

Fig. 1. Isolated Colonies from Soil Sample

(C1)

(C2)

(C3)

Fig. 2. Pure Culture of Isolated Colonies

TABLE 1. Biochemical Tests for Bacterial Identification

TEST	C 1	C 2	C 3
Gram's Staining	+ve Rod Shaped	+ve Rod Shaped	+ve Coccus
Endospore Staining	-	+	-
Catalase	+	+	+
Starch Hydrolysis	+	+	-
Indole	-	-	-
Methyl Red	+	-	-
VogesProskauer	-	+	+
Nitrate Reduction	+	-	-
Glucose Fermentation	+	+	+
Maltose Fermentation	+	-	-
Sucrose Fermentation	+	+	+
Mannitol Fermentation	-	+	+
6.5% NaCl growth	+	+	+
Motility	-	+	+
H_2S test	+	-	-
Alkaline Phosphate	+	+	+

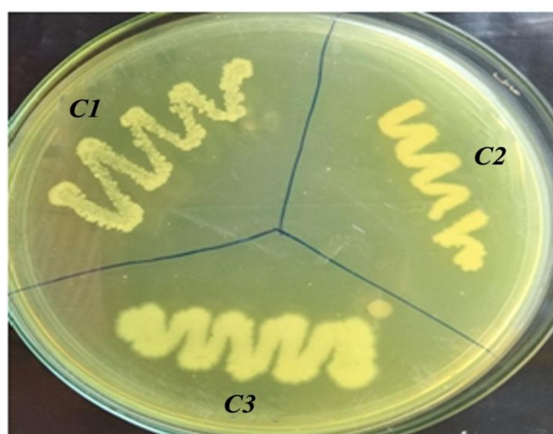

Fig. 3. Alkaline Phosphate Test

IV. DISCUSSION

Soil sample was collected from uranium mining site. Further microorganisms were isolated by serial dilution method and agar plating method [Udeani *et al*., 2009]. Three isolated cultures were purified by streaking techniques on nutrient agar and identified the structure of bacteria by Grams staining procedure. Purified cultures were characterized through biochemical activities and compared with bergey's manual [Udeani *et al*., 2009].

V. CONCLUSION

In uranium deposits, we have discovered microorganisms having ability to produce alkaline phosphate enzyme. These bacterial cells may be remove heavy metal toxicity from soil and water. Particularly, they can absorb amounts of uranium from soil. Further experiments to be need for analysis of heavy metal toxicity.

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