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ISOLATION OF CHROMIUM TOLERANT BACTERIA FROM TWO RECURRING OIL SPILL SITES IN BAYELSA STATE

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Abstract: **solation of chromium tolerant bacteria from two perennial oil spill sites in Bayelsa State was performed. This was done to identify the oliphilic bacteria in the soil samples and the possibility of their potential use in bioremediation of contaminated oil spill sites with heavy metal pollutants. Some bacteria tend to sequestrate and transform the heavy metals thus making them useful candidates in bacteria-assisted remediation strategies. Soil sample were collected from two recurring crude oil spillage sites. Each soil sample was collected from three equidistant points within the location that show clear physical impact of the crude oil spillage. The triplicate samples from each location were pooled to form a composite sample. The samples were then subjected to routine bacteriological and biochemical isolation and identification of bacteria using standard procedure. Part sample were also routinely digested and subjected to chromium analysis with the inductively coupled plasma-optical emission spectroscopy (ICPOES). Result reveal the occurrence of *Bacillus sp, pseudomonas sp, Staphylococcus sp, Enterobacter sp, Arthrobacter sp, micrococcus sp, Paenibacillus sp, Citrobacter sp and Salmonella sp.* Chromium analysis revealed varied concentrations ranging from 114.67 to 111.67 mg/kg and from 224.00 to 219.33 mg/kg in study site 1 and study site 2 respectively. Also, the chromium level identified was above the WHO/FAO recommended permissible limits of 100mg/kg. Thus the organisms have been identified as probable chromium resistant bacteria. It is recommended that the identified organisms be subjected to further studies to establish their potency with a view to deploying them as bacteria-assisted bio-remediation agents.**

Keywords: **Chromium, Tolerant Bacteria, Oil Spill, Sites, Bayelsa State.**

1. INTRODUCTION

As we continue the exploration and exploitation of crude oil in Nigeria, accidents will be inevitable. Accidents will occur through the production process or through the transport of crude either by tankers, piping or other means. Worse still, the theft of crude oil for local refinery operators will always result in oil spills of epic and uncontrolled proportions. It is impossible to extract, refine or transport crude oil without shedding a bit into our fragile ecosystem (Odu, 1977a). Therefore our lands have become perennial sites for crude oil pollution. Crude oil polluted lands are epitomized by low crude yield, destruction of bacterial flora, intrusion of toxins into portable waters and a loss of general aesthetics of surface soils. Allied with heavy metals, crude oil pollution possesses even greater concerns and nemesis for the environment. Fortunately, the cleanup of crude oil and heavy metal polluted sites is receiving global attention. Apart from manual or mechanical

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interventions, the use of bio-remediation techniques provides a classical option. The isolation and use of heavy metal and crude oil tolerant bacteria provide a better option in cases of heavy metal pollution from industrial plants and crude oil leaks during production and transport. Several bacteria in soil have been indicated as possible useful bacteria in the bio-remediation of polluted soils.

This study was therefore conducted to evaluate and possibly identify useful bacteria in the incidence of crude oil pollution in the Niger Delta.

2. MATERIALS AND METHOD

2.1 Study Location

Two (2) different study locations were chosen in the South-south of Nigeria. The GPS Coordinates of the sites are as follows: location 1 in Ikarama Area of Zarama Local Government Areas of Bayelsa State. Point 1 is located on Latitude 5°88.391'' N and Longitude 6°28'6.401'' E. Point 2 is located on Latitude 5°8'7.160'' N and Longitude 6°28'7.879''E. Point 3 is located on Latitude 5°8'7.946'' N and Longitude 6°28'8.056''E.

Location 2 also located in Ikarama Area of Zarama Local Government Areas of Bayelsa State. Point 1 is located on the Latitude 5°8'15.657'' N and Longitude 6°27'59.287'' E at Akala-mini road, Akala-mini community. Point 2 is located on Latitude 5°8'16.896'' N and Longitude 6°27'59.394''E at unnamed road Nigeria. Point 3 is located on Latitude 5°8'16.000'' N and Longitude 6°28'0.112''E at Akala-mini Road, Akala-mini community.

2.2 Collections of Soil Samples

Samples were collected from two randomly selected sites of crude oil spillage. A total of 18 samples were collected from two soil depths of 0-5cm in that location in a randomized manner. The samples were put in a black bag, thereafter taken to the laboratory .

2.3 Bacteriological Analysis

2.3.1 Sterilization/disinfection

All materials were sterilized by moist heat before the bacteriological analysis of the samples. The materials (glass petri dishes, culture media, cotton wool etc) were sterilized by autoclaving using protocol outlined by Cheesbrough, (2010). The materials that are not suitable for sterilization by moist heat sterilization using the autoclave were disinfected thoroughly with 70% ethanol. Additionally, the bench top was disinfected with 70% ethanol to ensure a clean and safe working environment (Cheesbrough, 2010).

2.3.2 Preparation of culture media

The following culture media were used;

- **Nutrient Agar-** For cultivation of less fastidious microorganisms

Other media used in the study was for the purpose of biochemical characterization of the bacterial isolates. These include; Kliger iron agar (for the fermentation of glucose, lactose, gas and hydrogen sulphide production), Simmon Citrate agar (for the detection of citrate utilizing bacteria as the sole source of carbon), Peptone water (for indole production test) and SIM, for detection of bacteria motility.

The powder media were weighed and dissolved in distilled water according to the manufacturer's instructions. The dissolved media were autoclaved at 121^0 C for 15 minutes, following standard operation procedures.

2.3.3 Preparation of samples

Ten gram of the soil sample was weighed with an electronic weighing balance. The weighed sample was put into a test tube containing 100ml of 0.85% normal saline to form the stock solution. Thereafter, a ten-fold serial dilution was done. From the stock solution, 1ml was transferred to the first dilution tube. The sample was diluted up to the 6^{th} dilution factor (1000000).

2.3.4 Cultivation of hydrocarbon/heavy metal tolerant bacteria

The cultivation of the total heterotrophic bacteria was done on nutrient agar using pour plate method. Inoculation was done with the 4th dilution in three replicates. 1ml of the inoculum was aseptically collected with a pipette and poured into a sterile petri-dish. Then 20 ml of the molten nutrient media (nutrient agar) was poured into the petri-dishes. The plate was swirled to distribute the inoculum evenly in the medium, and was allowed to set/solidify. After solidification the plates were inverted and incubated aerobically for 24 hours at 37^{o}C. Thereafter, the number of colonies in the inoculated plates were counted and recorded appropriately.

2.3.5 Isolation of bacterial colonies

The colonies from the nutrient agar plates were randomly selected and picked off with sterile wire loop. The colonies were sub-cultured on fresh nutrient agar plates by streaking colonies on the agar surface using the three-loop method. Four parallel lines were dragged from the inoculum pool. This process was repeated until the three-loop pattern was completed. The sub cultured plates were inverted and incubated at 37^{o}C under aerobic condition to obtain pure isolates.

2.4 Gram staining technique and Biochemical tests

Gram Staining Technique, Catalase Test, Citrate Utilization Test, Kliger Iron Agar Slant Test, Indole Test were conducted using standard methods as described by Cheesbrough (2010)

2.5 SIM

10ml of the SIM was prepared. The Sulphide, indole and motility (SIM) medium was inoculated by stabbing the medium with the test isolates.

2.6 Preparation of soil samples for heavy metal analysis

In the laboratory, the soil samples from each sampling site were spread on brown papers to dry under room temperature. They were then ground, sieved, weighed, and packaged in small brown envelops and labeled. The labels included site, date of collection, and weight in grams.

2.7 Materials and Reagents (All reagents are free of metallic elements)

- Concentrated nitric acid (BDH Chemicals)
- Hydrogen peroxide solution (Sigma Aldrich)
- ICP-OES (Perkin Elmer)

2.8 Digestion Procedure:

- 10 mL of a concentrated HNO_3 solution to 25.0g of the sample in 150 mL Pyrex beaker. The solution was heated at 85 ^{o}C for 3 h. It was allowed to cool and 5 mL of a 30% (v/v) H_2O_2solution was added, and heating continued until clear solution is obtained and volume reduced to about 2 mL. The content was transferred quantitatively into 25 mL volumetric flask and make up to the volume with deionized water. This was aspirated into the into the ICP-OES for analysis

Calculation:

$$Analyte\ concentration\left(\frac{mg}{L}\right) = (S - Bl)\ \times DF$$

where:

S = Analyze concentration in analytical solution (mg/L)

Bl = blank (mg/L)

DF = dilution factor

Critical Control Points: Run sample blank for each batch of analysis

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3. RESULT

Table 1: Mean Chromium concentration in soil samples

SITE A	SITE B	WHO/FAO PERMISSIBLE
114.67	224.00	100
111.67	219.33	100

Source: Field work, 2022. WHO, 2004

Table 2(a): Total bacteria population

Samples	Plate A	Plate B	Plate C
Site A. P1	30	35	28
Site A. P2	16	28	33
Site A. P3	30	17	25
Site B1. P1	52	41	38
Site B2. P2	47	31	53
Site B3. P3	18	27	23
Site B3. P3i	38	24	30
Site B3. P3ii	10	17	8

Table 2(b): Biochemical tests and characterization of bacterial isolates in Site A. P1

Gram stain	catalase	Indole	citrate	H_2	Glucose	Lactose	Gas	Tantative bacteria
+ve rod	+	+	+	+	+	-	-	Pseudomonas aeruginosa
+ve cocci	+	-	+	+	+	-	-	Micrococcus sp
+ve cocci	+	-	+	-	+	-	-	Arthrobacter sp
-ve Rod	-	-	+	-	-	-	-	Salmonella sp

TABLE 3: Biochemical test and characterization of bacterial isolation in site A P2

Gram stain	catalase	Indole	citrate	H_2	Glucose	Lactose	Gas	Tantative bacteria
-ve rod	+	-	+	-	+	-	-	Serratia sp
-ve rod	+	-	+	+	+	+	+	Citrobacter sp
+ve rod	+	-	+	-	+	-	+	Bacillus sp
+ve Cocci	+	-	+	-	+	+	+	Streptococcus sp

TABLE 4: Biochemical test and characterization of bacterial isolation in site A P3

Gram stain	catalase	Indole	citrate	H_2	Glucose	Lactose	Gas	Tantative bacteria
+ve rod	+	-	+	-	+	-	+	Bacillus sp
+ve cocci	+	-	+	-	+	-	-	Arthrobacter sp.
+ve rod	+	-	-	+	+	-	+	Paenibacillus sp
+ve Cocci	+	-	+	+	+	-	-	Micrococcus sp

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TABLE 5: Biochemical test and characterization of bacterial isolation in site B P1

Gram stain	catalase	Indole	citrate	H_2S	Glucose	Lactose	Gas	Tantative bacteria
+ve cocci	+	+	+	-	+	+	+	Staphylococcus sp
-ve rod	+	+	+	+	+	-	-	Pseudomonas aeruginosa
-ve rod	+	-	+	-	+	-	-	Serratia sp
+ve Rod	+	-	+	-	+	-	+	Bacillus sp

TABLE 6: Biochemical test and characterization of bacterial isolation in site B2 P2

Gram stain	catalase	Indole	citrate	H_2S	Glucose	Lactose	Gas	Tantative bacteria
+ve rod	+	-	+	-	+	-	+	Enterobacter sp
+ve cocci	+	+	+	-	+	+	+	Staphylococcus sp
+ve rod	+	-	+	-	+	-	+	Bacillus sp
+ve rod	+	-	+	-	+	-	-	Serratia sp

TABLE 7: Biochemical test and characterization of bacterial isolation in site B3 P3

Gram stain	catalase	Indole	citrate	H_2S	Glucose	Lactose	Gas	Tantative bacteria
-ve rod	+	+	+	+	+	-	-	Pseudomonas aeruginosa
-ve rod	+	-	+	-	+	-	-	Serratia sp
+ve cocci	+	-	+	-	+	-	-	Arthrobacter sp
+ve rod	+	-	+	-	+	-	+	Bacillus sp

TABLE 8: Biochemical test and characterization of bacterial isolation in site B3 P3i

Gram stain	catalase	Indole	citrate	H_2S	Glucose	Lactose	Gas	Tantative bacteria
-ve cocci	+	-	+	+	+	-	-	Micrococcus sp
-ve rod	+	+	+	+	+	-	-	Pseudomonas aeruginosa
+ve rod	+	-	+	-	+	-	+	Bacillus sp
-ve rod	+	-	+	-	+	-	-	Serratia sp

TABLE 9: Biochemical test and characterization of bacterial isolation in site B3 P3ii

Gram stain	catalase	Indole	citrate	H_2S	Glucose	Lactose	Gas	Tantative bacteria
+ve cocci	+	-	+	-	+	-	-	Arthrobacter sp.
+ve cocci	+	-	+	+	+	-	-	Micrococcus sp
-ve rod	+	+	+	+	+	-	-	Pseudomonas aeruginosa
-ve rod	+	-	+	-	+	-	+	Shigella sp.

4. DISCUSSION

The study determined the isolation of chromium tolerant bacteria from two recurring sites in Bayelsa state. The total population of bacterial is shown in table 2. Result showed that *Bacillus sp., Pseudomonas sp, Staphylococcus sp, Enterobacter sp, Arthrobacter sp, Micrococcus sp, Serratia sp, Paenibacillus sp, Salmonella sp*, bacteria were isolated from soil samples of crude oil spillage sites and were studied for their colony morphology characteristics through the serial dilution method. An intense level of gram-positive rod and gram-negative cocci is indicated in the identified bacteria. Results of biochemical test indicating catalase + or -, indole + or -, citrate + or - , glucose + or -, lactose + or –, gas + or – from the soil samples. Table 4 shows the results of all the tests conducted. Results for chromium analysis vary from 114.67 to 111.67 mg/kg among the soil sample in site A and 224.00 to 219.33 mg/kg soil sample in site B. Results show that chromium permissible limits of is 100 by WHO/FAO was exceeded in this study.

This show that these bacteria identified exhibit high tolerance of chromium and can be used for bacteria-assisted bio-remediation. Chromium analysis is the concentration of heavy metal detected in the soil samples, also the chromium level identified was above the WHO/FAO recommended.

Results also revealed that the organisms are identified as probable chromium resistant bacteria.

5. CONCLUSION

Crude oil pollution in the Niger Delta is continuous and wide spread. So far efforts at remediating polluted sites are mainly mechanical or at best the use of oil detergents which themselves pose environment risk. Therefore, the use of biological agents or organisms to remediate oil polluted sites remain the best option. Sadly, not much is known about these biological agents that enhance the remediation process. The findings of this study therefore is a useful step in the efforts to identify these oliphilic bacteria with high resistance to heavy metals that may be deployed in the bioremediation of polluted lands.

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