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Screening for Surface-active Agent Producing Bacteria from Diesel Oil Polluted Tropical Soil

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Abstract: Diesel oil impacted tropical soil was screened for the presence of surface-active agent producing bacteria using the Drop-collapse, the red blood cell haemolysis test and the slide test. The total viable bacteria count of 8.6 x 10^4-5.3 x 10^5 cfu/g was recorded in diesel impacted soil while a range of 1.6 x 10^5-4.0 x 10^5cfu/g were recorded for unimpacted soil respectively. Oil utilizers were found to be abundant in impacted soil with a mean count of 3.5 x 10^5cfu/g. The highest count of surface active agent producers were recorded in unimpacted soil with a mean population of 1.2 x 10^5 cfu/g, compared to 3.0 x 10^4 cfu/g recorded in diesel impacted soil. The highest ratio of 80% surface active agent's producers among oil utilizers was recorded in diesel impacted soil. The genera of screened surface active agent producing bacteria and theirincident rates were *Bacillus* (38%), *Pseudomonas* (29%), *Arthrobacter* (11%), *Proteus* (7%), *Corynebacterium* (7%), *Micrococcus* (4%) and *Klebsiella* (4%).

Key words: Surface - active agents · Bacteria · Diesel - oil polluted soil · Biosurfactant

INTRODUCTION

Human activities have remained the major source of pollution of the environment especially the soil [1, 2]. The polluted or impacted soil would have to be restored to its natural or near natural state by remediation. Bioremediation is a viable option, that involve the use of microorganisms especially bacteria [3]. It involves the introduction of genetically engineered microorganisms or the augmentation of the activity of the native microorganisms to remediate the environment [3]. Most of these microorganisms produce surface-active agents, that facilitate nutrient uptake and the breakdown of interfacial tension that may exist in interacting with hydrophobic substances like hydrocarbons [4-6].

Hence, the study screened for the diversity and distribution of surface-active agent producing bacteria from diesel oil impacted soil that will be of potential use in the remediation of diesel oil polluted tropical soil.

MATERIALS AND METHODS

Soil samples were obtained from the premises of the diesel-run electricity generating plant of Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Samples were taken randomly at depth of 0-15, 15-30 and 30-45 cm with a surface sterilized soil auger. Soil samples collected in 3 replicates were taken to the laboratory in ice chests. Unpolluted soil samples (control) were taken from an undeveloped site rich in organic matter. The total heterotrophic count of bacteria in diesel impacted soil was determined using standard plate count method on Plate Count Agar (PCA) (International Diagnostics Group, UK). Five grams of soil samples was dispensed into Nutrient Broth containing 2g/l of Yeast extract. The mixture was kept on a rotary shaker (G 24 Environmental Incubator Shaker New Brunswick Scientific Co., Inc Edison New Jersey, USA) for 12hrs after which it was serially diluted and spread-plated on PCA. The oil utilizer bacterial count was carried out on Minimal salt medium (MSM) on which diesel oil was used as the sole source of carbon. The composition of the MSM is as adapted from [7]. Bacteria isolates growing on the medium were reported as oil (diesel) utilizers. The experiment was carried out in triplicate. Screening assay for surface-active agent was done on blood agar (Nutrient Agar containing 5% defribinated rabbit blood). Previously poured blood agar plates were inoculated using the spread-plate method [8]. After 48hrs colonies showing clear zones of red blood cell haemolysis (beta-haemolysis) were recorded as being

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surface-active agent producers [9, 10]. The drop-collapsed test was carried out as described by Bodour *et al*. [11]. Supernatant obtained from shake flask culture grown for 7 days on MSM supplemented with 1% diesel oil (as the only source of carbon) was used for the experiment. It was obtained by centrifuging the broth culture at 18,000rpm for 20 mins. 0.01ml of the supernatant was dropped on the surface of sterile liquid paraffin in microtitre wells. After an hour, the wells were observed for activity of the supernatant on the liquid paraffin. If the drop after getting to the bottom of well, collapsed, it is a positive test, but if it forms a ball and does not collapse, it is a negative test. Drops of 0.01 ml of MSM and Crude oil respectively were used as control. The glass slide test was as described by Persson and Molin [12]. Wire loop was used to pick a colony, which was thoroughly mixed with adroplet of normal saline (0.9% aqueous solution of NaCl). The slide was observed for flow of water droplet over its surface. Flow of water over the surface of the slide was recorded as positive. Characterization of bacteria isolates was done according to the Bergey's Manual of Systematic Bacteriology.

RESULTS

Table 1 shows the total count of heterotrophic bacteria from diesel impacted and unimpacted soil. The range of aerobic heterotrophs in impacted soil range from 8.6 x 10^4 to 5.3 x 10^5 cfu/g while in unimpacted soil it ranges from 1.6 x 10^5 to 4.0 x 10^5 cfu/g. The highest count of heterotrophs of 5.3 x 10^5 cfu/g was recorded in contaminated soil samples at a depth of between 15-30cm while the unimpacted soil recorded the least count of 8.6 x10^4 cfu/g. The uncontaminated soil is an undeveloped and uncultivated soil (Table 1).The total count of oil utilizing bacteria in soil samples shows a high population of oil utilizers in contaminated soil than in uncontaminated soil. In impacted soil and unimpacted soil, 7.3 x 10^4-3.9 x10^5 cfu/g and 4.7 x10^4-1.5 x 10^5 cfu/g of oil utilizers were recorded respectively. Equally soil samples were screened for the presence of bacterial isolates that can produce surface-active agents. Contaminated soil samples showed a high population of surface-active agent producing bacteria. The highest count of 1.2 x 10^5 cfu/g surface active agent producing colonies were recorded (Table 1). The percentage population of surface-active agent producing bacteria was compared with the total population of the heterotrophic bacteria.The highest population of 31% of surface active agent producers was recorded in unimpacted soil while the least population ratio of 4.3% was recorded in contaminated soil (Table 2).

Table 1: Bacterial population in hydrocarbon impacted and unimpacted soil

Depth	Mean Total Viable bacteria Count (TVBC) (cfu/g)	Mean Oil Utilizing Bacteria Count (OUBC) (cfu/g)	Mean surface Active Agent Producing Bacterial Count (SABC) (cfu/g)
Impacted soil			
0-15 cm	$8.6\times10^4\pm0.7$	$7.3\times10^4\pm0.8$	$2.67\times10^4\pm0.6$
15-30 cm	$5.3\times10^5\pm1.2$	$3.9\times10^5\pm1.9$	$3.00\times10^4\pm1.7$
30-45 cm	$1.9\times10^5\pm0.4$	$3.5\times10^4\pm1.6$	$2.00\times10^4\pm1.0$
Unimpacted soil			
0-15 cm	$1.6\times10^5\pm0.3$	$4.7\times10^4\pm2.3$	$1.00\times10^4\pm0.3$
15-30 cm	$4.0\times10^5\pm2.0$	$1.5\times10^5\pm0.5$	$1.20\times10^5\pm0.2$
30-45 cm	$2.4\times10^5\pm2.3$	$1.5\times10^5\pm1.5$	$1.03\times10^4\pm3.5$

Table 2: Percent surface-active agent producing bacteria among heterotrophs in soil

Oil	Mean TVBC (cfu/g)	Mean SABC (cfu/g)	SABC /TVBC(%)
Impacted soil			
0-15 cm	$8.6\times10^4\pm0.7$	$2.67\times10^4\pm0.6$	31.00
15-30 cm	$5.3\times10^5\pm1.2$	$3.0\times10^4\pm1.7$	5.70
30-45 cm	$1.9\times10^5\pm0.4$	$2.0\times10^4\pm1.0$	10.50
Unimpacted soil			
0-15 cm	$1.6\times10^5\pm0.3$	$1.0\times10^4\pm0.3$	6.25
15-30 cm	$4.0\times10^5\pm2.0$	$1.2\times10^5\pm0.2$	30.00
30-45 cm	$2.4\times10^5\pm2.3$	$1.03\times10^4\pm3.5$	4.30

Table 3: Percent surface-active agent producing bacteria among oil utilizing isolates in soil

Oil	Oil utilizers (cfu/ml)	Biosurf producers (cfu/ml)	%
Impacted soil			
0-15 cm	$7.3\times10^4\pm0.8$	$2.67\times10^4\pm0.6$	36.6
15-30 cm	$3.9\times10^5\pm1.9$	$3.00\times10^4\pm1.7$	7.6
30-45 cm	$3.5\times10^4\pm1.6$	$2.00\times10^4\pm1.0$	57.1
Unimpacted soil			
0-15 cm	$4.7\times10^4\pm1.3$	$1.00\times10^4\pm0.3$	21.3
15-30 cm	$1.5\times10^5\pm0.5$	$1.20\times10^5\pm0.2$	80.0
30-45 cm	$1.5\times10^5\pm1.5$	$1.03\times10^4\pm3.5$	6.9

Among the oil utilizers, the highest ratio and the lowest ratio of 80 and 6.9% surface-active agents producers were recorded in unimpacted soil at the 0-15 and 15-30 cm, respectively (Table 3).

Using the drop collapse, red blood cells haemolysis and slide tests the following genera were screened positive for surface active agent production and their incidence rate were *Bacillus* (38%), *Pseudomonas* (29%), *Arthrobacter* (11%), *Proteus* (7%), *Corynebacterium* (7%), *Micrococcus* (4%) and *Klebsiella* (4%)

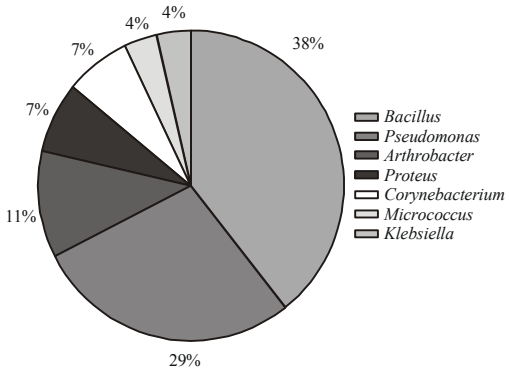

Fig. 1: Bacteria genera screened positive for surface active agent production

Serratia (19), *Arthrobacter* (20), *Corynebacterium* (21), *Micrococcus* (22) and *Klebsiella* (23)

DISCUSSION

The genera of bacteria isolated and screened for bisurfactant production has been reported by many workers viz: *Pseudomonas* [13, 14], *Bacillus* [15, 16], *Flavobacterium* [17, 18], *Serratia* [19].

The high range of aerobic heterotrophic counts recorded in unimpacted soil is due to the high level of organic matter usually present in fallow uncultivated tropical soil. The unimpacted soil is a loamy soil. This explains the preponderance or large population of microorganisms in the soil. This further explains the high heterotrophic bacteria population recorded from the soil. This agreed with Jennings and Tanner [10], that recorded a high number of heterotrophs bacteria from an uncultivated land rich in organic matter. Also Brady and Weil [20] had reported that organic matter stimulates the growth and increases the metabolic activities of microorganisms.

The highest number of oil utilizing bacteria recorded in the impacted soil is due mainly to the availability of the substrates which these organisms can utilize for their growth and other metabolic activities [4]. Hence, specialization will set in which leads to the preponderance of oil utilizing bacteria in the environment [21]. A lot of workers have reportedly isolated a large population of heterotrophic bacteria from hydrocarbon impacted soil [22]. The high population of surface-active agent producing bacteria recorded in the unimpacted soil is in concordance with the findings of Jenning and Tanner [10]. This suggests that surface active agents or the production of biosurfactants does not only occur in impacted soil but also in unimpacted soil. Also, since the agent can be found not only in oil polluted environments, hence it is an important product of this group of microorganisms necessary for their survival.

In conclusion, a preponderance of surface active - agent producing bacteria were recorded in the diesel impacted soil. These bacteria can be of great potential in the remediation of diesel oil impacted soil

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REFERENCES

1. Baker, J.M., 1976. Biological Monitoring Principles Methods and Difficulties: In Marine Ecology and Oil Pollution. Jennifer M. Baker (Ed.). Institute of Petroleum, London, pp: 41-52.
2. Tijani, M.N., S.A. Balogun and M.A. Adeleye, 2005. Chemical and Microbiological assessment of water and bottom - sediments contamination in Awba Lake (U.I) Ibadan, SW Nigeria, RMZ - Materials & Geo Environments, 52(1): 123-126.
3. Kosaric, N., 2001. Biosurfactants and their application for soil bioremediation. Food Technol. Biotechnol., 39: 295-304
4. Lin, S., 1996. Biosurfactants: Recent Reviews. J. Chem. Tech. Biotechnol., 66: 109-120.
5. Volkering, F., A.M. Breure and W.H. Rulkens, 1998. Microbiological aspects of surfactant use for biological soil remediation. Biodegradation, 8: 401-407.
6. Karanth, N.G.K., P.G. Deo and N.K. Veenanadig, 1999. Microbial production of biosurfactants and their importance. Current Science, 77: 116-126.
7. Fagade, O.E., 1990. Bacterial degradation of crude oil from the Niger Delta Area of Nigeria. Unpublished Ph.D Thesis, Dept of Botany & Microbiology, University of Ibadan, Ibadan, Nigeria.
8. Carrillo, P.G., C. Mardaraz, S.I. Pitta-Alvarez and A.M. Giuletti, 1996. Isolation and selection of biosurfactant producing bacteria. World J. Microbiol. & Biotechnol., 12 82-84.
9. Banat, I.M., 1995. Characterization of Biosurfactants and their use in pollution removal. State of the Art. Acta Biotechnol., 3: 251-267.
10. Jennings, M. and R.S. Tanner, 2000. Biosurfactant producing bacteria found in Contaminated and Uncontaminated soils. Proceedings of the 2000 Conference on Hazardous Waste Research, pp: 299-306.

11. Bodour, A.A., K.P. Drees and R.M. Maier, 2003. Distribution of biosurfactant producing bacteria in undisturbed and contaminated Arid south western soils. Applied & Environ. Microbiol., 69(6): 3280-3287.
12. Persson, A. and G. Molin, 1987. Capacity for biosurfactant production of environmental Pseudomonans and Vibronaceae growing on carbohydrates. Appl. Microbiol. Biotechnol., 26: 439-442.
13. Guerra-Santos, L.H., O. Kappeli and A. Fiechter, 1984. Pseudomonas aeruginosa biosurfactant production in continuous culture with glucose as carbon source. Appl. & Environ. Microbiol., 48: 301-305.
14. Hisatsuka, N.T., N. Sano and K. Yamada, 1971. Formation of rhamnolipid by Pseudomonas aeruginosa: its function in hydrocarbon fermentation. Agric. Biol. Chem., 35: 686-692.
15. Javaheri, M., G.E. Jenneman, M.J. McInnerney, and R.M. Knapp, 1985. Anaerobic production of a biosurfactant by *Bacillus licheniformis* JE - 2 Appl. & Environ. Microbiol., 50: 698-700.
16. Yakimov, M.M., K.N. Timmis, V. Wray and H.L. Fredrickson, 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniforms* BAS50. Appl. & Environ. Microbiol., 61: 1706-1713.
17. Bodour, A.A., C. Guerrero - Barajas, B.V. Jiorle, M.E. Malcomson, A.K. Paull, A. Somygi, L.N. Trinh, R.B. Bates and R.M. Maier, 2004. Structure and Characterization of Flavolipids, a novel class of Biosurfactant produced by *Flavobacterium* sp strain MTN II. Appl. & Environ. Microbiol., 70: 114 -120.
18. Bodour, A.A. and R.M. Maier, 2002. Biosurfactants: types, screening methods and application. In I.G. Bitton (ed) Encyclopedia of Environmental Microbiology. John Wiley and sons, New York.
19. Matsuyama, T., M. Sogawa and I. Yano, 1991. Direct thin-layer chromatography and rapid characterization of Serrattia marcescens mutants defective in production of wetting agents. Appl. & Environ. Microbiol., 53: 1186-1188.
20. Li, Z.Y., S. Lang, F. Wagner, L. Witte and V. Wray, 1984. Formation and identification of interfacial -active glycolipids fromresting microbial cells of *Arthrobacter* sp and potential use in tertiary oil recovery. Appl. & Environ. Microbiol., 48: 610 -617.
21. Copper, D.G., J.E. Zajic and D.F. Gerson, 1978. Production of surface active agent lipids by *Corynebacterium lepus*. Appl. & Environ. Microbiol 37: 4-10.
22. Das, M., 2001. Characterisation of de -emulsification capabilities of a *Micrococcus* sp. Bioresource Technology, 79: 15 -22.
23. Maier, R.M., 2006., Biosurfactant evolution and diversity in bacteria. Advan. in Appl. Microbiol., 52: 101 121
24. Brady, N.C. and R.R. Weil, 1999. The nature and properties of soil. Pp: 118-172. Upper saddle River, New Jersey. Prentice-Hall, Inc.
25. Atlas, R. and R. Bartha, 1997. Microbial Ecology. Fundamentals and Applications. 4th Edn. Addison Wesley Longman Inc., pp: 368.
26. Desai, J.M. and I.M. Banat, 1997. Microbial production of surfactants and their commercial potentials. Microbiology and Molecular Biology Reviews, 61: 47-64.