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ORIGINAL RESEARCH ARTICLE

Identification and Screening of Biosurfactant Producing Bacteria from Mechanic Workshops Soil in Gusau Metropolis, Nigeria.

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ABSTRACT
Biosurfactants are molecules that reduce interfacial tension. Their chemical composition can vary widely, but they have in common their amphiphilic or amphipatic nature and can thus be soluble in aqueous as well as in organic solvents. The study was carried out to identify and screen biosurfactant producing bacteria from mechanic workshops in Gusau metropolis. Eight (8) soil samples were collected at the depth of 0-7 and 8-15cm from selected mechanic workshops located at Gada Biyu, Taqama bye-pass, Birnin Ruwa and non-oil contaminated soil as control for analysis. The physicochemical parameters were analysed using standard procedures (blood heamolysis, drop collapse, oil displacement and emulsification index) methods were used to screen biosurfactant production by the isolates. Mineral salt medium supplemented with 1% Actual gasoline oil (AGO) as sole sources of carbon was used to isolate hydrocarbon degrading bacteria, while heterotrophic bacteria were isolated using nutrient Agar. The soil samples from the study area were characterised with smooth, grey to dark brown soil with an unpleasant smell as well as as well as high temperature and pH. The three mechanic workshops used for this study, shows relatively higher counts for Hydrocarbon Degrading Bacteria while higher heterotrophic bacterial count was obtained from the control site though there was no significant difference between individual mechanic workshop ($p>0.05$). The isolates are potential biosurfactant producers based on their performance especially blood haemolysis and emulsification index. The isolates identified belonged to the species of *Bacillus, Pseudomonas, Acinetobacter, Micrococcus* and *Serratia.*

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Biosurfactant, hydrocarbon degrading bacteria, physicochemical parameters, Actual gasoline oil (AGO).

INTRODUCTION
Microbial biosurfactants are surface-active molecules that reduce the surface tension at the air-liquid and liquid-air interfaces (Aminu *et al.,* 2022). They are primarily amphipathic chemicals made by aerobically growing microorganisms (Zhang *et al.*, 2010). These substances are generated by microbes while s growing in both water-soluble and water-insoluble substrates (Rosenberg and Ron, 2008). Numerous organic substances are used by microorganisms as sources of carbon and energy for their metabolism. Microorganisms, particularly bacteria, aid in the diffusion of carbon sources into cells when they are intractable substrates like hydrocarbons by producing ionic surfactants that emulsify the hydrocarbon substrates in the growth media. It has been shown that numerous bacterial species include *Pseudomonas, Bacillus*, and *Stenotrophomonas* are known to produce biosurfactants (Mulligan and Eekhari, 2003).

According to Banat *et al.* (2014), biosurfactants are often divided into low and high molecular mass polymers, with the former serving to reduce surface and interfacial tension and the latter serving as more effective emulsion-stabilizing agents.

In oil contaminated environments such as soil and water, biosurfactant encourages the biodegradation of organic wastes and the elimination of hydrocarbons (Ławniczak *et al.,* 2013).

In order to explain how biosurfactants alter the efficiency of microbial hydrocarbon breakdown, Li *et al.* (2019) devised a four-step model. First, the emulsification of crude oil products is mediated by surfactants. Next, the microbe responsible for the degradation adsorbs the emulsified hydrocarbon to its cellular surface. Third, the microbe takes the emulsified crude oil product

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intracellularly via endocytosis, active or passive transport. And finally, the emulsified crude oil product is degraded by enzymes. The identification of surfactant-expressing microbial strains that effectively breakdown petroleum hydrocarbons are thus one of the current areas of focus in successful microbial remediation (Tanzadeh *et al.,* 2020).

MATERIALS AND METHODS

According to the methods described by Aminu *et al.* (2022) and Adebajo *et al.* (2016), a total of eight (8) soil samples were collected using sterilized soil auger at the depths of 0-7 and 8-15 cm from three mechanic workshops located at Taqama bye-pass, Gada Biyu, and Birnin Ruwa all within Gusau metropolis, while agricultural soil was collected and serve as control . All the samples were put in a sterile polythene bags, labelled and transported aseptically to Microbiology laboratory of Federal University Gusau for analyses.

Determination of Physicochemical Parameters

The characteristics of the soil samples, such as colour and texture, were physically evaluated, while temperature and pH were analysed using thermometer and pH meter respectively in accordance with the approach described by Charan and Petal (2017).

2.2 Isolation of Heterotrophic Bacteria

One (1g) of each soil sample was weighed out and added to 9ml of distilled water. The mixture was then shaken for 24 hours at 180 rpm using an orbital shaker and 0.1mL of the sample was poured on a freshly prepared nutrient agar. The colonies were observed, counted and recorded as colony forming unit per gram of soil (CFU/g) after 24 hours (Aminu *et al.,* 2022; Kabir, 2019).

Isolation of Hydrocarbon Degrading Bacteria

One milliliter (1mL) of the diluents was aseptically spread on to sterile mineral salt medium (MSM), which contains 10% actual gasoline oil (AGO) as described by (Dukhande and Warde, 2016); Aminu *et al.*, 2022 and Kabir, 2017). MSM is composed of $NaNO_3$, KH_2P0_4, 1.8; $MgSO_4$ $7H_2O$, 0.2; $MnSO_4$, $CaCl_2$, 0.05; $(NH4)_2SO_4$, 1.2; and $FeSO_4$, 0.05 in g/L, the colonies were observed, enumerated and recorded as colony forming units per gram (CFU/g) of soil samples after 24 hrs at 37^0C.

2.4 Screening of Biosurfactant Expressing Isolates

The selected isolates were inoculated in the test tubes containing 10 ml nutrient broth each, and incubated for 24 hrs, the bacterial culture was then centrifuged at 3000 rpm for 15 minutes at room temperature before the screening (Kurniati *et al.*, 2019).

Haemolytic activity

Blood agar plates were prepared and the newly formed single colonies from the isolated cultures were streaked and incubate at 37 °C, for 24 hrs as described by Kabir (2019) and Kurniati *et al,.* (2019), the plates were then observed for a clear zone around the colonies.

Oil displacement

Fifteen (15µL) of AGO (actual gasoline oil) was poured onto petri dishes containing 10 mL of distilled water and 3 mL of cultured supernatant,. The separation of the AGO, which resulted in the formation of a clear zone, indicated the presence of biosurfactant. The non-separation of the AGO further revealed the absence of biosurfactant in the supernatant. The zones of displacement were seen throughout the test when chemical surfactants (detergents) were used as the positive control, according to Morikawa *et al.* (2000) and Sharma *et al.* (2022).

Emulsification Index

A two (2ml) volume of AGO was added to 2ml of the bacterial culture supernatant and the solution was vortexed at high speed for 5 mins in a test tube and left for 48 hrs. The height of the stable emulsion layer was measured and the emulsification index (EI24) calculated as the ratio of the height of the emulsified layer in centimetre and the total height of the liquid column in centimetre (Satpute *et al.*, 2010; Astuti *et al.*, 2019).

Equation 1 was used to calculate the emulsification index

$$EI24 = \frac{Hemulsion}{Htotal} \times 100\% \quad \text{..... Equation 1}$$

WhereEI24 = emulsification index after 24hrs.

$H_{emulsion}$ = the height of emulsion layer.

H_{total} = the total height of the liquid

(Kabir, 2019).

Drop collapse

A drop of bacterial culture supernatant was placed using sterile micropipette onto a clean glass slide coated with AGO. The presence of biosurfactant was indicated by the collapse of culture after 60secs while inability of the drop to collapse indicates negative result (Youssef et *al.*, 2004).

RESULTS

Physicochemical Parameters

The Physicochemical properties of soil samples studied are presented in table 1. Physicochemical parameters of the soil sample collected from different sampling sites. The Texture and colour were smooth and dark brown for all samples as opposed to control with coarse texture and pale brown colour. Temperature recorded ranges from 30^0C-35^0C and pH range were from 10.5-8.0

Table 1: The physicochemical properties of soil samples collected from mechanic workshops and control soil are presented in

S/N	Location	Sample code	Depth(cm)	Soil texture	Colour	Temperature(^{0}C)	pH
1	TB	A1	0-7	Smooth	Dark brown	35	10.5
		A2	8-15	Smooth	Dark brown	36	9.6
2	GB	B1	0-7	Smooth	Dark Brown	32	9.0
		B2	8-15	Smooth	Dark brown	33	10.2
3	BR	C1	0-7	Smooth	Dark grey	32	9.0
		C2	8-15	Smooth	Dark grey	30	8.8
4	C	D1	0-7	Coarse	Pale brown	29	8.0
		D2	8-15	Coarse	Pale brown	28	8.5

Key: TB= Taqama bye-pass GB= Gada Biyu, BR= Birnin Ruwa, C= Control

Total viable Counts for Heterotrophic and Hydrocarbon degrading Bacteria

The total bacterial count of the soil samples collected from the sampling sites shows the highest heterotrophic bacterial count (THBC) 77.0 x10^5 CFU/g obtained at location D1 and lowest count 3.3x10^5 CFU/g obtained at location A2 while the highest hydrocarbon degrading bacterial count (THDC) was 40.0x10^5 CFU/g obtained at location A1 and the lowest was 1.65x10^5 CFU/g from location D2 as presented in table 2.

Table 2: Total viable Counts for Heterotrophic and Hydrocarbon degrading Bacteria

S/N	Location	Sample code	Depth (cm)	THBC (x 10^5 CFU/g)	THDBC (x 10^5 CFU/g)
1	TB	A1	0-7	41.1 ±1.5	40.0±1.0
		A2	8-15	33.5 ±1.5	36.0±4.0
2	GB	B1	0-7	39.0±1.0	21.5± 1.5
		B2	8-15	37.5±2.5	21.0 ±2.0
3	BR	C1	0-7	38.0 ±4.0	30.5 ±9.5
		C2	8-15	30.0± 5.0	37.5±7.5
4	C	D1	0-7	77.0 ±10.0	14.0 ±1.0
		D2	8-15	49.5 ±5.5	10.0 ±1.5

KEY: THBC= Total Heterotrophic Bacterial Count, THDBC= Total Hydrocarbon Degrading Bacterial Count

Biosurfactant Expression

All the Sixteen (16) bacterial isolates were screened for biosurfactant expression ability, thirteen 13 (81%) isolates were positive for blood haemolysis and they were negative for drop collapse and oil displacement assays.

Table: 3: Biosurfactant expression ability of the bacteria isolates

S/No	Location	Sample code	Haemolysis	Drop collapse	Oil displacement
1	TB	A1	+	-	-
		A2	+	-	-
2	GB	B1	+	-	-
		B2	+	-	-
3	BR	C1	+	-	-
		C2	+	-	-
4	C	D1	+	-	-
		D2	-	-	-

Key: TB= Taqama Bypass, BR= Birin Ruwa, GB= Gada biyu, -=Negative,+=Positive

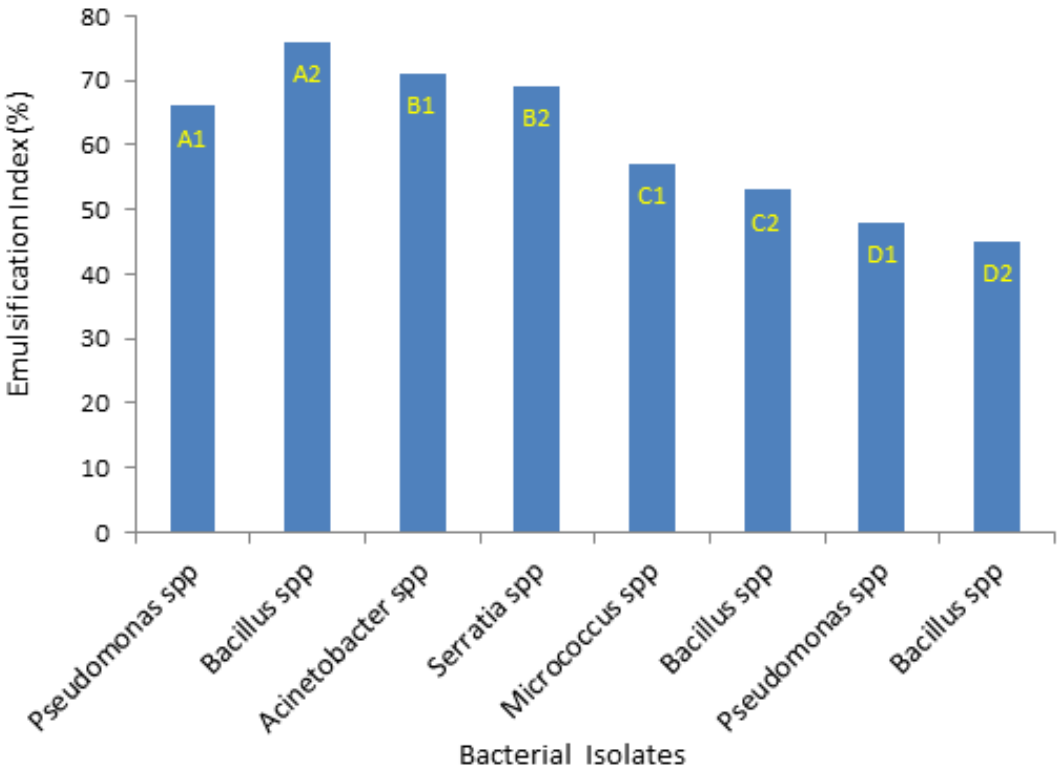

Fig 1:Emulsification index (EI) for the respective isolates. Isolates from the subsoil of Taqama bypass (TB) has the highest percentage of 76% and the isolates collected from Birnin Ruwa (BR) mechanic workshops shows the lowest percentage (53%) of EI. Nevertheless the isolates from the topsoil of control location recorded the lowest Emulsification Index (45%) compared to the corresponding subsoil layer (48%) of the same location.

Morphological characteristics, Gram stain and Biochemical tests results

The morphological characteristics of biosurfactant producing bacteria from soil samples obtained at different mechanic workshops within Gusau metropolis is presented in Table 4

Table 4: Morphological characteristics, Gram stain and Biochemical tests results of the isolated bacteria

S/N	Sample code	Colonial Morphology	Gram reaction	Biochemical Characteristics							Probable identity of bacterium
				Catalase	Citrate	Urease	Indole	MR	VP	Motility	
1	TB1	Small, greenish, rod, flat and undulate	-	+	+	+	-	-	-	+	*Pseudomonas spp*
2	TB2	Large, creamy, rod, entire and wrinkled	+	+	+	-	-	+	+	+	*Bacillus spp*
3	GB1	Medium, pale yellow round, raise and curled	+	+	+	-	-	-	-	-	*Acinetobacter spp*
4	GB2	Small, red, Irregular, convex and undulate	-	+	+	-	-	-	-	+	*Serratia spp*
5	BR1	Small, bright yellow, Circular, entire \and wrinkled	+	+	+	-	-	-		+	*Micrococcus spp*
6	BR2	Large, creamy, Rod , entire and wrinkled	+	+	+	-	-	+	+	+	*Bacillus spp*
7	CC1	Medium, greenish, Rod, flat, and undulate	-	+	+	-	-	-	-	+	*Pseudomonas spp*
8	CC2	Circular, creamy, rod, entire and undulate	+	+	-	-	-	-	+	+	*Bacillus spp*

Key: MR = Methyl Red, VP = Voges-Proskauer , - = Negative, += Positive ,Colonial morphology results are presented in this order: size, colour, shape, elevation and margin respectively

DISCUSSION

The physicochemical parameters of soils sampled at different mechanic workshops within Gusau metropolis revealed their textures as smooth; this could be related to the constant oil spillage due to mechanic activities which is native to the location. The soil colour observed from the collected samples were either dark brown or dark grey, except for control which appeared as pale brown this is in line with the finding of Lu *et al.* (2012) who reported that the surface particles of soil become smoother and retain the colour of oil when there is excessive oil spillage and contamination. The relatively higher temperature and pH recorded from mechanic workshops compared to the control sites could be as a result of complex processes involved in biodegradation of the petroleum contaminants carried out by the indigenous microorganisms and thereby causing heat generation and subsequent rise in temperature, this in line with the finding of Charan and Petal (2017) who reported that the daily maximum surface temperature of hydrocarbon-contaminated soils is frequently higher than that of nearby control sites. Likewise, the findings of Varjani (2017) reported that biodegradation of petroleum contamination is associated

on environmental factors such as high temperature and pH. According to report by (Zekri and Chaalal, 2005) stated that rise in temperature increases the rate of biodegradation of crude oil.

The bacterial colonies found in mechanic workshops in Gusau metropolis were relatively lower on nutrient agar compared to those from control sites; this could be as a result of the media lacking the necessary growth supplement in which the isolates is largely depended upon, while isolate from control site could survive in the nutrient agar without any hindrance. This finding corroborates the suggestion of Ojumu *et al.* (2004), who found that the microbial population in polluted soils is lower than in unpolluted soils. Akeredolu and Akinnibosun (2017) discovered that the microbial population of crude oil-polluted soil was reduced in comparison to control soil.

Nevertheless the bacteria count on mineral salt medium from mechanical workshops have the highest count compared to control site due to presence of hydrocarbon which serve as carbon source for the growth of hydrocarbon degrading bacteria while the media suppress the growth of bacteria found in control site. This is in line with the study of Zekri and Chaalal's (2005) who suggested that the higher bacterial counts discovered in the mechanic workshops are evidence of the active hydrocarbons-degrading strains.

The baseline range values (50-80%) in the result of Emulsification index proved the isolates as potential biosurfactant producers. This was also reported by Adebajo *et al.,* (2018) that emulsification activity of 41% is positive for biosurfactant production.

Negative results obtained might be partially attributed to factors such as the concentration of biosurfactant, its molecular weights and the type of biosurfactant. This is in line with the finding of Rodrigo, (2021) who reported that positive results of biosurfactants screening depend on the concentration and their molecular weight in the solution.

Gram reaction from the bacteria isolates revealed that gram positive bacteria make up the majority (60.5%) of the total isolates , this was similarly reported by Umar *et al.* (2020); Chikere, (2018); Mwamura, (2017), where they demonstrated that gram positive bacteria have been shown to be effective hydrocarbon degraders.

CONCLUSION

The soil samples from the study area (mechanic workshops) were characterised with smooth, dark brown and unpleasant smell and also has high temperature and pH compared to control site. Potentially biosurfactant producing bacteria were isolated from all sampling sites within Gusau Metropolis based on their screening performance, also relatively higher counts for hydrocarbon degrading bacteria as opposed to higher heterotrophic bacterial counts was obtained from the control site. The bacteria species isolated from both mechanic workshops and control site includes; *Bacillus, Pseudomonas, Serratia, Acinetobacter* and *Micrococcus,*

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