African Journal of Microbiology Research Vol. 4(9), pp. 730-734, 4 May 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Emulsifying bacteria in produce water from Niger-Delta, Nigeria

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Accepted 15 March, 2010

The study reports the diversity of emulsifying bacteria in produce water obtained from Bonny Oil Terminal, Niger Delta, Nigeria. Physico–chemical analysis of produce water showed a high chloride ion content of 650.0 mg/L. Total aerobic mesophilic bacterial count was 8.6 x 10^6 CFU/mL while the oil utilizing bacterial count was 1.32 x 10^6 CFU/mL. The predominant bacteria genera were *Bacillus, Pseudomonas, Serratia and Klebsiella*. The ratio of the surface active agent producers in the mesophilic bacteria was 0.34%, while 2.2% was recorded among the oil utilizing bacteria. The highest emulsification and de-emulsification indices of 65 and 50% recorded respectively were for *Pseudomonas mallei*. The result obtained was discussed in relation to the use of the emulsifying bacteria in microbial enhanced oil recovery (MEOR) in the Niger Delta, Nigeria.

Key words: Emulsifying bacteria, emulsification-index, surface-active agents, biosurfactants, MEOR, crude oil, Niger-Delta.

INTRODUCTION

Surface-active agents are substances that can act on surfaces thereby breaking down the surface tension (Fiechter, 1992; Desai and Banat, 1997). These agents can be produced industrially by chemical method or from biological origin. Among all the biological options, microorganisms give the most varied types of surface-active agents (Karanth et al., 1999). These surface-active agents are referred to as biosurfactants (Persson and Molin, 1987). They can breakdown the interfacial tension existing between polar and non-polar liquids in mixtures (Persson and Molin, 1987), thereby forming stable emulsions (Pruthi and Cameotra, 1997). Also some have been reported to have de-emulsifying properties (Akit et al., 1981).

The emulsification and de-emulsification attributes of these microorganisms have been reported to be of great potential in microbial enhanced oil recovery (MEOR) (Rocha et al., 1992; Pruthi and Cameotra, 1997; Akit et al., 1981). Hence, this study screened for the presence of emulsifying and de-emulsifying bacteria in produce water obtained from the Bonny oil terminal, Bonny, Niger Delta, Nigeria.

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MATERIALS AND METHODS

Produce water from crude oil loading terminal in Bonny, Niger Delta area of Nigeria was collected in sterile plastic containers and transported in cold boxes to the laboratory. The following physico-chemical parameters of the produce water were carried out: pH, colour, turbidity, conductivity, total dissolved solids, total suspended solids, alkalinity, total hardness, calcium hardness. The level of cations such as Ca^{2+}, Mg^{2+}, Fe^{3+}, Mn^{2+} and anions such as SO_7^{2-}, PO_4^{3}, F- and Cl^- were also determined according to Standard Methods (AOAC, 1984).

The total viable heterotrophic bacteria count was carried out using Standard Plate Count Agar (Oxoid, UK) as means of three replicates. The total oil utilizing bacteria count in the produce water was determined as described by Fagade (1990). The mineral salts

medium (MSM) was made up of basal salt medium and trace element solution. The basal contained (g/L): K_2HPO_4, 1.8; KH_2PO_4, 1.2; NH_4CL, 4.0; $MgS0_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 0.1 and $FeCl_2.4H_20$, 0.05 and trace elements solution contained: H_3BO_3, 0.1; $ZnSO_4.7H_20$, O.1; $CuS0_4.5H_2O$, 0.05 and $MnSO_4.H_2O$, 0.04 and 1% Bonny light crude oil (an internationally accepted form of crude oil from Bonny, Rivers State, Nigeria) was added as the carbon source. The pH was adjusted to 6.5 and 2% agar was added to solidify the medium. The basal salt medium and the trace element solution were sterilized separately. Ten milliliters of the trace element solution was added to the basal salt medium to make up one liter of the MSM. The isolates obtained were characterized and identified using Bergey's manual of systematic bacteriology (Krieg and Holt, 1984).

Ability of isolates to produce surface-active agent was determined as described by Lin (1996). Five percent defibrinated rabbit blood was added aseptically into sterilized nutrient agar. It was mixed thoroughly and poured into sterile Petri dishes. Using spread plate method, serially diluted samples of produce water was inoculated on to the blood agar plates and incubated at 37°C for 24 h. Zones of haemolysis of red blood cells were recorded as positive biosurfactant producers.

The potential biosurfactant producing bacteria were also screened using the drop-collapse test as described by (Bodour et al., 2003). The test was carried out using supernatant obtained from the culture broth of isolates by centrifuging the broth at 15,000 rpm for 25 min. The culture broth was obtained from MSM broth supplemented with glucose as the carbon source initially and later after 3 days 0.1% of Bonny light crude oil was added. The culture was kept on a rotary shaker (G 24 Environmental Incubator Shaker, New Brunswick Scientific Co., Inc. Edison, New Jersey, USA) at 180 rpm for 7 days. One milliliter of sterilized liquid paraffin was dispensed into the well of a sterilized clean white tile. Then 0.1 ml of the supernatant was dispensed into it. After 10 min, the well was observed visually for the break in the surface tension of the paraffin.

The emulsification index (EI) of cell free supernatant in kerosene was determined, using a high-speed Vortex mixer (Lab-line Illinois, USA) as described by Akit et al. (1981). Two milliliters of cell free supernatant was mixed with 3 ml of kerosene at high speed for 120 sec. The EI was recorded as a percentage of the height of the emulsified kerosene to the total height of the mixture after 24 h. The de-emulsification ability of the isolates on stable emulsion of kerosene-Tween 80 respectively was equally done as described by Akit et al. (1981). A mixture of sterile distilled water and Tween 80 containing 4% (0.08 ml) and 8% (0.16 ml) of Tween 80 respectively was placed in 2 ml of distilled water into which 3 ml of kerosene was added. It was vortexed for 120 s until a stable emulsion was formed. Then, 0.1 ml of isolate whole broth was added and vortexed again for 15 s. The stability of the emulsion was recorded after 24 h.

RESULTS

The mean count of aerobic mesophilic bacteria present in the produce water was 8.6×10^6 cfu/ml. The mean count of oil utilizing bacteria present in the sample recorded was 1.32×10^6cfu/ml. The surface active agent producers count based on the ability of the isolates to haemolyse red blood cells was 0.03×10^6cfu/ml. The incidence rate of 0.34% of surface-active agent producers was recorded amongst the bacteria isolates (Table 1). The physico-chemical analysis of the produce water showed an alkaline pH of 8.5 and a very high level of 650.0 mg/l chloride ion present in the sample (Table 1).

The ability of isolates to haemolyse red blood cell in agar culture showed that seven out of seventeen isolates grew on blood agar and lysed red blood cells. 41% of the isolates were positive for the haemolysis of red blood cell and the drop-collapse test. Ability to haemolyse red blood cells and the drop collapse were used to screen the isolates for emulsification activity and the selected ones were characterized and identified using the Bergey's manual of systematic bacteriology (Holt and Krieg, 1984).The isolates obtained were *Serratia rubidae*, (FMW 90)*; B. pantothenicus, (*FMW 98); *Pseudomonas syringae*, (FMW 99); *B. lentimorbus,* (FMW 102); *P. mallei*, (FMW 104); *B. pasteurii,* (FMW 105) and *K. terigena,* (FMW 106), (Table 2).

Isolates ability to emulsify kerosene showed that *P. mallei* had the highest emulsification index (EI) of 65% while *P. syringae*, *B. pasteurii* and *K. terigena* had the least emulsification indices of 2.5% after 24 h, respectively, (Table 3).

Table 4 shows the de-emulsification ability of isolates on a stable emulsion of kerosene-Tween 80, respectively. *P. mallei* showed the best de-emulsification ability by de-emulsifying 50% of kerosene. The least de–emulsification index of 14% was recorded by *B .pasteurii*.

DISCUSSION

Occurrence of Gram negative biosurfactants producing organisms has been reported by some workers (Rocha et al., 1992; Akit et al., 1981; Persson and Molin, 1987; Bodour et al., 2003). Biosurfactants-producing Gram positive rods have also been reported by many workers such as Copper et al. (1981); Yakimov et al. (1995) and Arima et al. (1968). Also recently, Tabatabaee et al., 2005 and Rashedi et al., 2005 reported the isolation of Gram positive and Gram negative emulsifying biosurfactant-producing bacteria.

The 17 bacterial isolates obtained from the produce water showed that the produce water supported the growth of a wide diversity of heterotrophic bacteria, despite the fact that produce water was obtained from the deep subsurface, microorganisms were found surviving there. This affirmed the ubiquity of microorganisms (Willey et al., 2008; Hunt, 1979).

The pH of 8.5 recorded in the study showed that many mesophilic bacteria are able to thrive at this depth (Prescott et al., 2003), hence the high microbial diversity and the high count of surface active agent producers. The unusually high level of 6500 mg/l chloride ions in the sample maybe from the drilling fluids or from the formation (that is, oil well).

Table 1. Physico–chemical properties and bacteria count of Bonny produce water.

Physico-chemical properties	Values
pH	8.5
Colour (pt. Co Unit)	20
Turbidity (FTU)	9
Conductivity (µS/cm)	151
TDS (mg/L)	98.15
TSS (mg/L)	4
Alkalinity (HC03-) (mg/l $CaCO_3$)	340
Total hardness (mg/l $CaCO_3$)	250
Calcium hardness (mg/L)	50
Ca^{2+}(mg/L)	80
Mg^{2+} (mg/L)	12.20
Fe (mg/L)	0.12
Mn (mg/L)	1.1
SO_4^{2}- (mg/L)	8
PO_4^{3}- (mg/L)	1.28
F (mg/L)	0.0
Cl- (mg/L)	650.0
Aerobic mesophilic bacteria count (AMBC) (CFU /mL)	8.6×10^6
Total oil utilizing bacteria count (OUBC) (CFU /mL)	1.32×10^6
Total surface active bacteria count (SABC) (CFU /mL)	0.03×10^6
SABC/ AMBC x 100	0.34%
OUBC/AMBC X100	0.24%

TDS–Total dissolved solids; TSS–total suspended solids; CFU/mL– colony forming unit per milliliter.

Table 2. Screening test for potential biosurfactant producers

Isolates	Haemolysis of red blood cell	Drop-collapse test	Probable Organism
FMW 90	+	+	*S. rubidae*
FMW 98	+	+	*B. pantothenicus*
FMW 99	+	+	*P. syringae*
FMW 102	+	+	*B. lentimorbus*
FMW 104	+	+	*P. mallei*
FMW 105	+	+	*B. pasteurii*
FMW 106	+	+	*K. terigena*

Table 3. Emulsification indices (%) of selected bacteria species on kerosene.

Isolates	24 h / (%)
Serratia rubidae	15 ± 0.0
Bacillus pantothenicus	0.0
Pseudomonas syringae	2.5 ± 0
B. lentimorbus	12.5 ± 3.5
P. mallei	65 ± 0
B. pasteurii	2.5 ± 0
Klebsiella terigena	2.5 ± 0.0

Table 4. De-emulsification indices (%) of isolates on stable emulsion of kerosene-Tween 80.

	0 h	24 h	
Isolates	**(%)**	**Kerosene-8% Tween 80 (%)**	**Kerosene-4% Tween 80 (%)**
S. rubidae	100	40	28.6
B. pantothenicus	100	30	28.6± 0.35
P. syringae	100	30	28.6
B. lentimorbus	100	30	28.6
P. mallei	100	50	42.9
B. pasteurii	100	43.7± 0.35	14
K. terigena	100	20	15.07± 0.35

Carillo et al. (1994); Lin (1996) and Banat (1995) have reported that isolates that haemolyse red blood cells are surface active agent producers (that is, biosufactants producers) hence, *S. rubidae, B. pantothenicus, P. syringae, B. lentimorbus*, *P. mallei, B. pasteurii* and *K. terigena* (Table 2) were biosurfactants producers. Jain et al. (1991) have reported that microorganisms that recorded negative drops-collapse test are not good emulsifiers; hence some isolates were not selected for further assay.

The ability to emulsify (kerosene) by the screened isolates point to the fact that the organisms are emulsifiers (Rosenberg, 1993) they reduced the surface tension of the water/oil mixture (Fiechter, 1992). The de-emulsification ability of genera *Bacillus* and *Pseudomonas* agreed with the works of Nadarajah et al. (2001, 2002). The various levels of emulsion stability recorded showed the emulsifying capability of the isolates. The 65% emulsification activity recorded for *P.mallei* was quite high when compared to the results of previous workers: 25, 20 and 30% (Pruthi and Cameotra, 1995) and 60% (Pruthi and Cameotra, 1997). The stable emulsion recorded for *P. mallei* showed that the organism could be of great potential for use especially in the oil industry in enhanced oil recovery (EOR).

The screened bacterial isolates from produce water obtained from Bonny, Niger Delta have shown sustained ability to emulsify hydrocarbon. All these attributes made the isolates, especially *P. mallei* to be good candidates for oil spill clean-up and tertiary recovery of oil in the Niger Delta, Nigeria using the microbial enhanced oil recovery (MEOR) approach.

ACKNOWLEDGEMENT

The provision of produce water used for this study by Shell Petroleum Development Company (SPDC), Nigeria is gratefully acknowledged.

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