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Production and Characterization of Biosurfactants from Abattoir Wastewater

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Abstract
Five genera of bacteria were isolated from abattoir waste water and identified as *Bacillus licheniforms, Bacillus alvei, Pseudomonas paucimobilis, Vibrio metschnikovii and Yersinia enterocolitica*. The isolates were screened for biosurfactant production using blood haemolysis test, emulsification test, oil spreading methods. The biosurfactants were characterized using GC-MS analysis. The isolates showed haemolytic activity and the highest emulsification index of 71% was observed from *Pseudomonas paucimobilis* followed by *Bacillus alvei* with emulsification index of 67% whereas *Bacillus licheniformis* has the lowest emulsification index of 11%. Also *Bacillus alvei* showed a high oil displacement of 20mm followed by *Bacillus licheniformis* with 18ml oil displacement whereas *Pseudomonas paucimobilis* has the lowest oil displacement of 15mm. The biosurfactant could be employed to enhance oil recovery bioremediation.
Keywords: Abattoir, *Bacillus, Pseudomonas,* Biosurfactants, Wastewater

INTRODUCTION

Biosurfactants are surface active biomeolecules that are produced by a variety of microorganisms (bacteria, yeast and filamentous fungi etc) when grown on water miscible or oily substrates (Chen *et al.,* 2007). These surfactants are surface active metabolites or amphiplilic components possessing both hydrophilic and hydrophobic moieties in one molecule. The hydrophilic moieties (water soluble) or polar group appear in variation such as amino acid or peptide, carbohydrates, phosphate, alcohol and carboxylic acid, while the hydrophobic moieties (oil soluble) or non polar, is frequently a hydrocarbon chain such as unsaturated fatty acid and saturated fatty acid (Raza *et al.,* 2007).

Unlike chemical surfactants, which are mostly derived from petroleum feedstock, these molecules can be produced by microbial fermentation processes using cheaper agro based substrates and waste materials such as municipal waste sludge (Gallert, 2002), In various industrial processes, they are potentially used as surface active agent, emulsifiers, wetting agents, spreading agents, foaming agents, functional food ingredients and detergents. The objective of this research is to exploit the possibility of producing these biosurfactants from abattoir wastewater.

MATERIALS AND METHODS

Sample collection and processing

Two litres of abattoir wastewater were collected in a 2 litre capacity plastic container from Sokoto Central abattoir along Garba Duba Road, Sokoto, Nigeria. The samples were immediately transported to the Microbiology laboratory of Usmanu Danfodiyo University, Sokoto, Nigeria for analysis. The sample was serially diluted and 1ml of the suspension was spread plated onto already prepared sterile nutrient agar plates. The plates were incubated at 37^{o}C for 24 hours (Oyeleke and Manga, 2008).

Screening for biosurfactant production

Blood haemolysis test

After the incubation period, the colonies were characterized and coded as DGI, DGII, DGIII and DGIV. The isolates were screened for biosurfactant production using the blood haemolytic test of Cooper, 1987 as follows: A loopful of the test isolates was streak-plated on blood agar media and at 37˚C for 24 hours. After, the appearance of transparent ring around the colony showed that the bacteria were heamolytic. The first screening test for biosurfactant production was haemolysis test. The isolated strains haemolytic activity was tested on blood agar containing 5% (v/v) human blood. The plates were then incubated for 48 hours at 37^{0}C (Bicca *et al.,* 1999).

Emulsification stability test

This was carried out according to the method described by Cooper *et al*. (1981). The emulsification stability test was carried out by suspending 0.3ml of culture broth into test tubes containing 2ml of mineral salt solution and was incubated for 48 hours. Two (2) ml of crude oil (bonnylight) was added to each tube after 48hours of incubation and mixture vortexed at high speed for a minute, and the test tubes containing the mixture was left to stand on bench for 24 hour. The isolates index is given as percentage of height of emulsified layer (cm) divided by total height of the liquid column (cm).

$$E_{24} = \frac{\text{height of emulsion}}{\text{height total}} \quad X\ 100\%$$

Complementary screening (oil spreading method)

The oil spreading technique was carried out according to the method described by Youssef *et al.* (2004). Fifty milliter of distilled water was added to petridishes followed by addition to 100microliter of crude oil to the surface water. The 10 microliter of cell free culture broth was dropped on the crude oil surface. The diameter of the clear zone on oil surface was measured and compared to 10 microliter of dislted water as negative control.

Production and extraction of biosurtantact

The whole culture broth was centrifuged at 400rpm for 1 minute. The supernatant was dispensed into sterile test tubes using a sterile pipette, and then 0.5ml of hydrochloric acid (HCl) was added. One mile (1ml) of the organic solvent (Chloroform-methanol) in the ratio of 2:1 (v/v) was also dispensed into the test tube and allowed to stay for 30 minutes and then centrifuge at 400rpm for 1 minutes. Then the supernantant was collected using a sterile pipette and dispensed into a sterile test tubes and then place in oven at 40^0C to obtain the dried crude biosurfactant.

Characterization of biosurfactants produced

GC-MS analysis was performed using GC-MS Q P2010 plus (Shimadzu, Japan) equipped with flame ionization detector (FID). The injection was conducted in split less mode at 250^0C for 3 minutes by using an in let of 0.75mm i.d to minimize peak broadening. Chromatography separations were performed by using DB-WAX analytical column 30m, 0.2mm, 0.25mm (J & W scientific, Folson C.A) with helium as carrier gas at a constant flow rate of 0.8ml/min. The oven temperature was programmed at 60^0C for 15 minutes, following by an increase (Held for 5 minutes), and finally at 10^0C/minutes to 280^0C (held for 10 minute). The temperature of the FID was 250^0C.

RESULTS

Blood haemolysis

All of the isolated bacterial strains were tested for haemolytic activity and the result indicated that only three (3) of the isolates showed haemolytic activity as shown in Table 1.

TABLE 1: Blood haemolysis of bacterial isolates

Isolate code	Isolate name	Haemolysis reaction
A	*Bacillus licheniformis*	β- haemolysis
B	*Bacillus alvei*	β- haemolysis
C	*Pseudomonas paucimobilis*	β- haemolysis
D	*Vibrio metschnikovii*	No- haemolysis
E	*Yersinia enterocolitica*	No- haemolysis

Emulsifying ability

The result for reduction of surface tension is presented in Table 2. The result indicated that out of the three (3) bacterial isolates that showed haemolytic activity and also screened for reduction in surface tension only 2 showed a significant emulsification index with 71.0% for *Pseudomonas paucimobillis* 67% for *Bacillus alvei* and. *Bacillus licheniformis* showed very low emulsification 11.0%.

TABLE 2: Emulsification capacity of bacterial isolates

Isolate code	Isolate name	Emulsification capacity (E)
A	*Bacillus licheniformis*	11%
B	*Bacillus alvei*	67%
C	*Pseudomonas paucimobilis*	71%

Complementary screening (oil spreading methods)

The three (3) isolates tested for oil spreading techniques represented in Table 4, showed that *Pseudomonas paucimobillis* displace oil by 15mm, *Bacillus licheniformis* displace by 18mm and *Bacillus alvei* displace oil by 20mm.

TABLE 3: Oil displacement of bacterial isolates

Isolate code	Isolate name	Oil displacement (mm)
A	*Bacillus licheniformis*	18mm
B	*Bacillus alvei*	20mm
C	*Pseudomonas paucimobilis*	15mm

Table 4: Characterization of biosurfactant produced by *Pseudomonas paucimobilis*

S/NO	Retention Time	Area	Name of compound Identified	Quality
1	16.05	2.10	Hexadecane	46
2	20.06	1.96	Heptadecane	90
3	21.54	3.21	1, 2-benzenedicarboxylic acid, butyl, 1,2-methyl propyl ester	38
4	21.54	3.21	1, 2- benzenedicarboxylic acid, bis (2-methyl propyl) ester	38
5	29.71	23.34	(3-Ethoxycarboxylmethyl-5-hydroxyclohexyl) acetin acid, ethyl ester	38
6	33.41	4.97	Phthalic acid, heptyl octyl ester	72
7	33.41	4.97	1, 2-benzenedicarboxylic acid, isodecyl octyl ester	64
8	33.41	4.97	1, 2-benzenedicarboxylic acid, diheptyl ester	64
9	33.41	4.97	1,2-benzenedicarboxylic acid, diundecyl ester	35
10	33.62	6.44	1,2-benzenedicarboxylic acid, diisocoolyl ester	95
11	33.62	6.44	1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	64

DISCUSSION

This study reveal that, five (5) bacteria were isolated from abattoir waste water were identified as *Bacillus licheniformis, Bacillus alvei, Pseudomonas paucimobilis, Vibrio metschnikovii* and *Yersinia enterocolitica*.. The occurrence of all these isolates in abattoir waste water could be attributed to the abundance of microorganisms found in the soil and their ability to utilize or degrade both organic and inorganic molecules also contained in the abattoir waste water. There are many reports of bacterial isolates from abattoir waste water with the potential of producing biosurfactants *Pseudomonas* spp and *Bacillus* spp are frequent specie that are frequently used in the study of biosurfabnctant producers. Jean Guy *et al.,* (1996), Nasr *et al.* (2009) identified and report that spp and *Pseudomonas* spp are good producers of biosurfactant. Yu-Hong *et al.,* (2009) used *Serratia* spp and *Pseudomonas* spp and reported them as good biosurfanctant producers.

Observation made from haemolytic activity which is regarded by some authors as indicatives of biosurfactant production and as a rapid method for bacterial screening was considered for its strong advantages including simplicity, low cost, quick implementation and the use of relatively common equipment that is accessible in almost every microbial laboratory, however showed β-haemolysis with *Bacillus licheniforms, Bacillus alvei* and *Pseudomonas paucimobilis* which may probably be attributed to the fact that cellular uptake of abattoir waste water (nutrients) may result in bacterial "hyper-respiration" leading to elaboration of haemolytic molecules such as hydrogen peroxide and haelolysis-causing lipids (Biosurfactant). This report is in agreement with Rashedi *et al.* (2005) who used blood haemolysis test for screening Biosurfactant produced by *Bacillus* spp, *Pseudomonas* spp and *Serratia* spp.

Oil spreading method showed that the activity of the biosurfactants diameter of the clearing zone on the oil surface correlates to surfactant activity also called oil displacement. Previous report by Huy *et al.* (2009) also used this method for biosurfactant activity by *Bacillus* spp (19mm) *micrococus* spp (21mm) *Pseudomonas* spp (15mm) and *Serratia* spp (22mm) which is in agreement with this study Urum *et al.* (2004) also reported similar result.

Emulsification activities indicated that *Pseudomonas paucimobilis* was the best organism for biosurfactants production, since the highest emulsification index was observed with *P. paucimobilis*. This could be as a result of the readily utilizable nutrients in the broth medium. This finding is in agreement with that of Yu-Hong Wei *et al.* (2008) who showed similar result in their study with *P. paucimobilis* as good emulsifiers and had an emulsion index of 72% whereas the emulsion index of *P. paucimobilis* in this study was 71%. Moderately low emulsification index observed with *Bacillus alvei* and very low emulsification observed *Bacillus licheniformis* could be attributed to unavailable nutrients in the broth medium and very low shear stress during agitation and aeration, which is in accordance with previous reports of Rughooputh *et al.* (2005) that showed emulsion index of 70% with *Bacillus alvei* and 18% with *Bacillus licheniforms*. As such *P. paucimobilis* could be harnessed for industrial production of Biosurfactant for a better yield.

The characterization of biosurfactant produce by *Pseudomonas paucimolibilis* carried out using Gas chromatography and mass spectroscopy (GC-MS). The result revealed the present of 11 compound of which seven (7) have the same chemical structure. The compound with the highest quality of as are 1, 2-

benzenedicarboxylic acid, diisoctyl ester having long hydrocarbon chain therefore produce active biological activity such as use as a softener, use in preparation of perfumed in cosmetic, use as plasticized vinyl seats on furniture in cars use in textiles as dye stuffs and in glass making. This finding is in agreement with that of Srinivae and Baboo, 2011 who showed similar result in their study of Schleichera Oleosa by (GC-MS) technique.

Conclusion

This study revealed that abattoir waste water samples used, harbours predominantly members of *Pseudomonas* spp*, Bacillus* spp*, Yersinia* spp*,* and *Vibrio* spp of which *Pseudomonas* and *Bacillus* spp have the capability of produce biosurfactants. As such abattoir waste water could be exploited as readily available source of biosurfactant producers, among which *Pseudomonas paucimobilis* showed more promising potentials. This study suggests that *Pseudomonas paucimobilis* could be harnessed for industrial production of biosurfactant..

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