Middle-East Journal of Scientific Research 27 (7): 560-564, 2019
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2019.560.564

Screening of Bacteria Species from Soil Treated With a Pesticide for Biosurfactant Production

[1]Adieze Ifechukwu, [2]Ikeanumba Michael, [3]Onwa Ndubuisi Collins, [3]L. Maduko Amaka, [2]I. Ugwuoti and [2]C. Kemka

[1]Department of Industrial Microbiology Federal University of Technology Owerri Imo State, Nigeria
[2]Biology Department, Alvan Ikoku Federal College of Education Owerri, Imo State, Nigeria
[3]Applied Microbiology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria

Abstract: The assessment of the biosurfactant producing potentials of Bacteria species from soil treated with the pesticide; Diclorvos; 2,2-dichlorovinyl dimethyl phosphate (an organophosphate insecticide) was carried out. The soil samples were contaminated with 1% v/w and 2% v/w respectively with the pesticide. Standard microbiological methods were employed in the isolation, enumeration and identification of the Bacterial species in the treated soils. Determination of the biosurfactant producing ability was investigated by the Collapse drop test, emulsification index (E_{24}) assay and the Haemolysis test. Result of the total bacteria count (TBC) of the soil treated with 1% v/w of the pesticide indicated that it ranged from for the day one (24hrs) to $0.9X10^2$ value on day four (96 hours). For soil treated with 2% v/w of the pesticide, the TBC was $0.8X10^3$ for day one and $0.5X10^2$ for day four. It was noted that the presence of the pesticide greatly reduced the bacterial load of the soil. With higher concentration of the pesticide, the bacterial load is further reduced. Three bacterial species were identified, namely *Bacillus species, Pseudomonas aureginosa* and *Serratia species*. Result of the screening tests showed that all the bacterial species gave a positive collapse drop test; all were able to haemolyse blood. Pseudomonas had the highest emulsification index of 20 followed by Serratia species 10 and *Bacillus species* had the lowest value of 8.5. The result of these tests indicated that the bacterial species have the ability to produce biosurfactants.

Key words: Biosurfactants · Bacteria · Pesticide · Soil · Organophosphate

INTRODUCTION

A vast number of pollutants and waste materials including heavy metals are disposed into the environment per annum. Approximately 6 x 10^6 chemical compounds have been synthesized, with 1000 new chemicals being synthesized annually [1]. Almost 60,000 to 95,000 chemicals are in commercial use [2].

According to Third World Network reports, more than one billion pounds (450 million kilograms) of toxins are released globally in air and water. The contaminants are causing ecological problems leading to imbalance in nature and are now of global concern. The environmentalists around the world are trying to find solution to this menace by several means. Although they are raising their voices at international platforms regarding the depletion of natural resources and proffering solutions, little attention is given to their words and many harmful substances are still used without recourse of the adverse effects to the environment [3].

Among these chemicals we can find pesticides, which are defined as any substance or mixture of substances which are used to control destructive pest such as insects, plant disease causing organisms and weeds, including many other living organisms such as nematodes, arthropods other than insects and vertebrates that endanger our food supply, health or comfort.

In particular, the term pesticide refers to chemical substances that alter biological processes of living organisms deemed to pest, whether these are insects, moulds or fungi, weeds or noxious plants. Pesticides are widely used in most areas of crop production to minimize

Corresponding Author: Adieze Ifechukwu, Department of Industrial Microbiology, Federal University of Technology Owerri Imo State, Nigeria.

infestations by pests and thus protect crops from potential yield losses and reduction of product quality [4].

Pesticides have made a great impact on human health, production and preservation of foods, fiber and other cash crops by keeping in check many species of unwanted insects and plants. However, the rate of increase in the use of pesticides in developing countries is considerably higher than that of the developed countries. Extensive and improper use of chemicals leads to greater health risks to plants, animals and human population which had been reviewed from time to time by several workers [5]. One of the major problems aside from toxicity and carcinogenicity of pesticides is their long persistence in nature that amplifies the toxicity and health risk problems in area of contamination [6].

A variety of physical and chemical methods are available to treat the soils contaminated with hazardous materials but many of these physicochemical treatments do not actually destroy the hazardous compounds but are bound in a modified matrix or transferred from one phase to another hence biological treatment is essential [7]. The biological treatment of chemically contaminated soil involves the transformation of complex or simple chemical compounds into nonhazardous form [8]. For biodegradation, ideally the target pesticide will be able to serve as sole carbon source and energy for microorganisms, including the synthesis of appropriate enzymes if needed. The specificity of enzymes active against xenobiotic compounds differs from one microorganism to another and this non-specific metabolism provides an important mechanism for xenobiotic degradation in the environment [9].

One of the ways that can improve xenobiotic degradation in the environment and which is being investigated is the use of biosurfactants (). Biosurfactants increases the solubility and dispersion of hydrophilic compounds and are produced by variety of microorganisms [10]. These biomolecules act on the interfaces of two liquids and alter their physical conditions (). Pollution caused by man-made, non-biodegradable organic chemicals, widely used in agriculture and industry has become a key issue of environmental concerns.

Accumulation and persistence of toxic materials in farm soils, irrigation and drinking water has become a threat today. Use of biosurfactants for degradation of pesticides in soil and water environment has gained momentum in recent times (). Surfactants can increase the surface areas of hydrophobic materials, such as pesticides in the soil and water environment, thereby increasing their water solubility and hence, the presence of surfactants may increase microbial degradation of pollutants. Several bacteria belonging to the genera *Pseudomonas, Flavobacterium, Acinetobacter, Rhodococcus, Bacillus, Arthrobacter* and Mycobacterium are known to be efficient pesticide degraders [11]. So, the present investigation was carried out to determine the biosurfactants producing ability of bacteria from soil contaminated with pesticide.

MATERIALS AND METHODS

Collection of Soil Samples: 100 grams of soil samples were collected randomly using auger from three different points from a fallow land within the gardens of Federal University of Technology Owerri, Imo State, Nigeria. They were collected from a depth of about 15cm.

Pesticide: The pesticides used in this study was Diclorvos; 2,2-dichlorovinyl dimethyl phosphate. It is an organophosphate insecticide widely used in developing countries to control household pests and stored products from insects. It is abbreviated as DDVP. It was purchased from the open market in Owerri, Imo State, Nigeria.

Contamination: The pesticide was mixed with the soil samples to obtain final concentration of 1% and 2% (V/W) pesticide in soil. The samples were then moistened with tap water to bring soil moisture level to about 80% of its water holding capacity.

Enumeration of Bacterial Populations in the non Pesticide Contaminated Soils: A ten-fold serial dilution of the soil samples were prepared with sterile distilled water and 1ml of the appropriate dilutions plated on plate count agar using the pour plate method [12]. The plates were incubated at 35°C after which colonies in the plates were counted after 24 hrs for four days. All the plates were prepared in triplicates and the average counts recorded and used for the calculation of colony forming units per gram (cfu/g) of soil.

Enumeration of Bacterial Populations in the Pesticide Contaminated Soils: From appropriate dilution, aliquots (0.1ml) of soil contaminated with the pesticide was plated in triplicates onto mineral salt medium (oxoid) and then incubated for 5 days at 35°C. Colonial counts were made every 24 hrs and average counts recorded.

Characterization of Isolates from Pesticide Contaminated Soil: Isolates from the mineral salt agar plates were identified using their biochemical and morphological characteristics up to the genus level. The tests performed included; motility, catalase, citrate utilization, indole, oxidase, oxidative fermentative (O/F) utilization of glucose, urease, methyl red (MR) and Voges-Proskauer (VP). Characterization of the isolates was performed following the procedures in the Bergey's manual of determinative bacteriology [13, 14].

Biosurfactant Production Screening Test: The cultures of isolates that grew in the pesticide treated soil were grown for 5 days in mineral salt medium with olive oil as sole source of carbon. After 5 days, the culture broths were tested for biosurfactant production using three methods described below:

Collapse Drop Test: The test was carried out as described by Shardendu Kumar *et al.* [15]. The supernatant of culture was obtained by centrifuging mineral salt broth culture at 18,000 rpm for 20 minutes. 0.1 ml of the supernatant was dropped on the surface of glass slide coated with olive oil. if the drop collapsed within 2 mins, it is a positive result (+), if it forms a ball and does not collapse, it is a negative result (-). A clean oil coated glass slide with a drop of water was used as a negative control.

Emulsifying Activity Assay: Emulsifying activity of the biosurfactant was measured by the addition of oil to the same volume of the mineral salt broth that produced biosurfactant in screwed-capped test tube. The test tube was vortexed for 2 minutes and left to stand for 24 hours.

The emulsification index at 24 h (E_{24}) was determined as the percentage of the height of emulsified layer, divided by the total height of the liquid column [16]. It was calculated as follows;

$$\text{Emulsification Activity } (E_{24}) = \frac{\text{height of emulsion}}{\text{Total height of mixture}} \text{ X } 100$$

Hemolytic Activity (Blood Hemolysis): The isolates that grew in the 1% and 2% pesticide were tested for beta hemolytic activity. Isolates were screened for on blood agar plates containing 5% (V/V) human blood and incubated at room temperature for 24 hours. Hemolytic activity was detected as the occurrence of a defined clear zone around colony [17].

Table 1: Total Heterotrophic Bacteria count of the non pesticide contaminated soil

	Time (hr) /No of colonies (cfu/g)			
Sample	24	48	72	96
A	$2.2x10^5$	$1.2x10^6$	$8.0x10^6$	$4.5x10^6$
B	$2.1x10^5$	$7.2.0x10^5$	$2.4x10^6$	$9.0x10^6$
C	$9.0x10^5$	$1.7x10^6$	$6.5x10^6$	$1.5x10^7$

Table 2: Total Bacteria count of the soil contaminated with 1% the pesticide

	Time (hr) / No of colonies (cfu/g)			
Sample	24	48	72	96
A	$3.0X10^4$	$1.2X10^3$	$2.0X10^2$	$0.9X10^2$
B	$1.4X10^4$	$6.3X10^3$	$4.0X10^2$	$2.6X10^2$
C	$2.6X10^3$	$1.1X10^3$	$5.7X10^2$	$1.8X10^2$

Table 3: Total Bacteria count of the soil contaminated with 2% of the pesticide

	Time (hr) / No of colonies (cfu/g)			
Sample	24	48	72	96
A	$4.0X10^3$	$8,7X10^2$	$2.5X10^2$	$0.3X10^2$
B	$6.2X10^3$	$1.3X10^3$	$3.0X10^2$	$0.5X10^2$
C	$0.8x10^3$	$2.9x10^3$	$2.0x10^2$	$0.3X10^2$

RESULTS AND DISCUSSION

Biochemical and Morphological Characterization of the Isolate: The Bacteria species isolated from the pesticide treated soils were identified based on their morphological and biochemical characteristics (Table 5). A total of three (3) bacteria species were isolated and identified from the samples. These include: *Bacillus* spp, *Pseudomona*s spp and *Serratia species* as shown in Table 2.

The total aerobic plate counts of the soil samples and the plate counts of soil samples contaminated with pesticide showed a marked difference in their numbers. The bacteria counts in soils contaminated with pesticide were very low (Tables 2 and 3), while the bacteria numbers in soils not contaminated with pesticide was high (Table 1). The low bacteria numbers in soils contaminated with pesticide may be attributed to the toxicity of the pesticide which may lead to bactericidal or bacteriostatic effect on the organisms and or the inability of the organisms to utilize the pesticide. It was also observed that the bacterial load of the soils contaminated with the pesticide reduces as the period of incubation increases.(Tables 2 and 3). This will continue until the bacterial species that are able to utilize the pesticide as their source of energy and carbon predominates and then begins to multiple.

Table 4: Morphological and Biochemical characteristics of the isolates from the pesticide treated soils.

	Morphological				Colonial characteristics	Biochemical Tests						
Gram Reaction	Cell Shape	Cell Arrangement	Colony Margin	Colony Elevation	Colony Configuration	Catalase	Oxidase	Citrate	MR	VP	Indole	Suspected Organism
-	cocci	singles	smooth	flat	smooth	+	-	+	-	+	-	*Serratia* spp
+	rod	clusters	smooth	flat	smooth	+	-	+	-	+	-	*Bacillus* spp
-	bacilli	clusters	wavy	raised	irregular	+	+	+	+	-	-	*Pseudomonas* spp

Key: MR= Methyl red, VP= Voges Proskauer, + = Positive reaction, - = Negative reaction

Table 6: Result of the Hemolytic Activity of the Bacteria species

Species	Hemolysis	Pigmentation
Bacillus spp	ß hemolysis	clear zone
Pseudomonas spp	ß hemolysis	clear zone
Serretia spp	ß hemolysis	clear zone

Table 7: Result of the Drop Collapsing Test

Organism	Drop Collapsing assay
Bacillus species	+ve
Pseudomonas species	+ve
Serretia species	+ve

Table 8: Result of the percentage Emulsification Activity E_A (Emulsification Index E_{24}) of the Bacteria species

Organism	% E_A
Bacillus species	8.5
Pseudomonas species	20
Serretia species	10

Morphological and biochemical tests on the isolates (Table 5) showed that *Bacillus spp, Pseudomonas spp.* and *Serretia spp* were isolated from the pesticide contaminated soils. Their isolation indicates that they are able to withstand the toxic effects of the pesticide [18]. It may also indicate their ability to degrade the pesticide and use it as a source of energy and carbon with the ability to produce biosurfactant. This is in line with the findings of Willumsen and Karlson [19], where species of Pseudomonas species was isolated from pesticide polluted soil with ability of producing biosurfactant. *Bacillus* species have been found to occur predominantly in pyrethrijn contained contaminated soil and have the ability to utilize pesticide [20]. This was also observed by other researchers [21]. The three isolates *Bacillus* species, *Serretia* specie and *Pseudonomas* species have also known to utilize olive oil as the best source of carbon for the biosurfactant production [22].

All the three bacterial species hemolysed human blood, which is an indirect indication of their ability to produce biosurfactant (Table 6).

The positive drop collapsing test by the three organisms showed that they can produce biosurfants. Aislabie, Richards and Boul [23] stated that there is a direct relationship between the diameter of the sample after a collapse drop test and concentration of the biosurfactant. *Pseudomonas* species had the highest value of emulsification activity (E_{24}) of 20%, followed by *Serretia* species with 10% and *Bacillus* specie had the least value of 8.5% (Table 8). This could be the reason why *Pseudomonas* species and *Serretia* species are always implicated in biodeterioration of hydrocarbon products [5]. This is in line with the findings of Holt *et al.* [7] in which they stated that biosurfactant production accounts for the natural adaptation of many degrading bacteria.

CONCLUSION

The production of biosurfactant is a desirable property of pesticide utilizers, it could be an important survival tool especially in polluted soils. *Bacillus species, Pseudomonas species* and *Serratia species* in this study, isolated from soils contaminated with the pesticide; 2,2-dichlorovinyl dimethyl phosphate had been shown to be potential biosurfactant producers. Hence, they may be investigated further for possible use as commercial producers of biosurfactants.

REFERENCES

1. Bodour, A.A. and R.M. Miller-Maier, 1998. Application of a modified drop collapse technique for surfactant quantification and screening of biosurfactant producing microorganisms. J. Microbial Methods, 32: 273-280.
2. Cooper, D.G., 1980. Biosurfactants Microbial. Sci., 3: 145-149.
3. Carriol, P.G., C. Mardaraz, S.I. Pitta-alvaez and A.M. Guiliet, 1996. Isolation and selection of Biosurfactant Producing Bacteria. World Journal of Microbiology, Biotechnology, 12: 82-84.
4. Cheesbrough Monica, 2000. District Laboratory Practice in Tropical Countries Part 2. Cambridge University Press, pp: 63-70.
5. Das, K. and A.K. Mukherjee, 2007. Comparism of lipopeptide Biosurfactant Production by Pseudomonas aureginosa strain in submerged and solid state fermentation systems using a cheap carbon source some industrial application of biosurfactants. Process Biochem, 42: 1191-1199.

6. Harold, J.B., 1989. Microbiological Applications. A laboratory Manual in General Microbiology 5th ed. WCB Brown Publishers USA, pp: 76-79.
7. Holt, J.G., N.R. Krieg, P.H.A. Sneath and S.T. Williams, 1994. Bergeys Manual of Determinative Bacteriology 8th ed. The Williams and Wilkins Company, Baltimore USA.
8. Karanth, N.G.K., P.G. Doe and N.K. Veenanding, 1999. Microbial Production of Biosurfactant and their importance. Curr. Sci., 17: 116-123.
9. Kosaric, N., 1992. Biosurfactants in Industry. Pure Appl. Chem., pp: 1731-1737.
10. Kosaric, N., 2001. Biosurfactants and their Application in Bioremediation. Food technol. Biotechnol., 39: 295-304.
11. Lai, C.C., Y.C. Hang, Y.H. Weir and J.S. Chang, 2009. Biosurfactant enhanced Removal of Pesticide from Contaminated soil. J. Hazard Mater., 167: 609-614.
12. Lang, S., 2002. Biological Amphiphiles (Microbial Biosurfactants). Curr. Opin. Colloid Interface Sci., 7: 12-20.
13. Lee, S.G., B.D. Yoon, Y.H. Park and H.M. OH, 1998. Isolation of a novel pentachlorophenol degradation bacterium, Pseudomonas sp Bu 34. J. Appl. Microbiol., 85: 1-8.
14. Mulligan, C.N. and B.F. Gibbs, 2004. Types, Production and Application of Biosurfactants. Proc. India Nat. Sci. Acad, 1: 31-55.
15. Shardendu Kumar, Garima Kaushik, Mohd Ashraf Dar, Surendra Nimesh, Irico Javier Lopez-Chukenu3 and Juan Francisco Villarreal-Chiu4, 2018. Microbial Degradation of Organophosphate Pesticide. A Review. Pedosphere, 28: 1-19.
16. Shelton, D.R., S. Khader, J.S. Kams and B.H. Pogel, 1996. Metabolism of twelve Herbicides by Streptomycetes. Biodegrad, pp: 129-136.
17. Volkering, F., A.M. Breure and Rulkens, 1998. Microbiological Aspects of surfactant use for biological soil remediation. Biodegradation, 8: 401-417.
18. Walker, A., N.R. Perekh, S.J. Roberts and S.J. Welch, 1993. Evidence of the enhanced biodegradation of napropamide in Soil. Pestic. Sci., 39: 55-60.
19. Willumsen, P.A. and U. Karlson, 2009. Screening of bacteria, isolated from PAH contaminated soils, for production of biosurfactants and bioemulsifiers. Biodegradation, pp: 415-423.
20. Desai, J.O. and I.M. Benart, 1997. Microbial Production of surfactants and their commercial potential. Microbiology. Biol. Rev., 61: 47-64.
21. Desia, E., G. Paquette, R. Villemur, F. Lepime and J.G. Bisallon, 1996. Biosurfactant production by a soil Pseudomonas strain growing on polycyclic Aromatic Hydrocarbon. Appl. Environ. Microbial., 62: 1908-1912.
22. Banat, I.M., R.S. Makkar and S.S. Cameotra, 2002. Potential Commercial application of microbiology surfactants. Appl. Microbial. Biotechnol., 53: 495-508.
23. Aislabie, J., N.K. Richards and H.L. Boul, 1997. Microbial Degradation of DDT and its residues. A Review. New Zealand Journal of Agricultural Research, 40: 269-282.