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Mosquito-Degradative-Potential of Cockroach and Mosquito Borne Bacteria

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The type of bacteria present on two insects (cockroach and mosquito) were investigated. These microorganisms were screened for microbial control of mosquito employing their degradative ability at various microbial cell loads. The degradation of the mosquito was observed spectrophotometrically for an incubation period of 5 to 7 days. Six bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus aureus* and *Aerobacter aerogenes*) were isolated from cockroach. Mosquito borne *E. coli*, *P. aeruginosa*, *B. cereus* and *Staph. aureus*. All the bacteria digested the mosquito with activity ranging from 0.02 to 1.27. Microorganisms associated with cockroach showed higher degradation activity (0.02-1.27) during the incubation than those obtained from mosquito (0.02-1.00).

Key words: Mosquito, biocontrol, single cell organism

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INTRODUCTION

Among animals that are facing human beings with major problems, insects are important because of their pestiferous activities to agricultural crops, stored products and health of man and animals particularly the domestic ones (Berenbaum, 2002). Mosquito, an insect, is the carrier of the causal agent of diseases such as dengue, malaria, filariasis and Japanese encephalitis which remain endemic in many tropical areas. Chemical pesticides such as dichlorodiphenyltrichloroethane (DDT), gammasxane, malathione and chlorodane applied with the aim of eliminating mosquitoes have given rise to other serious problems (Phillip, 2001). Not only have mosquitoes developed resistance against these chemicals, but the pesticides themselves pose threat to both human health and ecosystems (World Health Organization, 1995; Phillip, 2001). It is now generally accepted that insecticides can not provide final solution to the eradication of insect borne diseases (John and Catherine, 2002). The use of microorganisms (Biopesticides) for mosquito control has been successful in many applications. This use has many merits including being selective towards mosquitoes and non-harmful to humans and other non-target organisms. They retain their activity during storage and are effective over a wide range of salinity, temperature, relative humility and breeding site. They also require only one or few applications for a period of time (Scholte *et al*., 2004). Based on this circumstance, the possibility of utilizing biopesticedes as alternative to chemicals is now a major consideration.

Biopesticides compare considerably with conventional chemical pesticides in efficacy and cost (Kaya and Lacey, 2007). Bacteria and fungi have been shown to kill mosquitoes to varying degrees (Orduz and Axtel, 1991; Su *et al*., 2001). Bacteria isolated from mosquito showed high degradative ability (Megally *et al*., 2001). The toxins produced by pest bio-control microbes are also used for an effective control of insects (Lacey and Becnel, 2004). At present, *Bacillus thuringiensis* serovar israeliensis (BTI) and *B. sphaericus* are being used in worldwide field test designed to control the population of mosquitoes (Phillip, 2001; Shilulu *et al*., 2002). However, the utilization of these bacteria has been limited to several disadvantageous biological properties that they exhibit. The mosquito larvicidal crystals of the bacteria do not persist for long periods in warm environment due to their rapid inactivation by sunlight or other degradative agents, while crystals and spore crystal complexes rapidly sediment from water surface which is the predominant larval feeding zone. The direct use of BTI cells also has its drawbacks as its cells do not exhibit stable habitation in the environment. In addition, the strain of mosquito existing in a particular region appears to differ from that in another place. Thus, one BTI may not be very effective for use in all regions where mosquitoes are problematic. Therefore, the isolation and/or development of bacterial strains with larvicidal activity having a broad host range specificity, stable habitation and non-harzadous properties is desired. In order to achieve these, we carried out a study, to investigate the type and mosquito-degradative ability of bacteria found on two insects (Cockroach and mosquito) commonly found in the tropics (Nigeria). These insects occupy the same habitat with human beings.

MATERIALS AND METHODS

Isolation and identification of bacteria: This study was conducted in 2007 at the Federal University of Technology, Akure, Nigeria. Cockroaches and mosquitoes were collected into sterile containers from two natural breeding habitats (cupboards for cockroach and stagnant water for mosquito) in a house at Alaba layout area of the Federal University of Technology, Akure, Nigeria. In the laboratory, adult cockroaches were placed inside a sterile petri dish containing 10 mL of sterile water each (in triplicate). The Petri dish was properly shaken to ensure good washing away of particles that were on the cockroaches. One milliliter was taken from the wash water, serially diluted to 10^{-4} and 0.1 mL of the 10^{-4} serial dilution was pour plated using molten nutrient agar. Incubation was done at 37°C for 24 h and the plates were observed for growth. Identification of the isolates was done using cultural, morphological and biochemical characteristics according to the methods described in Bergeys manual (Holt *et al*., 1994). Elevation, colour and shape of colonies on agar were studied. The cells were Gram stained, tested for motility, spore and enzymes (catalase, coagulase, oxidase) production and the use of sugars including lactose, sucrose and glucose. The same procedure was repeated using adult mosquitoes.

Cultivation of bacteria: A basal medium was used. The medium contained K_2HPO_4 (17.4 g), NH_4SO_4 (1.98 g), $MgSO_4$ (0.48 g), $FeSO_4.7H_2O$ (0.0025 g) and glucose (2.0 g) in 100 mL of sterile distilled water. Each isolate was inoculated into 10 mL of sterile basal medium, incubated at 37°C for 24 h. The cells were centrifuged at 12.168×10^3 g for 15 min (Centrifuge MSE Minor 35) and resuspended into 2 mL sterile water. The cells were counted and diluted. At inoculation onto mosquito, the diluted cells were pour plated into nutrient agar, incubated and counted using colony counter.

Test for degradation of mosquito: Ten mosquitoes belonging to the genus, *Anopheles* were used for this experiment. Each of the mosquitoes was surface sterilized in separate Petri dishes using 75% alcohol and rinsing with sterile water. Each mosquito was inoculated with the cells of each bacterial isolate at various cell loads ranging from 0 to 1.0×10^6 cells. Incubation was carried out for 5 to 7 days during which the degradation of the mosquito was monitored by reading optical density of the culture medium at 540 nm at 24 h interval.

RESULTS AND DISCUSSION

Different species of bacteria were associated with cockroach and mosquito. The bacteria showed various cultural, morphological and biochemical features (Table 1). Six bacterial species (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Serratia marcescens*, *Staphylococcus aureus* and *Aerobacter aerogenes*) were isolated from cockroach while four different bacterial isolates were obtained from mosquito. They are *E. coli*, *P. aeruginosa*, *B. cereus* and *Staph. aureus*. They showed varieties of cultural, morphological and biochemical features (Table 1).

Progressive increase in absorbance was recorded for all the bacteria when they were inoculated onto mosquitoes (Table 2-11). This indicates that the mosquitoes were degraded by the microbes. On the 5th day considering isolates from mosquito, the highest degradative activity was shown by *P. aeruginosa* (Table 2) followed by *B. cereus* (Table 5), *S. marcesens* (Table 7), *E. coli* (Table 3), *Staph. aureus* (Table 4) and *A. Aerogenes* (Table 6) having optical density of 0.03-1.00, 0.05-0.97, 0.04-0.85, 0.02-0.74, 0.02-0.59 and 0.03-0.50, respectively. Comparing the isolates from mosquito, *P. aeruginosa* (Table 8) and *B. cereus* (Table 11) ranked best with OD values of 0.93 and 0.92, respectively. They were followed distantly by *E. coli* (Table 9) having OD of 0.60 and *Staph. aureus* (Table 10, OD = 0.52) was the least. Cell population and incubation time affected the degradation. With increase in cell number and incubation time there was corresponding increase in absorbance (Table 2-11). Degradative effect of the total subtance released by bacteria common to cockroach and mosquito with increase in incubation time is shown in Table 12. This implies that there is need to increase the contact time to effectively eradicate mosquito particularly when low cell number is used. The digestion of mosquito was rapid when sufficient high cell number

Table 1: Cultural, morphological and biochemical characteristics of bacteria isolated from cockroach and mosquito

	Bacterial isolates					
Characteristics	A	B	C	D	E	F
Colour on agar	Green	Milky	Yellow	Cream	Milky	Red
Shape of colony on agar	Rough	Smooth	Rough	Smooth	Rough	Smooth
Elevation on agar	Flat	Flat	Raised	Flat	Flat	Raised
Gram reaction	-	-	+	+	-	-
Shape of cell	Rod	Short rod	Spherical	Rod	Short rod	Rod
Spores	-	+	-	+	-	-
Motility	+	+	-	+	-	+
Catalase	+	+	+	+	-	+
Coagulase	-	-	+	-	-	-
Oxidase	+	-	-	-	-	-
Indole reaction	-	+	-	-	-	-
Fermentation: Glucose	+	+	+	+	+	+
Lactose	+	+	+	+	+	+
Arabinose	+	+	-	+	+	+
Sucrose	+	+	+	+	+	-
Maltose	+	+	+	+	+	+
Identity	*P. aeruginosa*	*E. coli*	*Staph. aureus*	*B. cereus*	*Aerobacter aerogenes*	*Serratia marcescens*

-: Negative or absent; +: Positive or present, *P. aeruginosa*, *Pseudomonas aeruginosa*; *E. coli*, *Escherichia coli*; *Staph. aureus*, *Staphylococcus aureus* and *B.cereus*, *Bacillus cereus*

Table 2: Degradative activity of *Pseudomonas aeruginosa* isolated from cockroach

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
9.90×10^6	0.75	0.79	0.85	0.96	1.00	1.20	1.27
1.00×10^5	0.55	0.60	0.63	0.72	0.81	0.83	0.86
1.00×10^4	0.17	0.30	0.40	0.54	0.70	0.72	0.74
1.04×10^3	0.11	0.28	0.37	0.45	0.63	0.64	0.66
2.40×10^2	0.09	0.18	0.26	0.35	0.50	0.51	0.53
1.20×10	0.03	0.13	0.19	0.27	0.43	0.45	0.47
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 3: Degradative activity of *Escherichia coli* isolated from cockroach

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
9.91×10^{6}	0.50	0.55	0.60	0.68	0.74	0.76	0.80
9.78×10^{5}	0.30	0.40	0.50	0.56	0.60	0.68	0.72
1.01×10^{4}	0.19	0.30	0.38	0.43	0.49	0.53	0.58
1.03×10^{3}	0.10	0.20	0.33	0.40	0.43	0.47	0.50
2.09×10^{2}	0.06	0.13	0.25	0.27	0.23	0.30	0.33
2.40×10	0.04	0.05	0.09	0.12	0.18	0.22	0.23
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.06

Table 4: Degradative activity of *Staphylococcus aureus* isolated from cockroach

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
1.00×10^{6}	0.25	0.40	0.45	0.55	0.59	0.60	0.62
1.00×10^{5}	0.20	0.30	0.37	0.41	0.50	0.52	0.53
9.80×10^{4}	0.08	0.21	0.30	0.36	0.42	0.43	0.44
1.00×10^{3}	0.05	0.09	0.18	0.26	0.33	0.34	0.34
2.82×10^{2}	0.04	0.06	0.12	0.18	0.29	0.30	0.30
5.2×10	0.02	0.05	0.09	0.14	0.25	0.26	0.26
0	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 5: Degradative activity of *Bacillus cereus* isolated from cockroach

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
1.00×10^{6}	0.70	0.79	0.89	0.92	0.97	1.00	1.10
1.00×10^{5}	0.50	0.58	0.70	0.75	0.78	0.82	0.88
1.00×10^{4}	0.15	0.43	0.48	0.52	0.64	0.68	0.72
9.80×10^{3}	0.10	0.27	0.41	0.45	0.50	0.54	0.63
2.02×10^{2}	0.08	0.15	0.29	0.32	0.43	0.47	0.55
6.20×10	0.05	0.09	0.13	0.26	0.35	0.39	0.46
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 6: Degradative activity of *Aerobacter aerogenes* from cockroach

	Degradation (OD_{540} per day)				
Cell load (count)	1	2	3	4	5
1.00×10^{6}	0.20	0.29	0.36	0.42	0.50
1.00×10^{5}	0.15	0.20	0.30	0.38	0.41
1.02×10^{4}	0.10	0.17	0.25	0.31	0.37
1.10×10^{3}	0.08	0.14	0.19	0.26	0.32
2.05×10^{2}	0.15	0.12	0.16	0.20	0.27
3.00×10	0.03	0.07	0.09	0.17	0.22
0.00	0.00	0.01	0.02	0.02	0.03

Table 7: Degradative activity of *Serratia marcescens* from cockroach

	Degradation (OD_{540} per day)				
Cell load (count)	1	2	3	4	5
1.00×10^{6}	0.62	0.70	0.74	0.78	0.85
1.00×10^{5}	0.19	0.47	0.53	0.60	0.68
1.00×10^{4}	0.14	0.33	0.42	0.48	0.52
1.02×10^{3}	0.09	0.28	0.35	0.40	0.45
1.99×10^{2}	0.06	0.13	0.25	0.29	0.32
7.00×10	0.04	0.06	0.10	0.14	0.21
0.00	0.00	0.01	0.02	0.02	0.03

was used. Bacterial species isolated from cockroach exhibited higher values of absorbance than the same species harboured by mosquito. This implies that cockroach-associated-bacteria possess better degradative potential than the latter set of bacteria. This is because the organisms may be different strains of the same species; hence these isolates are genetically different.

Similar result was reported by various scientists that microorganisms isolated from insects can digest mosquito and other insects and pests. Lower absorbance recorded for mosquito borne bacteria indicates that these bacteria possess average quality for use in biocontrol of mosquito. Similar report was presented by Scholte *et al.* (2004). According to Megally *et al.* (2001), bacteria with

Table 8: Degradative activity of *Pseudomonas aeruginosa* isolated from mosquito

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
9.90×10^{6}	0.72	0.71	0.81	0.92	0.93	1.00	1.18
1.06×10^{5}	0.50	0.56	0.60	0.69	0.78	0.80	0.84
1.0×10^{4}	0.15	0.28	0.35	0.52	0.66	0.69	0.73
1.04×10^{3}	0.10	0.25	0.31	0.42	0.58	0.60	0.63
2.44×10^{2}	0.05	0.15	0.21	0.33	0.48	0.49	0.52
1.20×10^{1}	0.03	0.09	0.19	0.25	0.40	0.42	0.45
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 9: Degradative activity of *Escherichia coli* isolated from mosquito

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
9.90×10^{6}	0.40	0.44	0.50	0.55	0.60	0.63	0.70
9.78×10^{5}	0.25	0.36	0.40	0.47	0.52	0.58	0.61
1.01×10^{4}	0.20	0.25	0.31	0.38	0.45	0.50	0.52
1.03×10^{3}	0.08	0.14	0.18	0.25	0.30	0.38	0.44
2.09×10^{2}	0.03	0.09	0.14	0.20	0.26	0.29	0.33
2.40×10^{1}	0.02	0.05	0.07	0.10	0.15	0.19	0.21
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 10: Degradative activity of *Staphylococcus aureus* isolated from mosquito

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
1.00×10^{6}	0.20	0.30	0.40	0.48	0.52	0.56	0.60
1.00×10^{5}	0.15	0.23	0.30	0.39	0.43	0.47	0.50
9.98×10^{4}	0.06	0.15	0.24	0.30	0.36	0.40	0.42
1.00×10^{3}	0.04	0.07	0.14	0.20	0.27	0.31	0.32
2.82×10^{2}	0.03	0.05	0.09	0.14	0.21	0.25	0.28
5.20×10^{1}	0.02	0.04	0.07	0.10	0.15	0.23	0.24
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 11: Degradative activity of *Bacillus cereus* from mosquito

	Degradation (OD_{540} per day)						
Cell load (count)	1	2	3	4	5	6	7
1.00×10^{6}	0.65	0.70	0.80	0.88	0.92	0.98	1.00
1.00×10^{5}	0.40	0.47	0.57	0.66	0.72	0.79	0.82
1.00×10^{4}	0.12	0.38	0.43	0.50	0.57	0.63	0.72
0.98×10^{3}	0.08	0.27	0.39	0.44	0.48	0.50	0.59
2.02×10^{2}	0.05	0.11	0.22	0.30	0.37	0.43	0.50
6.20×10^{1}	0.05	0.06	0.10	0.20	0.32	0.36	0.41
0.00	0.00	0.01	0.02	0.02	0.03	0.04	0.05

Table 12: Degradative effect of the total substance released by bacteria common to cockroach and mosquito

	Degradation (OD_{540} per day)						
Bacteria	1	2	3	4	5	6	7
Escherichia coli	0.10	0.15	0.19	0.28	0.33	0.38	0.51
Staphylococcus aureus	0.05	0.11	0.16	0.21	0.26	0.31	0.42
Bacillus cereus	0.58	0.69	0.73	0.88	0.95	1.10	1.13
Pseudomonas aeruginosa	0.10	0.15	0.21	0.29	0.37	0.41	0.55

high degradative ability are good biocontrol agent. It then means that insects are good and cheap source of microorganisms capable of eradicating mosquito. The only limitation is that one type of microbe may not be very effective for use in all regions where mosquitoes are problematic. Therefore, the data obtained in this study will be useful in regions having similar environmental conditions as Nigeria.

CONCLUSION

In conclusion, the use of bacteria as biocontrol agent of mosquito is essential because they can provide short and occasionally, long term control. It affords minimal disturbance to non-target species and to the environment. Moreover, it is cheaper. Mosquito control can be more expensive and a hazardous process relying on toxic and

persistent insecticide. This preliminary study has shown that bacteria borne on cockroach and mosquito have good degradative ability and can be used as biocontrol. Further, study is being considered to examine the broad host range of these isolates using mosquitoes from other parts of the tropics.

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