JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2010. Vol. **4**(1), p. 23-30

Bio-control of *Anopheles* Mosquito Larvae with Bacteria Isolated from Housefly *(Musca domestica)*

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(Received: 04 September 2009; Accepted: 15 November 2009)

Five bacterial species namely: *Brahmella catarrhalis*, *Proteus vulgaris, Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from housefly (*Musca domestica*). At different populations, the active bacterial cells and their culture supernatants solutions. All the isolates expressed varying degrees of mortality. The mortality rate increased alongside increase in the bacterial cell load, concentration of supernatant and period of exposure of the larvae. Comparatively, in both active cells and supernatants, *P. aeruginosa*, *Staph. aureus* and *B. cereus* showed higher larvicidal activities than other bacteria. *Pseudomonas aeruginosa* displayed the highest larvae mortality than *Staphylococcus aureus* and *Bacillus cereus*.

Key words: Bio-control, Bacteria, *Anopheles* mosquito larvae.

Mosquitoes have been found to be a major agent in health problem in most parts of the world especially in the tropics. They are estimated to transmit disease to more than 700 million people annually. Worldwide, malaria fever, a disease caused by the *Anopheles* male mosquito is a leading cause of premature mortality, with around 1.4 million deaths annually particularly in children under the age of five years (Philip, 2001). Because mosquitoes are nuisance and life threatening insects, measures are taken to eliminate them. The major measures used today are chemical and bio-control methods.

Bio-control involves the use of living organisms to control pests, weeds and plant pathogens. Bio-control is an environmentally friendly technique of controlling pests (Terry *et al*., 2001; Kochler, 2003). Experiences in Central America show that biological control is effective, safer and cheaper than chemical control (Movimoride and Nicaraguan, 2001). The biology and mode of action of biological control organisms such as entomogenous fungi and entomopathogenic bacteria among other biological control organisms depend on taxonomy and evolution, life history, ecology, physiology, biochemistry, anatomy and genetics including

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genetic improvement and manipulation of bio-control agents (Hagler, 2000).

Bacterial pathogens often used for insects control are spore forming, rod shaped bacteria, mostly in the genus *Bacillus* (Weinziel and Whipps, 2004). They occur commonly in soil; hence, most insecticidal strains have been isolated from soil samples (Kochler, 2003). They are either obligate, facultative or potential pathogens which must be ingested by target organism to be effective, thus, they are not contact poisons. The *Bacillus* species which are currently being used for bio-control purposes include *Bacillus thuringiensis, B. cereus, B. popilliae, B. moritai* and *B. sphaericus* (Augus, 2000). The delta endotoxin produced by *B. thuringiensis* has the insecticidal property effective against black flies and many mosquitoes and can be used in malaria control (Sharman, 2005). *Bacillus popilliae* has been found to kill the Japanese beetle larvae (Weinziel and Whipps 2004, Singh and Seema, 2006).

Houseflies (*Musca domestica*) are insects which habitually feed on materials with odour and sugar. Their instinct for survival and attraction to odour lead them to open prive, garbage, decomposing materials, overflowing sewage disposal units and grossly sewage disposal units and heavy sewage-solid areas in search of partially digested or decaying foods. This behavioural and feeding habits cause them to contribute to gross microbial contamination (Akharaiyi and Omoya, 2005).

Plasmodium vectors are developing strains resistance to existing chemical control methods. There is no control measure developed in Nigeria, which may be very efficacious in eradicating mosquitoes. This could be because the efficacy of bio-control agents is dependent on the prevailing environmental factors. In order to effectively control malaria in the tropics and Nigeria particularly, indigenous strains of microbes need to be tested on mosquitoes for mortality. Recently, we reported that some bacteria isolated from cockroach and mosquito have the potential to degrade mosquito (Omoya *et al*., 2009). The present work aimed at isolating bacterial species from *Musca domestica* and testing their ability to cause mortality of *Anopheles* mosquito larvae.

METHODS

Source of Housefly

Houseflies (*Musca domestica*) were randomly collected from filthy environment at Federal University of Technology, Akure, Nigeria with an insect sweep net.

Basal medium

The basal medium used in this experiment contained H_2SO_4 (17.4g), $(NH_4)_2SO_4$ (1.98g), $MgSO_4$.(0.48g), $FeSO_4.7H_2O$ (0.0025g). Glucose (2g) was used as carbon source. These components were dissolved in 100 ml distilled water and sterilized in 10 ml lots at 115°C for 10 min.

Isolation and Purification of Microorganisms

The collected insects were transferred into universal bottles containing sterile saline solution. This was allowed to stay for 20-30 min. to dislodge the microbes from the insects into the saline water. The microbial suspension (1 ml) was diluted serially to 10^{-8} pour-plated with nutrient agar. The plates were incubated at 37°C for 24 h. The grown bacterial colonies were culturally observed and streaked on freshly prepared nutrient agar to obtain distinct pure colonies. The isolates were identified by the criteria of Holt *et al.* (1994). Positive control bacterium (*Bacillus thuringiensis* var. Israelensis HD522) was obtained from the Invertebrate Pathology and Microbial Pest Control Laboratory, School of Molecular and Cell Biology, University of the Witwalterstrand, Johannesburg, South Africa. This particular specie is known worldwide for its mosquito pathogenecity (Lonc *et al*., 2001; Maeda *et al.,* 2001). *B. thuringiensis* (BTI) was also subjected to identification tests along with the isolates for comparison.

Determination of Mortality Effect of Isolated Bacteria and Control Bacterial Strain on *Anopheles* Mosquito Larva

Each of the bacterial isolates was inoculated into the basal medium and incubated at 30°C for 72 h on a shaker rotating at 200 rpm. Cells © and culture supernatant (Cs) were separated by centrifuging the active cultures at 8000 rpm for 20 min. The C and Cs were serially diluted. The number of cells in each dilution were enumerated by pour plate method. The cells and

Cs of individual bacterial culture were bio-assayed separately at different concentrations to screen for mosquito larvicidal activity. The bio-assay was conducted thus: third instar larvae (25) of *Anopheles stephensis* in 125 mL chlorine-free tap water were put into a wax coated paper cup for each isolate. Then 1 mL of C and Cs was added separately to the larvae. The basal medium served as negative control. After 48 h exposure of larvae to the culture, mortality was assessed by counting the number of dead larvae present in the respective cups.

RESULTS AND DISCUSSION

As much as man tries in keeping good hygiene practice for a clean environment, microorganisms still prevails even in the rejects of man. Housefly is one of the insects that compete a lot with man in its environment for daily food intake. Therefore, housefly as a habitual feeder, will densely be populated with microorganisms. This accounts for high number (five) of bacterial species isolated from the housefly. The morphology and biochemical characteristics of the five isolates and the positive control bacterium (BTI) are presented in Table 1. The five bacterial isolates were identified as *Staphylococcus aureus*, *Pseudomonas aeruginosa, Brahmella catarrhalis*, *Bacillus cereus* and *Proteus vulgaris.*

Screening of the microbes for bio-control potential on *Anopheles* mosquito larvae showed that cells and supernatants (Cs) of the bacteria established some degrees of mortality on the *Anopheles* mosquito larvae (Figs. 1 to 5). The supernatants (Cs) of the bacterial species exhibited larvicidal activity lesser than the bacterial active cells. Ability of the isolated microbes to kill the larvae is dependent on cell load, concentration of Cs and incubation period. Mortality of the larvae increased with cells population, concentration of Cs and the period of exposure from 0 h to 48 h. Irrespective of the studied bacterial cells population and concentration of supernatant, increased larvae mortality was observed along increase in the period of exposure. At the 48 h exposure, a higher larvicidal activity was observed than at lower period of exposure. This observation is similar to the trend of larvicidal potency exhibited by the Bti which proved that the higher the exposure period, the higher the larvicidal strength.

Among the bacterial isolates, three bacterial strains (*P. aeruginosa*, *Staph.aureus* and *B. cereus*) expressed considerable high larvicidal property than others (Figs. 3 and 4) although lower than that of the Bti (Figs. 1, 2 and 5). The *Pseudomonas aeruginosa* (Fig. 1) active cells and its Cs were found more larvicidal potent than *Bacillus cereus* (Fig. 5) and *Staphylococcus aureus* (Fig. 2). At 0 h of incubation, there was no mortality of the mosquito larvae. From 12 h incubation, larvae mortality of 5 – 10 % and 10 – 14% (depending on cell concentration) were observed in the active bacterial cells of *P. aeruginasa, Bacillus cereus* and *Staph. aureus.* Mortality rate of between 12 % and 26 % for these three bacteria were observed at 48 h with cell load of 0.85 x 10^2 cfu/ml. The larvae-killing ability of the isolated entomopathogenic bacterial species is dependent on their physiology and biochemistry (Hagler, 2000). Ability of the bacteria to cause larvae mortality highlights that the medium did adequately support survival, growth and enhanced the cells to multiply for increased population and release of larvicidal substance/s (active principles or toxins) required for the potency.

The death of mosquito larvae by the isolated bacteria, suggests that bacterial cells are able to adhere specifically to their host (Prescott *et al.,* 2005). Consequently, the bacterial species isolated and used in this work are capable of killing *Anopheles* mosquito larvae. It is known that bacterial cells must be ingested by target organisms to be effective. Therefore, it is evident that the mosquito larvae in this investigation either fed on the bacterial cells or absorbed the active principles contained in the culture supernatant which could have been released during incubation. Toxins from bacteria were known to kill insects. The delta endotoxin produced by *Bacillus thuringiensis* has the insecticidal property effective against black fly and many mosquitoes, and can be used in malaria control (Sharman, 2005). The metabolites produced by the tested bacteria in this work may however contain toxins which are lethal to the

mosquito larvae. Though the mode of action is not yet known, the activities of the bacterial species, is as a result of the active principles contained in the supernatant. Upon ingestion by susceptible mosquito larvae, toxin inclusions are released from supernatant, disrupted bacterial cells are then solubilized in the larval midgut lumens that are generally in alkaline pH. The soluble protoxins are subsequently activated by gut proteases to yield toxic fragments that are

Table 1. Comparative morphological and biochemical characteristics of isolates and the positive control strain

Cultural	Isolates					
Characteristics	1	2	3	4	5	6
Appearance on agar	golden yellow, smooth, raised 0.5-1.5 µm by	green, irregular, flat, 0.5-1 by 1.5-4µm	white, irregular flat, 3-4µm 1µm	grey, entire regular, flat	white, rhizoid, flat	grey, undulate flat
Gram reaction	+	–	+	–	+	–
Spore	–	–	+	–	+	–
Presence of crystal	–	–	+	–	–	–
Coagulase	+	–	–	–	–	–
Catalase	+	+	+	–	+	+
Oxidase	–	+	+	–	–	–
Indole	–	+	–	+	–	+
Urease production	–	+	–	–	–	+
Motility	–	+	+	–	+	+
Starch hydrolysis	–	–	+	–	+	+
Nitrate reduction	–	+	+	–	+	–
Sodium acetate	–	–	+	–	–	+
Methyl red	–	–	+	–	+	+
Voges proskauer	–	–	–	–	+	+
Citrate utilization	–	–	+	–	–	–
Growth at:						
5°C	–	+	–	+	–	+
30°C	+	+	+	+	+	+
50°C	–	–	–	+	+	+
60°C	–	–	–	–	+	–
Growth in presence of:						
2% NaCl	+	+	+	+	+	+
5% NaCl	+	+	+	+	+	+
7% NaCl	+	–	–	+	–	–
10% NaCl	+	–	–	–	–	–
Growth under:						
Aerobic	+	+	–	–	+	+
Anaerobic	+	w	w	w	–	–
Utilization of:						
Lactose	+	–	–	–	–	–
Mannitol	+	–	–	–	–	–
Maltose	+	–	+	–	+	+
Glucose	–	+	+	–	+	+
Sucrose	–	+	–	–	–	+
Ribose	+	–	+	–	–	–
	Staph. aureus	*P. aeruginosa*	*B. thuringiensis*	*Brahmella* catarrhalis	*Bacillus* cereus	*Proteus* vulgaris

+ = positive; - = negative; w = weak

relatively resistant to further proteolysis (Schnepf *et al*., 1981).

As early as in 1989, Gupta and Vyas reported a strain of *B. subtilis* capable of infecting and causing mortality of larvae of *Anopheles cullicifacies*, the primary vector of malaria in Central India. Recently, Das and Mukherjee (2006) reported two *B. subtilis* strains active against third instar larvae of *Culex quinquefasciatus.* The only bacterial bio-control agent known to exhibit mosquito pupicidal activity is a gram-negative bacteria *Pseudomonas flourescens* (Prabakaran *et al*., 2003). However, this is the first report where the supernatant of *Staph. aureus* and *P. aeruginosa* is shown to have potent activity against larvae of *Anopheles* mosquito. This report therefore suggests that other bacterial species (besides those reported before) having larvicidal potency could be available, and unless when tested, would not be discovered.

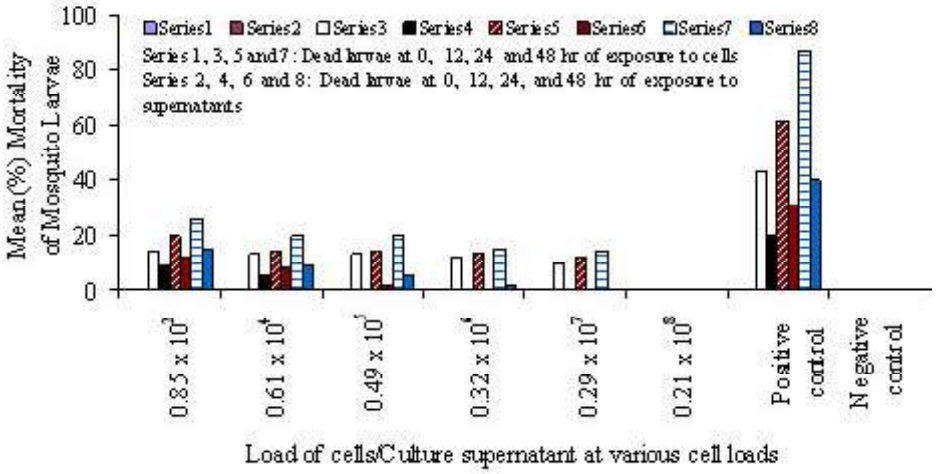


Fig. 1. Mortality of mosquito larvae at different concentrations of *Pseudomonas aeruginosa* cells and growth medium supernatants

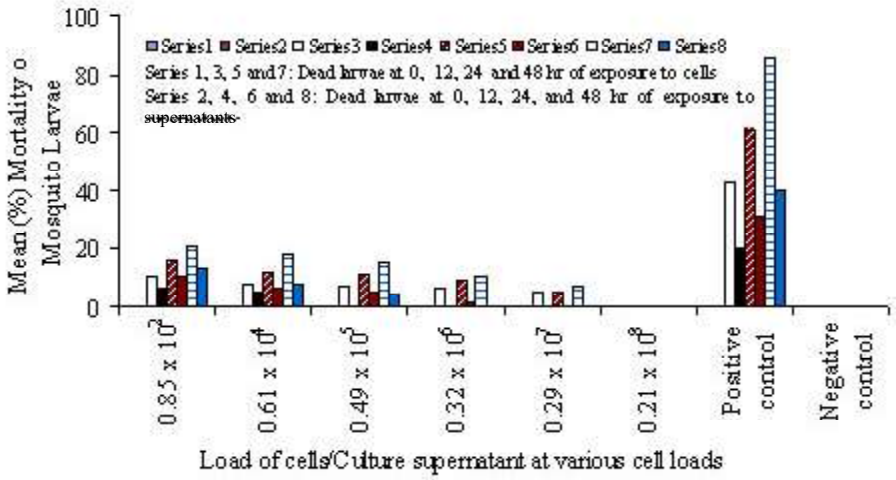


Fig. 2. Mortality of mosquito larvae at different concentrations of *Staph. aureus* cells and growth medium supernatants

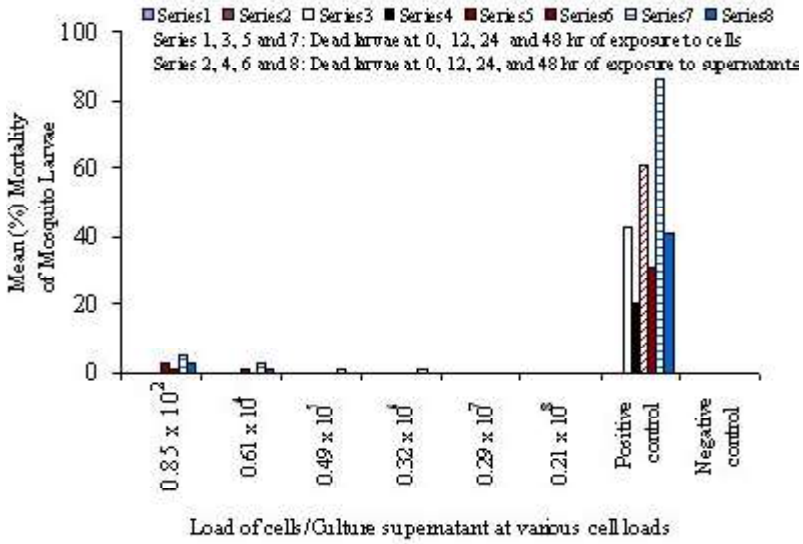
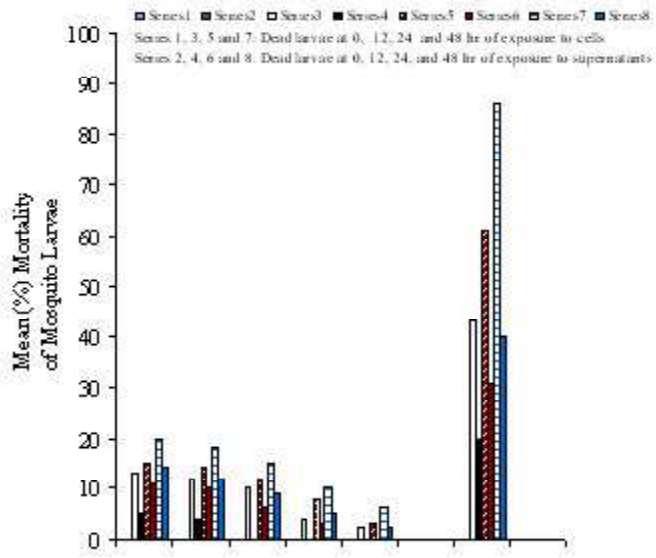


Fig. 3. Mortality of mosquito larvae at different concentrations of *Brahmella catarrhalis* cells and growth medium supernatants



Mortality of mosquito larvae at different concentrations of *Proteus vulgaris* cells

Fig. 4. Load of cells/Culture supernatants at various cell loads and growth medium supernatants

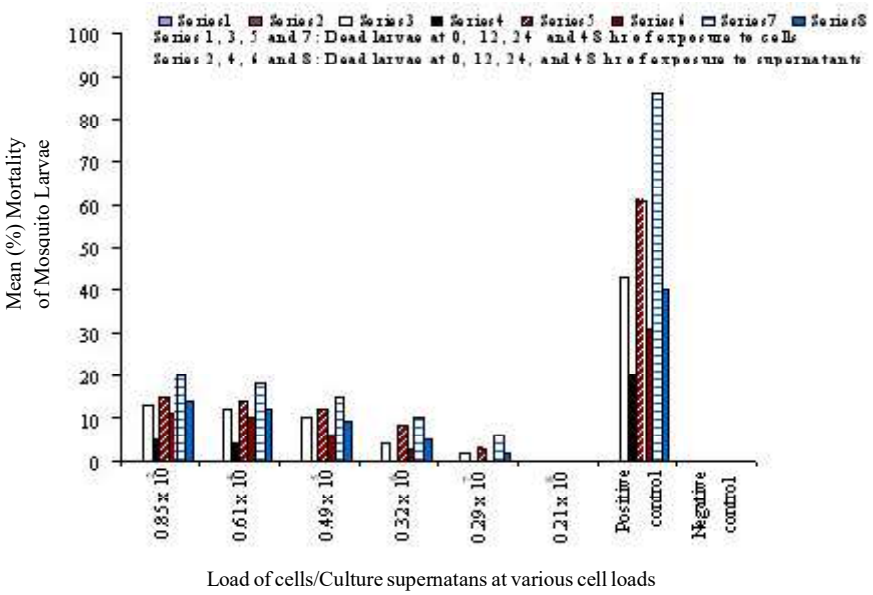


Fig. 5. Mortality of mosquito larvae at different concentrations of *Bacillus cereus* cells and growth medium supernatants

CONCLUSION

As shown by the current experimental study, the bioavailability of toxin potent substance in the active cells of *P. aeruginosa, B. cereus*, *Staph. aureus, Brahmella catarrhalis* and *Proteus vulgaris* for mosquito larvae appears to result from the complex interaction among several factors: time of exposition and load of bacterial cells. As those bacterial cells may be of dietary and / or toxicological interest, knowledge of such interactions may allow us to better understand, the nutritional and toxicological ecology of *Anopheles* mosquito larvae, in order to improve control strategies as part of malaria eradication programs. For this reason, these organisms can be cultured and used for mosquito control safety with special attention given to human pathogenecity and environmental influence of the bacteria.

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