Original Research Article

Incidence of entomopathogenic *Bacillus* spp. in some plantations in Ibadan, Nigeria

T.A. Adelani-Akande¹,²*, O.E. Fagade¹ and B.I. Aderiye²,³

¹Department of Microbiology, University of Ibadan, Ibadan, Nigeria
²Microbiology Unit, Biological Sciences Department, Landmark University, Omu Aran, Kwara State, Nigeria
³Microbiology Department, Ekiti State University, Ado-Ekiti, Nigeria

*Corresponding author

ABSTRACT

Soil samples from some agricultural research plantations (IITA, CRIN, IAR&T, NIHORT and FRIN) in Ibadan metropolis, Nigeria were screened for the presence of entomopathogenic strains of *Bacillus* species. The loamy-sand and sandy-loam soil types were typical soil texture of these locations with soil pH ranging between 4.9 and 6.1. The loamy-sand soil type recorded the highest number of *Bacillus* spp. and the history of pesticides applied on the sampling locations did not seem to affect the distribution of *Bacillus thuringiensis* and other entomopathogenic *Bacillus* spp. in the different soils. Eighty-two strains of *Bacillus* spp. were isolated with only seven of these being entomopathogenic. The seven strains of *Bacillus* spp. were toxic to *Anopheles* mosquito larvae exhibiting between 5% and 15% mortality within 72h.

Introduction

Some *Bacillus* species have been recognized for their effectiveness against larvae of some major insects which are either plant pests such as *Spodoptera frugiperda*, *S. littoralis*, *Lymantria dispar*, *Drosophila melanogaster* (Al-Momani and Obeidat, 2012; Aly 2007; Broderick et al., 2000; Manju et al., 2009; Valicente et al., 2005) or vectors of diseases such as *Anopheles gambiae*, *Culex* and *Aedes* (Gbehou et al., 2010; Majambere et al., 2007). *Bacillus thuringiensis* (Bt.) and *Bacillus sphaericus* are well known but *B. sphaericus* is reported to be slow in its action on insect larvae and thus Bt. has been widely explored for its insecticidal ability (Chatterjee et al., 2007; Surendran and Vennison, 2011). In Africa and particularly Nigeria, malaria is widespread and the *Plasmodium* parasite is transferred to humans by the female *Anopheles* mosquito. *Bacillus thuringiensis*, a Gram positive, toxin producing species of the genus *Bacillus*
has been widely known because of the insecticidal prowess resulting from its ability to produce parasporal protein bodies (Prescott et al., 2010). *B. thuringiensis* belongs to the group 1 bacilli which include *B. thuringiensis*, *B. cereus* and *B. anthracis*; however, *B. thuringiensis* does not cause food poisoning. *Bacillus thuringiensis* serovar *israelensis* is known for its efficiency against the larvae of mosquitoes (Charles and Nielson-Le Roux, 2000).

*B. thuringiensis* is able to produce an intracellular protein crystal during sporulation which is toxic to mosquitoes. The protein crystals so produced are solubilized in the mid-gut of the insect to form proteins called delta-endotoxins (Ammouneh et al., 2011) that are toxic in low concentrations to insects that have specific receptors located in the mid-gut epithelium (Tyrell et al., 1979). Although *B. thuringiensis* has no adverse effects on variety of vertebrates and invertebrates animals, however under certain conditions, it was reported to be pathogenic to earthworm *Lumbricus terrestris* (Zwahlen et. al., 2003). Isolation of *Bacillus thuringiensis* from soil used to be cumbersome however the development of better isolation techniques and media has enhanced research on *B. thuringiensis* (Travers et al., 1987; Andrzejczak and Lonc, 2008).

*Bacillus sphaericus* has also been shown to be insecticidal to mosquito larvae (Charles and Nielson-Le Roux, 2000). Surendran and Vennison (2011) isolated *B. sphaericus* from soils obtained in different ecological zones in Devakottai, India and tested for larvicidal activity on *Culex quinquefasciatus*. They reported a significant level of variation in larvicidal activity of the *B. sphaericus* isolates.

Valicente et al. (2005) reported the wide spread of *Bacillus thuringiensis* in soils in Brazil indicating that its occurrence was higher in the West-Central and Southern regions of the country. Occurrence of *Bacillus thuringiensis* has also been revealed in various regions of the United States (Gbehou et al., 2010) as well as in 12.5% of soil samples collected from various regions of Syria (Ammouneh et al., 2011).

Studies on the use of *B. thuringiensis* and other entomopathogenic *Bacillus* spp. against mosquito larva include the report by Majambere et al. (2007) where the third instar larvae of *Anopheles gambiae* were susceptible to water-dispersible and corn granule formulations of the *Bacillus sphaericus* strain. In Brazil, Valicente et al. (2005) also documented the incidence of *B. thuringiensis* in soil using a modified form of Luria Bertani broth and its ability to effectively control the maize pest, *Spodoptera frugiperda* (fall armyworm). No further application of the chemical insecticide was required after the application of *B. thuringiensis*; this further confirmed the efficacy of this organism as a biocontrol agent. Ammouneh et al., (2011) confirmed the toxicity of *B. thuringiensis* to the larvae of *Ephesia kuehniella*, *Phthorimaea operculella*, and *Cydia pomonella* but those of *Culex quinquefasciatus* were not susceptible.

Biocontrol methods have proved to be safer compared to chemical control of pest (Majambere et al., 2007). This study therefore seeks to isolate and screen the different strains of *Bacillus* spp. obtained from some soil samples in Ibadan metropolis and document the occurrence of entomopathogenic *Bacillus* spp. within this region.
Materials and Methods

Sample Collection

Soil samples were collected from farms and forests located in research institutes within and around Ibadan, Oyo State of Nigeria namely: International Institute of Tropical Agriculture (IITA), Moniya; Cocoa Research Institute of Nigeria (CRIN), Idi Ayunre; Institute of Agricultural Research and Training (IAR&T), Moor Plantation; Nigerian Institute of Horticulture (NIHORT), Idi Ishin and Forestry Research Institute of Nigeria (FRIN), Idi-Ishin.

Soil samples were collected using soil auger, hand trowel, shovel and sampling bags as required in each location. The samples were collected within a range of 0 - 15 cm depth. The bulk sample collected from each farm/ forest was thoroughly mixed and a representative sample was taken for further analysis.

Table 1 shows details of the farming practices employed in each location. All the soil samples had been treated with one or more pesticides in the past couple of years except those obtained from the Forestry Research Institute of Nigeria (FRIN), Idi-Ishin, where there was a fire incidence in 2008. However, there were no indications that pesticides were applied on the plantation.

All the soil samples were treated with one type of pesticide or another except the FRIN soil samples. The soil samples from IAR & T were treated with different pesticides such as Karate, Kochem, Furadan, Paraquat, Primextra, Glyphosphate, depending on the type of crops planted (Table 1). At different sites in the cocoa plantation at CRIN, Ultimax, Ridomil Plus and Kocide were applied to the pods at the recommended doses. Soils from the cassava plantation at IITA and the vegetable farms at the Nigerian Institute of Horticulture (NIHORT) were also treated with one form of pesticide (not disclosed).

Sterilization and Media Preparation

All apparatus used were properly sterilized. The media were prepared according to the manufacturer’s direction and sterilized by autoclaving at 121°C and 1.05Kg/cm² for 15 minutes.

Isolation of Bacteria from Soil Samples

Isolation was done using the modified method of Travers et al. (1987). A portion of each soil sample was spread evenly on aluminum foil and heated at 80°C for 30 minutes in an oven. Serial dilution was carried out and samples were plated out on tryptone soy agar. The plates were incubated at 27°C ± 2 for 24 h. The total viable bacteria count in each sample was determined. Pure cultures were obtained by streaking isolates on tryptone soy agar using a wire loop.

Morphological and Biochemical Characterization of isolates

Morphological and biochemical characteristics of the isolates were determined using the standard procedure described by Harrigan and McCance (1966).

Identification of Isolates

The isolates were identified according to Bergey’s Manual of Determinative Microbiology, 8th edition (Buchanan and Gibbons, 1974) and compared with standard microbial cultures from our laboratory.
### Table 1 Summary of crops and pesticide application on sampling sites

<table>
<thead>
<tr>
<th>SAMPLE LOCATION</th>
<th>CROPS PLANTED</th>
<th>PESTICIDE APPLICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>IITA, Moniya</td>
<td>Cassava</td>
<td>Yes</td>
</tr>
<tr>
<td>CRIN, Idi Ayunre</td>
<td>Cocoa</td>
<td>Ultimax, Ridomil Plus, Kocide</td>
</tr>
<tr>
<td>IAR &amp; T, Moor Plantation</td>
<td>Maize, Pigeon pea/ local beans, yam/ cassava</td>
<td>Karate, Kochem, Furadan, Paraquat, Primextra, Glyphosphate</td>
</tr>
<tr>
<td>NIHORT, Idi Isin</td>
<td>Vegetable, Okra, water melon</td>
<td>Yes (except in one of the farms)</td>
</tr>
<tr>
<td>*FRIN, Idi Isin</td>
<td>Pine, Gmelina</td>
<td>Not Determined</td>
</tr>
</tbody>
</table>

*Fire incidence around 2008

### Bioassay of Isolates on Mosquito Larvae

Newly emerging *Anopheles* larvae were harvested from stagnant water bodies at Ojoo, in Ibadan. Identification of the insect larvae was carried out at the Department of Zoology, University of Ibadan. The larvae were allowed to acclimatize in distilled water for 18h before the bioassay. The third and fourth instar of *Anopheles* larvae were used for the bioassay. Only seven isolates of the *Bacillus* spp. were screened for their ability to produce toxins harmful to larvae of *Anopheles* mosquito.

The bacteria isolates were grown in nutrient broth supplemented with MnSO₄ for 48 h at 27°C ± 2 (Ammouneh et al., 2011). The cells where harvested in a refrigerated centrifuge at 2,000 rpm for 15 min, later rinsed and adjusted with distilled water to a known concentration. Twenty (20) third and fourth instar larvae of field collected *Anopheles* mosquito were introduced into 10 ml of known concentration of each isolate. The control contained only distilled water and the mosquito larva. The effect of each *Bacillus* spp. on the mosquito larvae was monitored over a period of 72 h at 27°C ± 2°C, checking for mortality at 12h, 18h, 24h, 36h, 42h, 48h and 72h.

### Result and Discussion

Considering the soil texture, a very high proportion of sand (ca. 69.2% - 83.2%) was observed in all the samples compared to the quantity of silt (10% - 14%) and clay (6.8% - 16.8%) (Fig.1). The texture of the soil samples obtained from IITA, IAR&T and NIHORT were loamy-sand while those from CRIN and FRIN were of sandy-loam nature. Soil samples from IAR&T had the highest sand content (83.2%) while the CRIN soil samples had low sand content (69.2%). The percentage of silt (14.0%) and clay (16.8%) on the cocoa farm was relatively high compared to other soil samples [Fig. 1]. The soil sample from IAR&T had the least clay content (6.8%).

The pH of soil samples ranged between 4.9 and 6.1, mainly of acidic nature (Fig. 2). The soil sample obtained from the fruit and vegetable plantation (NIHORT) was the most acidic (4.9) while those samples collected from the cassava (IITA) and the cocoa farms (CRIN) were weakly acidic (pH 6.0 and 6.1 respectively).

Fig. 3 shows the total viable bacteria count (VBC) of the different soil samples. The IAR&T soil samples revealed the highest VBC of $1.87 \times 10^3$ CFU/ml while the CRIN soil samples recorded the least total
VBC (7.7 \times 10^2 \text{ CFU/ml}). The soil samples from IITA and IAR&T showed very high incidence of bacteria (values above 1.50 \times 10^3 \text{ CFU/ml}).

A total of eighty-two isolates of Bacillus spp. were obtained from all the soil samples, with sixty from the loamy-sand and twenty-two from the sandy-loam soil (Table 2). Very high number of isolates were obtained from the soil samples in IITA, IAR&T and NIHORT (73.1%). Soils from IITA and IAR&T had the highest incidence of Bacillus spp. (>50%) while those from CRIN and NIHORT recorded average occurrence of Bacillus spp. (less than 40%) and FRIN recorded very low incidence of Bacillus spp. Only few isolates (8.5%) of Bacillus spp. were recovered from the soil of the forest plantation (FRIN). For further work, only seven strains of Bacillus spp. were screened for entomopathogenesis.

All the Bacillus spp. displayed some degree of mortality within the first 12h of the bioassay. Bacillus sp. TAA1018 displayed 10% mortality within 12h, and this remained constant throughout the 72h bioassay (Table 3). The same trend was observed with Bacillus sp. TAA1024 which recorded a final mortality of 10%. After 12h of bioassay with Bacillus sp. TAA1019, a 10% mortality of Anopheles larvae was recorded, this was followed by a 5% increase after 36h.

The final percentage mortality after 72h was 15%. The Bacillus sp. TAA1022 obtained from CRIN recorded a percentage mortality of 5% within 12h and maintained the same potency until 72h of the bioassay. Within 12h of the bioassay, Bacillus sphaericus (TAA1025) and Bacillus sp. TAA1031 showed 5% mortality. Their potency increased to 10% mortality after 18h and the value remained constant until 72h. Bacillus thuringiensis (TAA1033) on the other hand revealed 5% mortality after 12h and after 24h there was an increase to 10%.

The control did not show any mortality during the first 42h of the bioassay but 5% mortality was recorded after 48h of the bioassay (Table 3). At the end of the 72h bioassay, only one of the Bacillus sp. TAA1022 displayed a 5% mortality, five Bacillus spp. including Bacillus thuringiensis TAA1018, Bacillus thuringiensis TAA1033 and Bacillus sphaericus TAA1025 revealed a percentage mortality of 10% while Bacillus sp. TAA1019 exhibited a mortality of 15%.

The incidence of Bacillus spp. varied with the soil texture of the different plantations. Similarly, the soil pH had an effect on the diversity of the microbes. But Lauber et al. (2008) stated that the soil texture, total carbon and extractable phosphorous differed significantly between land use types while noting that the soil pH did not vary significantly. It is not clear from this study if the application of the known pesticides affected the occurrence of Bacillus spp. However, it is evident that the outbreak of fire in 2008 at FRIN had an impact on the incidence of these microbes in the pine and Gmelina plantations.

Sixty Bacillus spp. were obtained from the loamy-sand while only twenty-two isolates were recovered from the sandy-loam soil. At the time of sampling, cassava was planted on all the sampling locations in IITA while yam and cassava were cultivated on one of the farms in IAR&T. The high incidence of Bacillus spp. in IAR&T, FRIN and IITA is likely due to high sand content in this soil samples.
Fig. 1 Average proportion of sand, silt and clay in sampling locations

Fig. 2 Average pH of soil samples

Fig. 3 Total Viable Bacteria Count
### Table 2 Occurrence of Bacillus spp. in the plantations

<table>
<thead>
<tr>
<th>Sample location</th>
<th>Soil Texture</th>
<th>No. of Bacillus spp.</th>
<th>% Occurrence in Soil types</th>
</tr>
</thead>
<tbody>
<tr>
<td>IITA</td>
<td>Loamy-sand</td>
<td>22</td>
<td>26.8</td>
</tr>
<tr>
<td>CRIN</td>
<td>Sandy-loam</td>
<td>15</td>
<td>18.3</td>
</tr>
<tr>
<td>IAR&amp;T</td>
<td>Loamy-sand</td>
<td>21</td>
<td>25.6</td>
</tr>
<tr>
<td>NIHORT</td>
<td>Loamy-sand</td>
<td>17</td>
<td>20.7</td>
</tr>
<tr>
<td>FRIN</td>
<td>Sandy-loam</td>
<td>7</td>
<td>8.5</td>
</tr>
</tbody>
</table>

### Table 3 Percentage *mortality of seven strains of Bacillus spp. screened for entomopathogenesis

<table>
<thead>
<tr>
<th>Location of isolate</th>
<th>Bacillus spp.</th>
<th>Percentage Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12h</td>
</tr>
<tr>
<td>IITA</td>
<td>Bacillus thuringiensis TAA1018</td>
<td>10</td>
</tr>
<tr>
<td>IITA</td>
<td>Bacillus sp. TAA1019</td>
<td>10</td>
</tr>
<tr>
<td>CRIN</td>
<td>Bacillus sp. TAA1022</td>
<td>5</td>
</tr>
<tr>
<td>IAR &amp; T</td>
<td>Bacillus sp. TAA1024</td>
<td>10</td>
</tr>
<tr>
<td>IAR &amp; T</td>
<td>Bacillus sphaericus TAA1025</td>
<td>5</td>
</tr>
<tr>
<td>NIHORT</td>
<td>Bacillus sp. TAA1031</td>
<td>5</td>
</tr>
<tr>
<td>NIHORT</td>
<td>Bacillus thuringiensis TAA1033</td>
<td>5</td>
</tr>
<tr>
<td>CONTROL</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*Average of 20 mosquito larvae were tested in each case
Chau et al. (2011) reported that the richness of bacterial species increased with the coarseness of the soil which was measured by the percentage of sand content of the soil.

The optimum pH for growth of Bacillus thuringiensis and B. sphaericus was reported to be between 5.5 - 7.5 (Kheseli et al., 2012; Khurshed, 2003; Saleh et al., 1969). Saleh et al. (1969) reported that the optimum growth of B. thuringiensis occurred at pH of 6.4 to 6.7. In this study a gradual reduction of the incidence of the organism occurred as the pH reduced to 4.4. Some entomopathogenic Bacillus species were obtained from some soil samples with pH ranging between 4.9 and 6.1. However, unlike Saleh et al. (1969) the detrimental effect of some strains of Bacillus thuringiensis occurred at pH of 4.9 and 5.1.

The acidic nature of the soil may also have affected the total viable bacteria count in the soil samples except for the samples collected from the cocoa farm which showed very low total VBC even though the pH of the soil tended towards neutrality (6.1). Rousk et al. (2009) reported a five-fold decrease in the bacterial growth and a five-fold increase in fungal growth with lower pH (tending towards pH 4). Lauber et al. (2008) on the other hand reported that the soil texture affected the composition of bacteria in soil.

It is possible that the type of crop planted affected the viable bacteria count in these locations because higher VBC was obtained in soils collected from the farm lands on which root and tuber crops had been grown. The IAR&T soil sample with the highest VBC (1.87 X 10^3 CFU/ml) was collected from various farms including the yam/cassava farm while the only crop cultivated on the IITA plantation was cassava with an average soil microbiota load of 1.58 X 10^3 CFU/ml. The diversity and concentration of bacteria in CRIN soil samples was the least (7.7 X 10^2 CFU/ml) possibly due to the use of different pesticides on cocoa yearly.

Despite the use of pesticides on most of the sampling locations, the presence of Bacillus spp. was still reported in the IITA, IAR&T and NIHORT soil samples. One of the cocoa farms (CRIN) was not sprayed with any pesticide, yet some of the soil samples still showed the presence of some cells of Bacillus spp. Details of the pesticide (s) applied on the soil at the forest plantation were not made available. However, the low incidence of Bacillus spp. in the samples cannot be attributed to pesticide application.

B. thuringiensis, Bacillus sphaericus and some other entomopathogenic Bacillus spp. were obtained from four of the five locations sampled. The ratio of occurrence of B. thuringiensis, Bacillus sphaericus and the other entomopathogenic Bacillus species in the soil samples from the different locations was 4: 1. Our report affirms the finding of Valicente et al. (2005) that in Brazil B. thuringiensis was obtained in all the regions of the country. Only the FRIN soil samples collected from the pine and Gmelina plantations did not have any strain of entomopathogenic Bacillus spp. This however contradicts an earlier report on the incidence of B. thuringiensis in Virgin woods (DeLuca et al., 1981). The low incidence/isolation of Bacillus spp. from the FRIN soil samples may have been due to the fire incidence reported in 2008. The microbial flora of the forest location may have been destroyed by the fire leading to on set of a
new microbial community which may be devoid of entomopathogenic Bacillus spp. Bacillus sp. TAA1019 which exhibited the highest mortality (15%) may be a subspecies of Bacillus thuringiensis probably B. thuringiensis var israelensis which is documented in literature as one of the most potent strains of Bacillus thuringiensis. This however needs to be confirmed by molecular identification of this strain of Bacillus sp. TAA1019.

Use of biocontrol measures against both plant pests and disease vectors is popular in many countries because it is a safer alternative to chemical insecticides. The incidence of entomopathogenic strains of Bacillus spp. in soils within Ibadan metropolis and the larvicidal activities of these indigenous isolates against Anopheles larvae has been confirmed. Entomopathogenic strains of Bacillus spp. should be exploited for large scale production of biopesticides in Nigeria; also, their use against vectors such as mosquitoes and other plant pests should be encouraged within the country.

References


Kheseli, P. O., J.O. Ochanda and Okoth, S. A. 2012. Isolation and characterization of Bacillus thuringiensis from coastal intertidal brackish sediments and from farmlands in Machakos District Kenya. from


