RESEARCH PAPER | Asian Journal of Bio Science, Volume **8** | Issue 2 | October, 2013 | 221-224
Received : 27.06.2013; **Revised :** 08.06.2013; **Accepted :** 05.09.2013

Evaluation of local *Bacillus thuringiensis* from the soils of Western Ghats, Karnataka and their biocontrol potential against white grub, *Holotrichia serrata* (F.) (Coleoptera) and house fly, *Musca domestica* L. (Diptera)

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The Western Ghats of India is one of the world's "biodiversity hotspots" that runs along the western part of South India through four states including Karnataka. As a result, Western Ghats are expected to yield high diversity of any taxon. With a view to understand this aspect study on the diversity of *Bacillus* spp. in the soils of Western Ghats was conducted. A total of 292 *Bacillus* isolates were identified as *Bacillus thuringiensis* which were recovered from 35 soil samples collected from different habitats of Western Ghats of Karnataka. Soils of different habitats varied tremendously in the natural load of *Bacillus* CFUs. Lowest CFU load was observed in soil W15 (2.6×10^6) whereas the soils W13, W20, W24, W29 (8.1×10^6) yielded the highest number of *Bacillus* CFUs/g of soil, with an overall mean of 6.07×10^6 CFUs per g of soil. On an average, 8.34 (± 1.95) colonies were picked from each soil sample. These colonies were subjected to standard biochemical tests to identify the *B. thuringiensis* colonies. On an average, 5.6 (67.12 %) of the picked colonies per soil sample were observed to be *B. thuringiensis* colonies. Tests of activity of these isolates against a species of white grub, *Holotrichia serrata* (F.) and a fly pest, *Musca domestica* revealed 14 isolates to be active against *H. serrata* and 10 against *M. domestica*, with three of these against both the species. The study thus demonstrated that there is potential for the use of these isolates in pest management.

Key words : *Bacillus thuringiensis*, Endospores, Toxicity, *Holotrichia serrata, Musca domestica*

How to cite this paper : Kumari, Manju, Manjulakumari, D. and Kumar, A.R.V. (2013). Evaluation of local *Bacillus thuringiensis* from the soils of Western Ghats, Karnataka and their biocontrol potential against white grub, *Holotrichia serrata* (F.) (Coleoptera) and house fly, *Musca domestica* L. (Diptera). *Asian J. Bio. Sci.,* **8** (2) : 221-224.

INTRODUCTION

Bacillus thuringiensis is a gram positive, spore-forming bacterium, which during the sporulation phase produces a protein crystal. The crystal proteins (cry and cyt) are toxic against a large number of insects, mainly species of Lepidoptera, Diptera and Coleoptera (Feitelson *et al*., 1992; Schnepf *et al.,* 1998), some of which are important pests in agriculture or vectors of human diseases. *B. thuringiensis* has been isolated from different natural sources such as soil (Arango *et al.,* 2002), dead or sick insects (Bernhard *et al.,* 1997), stored plant products (Hongyu *et al.,* 2000), phylloplane (Maduell *et al.,* 2002) and other natural sources (Iriarte *et al.,* 1998). Further, it is anticipated that it is highly diversified in areas where the insect pests' diversity is high. Considering this, Western Ghats being one of the important mega diversity centers of the world, soil samples were collected from these areas. *Bacillus* spp. were isolated and evaluated from these soils against two insect pests, *viz.*, *Holotrichia serrata* (F.) (Coleoptera: Scarabaeidae) and House fly, *Musca domestica* (L.) (Diptera: Muscidae) were carried out.

RESEARCH METHODOLOGY

Sample collection :

Ten gram of soil sample was taken from top 5cm depth, after gently removing the debris in the top soil, using a sterile spatula. The sample was then placed inside the sterile polythene covers (10cm x 5cm). Labels showing the details of

date of collection, place of microhabitat were placed inside the polythene covers and secured properly. The soil samples were stored at 4°C until further use.

Isolation of *Bacillus thuringiensis* :

A total of 35 soil samples were collected from different locations of southern parts of Western Ghats of Karnataka. *Bacillus* species were isolated employing sodium acetate method (Travers *et al*., 1987) from the soil samples. The colonies that were formed on T3 (Tryptose 3g, Tryptone 2 g, Yeast extract 5g, Magnesium chloride 0.005 g) medium were picked up for further studies. Depending on the variations in the colony morphology, a maximum of ten isolates were picked from each soil sample leading to 292 isolates from 35 soil samples. Bacilli were confirmed by the presence of spores. The isolates obtained were serially numbered with the prefix *SBT i.e* Soil Bacteria. Bacilli with endospores were sub cultured individually and incubated at 30°C for 48h and were further tested for growth on Luria agar and Nutrient agar media. All the isolates were grown in liquid media and conserved as pellets frozen at -80°C, following the standard laboratory protocol.

Characterization :

In this study, the discrimination of *Bacillus* isolates was done according to their colony morphology. After that, the cellular morphology of the isolates randomly chosen was observed to identify *B. thuringiensis* isolates by light microscopy. *Bacillus* isolates were subjected to Gram staning, endospore staining and biochemical tests *i.e.* catalase activity, starch and casein hydrolysis following Bergey's manual protocols (Holt, 1984).

Bioassays :

The isolates were tested for the biological activity against the second instar larvae of the white grub, *H. serrata* (F.) (Coleoptera) and the neonate maggots of the house fly, *M. domestica* (L.) (Diptera). For testing against *H. serrata* the broth medium along with the spores was added to the soil (1:10) and potato pieces were provided as food for the larvae. Individual field collected larvae or laboratory reared larvae were reared in the laboratory on potatoes and followed to obtain second instar larvae. Freshly moulted second instar grubs were used for all the tests. The individual larvae placed in a 50 ml vial with 40 g soil served as the replication and four such larvae were used for each isolate of *Bacillus* spp. Untreated controls were kept separately following similar protocols for every set of isolates. Mortality data was recorded at weekly intervals and the food was replaced regularly.

The larval rearing diet (Wheat bran 30.8 g, Milk powder 1.00 g, Sodium benzoate 0.10 g, Sugar 5.00 g, Nipagen 0.10 g, Brewer's yeast 3.0 g, Water 60 ml) of Ruiu *et al.* (2006) was used in the bioassays against the house fly. The insecticidal activity of *B. thuringiensis* spore-crystal complex along with broth medium was assessed by adding the five day old culture of the bacterial isolate incubated at 30°C for testing their insecticidal activity against house fly maggots. One part of bacterial culture and two parts of diet (1: 2) were placed in Petri dishes containing sterile filter paper. Ten neonate maggots were transferred onto the diet in the Petri dish. Three replicates were maintained. The mortality data were recorded at an interval of 24 h. Each isolate was individually screened to identify the toxic isolates and the larvicidal activity was assessed by comparing with the control. The mortality data obtained were corrected using Abbot's formula (1925).

Statistical analyses :

The data were analyzed by using SPSS software (SPSS 10.0) for analysis of variance and means were separated by Tuky's HSD test. Suitable statistical analyses were carried out on MS Excel for the data generation and graphic illustrations.

RESEARCH FINDINGS AND ANALYSIS

Soils of different habitats (Table 1) varied tremendously in the natural load of *Bacillus* CFUs reaching up to 100 folds. Lowest CFU load was observed in soil W15 (2.6×10^6) whereas the soils W13, W20, W24, and W29 (8.1×10^6) yielded the highest no. of *Bacillus* CFUs /g of soil (Table 1). Travers *et al.* (1987) tested 37 strains of spore-forming bacteria in four sodium acetate concentrations (0.06M,

Table 1: Density of Bacillus (CFUs/g) of soil from different soil samples of Western Ghats

Place of collection	No. of samples	Sample code	CFUs/g soil (Mean± S.D) $x10^6$
Annual crop	5	W2, W3, W6, W9, W11	4.2±1.48 [a]
Areca garden	20	W16, W17, W18, W19, W20, W21, W22, W23, W24, W25, W26, W27, W28, W29, W30, W31, W32, W33, W34, W35	7.8±2.7 [a]
Coconut garden	2	W4, W7	5.0±00 [a]
Fallow land	4	W1, W10, W12, W13	7.5±5.06[a]
Forest land	2	W14, W15	3.0±0 0[a]
Grass land	2	W5, W8	7.5±3.53 [a]

Values with the same letter are not significantly different from each other ($p<0.05$)

0.12M, 0.25M and 0.5M) in order to determine their ability to germinate in acetate buffered medium.

They reported that the germination of *B. thuringiensis* strains was usually inhibited by 0.25M sodium acetate concentration, while other spore-formers germinated. Similarly, in this study, sodium acetate concentration of 0.25M was used to increase the efficiency of the isolation rate of *B. thuringiensis* strains from soil samples. Pasteurization of samples at 80°C for 10 minute duration was performed to kill vegetative cells of other spore-formers and to eliminate non spore formers. Both sodium acetate/heat treatment and negative control with heat treatment gave similar isolation rate of *B. thuringiensis*. However, more of the other spore formers were isolated with negative control and overall 292 *Bacillus* isolates were obtained from 35 soil samples.

Further, the colony morphology of *B. thuringiensis* is rough, white and spread out over the plate, and the cellular morphology rod shaped. On an average, 8.34 (± 1.95) colonies were picked from each soil sample. The biochemical characterization of the isolated bacilli revealed 196 of the 292 cultures were *B. thuringiensis*. Number of *B. thuringiensis* cultures among the bacilli ranged from 10 to 100 per cent in different locations (Fig.1) and the recovery of *B. thuringiensis* was independent of the bacilli load observed in different locations sampled.

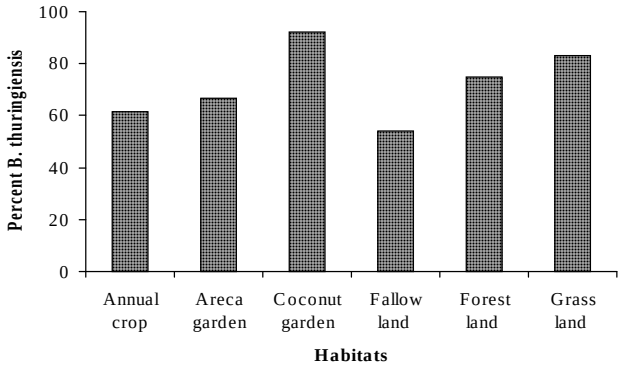

Fig. 1: Percentage of isolates identified ad *Bacillus thuringiensis* from different soil samples of Western Ghats

According to the colony and cellular morphological observations, 196 of 292 (67.12%) isolates were provisionally identified as *B. thuringiensis*. The results are in accordance with the studies of Martin and Travers (1989) who reported that the normal habitat of *B. thuringiensis* is soil, since the spores of the organism persist in soil for long periods of time. They studied 44 soil samples from agricultural areas and most of the *B. thuringiensis* strains were isolated from soil samples.

As many as 32 isolates identified were assessed for their insecticidal activity against white grubs and also against the house fly maggots. Against the house fly, it was observed that the mortality due to the isolates ranged from 0 to 93 per cent (Fig.2). Only one culture provided 93 per cent mortality, while a majority of the isolates were non-toxic (13 isolates).

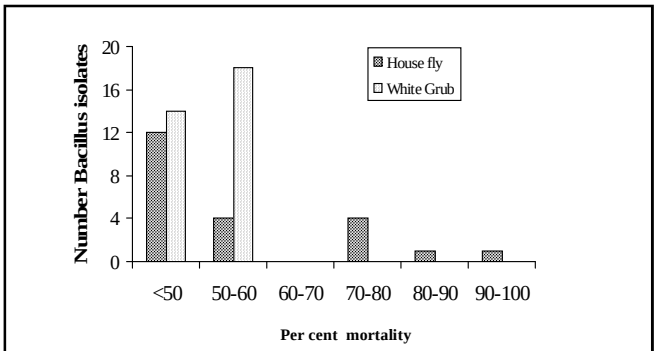

Fig. 2: The frequency distribution of insecticidal activity of *Bacillus* isolates against larvae of house fly, *Musca domestica* and white grub, *Holotrichia serrata*

In order to overcome the problem of identifying the toxic and non-toxic isolates a fifty per cent rule was followed. Accordingly, 8 of the isolates were considered toxic and with two additional isolate causing exact 50 per cent mortality. 31 per cent of the isolates were toxic to house fly. This value is much higher than what is generally expected for the Diptera, *i.e.*, 6 to 20 per cent (George, 2009). An interesting feature is that three out of ten cultures were also found toxic to white grubs (Fig. 3). Eighteen isolates were found toxic to *H. serrata* (Fig.3). They provided up to 50 per cent mortality and 44 per cent of the cultures were toxic. Relatively lower mortality observed could be a factor associated with the age or stage of the grubs, the activity in the present study was assessed only against the second instar grubs.

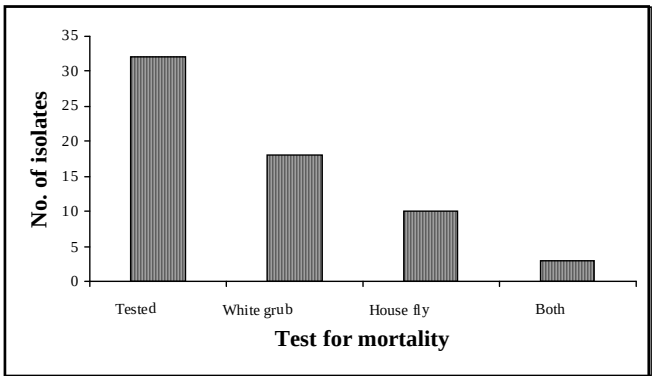

Fig. 3: Biocontrol potential of Bacillus isolates toxic against white grub, *Holotrichia serrata* and house fly, *Musca domestica*

Conclusion :

The study demonstrated that a considerable proportion

of the isolates from the soils of Western Ghats were active against either the white grub or the house fly and the activity was further compared to what is reported earlier (George, 2009). However, the activity was much lower than what was expected against *Holotrichia serrata*, while that of house fly was in tune with the general expectations. The relatively high percentage of the active isolates clearly suggests the possibility of finding *Bacillus thuringiensis* isolates with greater virulence if the soils of Western Ghats are intensively searched. This would be of particular importance as it is difficult to use insecticides for managing house flies and there are no suitable insecticides available to manage the white grubs under field conditions. Hence, an intensive search for the ideal isolates would greatly enhance our chances of managing these pests, which would also be ecologically viable and economical.

Acknowledgement :

We are thankful to Mr. Balaraju, Mr. M. Papanna and Mr. K.V. Prakash for their help in the maintenance of insect cultures and bioassays. Dr. K. Chandrashekara provided valuable inputs at various stages of the study. The laboratory facilities required for the study were set up from various grants from DST and DBT, Govt. of India to Dr. A.R.V. Kumar and to Dr. K. Chandrashekara. The two agencies also provided the partial financial support for the present study.

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