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Genotypic Diversity of Mosquitocidal Bacteria: An editorial

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The genus *Bacillus*encompasses Gram-positive rod shaped, endospore–forming or facultatively anaerobic bacteria, and is a phenotypically and phylogenetically diverse taxon [1]. *Bacillus*species are distributed widely in nature. Members of this group can be isolated from soil, plants, insect guts, foods and diseased animals. The *Bacillus cereus*(*Bc*)group comprises closely related gram-positive bacteria that exhibit highly divergent pathogenic properties. Many bacteria classified as *B.cereus*are widely distributed in the environment, with probable reservoirs in the soil, and as commensal inhabitants of the intestines of insects. Occasionally they are associated with food poisoning. This is due to mainly four main enterotoxins, named cereulide, hemolysin-T, non-hemolytic enterotoxin complex and cytotoxin-K. *B.thuringiensis*are the other members of insect pathogens and it is a gram positive bacteria that synthesizes crystal inclusions during sporulation constituted by δ endotoxins (M.wt: 134, 127, 68 and 27 kDa) [2,3] encoded by the *Cry*and *Cyt*genes. The geometry of these crystals allows a first classification system of *Bt* strains. In addition, *Bt*has other insecticidal proteins that are secreted during its vegetative cycle. The Cry toxins of *Bt*have specific activities against insect species of the orders *Lepidoptera*, *Diptera*, *Coleoptera*, *Hymenoptera*and nematodes [4]. Thus, *B. thuringiensis*produces crystalline protein inclusions with insecticidal properties. When insects ingest toxin crystals, the alkaline pH of their digestive tract activates the toxin. Cry toxin gets inserted into the insect gut cell membrane, forming a pore. The pore results in cell lysis and eventual death of the insect. Occasionally, *Bt* strains are responsible for human infections, similar to those caused by strains of *B.cereus*. *B.thuringiensis*subsp. *kurstaki*(*Btk*)is another subspecies that produces crystal proteins and are pathogenic to agricultural pests.*B. sphaericus* (*Bs*) is also aerobic, mesophilic, spore-forming and gram-positive bacterium, commonly isolated from soil [5]. Most of the strains are pathogenic to mosquito larvae and have been widely used as biocontrol agents for disease transmitting mosquitoes. The larvicidal activity of *Bs*mainly originates from the binary toxins (Bin proteins), which are produced during sporulation a major toxic component of commercial *Bs*strains. A new component of Cry toxins (Cry48Aa and Cry49Aa) with high specificity against *Culexquinquefasciatus*has also been reported [6].

In the application of the above mentioned *Bacillus*species for insect biological control, only limited effort has been directed towards identifying genetically diverse strains that have novel toxic activities towards insect pests. Since there is always a demand for selecting the most promising and most potential bacterial strains for producing biological insecticides, genetically diverse collections of strains can have practical importance in the development and formulation of these organisms for medicinal and agricultural applications [7]. Considering the view of usefulness of genetically diverse microbes in insect biological control, there has not been a major focus on this in the recent years; therefore, such an approach deserves attention. The genotypic analysis of *Bacillus*species (*B.cereus, B. thuringiensis*and *B. sphaericus*) has been studied using Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) to identify similarities and dissimilarities that exist between the various naturally isolated strains. There are 30 bacterial strains of *Bacillus* sp., which has been isolated from samples collected from various natural sources including marine soil, excreta from birds, fish gut and soils from rural villages around UT of Puducherry, India. Seventeen previously isolated cultures obtained from various researchers were also used for comparative genome analysis; these included 7 *B.sphaericus*, 9 *B.thuringiensiskurstaki*and 1 *B.thuringiensisisraelensis*.

Thirty *Bacillus sphaericus*(*Bs*), *B.thuringiensis*(*Bt*) and *B. cereus*(*Bc*) strains collected from natural sources were used. Ten of them were isolated from the bird excreta and the remaining isolates were collected from fish gut, marine and terrestrial soils (11, 4 and 5 isolates respectively). All the strains had been identified as new bacterial isolates and 16s rDNA sequences had been recorded in NCBI. Seventeen reference strains were included as comparative material.

The development of Random Amplified Polymorphic DNA (RAPD) or arbitrarily primed PCR fingerprinting gave an advantage in which molecular preliminary information of the species studied is not necessary and polymorphism pattern obtained usually varies among the species. In earlier studies, RAPD-PCR has been successfully used for genetic fingerprinting of human, animals, plant, and microorganism such as *Lactobacillus*, *Salmonella*, *E. coli*, yeast and *Bacillus*. Genetic diversity from *Bacillus* strains collected from various natural sources around UT of Pondicherry in comparison with standard strains was analyzed. The isolates were primarily environmental and were collected from diverse natural sources. Results showed that broad diversity occurred among the strains and cluster analysis revealed several associations among isolates based on their source of origin with variation in mosquito toxicity. Phylogenetic analysis based on 16S rRNA sequence revealed high degree of similarity existing among the species, while the analysis of RAPD pattern revealed variation with respect to toxicity.

Among the 15 primers used, OPE-14, OPE-19, OPA-10 were found to be the most discriminatory and gave the highest level of polymorphism for most of the strains of *Bacillus*. Similarity coefficients among the studied isolates were estimated after pooling all the gained data. We used average similarity co-efficient value of 0.36 which increased the chances of generation of clusters thus enabling a more diversified approach of analysis. The results revealed high levels of polymorphism among the studied isolates and the generated dendrogram clustered the 47 isolates into 28 different clusters. Cluster analysis revealed the existence of distinct genetic lineages corresponding to their source of isolation. All *Bt* isolates from bird excreta formed a single cluster (cluster1), which showed phylogenetic relatedness among isolates from the same source. Further the dendrogram separated most of the *Bs*samples from the fish

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source into a distinctcluster (cluster 14). Similarly all *Bs*isolates from marine soil, other than *Bs*VCRC-B587(JQ685231)formed a distinct cluster (cluster20). Bacteria obtained from terrestrial soil also showed significant differences between the isolates and formed different groups. Thus the high similarity among various *Bacillus*isolates reflected their different origins or genetic relatedness among the same species. These results are in agreement with previous study that also found genetic relationship with source of origin of *Bacillus*strains.

Further similarity co-efficients between the species that has been generated as a result of cluster analysis of the RAPD profiles can be screened for any particular band of DNA that is present in all the mosquitocidal strains under investigation. The presence of this unique band pattern for all the strains can thus be inferred as a marker that is specific only to the mosquitocidal species of *Bacillus*. The band which corresponds to this unique pattern between the strains can then be sequenced to find a genetic marker that is diagnostic of the mosquitocidal activity of any species of *Bacillus*, even unknown. This marker gene can be used to probe the genome of the *Bacillus*species and the presence of the gene in the genome of the test organism after amplification leads to the only conclusion that the genome belongs to a mosquitocidal bacterial species. This eliminates the necessity to test the species for its mosquitocidal activity through bioassay experiments. In the view of vector control, the characterization of the diversity between the different strains of *Bacillus*will enable us to identify a marker sequence which can be efficiently used to screen a wide variety of unknown mosquitocidal *Bacillus* species from the natural environment in the future, using this marker sequence for conveniently constructing the genetic maps of unknown mosquitocidal species. So, the bacterial isolates were primarily environmental and were collected from diverse natural sources. Data presented here demonstrate that broad diversity occurred among the strains and cluster analysis revealed some associations among isolates based on their source of origin. Therefore, the result revealed that high levels of polymorphism occurred with distinct genetic lineages consequent to source of origin of bacterial strains.

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