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A highly mosquitocidal *Bacillus thuringiensis* var. *thompsoni*

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A strain of *Bacillus thuringiensis* isolated from a soil sample in Pondicherry was examined for its flagellar antigenicity, mosquito larvicidal activity and protein composition. This strain was found to agglutinate with the antisera belonging to *B. t. thompsoni* and exhibit high toxicity towards the larvae of the species of *Culex quinquefasciatus, C. tritaeniorhynchus, C. sitiens*, *Anopheles stephensi* and *Aedes aegypti*, respectively. The 28 kDa protein which has been reported to be predominantly present in the mosquitocidal strains of *B. thuringiensis* was found to be present in a higher amount in this strain. Also, the mosquito larvicidal activity and electrophoretic profile were comparable to that of a strain of *B. t.* var. *israelensis* which is known to be highly toxic to mosquitoes.

THE use of bacterial agents for mosquito control, especially *Bacillus thuringiensis* var. *israelensis* (*Bti*) is gaining widespread importance[1–4]. Among various species of *B. thuringiensis,* serotypes *B. t. israelensis*[5], *B. t. darmstadensis*[6], *B. t. kyushuensis*[7], *B. t. morrisoni*[8], *B. t. fukuokaensis*[9], *B. t. medellin*[10], *B. t. canadensis, B. t. shandongiensis*[11], *B. t. amagiensis*[12], *B. t. jegathesan*[13] and *B. t. higo*[14] are reported to be toxic to mosquitoes. However, among all these varieties, *Bti* holds its place as the highly toxic mosquitocidal serotype. At the Vector Control Research Centre, Pondicherry a survey for the isolation of mosquito larvicidal bacterial agents was undertaken and during this programme several strains of *B. thuringiensis* were isolated from various sources, i.e. soil, water, larvae and roots of aquatic weeds representing diverse habitats. Among these, one strain of *B. thuringiensis* belonging to the serotype *B. t. thompsoni* (H-12) was found to be highly toxic to different species of mosquitoes.

This strain (VCRC Accession No. B175) was isolated from a soil sample collected near Pondicherry[15]. Water dispersible powder (WDP) was prepared from spore-crystal complex (SCC) of this strain and mosquito larvicidal activity determined as described by Manonmani *et al.*[15]. For comparison, a strain of *Bti* (VCRC Accession No. B17) was included in all the studies. The *B. t. thompsoni* strain was found to be toxic to 5 species of mosquitoes tested, namely *Culex quinquefasciatus, C. tritaeniorhynchus, C. sitiens*, *Anopheles stephensi* and *Aedes aegypti* and the LC_{50} values of this strain for these mosquito species were 250, 220, 300, 790 and 600 ng/ml, respectively. The corresponding values for *Bti* strain were almost similar to those of *B. t. thompsoni*. As per this, the relative susceptibility of the different mosquito species to the two *B. thuringiensis* strains, can be arranged as *C. tritaeniorhynchus* < *C. quinquefasciatus* < *C. sitiens* < *A. aegypti* < *A. stephensi*. Thus, the LC_{50} values were lower for culicines than for anophelines (Table 1). This is in agreement with the findings of several other workers[16–19].

The protein profiles of the SCC of the two subspecies of *B. thuringiensis* were compared. Proteins were extracted as per the procedure of Yamamoto *et al.*[20] and SDS electrophoresed on 10% polyacrylamide gel[21]. The proteins of both strains were found to resolve into 9 bands (Figure 1). However, 6 were present in major amounts in *Bti* (MW: 12, 24, 28, 40, 53 and 65 kDa), whereas only 4 were seen in *B. t. thompsoni* (MW: 12, 24, 28 and 40 kDa).

Although solubilization of the crystalline parasporal inclusions from final whole culture of the 2 strains gave a complex profile of proteins, a 28 kDa protein reported

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Table 1. Larvicidal efficacy of different mosquito species to water dispersible powders of *B. thuringiensis* strains, B175 and B 17

Species	*a*	*b*	LC_{50} (95% CI)	χ^2
B175				
C. quinquefasciatus	–4.70	1.75	250.72 (227.25–276.62)	1.87
C. tritaeniorhynchus	–21.87	4.98	220.04 (212.50–227.84)	0.55
C. sitiens	–34.91	6.99	300.96 (293.54–308.58)	0.71
A. stephensi	–80.55	12.82	790.61 (779.98–801.37)	3.26
A. aegypti	–19.63	3.85	600.03 (572.62–628.75)	9.53*
B17				
C. quinquefasciatus	–4.27	1.69	240.82 (217.25–266.95)	7.27
C. tritaeniorhynchus	–19.88	4.61	220.82 (212.78–229.17)	0.44
C. sitiens	–43.58	8.63	278.61 (272.31–285.05)	2.73
A. stephensi	–106.28	16.84	740.47 (730.77–750.31)	1.21
A. aegypti	–12.67	2.83	514.34 (481.21–549.76)	3.44

$Y = a + b \ln X$; Y = % mortality; X = dose in ng/ml; *Heterogeneity at 5% level of significance.

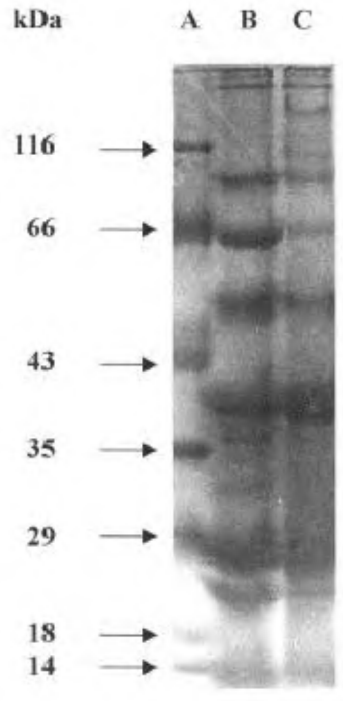

Figure 1. SDS–PAGE of proteins extracted from spore crystal complex of *B. thuringiensis* strains. Lane A, Molecular weight markers (116, beta galactosidase; 66, bovine serum albumin; 43, ovalbumin; 35, pepsin; 29, carbonic anhydrase; 18, beta lactoglobulin and 14, lysozyme), lane B, B17 and Lane C, B175.

to exhibit mosquitocidal activity in *Bti* strain[22–24] was found to be the major crystal component[25] and present in a higher quantity in this strain. Many authors[26,27] have observed positive synergism between the 28 kDa protein and 65 kDa protein and that mixtures of these two proteins from *Bti* were more toxic than expected on the basis of their individual toxicities. Reports on the protein profile of *B.t. thompsoni* are scanty. Rang[28] showed that the simultaneous production of a 34 kDa and a 40 kDa protein was found to be required for the formation of inclusion bodies in *B.t. thompsoni*, while Nickerson[29] has shown the presence of 2 low molecular weight bands of 40 and 47 kDa in size. However, the 47 kDa protein was not observed in the present investigation. This might be due to loss of a specific plasmid or due to chromosomal mutation with a pleiotropic effect, as shown Gonzalez and Carlton[30].

Thus the strain examined was found to be relatively similar to *Bti* in terms of its mosquitocidal activity and presence of mosquito-active toxic proteins, even though it belonged to a different serotype, i.e. *B.t. thompsoni*. Hence this strain of *B. t. thompsoni* could be an alternative bacterium for mosquito control programmes in case mosquito larval resistance emerges to *Bti* toxins.

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Investigation of outbreak of malaria in tribal area of Visakhapatnam, Andhra Pradesh

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The findings of an epidemiological investigation undertaken in Paderu division of Visakhapatnam district, Andhra Pradesh are reported in the present communication. The slide positivity rate (SPR) was still high (maximum SPR was 70) in spite of intensive surveillance and fever radical treatment in the affected areas. The vector, *Anopheles culicifacies*, was found resistant to DDT, but susceptible to Malathion and Deltamethrin. However, the finding of *An. culicifacies* in only 4 villages out of 12 tribal villages surveyed, and that too with highest density of 13.3, indicated that DDT was still having some impact. In a small sample, *Plasmodium falciparum* parasite was found resistant to chloroquine. The possible reasons of outbreak may be: (i) lack of surveillance and expertise in detection of malaria parasite; (ii) ineffective radical treatment as indicated by resistance in *P. falciparum*, and (iii) improper coverage of indoor residual DDT spraying in 1998. Advanced rains in the month of May 1999 also added to the hindrance in surveillance and control measures in the hilly terrain of the affected area. Suggestions for management of such outbreaks in future are discussed.

A tribal area under Paderu division of Visakhapatnam district, Andhra Pradesh (AP) started reporting fever-related deaths since March 1999. The majority of deaths occurred in May and June 1999, affecting all the 15 Primary Health Centres (PHCs) of Paderu division, comprising mainly a tribal population of 5,68,495 residing in 3370 villages. The number of blood slide collections for detection of malaria parasites also started rising from March 1999. Keeping in view high fever incidence and reported fever-related deaths in Paderu division, the State Government had drawn an epidemic action plan from 4 June 1999 till the time of survey wherein deployment of medical and paramedical staff from different parts of AP intensified surveillance and fever radical treatment and vector control measures were strengthened. In 1998, the first round of DDT spraying was undertaken from 15 April to 31 July 1999 and the second round from 5 August to 28 November 1998. In 1999, a special round of DDT spraying was undertaken in Paderu division from 1 February 1999 to 28 February 1999 except Pedabailu PHC. Intensive DDT spray operations were taken up from June 1999 and till 17 July 1999, 2022 villages (priority villages based on high fever incidence and reported death rate) with a population of 2,43,742 in 15 PHCs were covered. Fogging operations with Malathion/Pyrethrum were also done in priority villages. An epidemiological survey was therefore planned in different PHCs of Paderu division for rapid assessment of the situation, the results of which are reported here.

Visakhapatnam, the north coastal district of AP, is located between 17°15′ and 18°32′ north latitude and 18°54′ and 83°30′ east longitude. It is bounded in the north partly by Orissa and Vizianagaram district, in the south by East Godavari district, in the west by Orissa and in the east by the Bay of Bengal. The temperature ranges from 18 to 34°C throughout the year. The district receives most of the rainfall from south-west monsoon and annual rainfall ranges from 1000 to 1500 mm. The Paderu division is hilly with undulating terrain covered by Eastern Ghats and the altitude ranges from 900 to

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