Israel Journal of Entomology

Vol. XXIII(1989) pp. 3-8

BIOLOGICAL CONTROL BY *BACILLUS THVRINGIENSIS* SUBSP. *ISRAELENSIS (B.T.I.);* HISTORY AND PRESENT STATUS

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ABSTRACT

Bacillus thuringiensisvar. israelensis (B.t.i.), an aerobic, spore-forming bacterium, was first isolated and recognized as a highly pathogenic strain for mosquito larvae in 1976. It was isolated from a stagnant pond located in theNahal Besor desert river basin near kibbutz Zeclim in the Northwestern Negev desert of Israel. In addition to its high toxicity, *B.t.i.* is primarily specific for mosquitoes and blackflies and safe to vertebrates and other non-target organisms — an important attribute for a biological control agent. Ultrastructural studies of *B.t.i.* revealed that the parasporal body contains three major inclusion types. Purified inclusions consisted of a 65 kilodalton protein contaminated with minor quantities 38- and 28-kDa proteins. Different studies have shown that the crystalline parasporal body contains mainly 28, 68, 125 and 135 kilodalton polipeptides. However, the precise mosquitocidal activity of each of these polipeptides is still disputed. Studies on mode of action of the delta endotoxin which is elaborated by a large plasmid (72 MD) causes rapid cytolysis of the gut epithelium. The cellular target being the plasma membrane phospholipids leading to a rearrangement of these lipids resulting in a disruption of membrane integrity and cytolysis. A gene from *B.t.i.* was cloned from the large plasmid and was shown to code for a mosquitocidal polipeptide, The sequences of the toxin have been reported by different groups in the US, Europe and in Japan. In pursuit for better control strategies and in attempt to overcome the short duration of activity in the natural habitat, the B.t.i. toxin gene has been cloned into a variety of microorganisms, including blue-green algae. Thus, through the combined efforts of geneticists, microbiologists and insect pathologists, improved control methods of insect pest will be forthcoming.

It is estimated thal after nearly half a century of synthetic pesticide application, mosquito-bomc epidemic diseases such as malaria, filariasis, yellow fever, dengue and encephalitis are still affecting over half a billion people resulting in 90 million cases of malaria per year, including over one million deaths (WHO 1982a). The introduction of synthetic pesticides and prophylactics initially resulted in a drop in malaria cases. However, resistance of mosquitoes to DDT and other synthetic insecticides coupled with resistance developed by the malaria causing agent, the plasmodium, to various anti-malaria drugs, resulted in a dramatic increase of malaria in the tropical world (WHO 1982b).

The very properties that made the chemical pesticides useful — long residual action and toxicity to a wide spectrum of organisms, have brought about serious environmental problems. The emergence and spread of insecticide resistance in many species of vectors, the concern with environmental pollution, and the high cost of the new chemical insecticides, made it apparent that vector control can no longer depend upon the use of chemicals.

Thus, increasing attention has been directed toward natural enemies, such as predators, parasites and pathogens. Unfortunately none of the predators or parasites can be mass produced and stored for long periods of time. They all must be reared *in vivo*. It became evident that there was an urgent need for a biological agent that would possess the desirable properties of a chemical pesticide, i.e.,

it must be highly toxic to the target organism while being safe to non target organisms, able to be mass produced on an industrial scale, have a long shelf life and be transportable.

In the summer of 1976 as a part of an ongoing survey for mosquito pathogens we came across a small pond in a dried-out river bed in the north central Negev Desert near Kibbutz Zeelim (Goldberg and Margalit, 1977; Margalit and Dean, 1985). A very dense population of exclusively *Culex pipiens* complex larvae were found to be dead and the dying larvae produced a "thick carpet" on the surface. This appeared to be an epizootic situation. Later the etiological agent was identified and designated by Dr. de Barjac of the Pasteur Institute of Paris, as a new serotype, *Bacillus thuringiensis* subsp. *israelensis* (*B.t.i.*) serotype-H14 (de Barjac, 1978).

The species *Bacillus thuringiensis* Berliner 1915, is an aerobic, spore-forming saprophyte complex that lives in various soil and aquatic habitats (Dulmage, 1981). It consists of over twenty varieties or serotypes, all of which produce during sporulation, proteinaceous crystals known also as parasporal bodies or δ-endotoxins that are toxic to insect larvae upon ingestion (Fast, 1982). The *B.t.i.* serotype was tested on many species of mosquito and black fly larvae and found to be much more effective than any previously known variety (Margalit et al., 1984; Lacey and Undeen, 1986).

Serotypes of serovars are based on comparison of antibodies to flagellar (or "H" antigens of the vegetative bacterial cells. Although three other serotypes — (*B.t. darmstadiensis*, H10; *B.t. morrisoni* H8a, b (Lacey and Undeen, 1986) and *B.t. kyushuensis*, H11a, b) Ohba and Aizawa, 1979) have been found to be toxic to mosquito larvae — *B.t.israelensis* remains the most toxic and the most commonly used subspecies in mosquito and black fly control practices throughout the world (Gangler and Finney, 1982; Undeen and Lacey, 1982; Margalit and Dean, 1985). Due to its specificity to mosquitoes and black flies, the safety of application to the environment is remarkable. *B.t.i.* is not toxic to non-target organisms, except for a few filter-feeding nematocerous Diptera and this only when exposed to much higher than usually applied operational rates of *B.t.i.* (Garcia et al., 1980; Mulla et al., 1982; Margalit et al., 1984; Mulla, 1990). A second *Bacillus* species, *B. sphaericus*, has a good potential as mosquito larvicide, but it has a considerably narrower host range, being toxic mostly against *Culex* and much less against *Aedes* and *Anopheles* species (Davidson, 1985).

In most varieties of *B. thuringiensis* the parasporal body is a bipyramidal protein crystal that consists of one or more peptides of approximately 130–160 kDa toxic to species of Lepidoptera (Dulmage, 1981). *B.t.i.* is unusual among *B. thuringiensis* strains in several ways. The crystals are of irregular shape and serologically are quite distinct from the diamond shape *B. thuringiensis* varieties toxic to Lepidoptera; the toxin has few antigenic determinants in common with the toxins active in other insect groups (Charles and de Barjac, 1982). The crystals also differ in amino acid composition. Serotype H-14 crystals contain three times more lysine and 3½ times less arginine than the crystals of the Lepidoptera active *B.t.* var. *kurstaki* (Armstrong et al., 1985).

The crystalline parasporal body is plasmid-mediated. Out of 9 plasmids existing in *B.t.i.*, the 72 Md plasmid is responsible for encoding the toxin-crystal. This has been shown by curing (Clark and Dean, 1983; Kamdar and Jayaraman, 1983; Ward and Ellar, 1983) and by mating (Gonzalez and Carlton, 1984). The crystals are composed of a mixture of four major peptides: 27, 67, 128 and 135 kDa (Armstrong et al., 1985; Tyrell et al., 1979) which reside in three electron dense inclusions in the form of protoxin (Federici et al., 1990). This protoxin is very rapidly solubilized and converted to smaller toxic subunits in the presence of high pH and suitable enzymes in the gut of susceptible hosts (Ellar et al., 1985). When the paraspore is lysed, new proteins are formed as breakdown products of the four peptides (Eldridge and Federici, 1988). For example, 25 kDa fragment of the 28 kDa toxic protein was found by Davidson and Yamamoto (1984) to be responsible for the mosquitocidal activity. Other toxic effects include cytolysis of cultured insect cell lines, hemolysis of erythrocytes, and lethality for mice (Thomas and Ellar, 1982a and b). There is a general agreement that the 27 kDa peptide causes cytolysis and hemolysis.

There are conflicting reports concerning the precise identity of the toxic peptide responsible for

the specific toxicity to mosquito larvae. Davidson and Yamamoto (1984), Ward et al. (1984) and Armstrong et al. (1985) reported that the 25–28 kDa protein was responsible for the larvicidal activity. However, Hurely et al. (1985) showed that the 65 kDa protein was the larvicidal principle. Wong and Chang (1986) reported larvicidal activity of the 130 kDa protein, and further suggested that the simultaneous presence of at least two proteins was required. Eldridge and Federici (1988) suggest that the 65, 128 and 135 kDa proteins of *B.t.i.* are the precursors of the polypeptides that are toxic to mosquitoes, and currently it appears that none of these proteins alone is as toxic as in the concerted or synergistic effect of the mixture of them found in the parasporal body.

Studies on mode of action revealed that the primary target organ is the midgut epithelium, where enzymic systems transform the protoxin into the actual toxin under alkaline conditions (Eldridge and Federici, 1988). Histopathological and biochemical studies of the toxicity of activated toxin on cultured insect cells have provided evidence that the cellular targets of the 26 kDa cytolytic toxin are the plasma-membrane liposomes containing phospholipids (Ellar et al., 1985). Toxin-lipid binding leads to detergent-like rearrangement of these lipids, resulting in hypertrophy, disruption of membrane integrity and eventually cytolysis.

The narrow spectrum of activity of the *B.t.i.* δ-endotoxin of is probably due to the absence in most invertebrates of the specific enzymes transforming this protoxin into the toxin. Even groups closely related to mosquitoes and black-flies are not susceptible or have a low susceptibility which results in the safety of this bacterial pest control agent to non-target organisms.

The genes encoding the 135, 128, 72 and 27 kDa peptides have been cloned from plasmid DNA and the DNA sequences have been determined (Waalwijk et al., 1985; Thorne et al., 1986; Ward and Ellar, 1987; Sen et al., 1988). DNA sequence analysis suggests that the 58 kDa peptide (probably derived from a 72 kDa precursor) and a peptide encoded in an adjacent open reading frame may be evolutionarily related to lepidopteran toxin (Whiteley and Schnepf, 1986). The gene product of the 27 kDa is hemolytic and has an initial stretch of 45 hydrophilic amino acids, followed largely by hydrophobic residues. A gene coding for the 72 kDa peptide has also been cloned from *B.t.i.* into *E. coli* and *B. subtilis* (Waalwijk, 1985; Thorne et al., 1986). The 130 kDa toxin gene from *B.t.i.* consisted 3408 bp, encoding 1136 amino acids delta-toxin. The same toxin gene was transferred into the cyanobacterium *Synechocystis*. The 130 kDa protein was expressed and the transformed cyanobacteria were toxic to *Aedes aegypti* larvae (Peferoen et al., 1990).

Recombinations could lead to toxins with increased potencies or with altered insect spectra. However, we have to be aware that continuous presence of transgenic organisms harboring delta-endotoxin within the mosquito breeding environment may induce resistance. So far only one instance of enhanced resistance of insects to a lepidopteron toxin has been reported to date (McGaughey, 1985).

Studies investigating the fate of *B.t.i.* after application to the mosquito breeding habitats show that the larvicidal efficacy lasts less than 24 hours (Ramoska et al., 1982; Margalit and Bobroglio, 1984). However, the toxin remains in the sediment and is active for at least 22 days although unavailable to filter-feeding mosquito larvae (Ohana et al., 1987). Although sediments may remain insecticidal, their toxicity is evident only after resuspension which does not occur typically in nature (Mulla, 1985).

In small containers in the laboratory, it appears that cannibalism of *Bacillus* infected cadavers may serve as a mechanism by which the recycling takes place (Aly, 1983; Larget-Thiery, 1984; Zaritsky and Kawaled, 1986).

Presently *B.t.i.* is applied in all continents. In 11 West African countries nearly one million liters of *B.t.i.* are applied annually against the black flies — vectors of onchocerciasis. In Europe along the Upper Rhine Valley over 100 communities which include 2.5 million people are protected through mosquito abatement programs utilizing *B.t.i.* In the United States and Canada *B.t.i.* is being used in numerous mosquito abatement districts as well.

In summary, *B.t.i.* being environmentally safe exhibits also many advantageous properties of a

chemical pesticide, i.e. it is highly toxic to the target organism, mass-produceable on an industrial scale, has a long shelf life and is easily transportable. Moreover, its genetic plasticity will hopefully allow, through the combined efforts of geneticists, microbiologists and insect pathologists, to bring about improved control of insect vectors.

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