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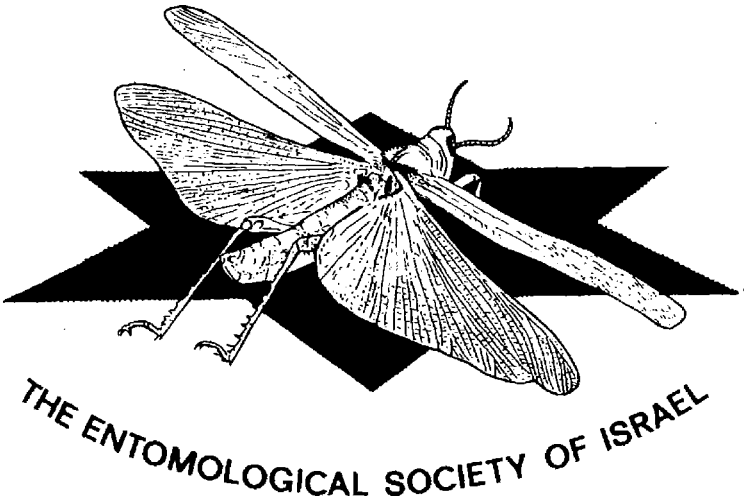

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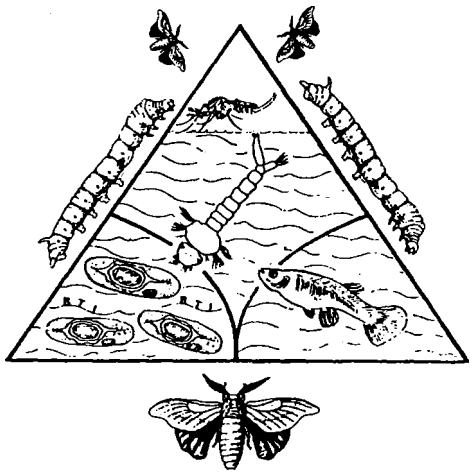
Proceedings of the Second En Gedi Conference on Bacterial Control of Agricultural Insect Pests and Vectors of Human Diseases *highlighting* The 20th Anniversary of the Discovery of *Bti*

held at

Shoresh, Israel

August 12–16, 1996



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CONTROL AND ECOLOGY

ISOLATION AND CHARACTERIZATION OF STRAINS

GENE EXPRESSION

MOLECULAR BIOLOGY AND GENETIC ENGINEERING

Participants of the Second En Gedi Conference on Bacterial Control of Agricultural Insect Pests and Vectors of Human Diseases held at Shoresh, Israel (12–16 August, 1996), visiting the site where *Bti* was discovered in the summer of 1976 — a small pond in a dried-out river-bed near Kibbutz Ze'elim in the northern central Negev Desert, Israel [Goldberg, L.J. and Margalit, J., *Mosquito News* 37:355–358 (1977)].

THE USE OF *BTI* IN SWITZERLAND: APPLICATION TECHNOLOGY AND COST EFFECTIVENESS *(Abstract only)*

P. LÜTHY

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The availability of the most advanced technology is a prerequisite for successful mosquito control with *Bacillus thuringiensis* subsp. *israelensis* (*Bti*). In Switzerland, as in other parts of the temperate zones of the Northern hemisphere, the main use of *Bti* is for the control of *Aedes vexans,* the floodwater (vexans) mosquito.

In-depth monitoring and planning is required well ahead of the treatments. Accurate weather forecasts will give trends of the development of flooding situations, and they are an important planning instrument to determine the optimum time for the treatments.

The elimination of more than 95% of the mosquito population is necessary in order to provide an effective reduction in biting activity and thus a noticeable relief for the people. This goal is achieved only by a complete coverage of all the existing breeding sites.

Treatments by helicopter have proved most effective. Ground applications are recommended only for spot treatments of well defined small sites.

Existing *Bti* formulations have major drawbacks. Their mosquitocidal activity is only short-lived; especially granular formulations tend to be adsorbed very quickly to solid organic material and are therefore removed from the feeding zone of the target insects.

Bti applications are expensive. They can be justified only in regions where the cost-benefit is in balance. Such areas include nature reserves near densely populated zones, especially tourist resorts.

NEW FORMULATION TECHNOLOGY FOR *BACILLUS THURINGIENSIS (Abstract only)*

S.H. BOK, K.H. SON, H.W. LEE, D.C. KIM AND S.U. KIM

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An efficient bioencapsulation technique was developed for target directed delivery of microbial pesticides. As a model a *Bt* toxin producing *Bacillus thuringiensis* HD-1 strain was bioencapsulated in an edible biopolymer gel matrix, dried, and tested in the crop field for its efficacy. These spore-forming microorganism were stable for over one year at room temperature. Microbial cells entrapped in the biopolymer matrix were protected from environmental damage caused by sunlight, rainfall etc. They grew inside the biomatrix and produced useful toxins under suitable field conditions. They were able to prevent or kill harmful insects such as diamondback moth, spruce budworm, and apple leafroller. The effective killing power of the bioencapsulated *Bt* under field conditions lasted over 3 weeks. Application of this bioencapsulation technique may open a new, low cost production method for biocontrol agents and contribute to the replacement of some toxic agrochemicals with non-toxic biocontrol agents encapsulated in an edible biopolymer matrix. Some of the existing encapsulation techniques together with our bioencapsulation technique which shows considerable preliminary promise, were reviewed.

GRANULAR *BACILLUS THURINGIENSIS*-FEEDING BAIT FORMULATIONS FOR THE CONTROL OF MOTH PESTS *(Abstract only)*

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Granular *Bacillus thuringiensis* (*Bt*)-feeding bait formulations based on wheat flour as a carrier were developed. The *Bt* in the formulations was a commercial product of subsp. *kurstaki,* 'Bio-Ti' (Zohar-Dalia, Israel), concentrated liquid, with a potency of 8,000 IU/mg. Yeast extract and wheat germ oil were feeding stimulants to *Helicoverpa armigera* larvae. The *Bt* material was embedded in the carrier. Then the carrier was dried and ground to a fine powder of 50–150 μ. The formulations were produced by Yewnin-Yoffe Industries Ltd. In field experiments, the formulation was dusted against: *Batrachedra amydraula* in dates, *Heliothis armigera* and *Chrysodeixis chalcites* in tomato for processing and *Earias insulana* in cotton. The dust formulation was more effective in managing the pests than a *Bt* spray or several chemical insecticides. Another formulation made of large granules sized 2-3 mm^3 was effective in soil application against mature larvae of *Agrotis ipsilon* in vegetables.

PRODUCTION

EXPERIMENTAL PRODUCTION AT INDUSTRIAL LEVEL OF BIOINSECTICIDES BASED ON *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* (*BTI*) AND *BACILLUS SPHAERICUS* (*BS*) 2362 *(Abstract only)*

L. RABINOVITCH,[1] C.M. BRAZÃO E SILVA,[2] R.S. DE A. ALVES,[2] R.A.G.B. CONSOLI,[3] M.A. LAMOUNIER,[2] B. DE S. SANTOS,[2] V. FERREIRA,[4] M. ATARASHI,[4] J.T. DA COSTA[4] AND R.O. CARDOSO[4]

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Bioinsecticides based on *Bti* and *Bs* have been produced routinely on laboratory scale (fermenters of 14 liter) at LFB, FIOCRUZ-RJ, using a culture medium that has soy flour as the main raw material. The process scale-up was done together with CIBRAN, using 3,000 liter capacity fermenters. Three different batches resulted in 1,024 kg of *Bti* and two batches produced 359 kg of *Bs*. The biomasses obtained were harvested by centrifugation, then formulated so that a emulsified liquid concentrate was obtained. The toxic activity of the products was evaluated by bioassays (WHO method) against *Aedes fluviatilis* larvae (in the case of *Bti*) and against *Culex quinquefasciatus* larvae (in the case of *Bs*). The mean values of LC_{50} obtained were 15.8 ± 14.6 µg/L and 0.57 ± 0.52 µg/L for *Bti* and *Bs*, respectively. The success achieved at this scale-up stage is one more step reached in making possible the production of these bioinsecticides on an industrial scale and their use in control programs of tropical disease vectors [INPAL-FIOCRUZ agreement].

TEMPERATURE SENSITIVITY OF *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* AND *BACILLUS CEREUS* DEPENDING ON AGE OF SPORE CULTURES *(Abstract only)*

R. SINAI, M. MYASNIK, Y. MARGALITH AND Z. BARAK

Department of Life Sciences, Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel

One of the most striking properties of spores is their extreme resistance to heat. Short heat treatments (70°C for 15–20 min) are often used to select for *Bacillus thuringiensis* (*Bt*) among other bacteria, and to stimulate spore germination. In this study we compared temperature sensitivity of vegetative cells and spores of *Bt* subsp. *israelensis* (*Bti*) to those of its close relative, *Bacillus cereus* (*Bc*). Heat sensitivity was measured by D_x values, which are defined as the time needed to kill 90% of bacterial population at temperature of x. *Bti* cells and spores were both more sensitive to 60°C than *Bc*. However, these differences decreased and almost disappeared at 70° and 80°C. Heat resistance of *Bti* spores, produced at 32°C in LB rich medium, increased with spore age. D_{60} after 24, 48, 72 and 96 hours of growth under standard conditions were 4.5, 5, 30 and ∞ minutes, respectively. Similar correlation was observed for D_{70}, i.e. 2.5, 7 and 22.5 min for 24, 48 and 72 hours old cultures, respectively. Growth in Lewis sporulation medium did not change this behavior of *Bti* spores. *Bc* spores also showed dependence of heat-resistance on culture age. It is concluded that in order to achieve a fully mature spore culture of *Bti* one should grow cultures for at least 96 hours after inoculation.

ENHANCEMENT OF LARVICIDAL ACTIVITY OF ENTOMOPATHOGENIC BACTERIA BY PROTOZOAN BIOENCAPSULATION *(Abstract only)*

R.R. AZIZBEKYAN, L.A. GANUSHKINA, T.M. GRIGOREVA, I.V. KUKINA, V.P. SERGIEV, T.A. SMIRNOVA AND V.Y. YUKUBOVICH

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Mosquitoes are the most important insects of public health concern due to their capacity to transmit human and animal diseases. During the past years vector control strategy was based on the use of chemical and biological insecticides. Bioinsecticides have been created on the basis of *Bacillus thuringiensis* and *B. sphaericus*. The limitation of entomopathogenic bacteria for use in mosquito control is the short residual activity of their spore-crystal complex.

To increase the persistence of the larvicidal activity Zaritsky et al. proposed the bioencapsulation of *B. thuringiensis* by a protozoan. We use *Tetrahymena pyriformis* for the enhancement of the toxicity of *B. thuringiensis, B. sphaericus* and recombinant strain *Methylobacillus flagellatum* with cloned *cry4B* gene from *B. thuringiensis*. Bioencapsulation of *B. thuringiensis* improved the toxicity against different species of *Anopheles* (*An. stephensi, An. atroparvus, An. pulcherimus, An. superpictus, An. sacharovi*). The difference between the various species of *Anopheles* to larvicidal activity of bioencapsulated *B. thuringiensis* was maintained. The mortality rate was shortened 2–3 fold.

Bioencapsulation of *B. sphaericus* enhanced the larvicidal activity against *An. stephensi*. The mixture of *B. sphaericus* and *T. pyriformis* (relation 1000:60) killed 60% of the larvae after 24 h; however, *B. sphaericus* alone does not result in mortality. After 24 h of incubation a slight difference in mortality of *Culex pipiens* larvae was found between *Cx. pipiens* treated with *B. sphaericus* loaded *T. pyriformis* and *B. sphaericus* alone. After 48 h mortality was the same. Bioencapsulation of *B. sphaericus* permitted a decrease in the dose of *B. sphaericus* to a level which can be used for the control of *Culex*.

Previously we had cloned the *cry4B* from *B. thuringiensis* strain and demonstrated that the product of this gene had specifically high toxicity for *Anopheles* mosquitoes. *M. flagellatum* bacteria are ecologically safe and they utilize cheap media, which ensures profitability of their production. The high-level expression of the *cry4B* gene was achieved in the cells of *M. flagellatum* when the gene was cloned using a wide-range vector under the control of the *recA* promoter of *M. flagellatum*. The recombinant strain produced small crystal-like inclusions.

The mortality of *An. stephensi* larvae caused by exposure to recombinant strain of *M. flagellatum*-loaded *Tetrahymena* was compared to those caused by the recombinant strain alone. The mixture resulted in 100% mortality after 8 h of incubation, whereas the recombinant strain alone did not result in mortality. By use of *An. atroparvus* which was more resistant to Cry4B toxin than *An. stephensi*, it was found the larvicidal activity of the mixture was 2–8 times higher than that of the recombinant strain alone.

SALT-GLUCOSE MINIMAL MEDIUM FOR *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS (Abstract only)*

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Chemically-defined media are essential for physiological and genetical studies, as well as for isotope labelling of bacterial products. Previously described minimal media for *Bacillus thuringiensis* (*Bt*) could not support growth of *Bt* subsp. *israelensis* (*Bti*). A new minimal medium was developed here for this variant that, unlike the previous *Bt* minimal media, does not contain any organic material beside glucose. *Bti* grew in this salt-glucose medium (SGM) in the liquid as well as in the solid form. The colonies developed on this new medium were all of a rough form, whereas those on LB-rich medium were smooth. Division in the solid medium started after 3.5–4 hr of incubation, compared to 1.5 hr in LB. Growth in the liquid SGM was in aggregates, that could not be prevented by addition of EDTA (0.01%). Generation time was approximately 2 hr and maximal bacterial concentration reached 3×10^7/mL, compared to 0.5 and 3×10^8/mL, respectively, in LB. In our standard cultures cells divided about 10 times before cessation of growth. In the cultures 100% sporulation and maximal larvicidal activity were obtained at 40 hr. The LC_{50}s of the cultures in our SGM were 200–500 spores/mL. Ions of heavy metals, that had been supplemented to previous minimal media, inhibited *Bti* growth in our SGM. We hope our new SG minimal medium will support efficient growth of other *Bt* variants as well.

THE MEDIUM OPTIMIZATION OF *BACILLUS THURINGIENSIS* MP-342 *(Abstract only)*

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An approach which combined single factor selection, the steepest uprising path method and the orthogonal- rotating-combination-design was incorporated in the medium optimization of *Bacillus thuringiensis* (*Bt*) MP-342. The bioassay data with *Heliothis armigera* as testing insects, were used as a parameter. Five carbon and eleven nitrogen sources were first evaluated by using single factor selection. Corn starch, soybean cake flour, fodder yeast and corn steep liquor proved to be suitable carbon and nitrogen sources. The steepest uprising path method was then used to work out a composition of the four nutrient ingredients which reached the optimized region. Based on the optimized composition, the orthogonal-rotating combination-design was finally used to obtain the response-plane equation from matrix algorithm. The response values could be obtained from isohypse-plane with different combinations. K_2HPO_4, $CaCl_2$, $MgCl_2$ and $MnCl_2$ content was optimized by using the steepest uprising path method. An optimized condition in shake flasks was obtained which doubled the toxicity to both *Heliothis armigera* and *Plutella xylostella,* compared with the production medium of MP-342. This approach proved to be a very efficient method for medium optimization of *Bacillus thuringiensis*.

CONTROL AND ECOLOGY