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Microbiology 2020: Mosquito-larvicidal activity of bacterial extracts produced by Colombian strains- Agudelo-Restrepo Manuela- National University of Colombia

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Management of mosquitoes with biological insecticides, such as Bacillus sp. Toxins have been commonly used in many countries. However, rapid sedimentation away from the mosquito larvae feed zone causes a low residual effect. In order to overcome this problem, it has been proposed that Bacillus toxin genes be cloned in aquatic bacteria capable of living in the upper part of the water column. Two strains of Asticcaulis excentricus were chosen to introduce B. The sphaericus binary toxin gene and the B gene. The Thuringiansis subsp. Medellin cry11Bb gene cloned in the appropriate vectors. In feeding experiments with these aquatic bacteria, it has been shown that Culex quinquefasciatus, Aedes aegypti and *Anopheles albimanus* larvae have been able to live on a diet based on this wild bacterium. A. The excentricus recombinant strains were able to express both genes, but the recombinant strain B expressed. The binary toxin of sphaericus was toxic to mosquito larvae. Crude A protease. Excentricus extracts did not degrade the toxin of Cry11Bb. The floatability studies indicated that the recombinant was A. Throughout the upper part of the water column, the excentricus strains persisted longer than the wild Bacillus strains.

Bacillus thuringiensis, a gram-positive bacterium, produces toxins that are active against certain insect species belonging to the orders Coleoptera, Diptera and Lepidoptera. Several highly toxic B strains. Thuringiensis has been recorded for mosquito control. Classification of active strains of mosquitoes in three groups, potentially important strains for mosquito control in group 2. These include two B strains. Thuringiensis subspecies medellin, subspecies strain 367. Jegathesan, and Clostridium bifermentans of serovar. Malaysia, guy. These four strains have a crystal protein pattern different from that found in B. The Thuringiansis subsp. Israelensis, while they're almost as active. Polyacrylamide gel electrophoresis (SDS-PAGE) study of pure crystalline parasporal inclusions of B. The Thuringiansis subsp. Medellin strain CIB 163-131 has a polypeptide of approximately 94 kDa, multiple bands between 80 and 65 kDa, and two doublets at 40-41 and 28-30 kDa. Sequence of the 94 kDa B toxin gene. The Thuringiansis subsp. Medellin encoding the protein Cry11Bb1 and its genetic organization has been reported.

Parasporal inclusions containing mosquitocidal toxins quickly settle down at the bottom of the ponds, away from the mosquito larvae feeding zone. In order to overcome this issue, it was proposed to clone active mosquito toxin encoding genes in aquatic bacteria, such as gram-negative and cyanobacteria. Several attempts have been made to clone B. The Thuringiansis subsp. Israelensis toxin genes in cyanobacteria obtained complex findings. At the same time, gram-negative bacteria such as Asticcacalis excentricus, Caulobacter crescentus and Ancylobacter aquaticus have been used to clone and express B. The Thuringiansis subsp. Israelensis toxin genes, providing better expression of gene products, and enhanced control by keeping toxins in larval environments at 105 to 106 cells / ml. Feeding Experiments-The growth of mosquito larvae was substantially different between controls (with and without regular food) and A-containing treatments. Excentric cells as larval food in the three species of mosquitoes tested; however, A. Excentricus lower concentrations (107 and 108 cells / ml) of larvae exceeded only the second instar after five days of treatment with 109 A. Excentricus cells / ml, 7.5, 8.2 and 4.5 third instar larvae have been observed for Cx. Ae, quinquefasciatus. Aegypt, and *A. Albimanus* and, in the negative control (no food added), no mosquito larvae had reached the second instar in any of the mosquito species tested.

Transformation of the efficacy and expression of A toxin genes. Excentric cells-The transformation of native A cells. Excentric C2 cells with plasmid pEA1 or pSOD2 were obtained only in cells treated with EMS. Efficiency transformation of A. Excentricus C2 with plasmid pEA1 after treatment with EMS was 5.8x102 to 2.8x103 transformants / μg of DNA, 2 to 9 times lower than A. The 4724 strain of excentricus. However, A 's transformation efficiency. Excentric cells with plasmid pSOD2 were 2.2x102 and 2.9x102 transformants / μg of DNA in A. Excentricus strain 4724 and C2 respectively.

We have cloned the cry11Bb gene of B in this study. The Thuringiansis subsp. Medellin and the B toxin gene. The sphaeric in the A. Excentricus strain isolated from mosquito larvae breeding ponds in Colombia and presents data on the expression and toxicity of recombinant cells to A. Albimanus, Ae, guy. Aegypt, and Cx. Quinquefasciatus first instar larvae, as well as information on the floatability properties of recombinant strains.

Results of A floatability experiments. The excentric recombinant cells suggested that this trait may possibly be due to the cell division that occurred during the experiment and/or the movement mediated by flagella in the cells that enter the stage of the movil. A change. Excentricus strains, and B expression. The floatability properties of this bacterium have not been impaired by the binary toxin sphaericus by these recombinants.

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