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BIOLOGICAL CONTROL OF MOSQUITOES BY BACILLUS SPHAERICUS

PROGRESS REPORT: AID-CDR PROJECT NO. PDC-5544-G-SS-5098-01

Towards the last year of the program we have embarked on two major aspects which are the following:

1) Establishment of field trials in Guinea
2) Improvement of the larvicidal activity of the recombinant B.t.i./B. sphaericus

1) FIELD TRIALS IN GUINEA:

Last November (1988) Dr. M. Keren-Zur who is a senior scientist at FRM Agricultural Sciences Partnership travelled to Guinea as a representative of the Israeli team. The purpose of his visit was to help the Guinean team (whose group leader is Dr. S. Mane of the Health Ministry) in setting-up their bioassay laboratories and to disusss plans of field trials with them. The impression was that our collaborators were fairly equipped to run bioassays on Culex, Aedes and Anopheles species of mosquitoes. (They have sent a scientist to a research station in Kenia to practice the methodoly of such bioassays including aritifical rearing of mosquitoes). We, therefore, intend to send samples of B.t.i., B. sphaericus and eventually of the recombinant microorganism for laboratory testing in their set-up.

Intensive discussions on field trials were followed by field excursions whereby adequate sites were selected. As soon as we finish all procedural and scientific preparations, we will produce semi-industrial quantities of the various larvicides and those initiate field trials.

PARTNERSHIP BETWEEN FIRST MISSISSIPPI CORPORATION OF DELAWARE
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2) IMPROVEMENT OF THE RECOMBINANT B.T.I./B. SPHAERICUS:

Introduction:

The goal of this project is to clone B.t.i. δ-endotoxin DNA into B. sphaericus, in order to produce a broad spectrum mosquito larvicide which is more persistent than B.t.i. We have previously reported on successful cloning of B.t.i. δ-endotoxin genes in B. sphaericus 2362.

Several clones of the recombinant microorganisms constistently express the genes coding for the 28 kDa and 65 kDa proteins of δ-endotoxin and their toxicity against A. aegypti larvae is significantly higher than the wild type of B. sphaericus, but it is still 10-20 times less toxic than B.t.i. This level of toxicity may be partly due to low copy number of the B.t.i. genes in the recombinants or to a tendency of the recombinants to lose the genes coding for the δ-endotoxin.

We have assumed that some of the clones that consistently express the δ-endotoxin genes have undergone insertion of the genes into the chromosome by an act of transposition, a well known phenomenon in Bacillus species.

We have since explored the possibility of producing more toxic recombinants by insertion of a complete copy of δ-endotoxin genes into the chromosome via homologous recombination between the previously inserted copy of the δ-endotoxin genes and a new one, for the reinsertion we used the 9.7 kb B.t.i. toxin DNA-fragment ligated to the pPL603E, (the plasmid which was used previously for transformation). The concept was tested by transformation of the appropriate protoplasts afer optimization of the transformation and fusion conditions in each case. Another approach to enhancement of copy numbers of the genes was to induce amplification by exposure of the stable clones to selective pressure by increasing concentrations of kanamycine. We are now engaged in the phenotypic and genotypic characterization of the new transformants and we hope to find among them clones with improved larvicidal activity.

Methods:

1) The optimal conditions for protoplast transformation and protoplast fusion have been defined for each case, but the basic procedures were as reported before.
2) We used clone #62 for retransformations and fusion.
3) The maximal concentration of kanamycine in experiments with increasing selection pressure was 30µg/ml.

Results: Table 1: Genotypic and phenotypic properties of wild type as well as recombinant isolates

Type of treatment	% P		% R	% K+		% H+		% C+		% AT	
				B	E	B	E	B	E	B	E
Transformation of B.sph.2362 protoplasts with pPL603E	95		83	70	50	0	0	0	0	0	0
Transfrm.of B.sph.2362 protoplasts w. pPL603E ligated to 9.7Kb-fragment of B.t.i.	95		80	60	5	~0	~0	≤1	≤1	8	1
Self-fusion of protoplasts of clone #62 - (one of the most active & stable clones)	85		70	40	10	8	4	≤2	≤1	n.c.	n.c.
Transfrm.of protoplast of clone #62 with 9.7-fragment of B.t.i.	80		80	70	50	15	13	≤5	≤4	n.c.	n.c.
Fusion of protoplasts of clone #62 w.protoplasts of B.t.i.	Clone 85	Bti 50	60	45	5	3	1	≤1	≤1	n.c.	n.c.
Fusion of protoplasts of B.sph.-clone previously transformed w. pPL603E w. protoplasts of B.t.i.	85	50	60	50	40	1	0	≤3	≤2	n.c.	n.c.
Selective pressure on clone#62 with elevated amounts of kanamycine	-	-	--	20	10	3	1	≤2	≤2	n.c.	n.c.

Legends:

B Results immediately after selection

E Results after more than 10 passages

%K+ Percent clones resistant to Kanamycine 10µg/ml

%H+ Percent clones hemolytic on plates

%C+ Percent clones with crystals of B.t.i.

%AT Active Transformants:
Percent clones toxic to A. aegypti at least 5 times more than B. sph. 2362

%P Percent bacterial cells turned into protoplasts

%R Percent bacterial cells which successfully regenerated

n.c.-not checked

Conclusions:

1.- Although we have so far tested only about 3% of the new transformants, it seems that among the clones there are some with improved larvacidity.

2.- There are clear phenotypic differences among the different clones, including stability, level of expression of the δ-endotoxin genes and sensitivity of the cell wall to lysozyme.