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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 9/16/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. CS-175

Attachment

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PD-AAW-657

C5-175

Annual Report - July 1987
US-ISRAEL AID grant No. DPE-5544-G-5506042-00

Title: Development of a Microbial Preparation for Biological Control of the African Armyworm, Spodoptera exempta.

Submitted by:

Prof. Baruch Sneh and Dr. Meir Broza
Tel-Aviv University, ISRAEL.

and

Dr. Wellington A. Otieno and Dr. Maurice O. Odindo
I.C.I.P.E. KENYA

The system in ICIPE can not allocate money for research from its own sources and subsequently claim the expenses from the grant funds, as required by the AID programme. The administrative arrangements required to provide an advanced payment for the lab. in Kenya were impossible at the beginning of the research period, and complicated at the later stage. It was initially planned that the cooperative investigator from Kenya (Dr. Otieno) will come to the Israeli lab. to work and get acquainted with the methodology of bacterial isolation, insect rearing, bioassay procedures etc., and then, after a month Prof. Sneh and his technician from Israel, were to join the work for 1 and 3 months respectively. As the money could not be available for a long time in Kenya, It was impossible for ICIPE to approve the travel of Dr. Otieno.

The work in Israel was initiated in time with collection of soil samples from Israel and from Kenya. Isolations and initially screening on larvae of S. littoralis (S. exempta can not be introduced to Israel). After many delays in the arrival of Dr. Otieno to Israel, it

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was decided that Prof. B. Sneh and Mr. M. Hamal will travel first to Kenya and provide the training to the scientists in ICIPE. At that time, the advanced payment for the first half of the year for the lab. of ICIPE was made available after spending time and efforts, and was delivered personally (by Sneh) to Kenya. The arrangement that the money has to go through Israel instead of directly to Kenya costs us 15% tax instead of being available for the research expenses. The administration of the AID should try to find a way to save these considerable sums of money for the research activities instead of wasting them on tax.

The investigators in ICIPE have initiated (before the advanced payment had arrived), a massive rearing of larvae of S. exempta for bioassays and collected soil samples from various locations there, some of which were sent to the laboratory in Israel. During the 2 weeks before the arrival of Sneh and Hamal to Mbita Point ICIPE, the massive populations of the reared larvae became heavily infected with NPV which wiped out most of the larvae. The outbreak happened due to inappropriate handling, sanitation, high density and excess of moisture. The conditions and handling were changed according to the experience of the rearing procedure in the lab. at Tel Aviv University. The larval population health was restored and all became ready for the bioassay experiments.

Currently, Mr. Hamal who brought with him a considerable numbers of new Bacillus thuringiensis isolates, is training a student and a technician all the procedures required for the screening experiments. These include: Insect rearing (on leaves and on artificial diet), preparation of media, microbial work, isolation and identification of Bt from soil and larval samples, growing the isolates in broth, culture preparation for bioassy and bioassay procedure. During the 3 months he intends to screen a great number of isolates including the new ones.

Isolation procedure of B. thuringiensis from soil samples and larvae.

One hundred grams of soil samples were collected from various fields in Israel and Kenya. Each sample was well mixed and sieved through a 2m sieve. One gram of each sample was transferred to 20 ml. of 0.5% peptone solution in a 100 ml erlenmyer flask for enrichment of Bacillus spp. After 48 h, incubation at 32°C in a rotary shaker, the suspension was passed through 3 layers of sterile gauze. one ml of the culture broth was transferred to a new erlenmyer flask containing 20 ml of 0.5% peptone solution and 50μg/ml ampicillin. After incubation for an additional 48 h, aliquots of 2ml suspension were transferred to sterile test tubes which were immersed in a water bath at 55°C for 5 min to kill the non-spore forming bacteria.

The suspensions were streaked on nutrient agar (NA) plates. After 48 h incubation at 32°C The Bacillus type colonies were observed under the microscope for crystal formation. The crystal forming colonies were streaked twice for confirming the isolation, and maintained in stab cultures in screw cup tubes with (1% agar) NA.

A modification of the isolation method was also used:
Two g of the soil sample were placed in a plate with 7ml of 0.5% peptone solution containing 100μg/ml ampicillin. After incubation at 32°C for 48h, 2ml of the supernatant were transferred to a test tube and placed in a water bath at 55°C for 5min. The suspension was diluted to 10^{-4} and aliquots of 0.1ml were spread on NA plates with a drigalski rod. Colonies of Bacillus spp. were inspected under the microscope for the presence of crystals and isolated as previously described.

Growing bacterial isolates for bioassays.

Isolates of B. thuringiensis were inoculated into 2 ml tryptic soy broth (TSB) (20g Difco tryptic soy powder/liter) in test tubes which were transferred to 20 ml B. thuringiensis sporulating broth

(BTSB) (Goldberg et al 1980. Biotechnology letters 2: 419-426) in 100 ml erlenmyer flasks and shake incubated for 48-56 h at 32°C. Viable cell counts were made using the drop plate method (Reed and Reed 1948. Can. J. Res. 26: 317-326). The spore crystal suspensions were harvested by centrifugation at 12000 rpm at 4°C for 10 min and washed with sterile distilled water (to discard soluble toxins other than the endotoxin). The pellet was resuspended in the original volume.

Bioassay procedure for B. thuringiensis on larvae of Spodoptera.

For the first mass screening of isolates, the crystal suspension in the artificial diet was 10^7 cfu/ml (colony forming units). The isolates inducing relatively high larval mortality were tested in a dosage response assay, using the following concentrations: $3x10^6$, 10^7, $3x10^7$, 10^8 cfu/ml artificial diet. The artificial diet consisted of: agar - 2g; sucrose - 2.5g; yeast extract - 1g; alfalfa meal - 1.5g; wheat germ - 3g; cholesterol - 40mg; sorbic acid - 100mg; methyl paraben - 100mg; chloramphenicol - 10mg; and H_2O - 100ml.

The artificial diet was divided into small test tubes (1.8ml in each) kept in a heating block at 45°C. The spore crystal suspension was diluted as desired and 0.2ml aliquots were mixed well with the 1.8ml diet in the tubes and poured on plastic plates. Each sample was divided after solidification into 4 portions and placed on 2 layers of moist whatman No. 4 filter paper in plates. Ten second - instar larvae (2-3 days old) of Spodoptera exempta (littoralis in Israel) previously starved for 3-4h, were placed in each plate (40 larvae/treatment). After 4 days incubation at 25°C and 60% R.H., mortality counts were made.(as described by Yawetz et al 1983. J. Invertebr. Pathology 42: 106-112.)

In Kenya, The first screening bioassays were carried out on sorghum leaves (the natural diet of the African armyworm), similar to the method described previously by Sneh et al (1981. Entomophaga 26: 179-190.) Sorghum leaves cut to $2x2cm^2$ were dipped in the dilution

series of the spore crystal suspensions containing 0.01% triton as a detergent (To provide an even coverage of the leaves surfaces) were placed on the moist filter papers in the plates, and second instar larvae were fed on them for 4 days.

Results of isolations and bioassays made in Israel on *S. littoralis*.

1. Isolations from soils collected from fields around Cfar Saba.

From 10 soil samples, 16 isolates were identified as *Bacillus thuringiensis*. designated as M1 - M16. After 3 successive bioassay tests, isolates M14 and M16 have demonstrated high insecticidal activities (Tables 1 & 2) while the rest were relatively low. The isolate Bt24 (Sneh et al 1981. Entomophaga 26: 179-190) which was developed as a commercial preparation for the control of *S. littoralis* was used in all the tests as a positive control.

Table 1. Insecticidal activities (based on cfu/ml) of the best 2 out of 16 Bt isolates (isolated from fields around Cfar Saba) on second instar larvae of *Spodoptera littoralis**.

	Mortality, (%)				
	CFU/ml			CFU/ml	
Isolate No.	$3x10^6$	10^7	$3x10^7$	LC 50	LC 90
Bt 24	15.0	35.0	82.5	$9.8x10^6$	$3.8x10^7$
M 14	17.5	37.5	82.5	$1.0x10^7$	$4.3x10^7$
M 16	10.0	20	62.5	$2.1x10^7$	$1.3x10^8$

* The data are the average of three separate experiments.

The bioassay method using cfu/ml does not take in consideration the biomass capacity of each isolate. Using dilution series of the culture broth relatively to the original broth (as presented in Table 2) provides the relative production of insecticidal activity of a unit of culture broth.

Table 2. Insecticidal activities (based on dilutions relative to the original culture broth) of 2 out of 16 Bt isolates (from fields around Cfar Saba) on second instar larvae of Spodoptera littoralis .

	Mortality, (%)		
	Relative dilution,		
Isolate No.	1:500	1:100	1:10
Bt 24	0.0	20	72.5
M 14	12.5	20	57.5
M 16	12.5	15	75.5

Isolations were made from 45 soil samples introduced from Kenya to Israel. Only two isolates of Bt were obtained from this set of soil samples. Their insecticidal activity against S. littoralis was close to that od Bt 24(Tables 3&4).

Table 3. Insecticidal activity of 2 isolates obtained from soil samples of Kenya (based on cfu/ml) on second instar larvae of S. littoralis.

	Mortality, (%)					
	cfu.ml				cfu/ml	
Isolate No.	10^6	$3x10^7$	10^7	$3x10^7$	LC 50	LC 90
Bt 24	0.0	15.0	35.0	82.5	$3x10^6$	$3.8x10^7$
K 30-3	27.5	47.5	85.0	100.0	$3x10^6$	$1.2x10^7$
K 30-3'	7.5	65.0	85.0	100.0	$7x10^6$	$1.3x10^7$

Table 4. Insecticidal activities (based on dilutions relative to the original culture broth) of the isolate obtained from a soil sample from Kenya on second instar larvae of Spodoptera littoralis.

	Mortality, (%)		
	Relative dilution.		
Isolate No.	1:500	1:100	1:10
Bt 24	0.0	20	72.5
K 30-3	2.5	22.5	60.0

From the area of Gilboa (Israel), from 25 soil samples, 32 Bt isolates were obtained, some of which have demonstrated somewhat lower

insecticidal activity than Bt 24 against S. littoralis (Table 5).

Table 5. Insecticidal activities (based on dilutions relative to the original culture broth) of the isolates obtained from a soil sample from Gilboa, on second instar larvae of Spodoptera littoralis.

Isolate No.	Mortality, (%) 1 : 100
Bt 24	50.0
Gl 13-1	27.5
Gl 13-2	37.5
Gl 13-3	30.0
Gl 13-4	25.0
Gl 20-4	0.0
Gl 24-1	0.0

Recently, from 55 soil samples collected in the Sharon region, 9 isolates of Bt obtained had a low insecticidal activity against S. littoralis.

All The isolates were taken with us to Mbita point Kenya together with many others of our culture collection are currently bioassayed there for their insecticidal activity against the African armyworm, S. exempta. In addition, more soil samples collected from various locations in Kenya will be used for the isolation of more isolates of Bt for screening.

In the recent annual meeting of the Society of Invertebrate Pathology (July 1987, Florida) Martin et al have presented a method

for selective isolation of Bt from soil which yields many more islates from each soil sample. The method is published in BioTechniques 3: 386-392. We intend to use this technique which will possibly yield many more isolates also from soil samples that have not yielded any in previous attempts.

During the visit to ICIPE, We have spent one day in the Desert Locust Research Center in Nairobi in the lab. of Dr. Rose and Dr. Page who introduced us to their comprehensive biological and ecological studies on populations dynamics of the African armyworm in the East African countries. The studies of this research group are of great importance for the subsequent steps of the present project. They have shown great interest in the success of our project and are ready to provide their experience for the strategy, planning and application of the preparation to be developed in our research.

Next year, It is planned to screen all the isolates accumulated for their insecticidal activity against the African armyworm. The best isolate will be used for the development of the preparation for application. Dr. M. Broza (the entomologist) will travel to Kenya during the outbreak season of the Armyworm to get acquainted with the population dynamics of the armyworm, for planning the field application of the Bt strain that will prove to be effective.