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DEVELOPMENT OF POTENCY BIOASSAYS FOR SELECTING *BACILLUS THURINGIENSIS* PREPARATIONS AGAINST AGRICULTURAL INSECT PESTS

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The potency bioassay was developed to replace the spore count by an insect assay screening activities of the entire spore-crystal toxin complex. The technique was based on titration of the *B.t.* activity in comparison with a reference strain against a test insect. National needs to register industrial preparations led to the use of the following microbial standards and bioassay insects: Serotype 1, van *thuringiensis,* strain E61 against *Anagasta kuhniella* in France (Burgerjon and Yamvrias, 1960) and Serotype 3a, 3b var, *kurstaki* strain HD-1 against *Trichoplusia ni* in the US. (Dulmage et al., 1971).

These two standards, each based on a different serovar, became international references tested against specific bioassay insects. However, the increasing knowledge of *B.t.* specificity in insects suggested that the scope of potency selections with these bioassay procedures is limited. Furthermore, the urgent need to solve pest problems by microbial control, has challenged insect pathologists and entomologists to adjust the bioassay for screening of microbial strains against native insects. In this way, target pests were used instead of the original bioassay insects. This approach was adopted in our Department of Entomology as a part of the efforts to replace chemical insecticides by entomopathogens.

The basic requirements for developing *B.t.* screening programs against a given insect may follow some common principles as regards the assay methodology and applications.

1. The insect colony

Practical mass rearing techniques on an artificial diet is the common tool to establish an insect colony for the use of young larvae for the bioassay. Simple diets have been developed against the following field crop pests: *Spodoptera littoralis* (Navon, 1985), *Heliothis armigera,* (Navon, in preparation), *Earias insulana* (Klein et al., 1983), *Ostrinia nubilalis* and *Sesamia nonagrioides* (Melamcd-Madjar and Raccah, 1979). The diets consisted of common food products such as beans, and meals of alfalfa, com and cotton. Ascorbic acid was required in the diet to maintain larvae feeding; yeast was the source of B vitamins in these media. Mould inhibition in the diet was obtained by methyl-p-hydroxy benzoate and sorbic acid, whereas bacterial contamination was prevented by antibiotics. Formaldehyde was used in the diet to avoid outbreaks of baculovirus diseases in the colony and as a general anticontaminant.

The diets were based on agar but an alinate gel system as an agar substitute was useful for rearing *S. littoralis.* In this system sodium alginate entered ionic transformation with calcium in acid pH to produce a calcium-alginate gel. The use of this gelling agent did not require healing and was less costly than agar. The alginate gel was applicable also for the potency diet (Navon et al., 1983).

TABLE 1
The modifications made in the *H. armiera* colony diet for composing the potency diet

Ingredient	Colony diet	Potency diet
Beans (*Phaseolus vulgaris*)	+	+
Alfalfa meal	+	+
Torula yeast	+	–
Cholesterol	+	+
Ascorbic acid	+	+
B vitamin solution*	–	+
Choline chloride	–	+
Synthomycetine	+	–
Methyl-p-hydroxybenzoate	+	+
Sorbic acid	+	+
Formaldehyde (37%)	+	±**
Agar	+	+
Distilled water	+	+

* i — inositol, panthotenic acid, niacin, p-aminobanzoic acid, riboflavin, pyridoxine, folic acid, biotin, vit. B12., dissolved in distilled water.
** up to 0.24% v/w in the diet.

The potency diet

In this diet the full insecticidal activity of the bacterial products should be expressed. For this purpose, modifications in the composition of the insect colony diet were essential. The antibiotics were omitted in order to maintain spore activity. The yeast was replaced by the synthetic B vitamin mixture, i-inositol and choline chloride. This change was made to eliminate possible adverse affects of the yeast against the *B.t.* exotoxins produced by the germinating spore in the larval midgut (Dulmage, 1981). For example, yeast inhibited a beta-exotoxin activity in flies (Peron and Benz, 1968). This exotoxin activity may play an insecticidal role in the septicemia developing in certain insects within the seven days of the bioassay. Formaldehyde at a concentration of aup to 0.24% in the diet did not affect the microbial activity in the bioassay. Qualitative changes in the nutrients other than replacing the yeast may cause undesired feeding effects due to nutritional adaptations, and therefore not recommended.

The modifications made in the colony diet for composing the potency diet are demonstrated in the work with *H. armigera.*

3. The bioassay technique

Young larvae at second or third instar were fed signly in jelly trays on the potency diet for six days. Mortalities caused by the *B.t.* strains and by the standard, Hd-1-S-80 (16,000 IU/mg), were analyzed in a Probit Dose program. The LC50 values were used to calculate the potencies according the the international formula. It was useful to determine the activity of the standard reference against each of the target insects, before operating the microbial screening programs.

The information in Table 2 indicated that, as opposed to other insects, *S. littoralis* was not susceptible to the standard *B.t.* This, in turn reduced the accuracy and the sensitivity of the bioassay. It seems therefore that the HD-1-S-80 standard would not be a suitable *B.t.* reference against this insect. Possibly a strain of serovar 7, var. *aizawai*, or 6, var. *entomocidus*, could become another international reference for this insect and for others not affected by the var. *kurstaki* standard. An alternative technique would be to use neonate larvae which, unlike mature larvae, would be affected in the bioassay at low levels of the HD-1 standard.

TABLE 2
Activity of the standard *B.t.*, HD-1-S-80, against five field crop pests

The insect	LC50 µg/g	95% fiducial limits lower	upper
Spodoptera littoralis	500.0	250.0	1800.0
Heliothis armigera	1.9	0.7	4.9
Earias insulana	6.5	1.0	45.0
Ostrinia nubilalis	19.0	5.6	35.6
Sesamia nonagrioides	25.0	5.0	70.2

THE APPLICATIONS OF THE POTENCY BIOASSAY

1. Selection of promising *B.t.* serovars and strains

In *S. littoralis* serovar 7, aizawai was more potent than serovar. 3a, 3b *kurstaki.* (Navon et al., 1983). As opposed to this, in *O. nubilalis* (Navon and Melamed-Madjar, 1986) and in *Heliothis zea* (see Table 1.), serovar 3a, 3b *kurstaki* strain HD-1 was highly potent.

Recently, two strains of *B.t.* serovar 3a, 3b *kurstaki* were screened against *H. armigera.* They were produced in gram amounts at the fermentation unit at The Biotechnology Unit, The Hebrew University, Jerusalem. (A screening program supported by Biotechnological Applications (B.A.), Jerusalem). The mortalities and potencies are detailed in Table 3.

The strain HD-263 was four times more active than the HD-1 standard. This record indicates on the promising prospects to improving potencies of commercial strains. Similarly, potent strains could be selected in microbial isolates from diseased larvae on the crop.

2. Potency tests in industrial formulations of *B.t.*

There is a practical need to screen potencies in industrial formulations of *B.t.* because: a. The American and French products differ in their potency systems and therefore these potencies would have to be titrated by a bioassay to recommend the effective field rate for pest control. b. Microbial formulations may have different shelf lives and therefore checking the potency of products by the bioassay before application could save pest control failures due to low spore-crystal activity. The industrial preparations were samples from the annual products of the various microbial insecticide producers and screened against *H. armigera* larvae.

Although the potency of Bactospeine is expressed in equivalents of the French references, the potency against *H. armigera* is close to that of the American standard. The potency of the other three

TABLE 3
Potencies of *B.t.* serovar 3a, 3b *kurstaki* strains bioassayed against *H. armigera* larvae

B.t. strain	LC50 µg/g	95% fiducial limits lower	upper	Slope ± SE	Potency IU/mg
HD-1-S-80	1.86	0.7	4.9	0.51 ± 0.04	16,000
HD-251	1.77	0.8	5.2	0.52 ± 0.04	16,813
HD-263	0.47	0.3	0.7	0.94 ± 0.11	63,319

TABLE 4
Potencies of commercial *B.t.* serovar 3a, 3b *kurstaki*, formulations bioassayed against *H. armigera*

B.t. product	Producer	Formulation	LC50 µg/g	Formulated potency	Potency in *H. armigera*
HD-1-S-80	USDA	Powder	1.20	16,000	16,000
Dipel 2X	Abbott	Wett. Powder	0.61	32,000	31,457
Bactospeine	Biochem. Pr.	Flowable conc.	1.31	8,500*	14,656
Thuricide	Sandoz	Wett. powder	1.21	16,000	15,868

*According to the French standard, E61 = 1000 IU Ak/mg.

formulations were about the same against *H. armigera*, as expected for freshly produced commercial *B.t.* preparations. This procedure would be useful to confirm potencies of microbial products before application on the crop.

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