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RETROSPECTIVE VIEW OF THE CONTRIBUTIONS OF DR. HOWARD T. DULMAGE TO *BACILLUS THURINGIENSIS* SUBSP. *ISRAELENSIS* RESEARCH *(Memorial lecture)1*

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ABSTRACT

The scientific production of Dr. Howard T. Dulmage comprised mostly research, development and production of *Bacillus thuringiensis (Bt)* varieties toxic to lepidopteran pests of agronomical crops. However, since the *Bt* subsp. *israelensis (Bti)* was discovered, Dulmage, perceiving its great potential for the control of dipteran vectors, carried out several studies with this *Bt* serotype. Among his most important contributions on *Bti* research were: (1) Recovering over 60 strains of *Bti,* now deposited at several international culture collections. (2) Production of 102 *Bti* fermentation extracts, which are deposited in our institution. (3) Elaboration of an international guide for *Bti* production sponsored by the WHO. (4) Development of a *Bti* standard bioassay to measure toxicity of *Bti* powders. (5) Determination of an effective concentration for *Bti* formulations, ranging from 1 to 10 lb/acre, for mosquito larval control in the field. (6) Establishment of the HD-968-S-1983 strain as primary reference standard for the assessment of the potency of powders of *Bti,* with an arbitrary potency of 4,740 ITU/mg assigned. (7) Five international publications about *Bti* for dipteran vectors control.

Certainly, there was more work from Dr. Dulmage in this field, but the contributions presented here are representative of the interest of this scientist in the progress of *Bti* research.

KEY WORDS: *Bacillus thuringiensis* subsp. *israelensis, Bti* strain collections, *Bti* fermentations.

INTRODUCTION

Undoubtedly, a considerable part of the progress achieved over the last 30 years in the research and production of *Bacillus thuringiensis (Bt),* has its origins in Dr. Howard T. Dulmage's work. Early in his career, working at the U.S. Department of Agriculture (USDA) in the 1960's, he envisaged the

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1This paper is dedicated to the memory of Dr. Howard T. Dulmage, deceased on May 30, 1983.

Working at the USDA, he isolated a *Bt* strain with a toxicity 20 to 200 times higher than that of the known strains (Dulmage, 1970a). He set up the basis for the fermentation and formulation procedures of *Bt* culture extracts for their commercialization (Dulmage, 1970b; Dulmage and Rhodes, 1971). In addition, he established international standards for the toxicity evaluation and comparison of different isolates, while he developed more toxic powders (Dulmage et al., 1971; Dulmage, 1971, 1973a,b, 1975). Also, he searched for isolates with new insecticidal activities that would expand the potential of use of this pesticide (Dulmage and de Barjac, 1973). He developed methods for the recovery of spore-crystal complexes at the laboratory and the industrial level, which are still being used widely (Dulmage, 1970b; Dulmage et al., 1970), and he established better bioassay methods to assess the effectiveness of powders (Dulmage, 1971, 1973a,b; Dulmage et al., 1971, 1976). It is noteworthy that these accomplishments centered on the control of lepidopteran pests for several vegetable and field crops, mostly cotton. However, by the mid 1970's, Dulmage continued to the control of disease-transmitting mosquito larvae using lepidopteran-active isolates having some reported dipteran activity (Hall et al., 1977; Lacey et al., 1978). It is, therefore, not surprising that as soon as Dulmage became aware of the discovery of a new *Bt* subspecies capable of attacking dipteran larvae, especially simuliids (*Bacillus thuringiensis* subsp. *israelensis*) (*Bti*), he quickly perceived the great value of this discovery, because of the possibility to control dangerous human disease vectors, and began to extend his work toward this new subspecies. So, in principle, he alerted the scientific community about the opportunity of controlling destructive diseases, such as malaria, leishmaniasis, filariasis, and trypanosomiasis, by means of this microorganism (Dulmage, 1980, 1981; Dulmage et al., 1981; McLaughlin et al., 1984) and began to be involved in studies on *Bti* as dipteran biocontrol agent. In this paper, some of the most important aspects of Dr. Dulmage's contributions to *Bti* research are summarized.

Bti strain collections

Collection of Bt cultures at the U.S. Department of Agriculture
At the Agricultural Research Service of the USDA (ARS-USDA), in Brownsville, Texas, Dulmage established the largest *Bt* collection in the Americas during 1970–1980. Receiving multiple contributions from laboratories around the world, he collected more than 800 isolates, that were named using his HD code, belonging to 21 serovarieties. From these 800 isolates, 17 belonged to the H-14 serotype, corresponding to *Bti*. In 1982, Dulmage published a catalogue of strains, making available any one of these isolates (Dulmage and Beegle, 1982).

International Entomopathogenic Bacillus Centre, Pasteur Institute (IEBC-PI)
The Pasteur Institute in France had, in 1994, five *Bti* strains with the HD code, from a total of 488 strains belonging to this serovariety. These strains were given to the Centre by Dulmage during 1980–1989 (Lecadet, 1994).

Colección Internacional de Bacilos Entomopatógenos de la Facultad de Ciencias Biológicas, de la Universidad Autónoma de Nuevo León (CIBE-UANL)
This collection has 60 *Bti* HD strains given by Dulmage in 1989 from 67 of this serovariety in total (Rodríguez-Padilla and Galán-Wong, 1992).

Table 1 is a summary of the *Bti* HD code strains deposited at the three institutions.

TABLE 1
Bti HD code strains banked at three *Bt* International Collections

ARS-USDA (1982)	IEBC-PI (1994)	CIBE-UANL (1992)		
HD-500	HD-567	HD-500	HD-789	HD-918
HD-522	HD-658	HD-522	HD-790	HD-919
HD-563	HD-814	HD-563	HD-791	HD-920
HD-567	HD-815	HD-567	HD-792	HD-921
HD-648	HD-968	HD-648	HD-793	HD-968
HD-649		HD-649	HD-794	HD-997
HD-653		HD-653	HD-795	HD-998
HD-654		HD-654	HD-796	HD-999
HD-655		HD-655	HD-797	HD-1000
HD-656		HD-656	HD-798	HD-1001
HD-657		HD-657	HD-799	HD-1002
HD-658		HD-658	HD-800	HD-1003
HD-659		HD-659	HD-801	HD-1004
HD-661		HD-661	HD-802	HD-1005
HD-756		HD-756	HD-803	HD-1006
HD-757		HD-757	HD-814	HD-1007
		HD-785	HD-815	HD-1008
		HD-786	HD-816	HD-1009
		HD-787	HD-916	HD-1013
		HD-788	HD-917	

Bti fermentations

In 1970–1988 Dulmage performed a series of fermentation experiments with *Bt*, with the purpose of optimizing production, standardization and assay conditions of his strains. These fermentation extracts were given to the Microbiology and Immunology Department of the Biological Sciences Faculty at the University of Nuevo León, Mexico, in 1992. From all serotype fermentations obtained by him, 2.54% from a total of 4,019 extracts, belong to *Bti*. From a total of 60 *Bti* HD code strains, Dulmage performed fermentations with 34. In Table 2 and Fig. 1 the different strains fermented by him and the proportion from the total *Bti* extracts are shown.

Guidelines for local *Bti* production

One of the greatest contributions of Dulmage to *Bti* research was the compilaton of a protocol guide for *Bt* H-14 serotype local production. This guide was an extension of the procedures developed by him for the production, formulation and standardization of lepidopteran-specific serovarieties. These guidelines were presented and discussed in the informal consultation on local H-14 *Bt* production, in Geneva, Switzerland, in October 1982. The 128-page booklet was prepared by Dr. Dulmage, at the request of the Scientific Working Group on Biological Control of Vectors of the Special Programme for Research and Training in Tropical Diseases of the World Health Organization, and was published in 1983 (Vandekar and Dulmage, 1983).

TABLE 2
Dulmage's *Bti* fermentation extracts deposited at the Biological Sciences Faculty of the UANL

Strain	Number of Extracts	Year*	Strain	Number of Extracts	Year*
HD-522	3	1979–82	HD-789	1	1983
HD-563	4	1979–82	HD-790	1	1980
HD-567	25	1979–87	HD-792	2	1981–83
HD-500	24	1978–79	HD-793	1	1983
HD-654	3	1980	HD-794	1	1983
HD-653	3	1980–82	HD-795	1	1984
HD-648	1	1982	HD-796	1	1984
HD-649	1	1982	HD-798	1	1984
HD-656	3	1980–82	HD-799	1	1984
HD-655	3	1980–82	HD-800	2	1981–84
HD-657	2	1980–81	HD-816	1	1981
HD-658	2	1980–81	HD-916	2	1984–87
HD-756	1	?	HD-917	2	1983
HD-659	2	1980–81	HD-918	1	1984
HD-814	1	1981	HD-919	1	1983
HD-815	1	1981	HD-920	1	1984
HD-785	1	1983	HD-1006	1	1987

*Refers to production year.

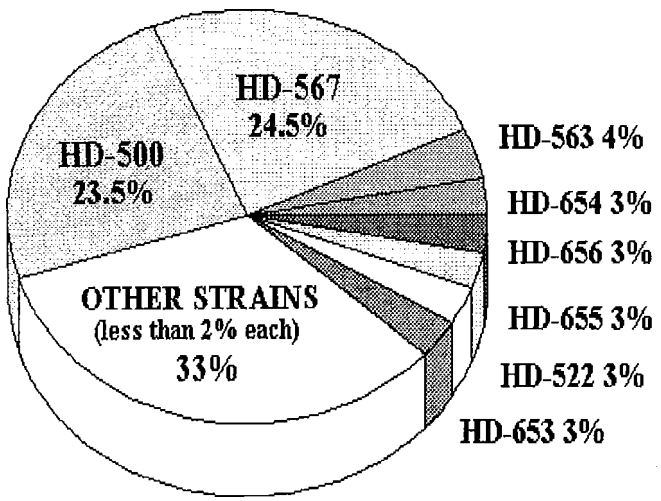

Fig. 1. *Bti* HD code ferment extracts performed by H.T. Dulmage.

The main objective of this handbook was to provide third-world administrators and scientists with a series of protocols for the development of research based upon *Bti*, with the final purpose of implementing local technologies for the massive production of this organism for the biological control of dipteran pests.

The guide contains methodologies ranging from the maintenance of the *Bti* cultures to their formulation for field application; it is divided into eight chapters:

1. Introduction
Describes what *Bti* is, its mode of action, target insects, classification, terminology of fermentation, and other basic concepts.

2. Bioassays of Microbial Insecticides
Details procedures for the toxicity assessment of *Bti* powders and defines international toxicity units.

3. Culture Maintenance and Preservation
Indicates the different technologies for the preservation of viable *Bti* spores and conservation of the genetic characteristics.

4. Submerged or Deep-Tank Fermentations
Analyses the equipment and recovery options in the *Bti* production, either at pilot-plant or industrial level in deep tanks.

5. Fermentation Procedures
Proposes strategies for the selection of culture media, preparation of inoculum, production conditions and recovery procedures.

6. Semisolid Fermentations
Discusses and presents options for producing *Bti* using this kind of fermentation technology.

7. Quality Control, Packaging and Distribution
Describes the necessary monitoring for the process quality control and the packaging and distribution strategies of the finished product.

8. Discussion: Ferment or Buy Microbial Insecticides?
Describes advantages and disadvantages of the local large-scale *Bti* production.

U.S. standard bioassay for the potency assessment of *Bti* H-14

In 1984, Dulmage participated in the establishment of a bioassay protocol for toxicity assessment of *Bti* powders. This protocol differs from the one previously suggested by WHO in the Guidelines for *Bti* Production regarding the follow aspects:

- Specifies a standard cup for larval exposure to *Bti* extracts.
- Establishes a number of 20 larvae per cup and three replications for the concentrations assayed.
- If a minimum of six extract concentrations is tested, a repetition of the assay is required.
- A computational probit analysis is required for evaluating the toxicity as LC_{50}.
- A mortality or pupation higher than 5% in the control invalidates the bioassay.

In addition, the study suggested a variability coefficient of less than 20% for each repetition. Dulmage, together with a team of colleagues, tested the validity of this protocol, and suggested

some considerations for the management of the reference standard strains and for the establishment of new ones (McLaughlin et al., 1984).

Efficacy and field evaluation of *Bti* against mosquitoes in California

In this study, Dulmage, in 1984, together with his working team, determined the effectiveness of the fermentation extracts of *Bti* and *Bacillus sphaericus* when applied in the field for the control of *Psorophora columbiae* and *Aedes melanimon*. *P. columbiae* was controlled at a level of 96–99% by granular formulations of *Bti* applied at rates from 1 to 10 pounds/acre, depending on the potency and formulation type (Mulla et al., 1985).

U.S. reference standard strain of *Bti*

In 1985, Dulmage and a research group proved and suggested the employment of a new strain of *Bti* to be used as reference standard for the potency assessment of *Bti* extracts. In this work, scientists from three ARS laboratories and three bioinsecticide-producing companies participated. The tested strain was *Bti* HD-968-S-1983, which resulted to be 4.74 times more potent than the standard used (IPS-78); the potency assigned to it was 4,740 ± 398 international toxicity units (ITU)/mg. They recommended the use of this strain as the potency reference standard for comparison with any *Bti* formulation, employing, of course, the previously suggested bioassay (see above). For determination of potency, they proposed to perform bioassays at parallel with standard and test powders and use the equation:

$$\text{Test extract potency (ITU / mg)} = \frac{\text{standard } LC_{50} \text{ [standard potency (ITU/mg)]}}{\text{test extract } LC_{50}}$$

The group widely recommended to employ the HD-968-S-1983 strain as reference standard because of facility of use, homogeneity and because of confidence in the results (Dulmage et al., 1985).

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