# SELECTION OF NEW MORE POTENT STRAINS OF *BACILLUS THURINGIENSIS* FOR USE AGAINST GYPSY MOTH AND SPRUCE BUDWORM

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The increased use of *B. thuringiensis* as an alternative to chemical insecticides has stimulated the search for more potent strains. Presently over 1100 strains have been isolated from worlwide sources. Some of these appear to be more effective than the commercially developed HD-1 strain against both the gypsy moth and spruce budworm. One strain in particular, when prepared as a standardized formulation was more effective than HD-1 against the eastern spruce budworm when compared under laboratory and field operational conditions. Laboratory bioassay data also indicates that against gypsy moth it may be approximately four times more potent than HD-1.

The selection of new *B. thuringiensis* (Bt) strains for development and use against specific insect pests can be a difficult and confusing task. The potency and toxin production of a given strain can be directly affected by the fermentation conditions used; not one single fermentation condition will be ideal for all isolates. Delta-endotoxins produced by different strains can differ quantitatively and qualitatively from each other and can have different activity spectra against a particular test insect. The spectrum of activity of a particular strain against different insect species, will vary such that a strain particularly potent against one insect species is not necessarily equally potent against another. Each strain has to be evaluated against each insect species.

Initial Studies: An understanding of the activity spectrum of Bt requires the evaluation of many strains against many different insect species. Because no single laboratory has the resources to conduct such an evaluation, several scientists from different laboratoris were organized into the International Cooperative Program on the Spectrum of Activity of *Bacillus thuringiensis* (Dulmage 1979). It was through the cooperation of this international program that about 300 uniformly prepared strains of Bt were recently evaluated against both the spruce budworm and gypsy moth. The results of this study will be reported here.

However, prior to this recent evaluation of Bt strains against the gypsy moth and spruce budworm the combined results of the first phase of this international research study, which included the screening of 350 strain preparations of Bt against 23 insect species was published in 1981 (Dulmage *et al.* 1981). The general conclusions were: (1) there is an interrelationship between insecticidal activity spectrum and strain serovar; (2) the same delta-endotoxin (crystal) can be produced by strains from more than one serovar; (3) clearly serologically different delta-endotoxins (crystals) i.e., *K-1* and K-73, can be produced by strains of the same serovar; (4) against specific insect species some strains were more potent than the commercially developed HD-1 strain.

Specifically, against the gypsy moth (Dubois 1984a), there is a closer relationship between the crystal serotype and relative potency than between serovar and relative potency. Approximately 5% of the 350 strain preparations that were screened were considered very potent, and 16% were moderately potent. Of the 19 strains considered very potent 17 had a *K-1* or *K-1+ mixed* crystal serotype and one each had a *gal* and *aiz* crystal serotype (from 64 and 35 representative strains respectively). Thirty three of the 57 strains that were considered moderately potent had a *K-1* or *K-1 + mixed* crystal type. The influence of the type of crystal on potency can be particularly appreciated when we consider that most of the strains with a *K-1* crystal type were of serovar $H_{3a\ 3b}$ var. *kurstaki* (49 strains), strains of the same serovar that produced a K-73 type of crystal (23 strains) were nonpotent or weakly potent. Forty-five strains of serovar $H_1$ variety *thuringiensis* that had a *thu* crystal type were also screened; only 9 of these strains were moderately potent and none were very potent. Numerous strains representing other serovars were also screened and most were weakly potent or nonpotent.

Relative to *Trichoplusia ni* and *Heliothis virescens*, strains that were very potent against the gypsy moth had a Tn/Hv ration of 1.91 which is a good indication that potency is associated with the *K-1* type of crystal (Dulmage *et al.* 1981). Also strains that were very potent against gypsy moth were also very potent against *T. ni* but the reverse was not necessarily true.

The initial screening program identified some Bt strains that were as potent or more potent than HD-1 against specific insects. Two of these strains were field tested against a gypsy moth infestation and the results were encouraging (Andreadis *et al.* 1982). Morris and Moore (1983) evaluated 50 strains against spruce budworm larvae. Their selection for evaluation was based on the spectrum of activity of the selected strains against the Douglas fir Tussock Moth; none were more potent than the presently commercially used HD-1 strain.

Current Studies: For the present study, the $LC_{50}$ (in µg/ml of diet) of 298 uniformly prepared strains was determined against both the gypsy moth and spruce budworm. Additionally the $LT_{50}$ (at specific doses) of the experimental strains against the spruce budworm was also determined. The $LC_{50}$ (and $LT_{50}$) of the HD-1 International Standard, HD-1S-1980, was determined against the same batches of larvae and used to compare the relative potency of the experimental strains.

The response of gypsy moth larvae to a specific preparation of Bt is generally consistent. Results of laboratory bioassays on

numerous batches of three generations of laboratory reared gypsy moth and spruce budworm larvae with the HD-1 standard, HD-1S-1980, are summarized in Table 1. The average $LC_{50}$ is reasonably consistent between the generations ($F_{23}$ to $F_{25}$) of gypsy moth that were used. Between batch and within generations variability (as measured by the C.V.) was higher than desirable but nonetheless acceptable. Slopes of the regression usually range between 4-5 (P = 0.05). Using the defined potency of 16,000 International Units of potency (IU)/milligram (mg) for the HD-1S-1980 standard, the overall $LC_{50}$ for the three generations ranged from a low of 75 to a high of 105 IU/milliliter (ml) of diet. This is in good agreement with an earlier estimate of 96.2 ± 5.4 IU/ml (Yendol et al. 1973).

**Table 1. Averages of the 1981, 1982, and 1983 bioassays with the standard HD-1S-1980 against laboratory stocks of instar II, Lymantria dispar L. and instar IV, Choristoneura fumiferana (Clem.)**

| Insect | YEAR | | |
|---|---|---|---|
| L. dispar | 1981 | 1982 | 1983 |
| No. of bioassays | 28 | 30 | 33 |
| $LC_{50}$ (µg/ml of diet) | 5.25 | 4.95 | 5.99 |
| ± 95% C.L.[a/] | 0.72 | 0.47 | 0.55 |
| S.E.[b/] | 0.35 | 0.23 | 0.27 |
| C.V. | 0.36 | 0.25 | 0.26 |
| C. fumiferana | | | |
| No. of bioassays | 6 | 18 | 22 |
| $LC_{50}$ (µg/ml) of diet) | 4.84 | 4.75 | 6.88 |
| ± 95% C.L. | 2.52 | 1.35 | 1.25 |
| S.E. | 0.98 | 0.64 | 0.60 |
| C.V. | 0.49 | 0.57 | 0.41 |

[a/] C.L. - confidence limits

[b/] S.E. - standard error

[c/] C.V. - coefficeient of variation

Mortality data obtained from spruce budworm bioassays is often heterogeneous. Frequently, variation about the $LC_{50}$ results in wide fiducial intervals which can actually be understated and will limit meaningful interpretation of the potency of a particular test strain relative to the standard. As such, estimates of the $LC_{50}$ of a particular preparation are usually discarded when the calculated "g" value exceeds 0.2 (Finney 1964). The mean $LC_{50}$ over three generations of spruce budowrm with HD-1S-1980 are also summarized in Table 1. Between generations, the $LC_{50}$ is fairly consistent however between batch (within generation) variability can be large (note the high C.V.) such that, with all generations considered, the $LC_{50}$ could actually range (P = 0.05) from 37 to 137 IU/ml of diet. Slopes of the regression usually vary between 2 and 3. Variability within and between bioassays with Bt against the spruce budworm has been a continuing problem for this and other investigators. Fast and Dimond (1984) and Fast and Régnière (1984) have reported higher $LC_{50}$ and lower slope values in their bioassays with the HD-1 standard, HD-1S-1971 (precursor to HD-1S-1980) than reported here with HD-1S-1980. Some of these discrepancies could be accounted for by differences in diet and larval rearing conditions and the bioassay procedure used. The observation that larvae recover after exposure to Bt and the possibility of cycling of feeding inhibition and recovery could also contribute to the variability observed in bioassays with this insect. These investigators also reported that there was no significant difference in susceptibility between instar IV to VI. On the other hand Dubois and Squires (1970) had reported that in gypsy moth the $LC_{50}$ of a Bt preparation could vary with larval weight and age.

The $LT_{50}$ of spruce budworm with HD-1S-1971 as reported by Fast and Régnière (1984) was also higher than reported here (Table 2). Their estimate did not vary with larval age but did vary with Bt dose and length of larval exposure on the test diet. Present estimates of the $LT_{50}$ with HD-1S-1980 over a two-fold dose range against instar IV larvae is remarkably consistent and within dose variability is very low.

**Table 2. $LT_{50}$ of different doses of the HD-1 standard, HD-1S-1980, against instar IV Choristoneura fumiferana (Clem.)[a/]**

| | Dose in IU (µg)/ml of diet | | |
|---|---|---|---|
| | 200(12.50) | 150(9.38) | 100(6.25) |
| $LT_{50}$ (days | 4.9 | 4.6 | 4.1 |
| 95% range | 4.3-5.6 | 2.9-6.7 | 3.6-4.7 |
| C.V. | .19 | .30 | .14 |

[a/] Larvae in constant contact for 8 days; 16:8 L:D photo period, 21°C. Average of 8 bioassays/

Most of the 298 strains evaluated in the present study were weakly potent or had no effect on the hosts. None of the 18 strains of serovar $H_{14}$ var. isrealensis or the 14 strains of serovar $H_{10}$ var. darmstadiensis had any effect on these two insect species. Numerous other strains had a limited effect on larval growth and effected very low mortality even at doses as high as 100 µg/ml of diet. Based on the response of these insects to the standard (Table 1) only those experimental strains with an $LC_{50}$ of ⩽ 1.99 ug (32 IU)/ml and those with an $LC_{50}$ between 2.00 and 10.00 µg (32 and 160 IU)/ml of diet are summarized in Table 3; these $LC_{50}$ values would indicate strains that are more potent than or equally potent as the HD-1S-1980 standard. The majority of the strains that are more potent than HD-1S-1980 against both insect species are primarily of serovar $H_{3a3b}$ var. kurstaki and have a K-1 type crystal. A few strains of serovar $H_{4a\ 4c}$ var. kenyae and serovar $H_7$ var. aizawae are also very potent against both insect species. Of all the strains that are considered more potent than the standard against each insect species (16 + 10, $LC_{50}$ ⩽ 1.99 µg/ml) only four are equally more potent against both insect species. These are HD-262 ($H_{3a-3b}$-K-1), HD-545 ($H_{3a3b}$-K-1), HD-551 ($H_{4a4c}$-ken) and HD-562 ($H_{3a3b}$ - ?). Against gypsy moth very

potent strains of other serovars worthy of mention are HD-287 ($H_{5a\ 5b}$ - K-1) and HD-854 ($H_7$ - ?) whereas against the spruce budworm HD-150 ($H_{5a\ 5b}$ - gal) and HD-120 ($H_1$ - K-1) are also worthy of mention as being specifically very potent. The observation that only four strains were equally very potent against both insect species and that there were a few strains uniquely potent against each insect further supports the observation made in the initial study that for each insect species there are some strains that are uniquely potent (Dulmage et al. 1981).

Table 3. Summary of the bioassay of HD strains considered more potent ($LC_{50} \leq 1.99$ µg/ml) or as potent ($LC_{50} \geq 2.00 \leq 10$ µg/ml) as the HD-1s-1980 standard against gypsy moth and spruce budworm.

| serovar | crystal serovar | Gypsy moth $LC_{50}$-(µg/ml) ≤1.99 | Gypsy moth ≥2.00 ≤10.00 | Spruce Budworm $LC_{50}$-(µg/ml) ≤1.99 | Spruce Budworm ≥2.00 ≤10.00 |
|---|---|---|---|---|---|
| 1 | thu | 0 | 5 | 0 | 1 |
| | K-1 + thu | 1 | 9 | 0 | 2 |
| | K-1 | 1 | 2 | 1 | 0 |
| | NI[a] | 0 | 0 | 0 | 1 |
| 3a | ale | 0 | 0 | 0 | 1 |
| 3a3b | K-1 | 5 | 19 | 5 | 6 |
| | K-73 | 0 | 1 | 0 | 0 |
| | NI | 1 | 6 | 2 | 0 |
| 4a4c | ken | 2 | 1 | 1 | 3 |
| | NI | 1 | 1 | 0 | 1 |
| 5a5b | K-1 | 1 | 0 | 0 | 0 |
| | gal | 0 | 2 | 1 | 0 |
| 6 sub | sub | 0 | 0 | 0 | 1 |
| 6 ent | ent | 0 | 1 | 0 | 0 |
| 7 | aiz | 1 | 6 | 0 | 5 |
| | NI | 1 | 2 | 0 | 2 |
| 8a8b | K-1 | 0 | 1 | 0 | 0 |
| | NI | 1 | 0 | 0 | 0 |
| 9 | tol | 1 | 3 | 0 | 1 |
| | NI | 0 | 2 | 0 | 0 |
| 12 | NI | 0 | 1 | 0 | 0 |
| Unk[b] | thu + K-1 | 0 | 1 | 0 | 0 |
| | aiz | 0 | 1 | 0 | 0 |
| | NI | 0 | 4 | 0 | 1 |
| Totals | | 16 | 68 | 10 | 25 |
| Other Totals[a] | | 214 | | 263 | |

[a] not identified

[b] serovar not determined

[c] strains non/or weakly potent

In addition to the 298 strains of Bt reported above, twenty other strains of Bt were also evaluated. These strains were isolated (Dubois unpublished data) from diseased spruce budworm larvae received as hibernaculum from FPMI at Sault Ste. Marie, Ontario. No other shipment of larvae received from FPMI either prior to or since that particular shipment exhibited such a high natural incidence of Bt infection. All the strains that were isolated except for one, were identified as serovar $H_{3a\ 3b}$ var. kurstaki; one strain was identifies as serovar $H_{5a\ 5b}$ var. galleriae (G. Donaldson USDA-ARS, Brownsville, TX 1983, personal communication). The serotype of the crystal of these new strain has not been determined. Three of these isolates labelled as NRD-8, NRD-10, and NRD-12, are 2 and 3.5 times more potent than a similarly prepared HD-1 (concentrate or formulated) against the spruce budworm and gypsy moth respectively. Also, unique with these three strains was their rate of kill against the spruce budworm. Regardless of the preparation, spore-crystal concentrate or standardized formulation, the $LT_{50}$ of the three strains was almost half that of a similarly prepared HD-1 or of the international standard, HD-1S-1980 when compared at similar doses of µg/ml of diet. One of these strains, NRD-12, was commercially formulated and labelled as SAN 415 32LV. This formulation, standardized at 32 BIU/gallon, was then compared to the registered HD-1 formulation, Thuricide 32 LV®, against a natural spruce budworm infestation. The favorable results were summarized in a preceding presentation (Dubois 1984b). The microbial and pathogenic characteristerics of this strain are summarized in Table 4. The higher potency of NRD-12 compared to HD-1 is also apparent from bioassay with H. virescens but not with T. ni.

Table 4. Summary of the microbial and pathogenic characteristics of the NRD-12 Bacillus thuringiensis strain.

Source: Isolated by N.R. Dubois from diseased Choristoneura fumiferana (Clem.) larva at the NEFES-USFS laboratory at Hamden, CT, January 1981. Larva had been received from FPMI Sault. Ste. Marie, Ontario in December 1980

USDA Bacillus thuringiensis Culture Collection No. for NRD-12 is HD-945. Serovar[a]: $H_{3a3b}$ variety kurstaki, crystal antigen unkown.

Bioassay Characteristics:

| | Comparative Potency (IU/mg) Trichoplusia ni | Heliothis virescens |
|---|---|---|
| HD-1 R641A: | 39,800 | 15,400 |
| NRD-12 (HD-945): | 30,300±8980 | 32,700±7280 |

| Lymantria dispar | NRD-12 (conc.) | HD-1S-1980 | Ratio |
|---|---|---|---|
| Slope: | 5.19 | 3.75 | |
| $LC_{50}$ (µg/ml) | 1.75 | 6.14 | 3.51 |
| $LC_{50}$ (IU/ml) | 28.00 | 98.2 | |
| **Choristoneura fumiferana** | | | |
| Slope: | 2.85 | 3.50 | |
| $LC_{50}$ (µg/ml) | 1.75 | 3.60 | 2.06 |
| $LT_{50}$ (days) (3.0 µg/ml) | 4.6 | 8.3 | 1.80 |
| (6.6 µg/ml) | 2.7 | 5.1[b] | 1.91 |

[a] Serovar, data for T. ni and H. virescens provided by G. Donaldson and H. Dulmage, USDA-ARS, Brownsville, TX.

[b] $LT_{50}$ based on a spore-crystal concentrate of HD-1: for $LT_{50}$ of HD-1S-1980 at comparable dose refer to Table 2.

This similarity to HD-1 relative to its serovar and its potency against T. ni was also independently confirmed by Hostetter (D. Hostetter, USDA-ARS Columbia, MO, personal communication Dec. 1983). However, its spectrum of activity (and presumably that of NRD-8 and NRD-10) relative to gypsy moth, spruce budworm and H. virescens certainly indicates some toxin composition different from HD-1. Furthermore, its rate of kill ($LT_{50}$) against the spruce budworm is significantly faster than with any preparation of HD-1( at comparable doses) reported by others (Fast and Régnière 1984, Fast and Dimond 1984), or reported in this study. This insecticidal characteristic may be particularly advantageous in operational use of Bt (Fast and Dimond 1984, Morris, 1983).

Evaluation of Bt strains against insect pests is a continuing effort to search for new more effective strains. Presently, approximately 1,100 strains have been isolated from worldwide sources, and as with the NRD-series more are continually being isolated. This influx of new strains will undoubtedly increase as the potency of variants generated from genetic engineering efforts need to be evaluated.

Aside from the large grouping of the specific activity of serovar $H_{14}$ against mosquitos and black flies, differences in activity among lepidopteran pests are more subtle. These differences such as the $LT_{50}$ differences between NRD-12 and HD-1 relative to spruce budworm may have a greater impact on field effectiveness than the two-fold difference in $LD_{50}$. Whereas the almost four-fold difference in $LD_{50}$ of these two strains relative to gypsy moth may be the insecticidal characteristic of greater significance. These observations along with the results of this survey, that only four strains were equally very potent against both insects and several strains were uniquely very potent against each insect species certainly suggest that serious efforts to improve the use of Bt by strain selection will require the evaluation of each strain against each insect pest.

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