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# Worldwide Abundance and Distribution of *Bacillus thuringiensis* Isolates

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**We found the insect control agent *Bacillus thuringiensis* to be a ubiquitous soil microorganism. Using acetate selection to screen soil samples, we isolated *B. thuringiensis* in 785 of 1,115 soil samples. These samples were obtained in the United States and 29 other countries. A total of 48% of the *B. thuringiensis* isolates (8,916 isolates) fit the biochemical description of known varieties, while 52% represented undescribed *B. thuringiensis* types. Over 60% (1,052 isolates) of the isolates tested for toxicity were toxic to insects in the orders Lepidoptera or Diptera. Soil samples were collected from various habitats, including those habitats with different numbers of insects. The current presence of insects did not predict the presence of *B. thuringiensis* in a particular soil sample. *B. thuringiensis* was most abundant in samples from Asia.**

Strains of *Bacillus thuringiensis* are used to control pest insects in the orders Lepidoptera, Diptera, and Coleoptera. Because *B. thuringiensis* does not ordinarily cause epizootics among insects (6), we hypothesized that the association of *B. thuringiensis* and these insects may be serendipitous, or at least uncommon. Dulmage and Aizawa (6) have suggested that the normal habitat of *B. thuringiensis* is the soil.

Early attempts to isolate *B. thuringiensis* from the soil suggested that *B. thuringiensis* is almost nonexistent in that environment (4). Our development of acetate selection to isolate *B. thuringiensis* from soil samples containing a high-level background of other soil microbes, including other *Bacillus* spp. (18), has shown otherwise. Other studies that have described the distribution of *B. thuringiensis* have concentrated on limited geographical areas. There have been efforts to isolate *B. thuringiensis* strains from soil samples in the United States (4), Japan (13), and the Philippines (15). In this study we examined soil samples from five continents (Africa, Asia, Europe, and North and South America) and their associated islands.

In any large-scale effort to isolate a species of bacteria, a method to distinguish isolates from one another is needed. Originally, *B. thuringiensis* strains were divided into subspecies or varieties based on their spectra of activity against insects and complementary biochemical tests (8). As more strains of *B. thuringiensis* were isolated, this method was too cumbersome and a serological shorthand method based on biochemical profiles was developed (2). For this study, serology was too cumbersome to identify thousands of isolates, so a rapid method based on biochemical tests was used (11).

Before this study, most *B. thuringiensis* strains were isolated in association with insects (1, 5–10). If the range of insects that *B. thuringiensis* affects is to be extended, more diverse *B. thuringiensis* strains and endotoxins are needed. This search for genetic diversity and for insect control and evolutionary and ecological studies were the initial purposes of this study.

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## MATERIALS AND METHODS

**Soil collection.** Soil samples were collected by scraping off surface material with a sterile spatula and then obtaining a 10-g sample 2 to 5 cm below the surface. These samples were stored in sterile plastic bags at ambient temperature. Additionally, 200 site borings from several locations were obtained from a local engineering firm. We attempted to collect soil from locations that were as diverse as possible. Some of the areas sampled were high-altitude mountains, tropical jungles, temperate and tropical caves, beaches, forests, agricultural fields, grasslands, scrub wilderness, and urban locations. Soil samples from foreign countries, as well as unusual locations, were treated as if they harbored human pathogens or agricultural pests.

**Selection.** Our soil samples from New Zealand were primarily from the North Island in park and wilderness areas near Wellington. Our soil samples from Asia were collected from uninhabited areas in Nepal (5 samples); agricultural land in Pakistan (4 samples) and India (2 samples); various areas in southern Vietnam (23 samples); and Hanoi, Vietnam (1 sample); an urban area in Beijing, People's Republic of China (1 sample); and agricultural land in the Republic of Korea (18 samples). Also included in the Asian count were 15 soil samples from Turkey.

The European soil samples came from inhabited areas of Spain (8 samples), Portugal (29 samples), France (5 samples), the Federal Republic of Germany (1 sample), The Netherlands (1 sample), England (10 samples), and Ireland (4 samples). We also tested four samples from forest soils in Poland. Soil samples from Norway (14 samples), Sweden (4 samples), and Iceland (11 samples) were collected in high-altitude forests or arctic tundra. Another six samples were collected in the Azores.

Most of our African soil samples were collected in Egypt (182 samples). Other sample sites included Morocco (1 sample) and rain forests in Cameroon (3 samples).

Samples from Mexico (2 samples) were collected in Baja California. Four samples were from caves in Jamaica. Samples from Guatemala (3 samples), Honduras (5 samples), and Nicaragua (6 samples) were collected on agricultural lands. Five samples were collected in the Peruvian rain forest, and one sample was from the Amazon basin in Brazil. A total of

36 samples were collected in Argentina in association with fire ants (*Solenopsis invicta*).

In the United States, we sampled soil from urban areas, agricultural lands, prairies, forests, and beaches. We found *B. thuringiensis* in backyards, cultivated soils, public parks, wilderness trails, lake sediments, archeological excavations, borings from construction projects, and all 37 states from which we had soil samples. A total of 400 of the 693 samples from the United States were taken from in and around the Washington, D.C., area. Included in this count were 189 samples from borings at local construction sites and 84 samples from other ongoing studies.

One gram of soil was subjected to acetate selection (18). Acetate inhibited the germination of *B. thuringiensis* spores; so other spores germinated, and then the growing cells and other, non-spore-forming bacteria were eliminated by heat treatment. After acetate selection, an average of 100 to 300 sporeforming colonies were recovered per plate. By using the agar dot method for further identification (11), we picked a sample of 32 colonies from these plates and placed them onto T3 (3 g of tryptone per liter, 2 g of tryptose per liter, 1.5 g of yeast extract per liter, 0.05 M sodium phosphate [pH 6.8], 0.005 g of $MnCl_2$ per liter) dots for sporulation (18). The different colony morphologies picked were representative of the proportion of each colony type present on the L-agar plates. There were some exceptions, which we note here. A total of 96 colonies were picked from 3 samples, and 64 colonies were picked from 60 others. These samples were collected from unusual places such as caves in Jamaica and mountains in Nepal, from which we did not expect to obtain samples again. Many of these samples were tested early in this study, when we thought it would be necessary to screen a large number of colonies in order to recover any *B. thuringiensis* isolates. In 400 other soil samples, there were less than 32 colonies after acetate selection, and therefore, all colonies were picked for further characterization. The numbers of *B. thuringiensis* isolates are expressed as a decimal fraction or index of the number of colonies examined. This index represents the fraction of *B. thuringiensis* (crystal formers) in the total acetate-selected bacterial population examined. This index also indicates how easily *B. thuringiensis* was isolated. The other bacteria which made up the total acetate-selected population were *Bacillus megaterium*, *Bacillus sphericus*, and other unidentified members of the genus *Bacillus*. In some samples *Bacillus cereus* was coisolated, but not often (18).

**Biochemical identification.** When the isolates were placed on T3 dots, they were allowed to sporulate and were determined to have the *B. thuringiensis* biochemical profile. Putative *B. thuringiensis* colonies were screened by light microscopy for crystal morphology. Subdivisions within the *B. thuringiensis* species by biochemical type were also made at this time (11). Fourteen biochemical tests were performed to identify isolates. For this study, only the results of the following four (the most relevant) biochemical tests are presented: esculin utilization, acid formation from salicin and sucrose, and lecithinase production (16). This enabled us to divide the *B. thuringiensis* isolates into 16 biochemical types. The distribution of isolates in these groups was parallel to the distribution obtained by additional tests.

**Toxicity.** For qualitative toxicity testing, spore-crystal preparations were grown on T3 plates (18). The spores and crystals from the agar were floated on 10 ml of sterile water. The suspension was stored in sterile scintillation vials until it was tested.

The activity of *B. thuringiensis* strains against insects of the order Lepidoptera was tested by using either *Bombyx mori* (silkworm) or *Trichoplusia ni* (cabbage loopers). Third-instar silkworms were force-fed 4 μl of a spore-crystal preparation and allowed to feed on untreated white mulberry (*Morus alba*) leaves. A positive test left the mulberry leaves uneaten and the silkworms dead in 16 to 24 h. To test *B. thuringiensis* activity against cabbage loopers, 100 μl of the spore-crystal mixture was spread onto petri dishes (diameter, 100 mm) containing 25 ml of artificial diet (17). When dry, 10 neonatal cabbage loopers were added. A positive test left all 10 cabbage loopers dead at 48 h. When HD-1 (a standard lepidopteran toxic strain) was grown and tested in the same manner, all cabbage loopers died when the original preparation was tested. All cabbage loopers also died at a 1:10 dilution of this preparation. At the 1:100 dilution of this preparation, less than 50% of the cabbage loopers usually died.

The activity of *B. thuringiensis* strains against mosquitoes was tested by using *Culex pipiens*. Ten fed, third-instar mosquitoes were added to 10 ml of sterile water in 20-ml scintillation vials. Then, 50 μl of a spore-crystal preparation was added. A positive test left all mosquitoes dead at 24 h. When ONR-60A (a standard dipteran toxic strain) was grown and tested in this manner, all mosquitoes died. A 1:10 dilution of this preparation often did not kill all mosquitoes in 24 h.

TABLE 1. Distribution of *B. thuringiensis* in soil samples, by region

| Location | No. of samples examined | % of samples with at least one *B. thuringiensis* isolate (no. of samples) | *B. thuringiensis* index[a] (no. of *B. thuringiensis* isolates) |
|---|---|---|---|
| New Zealand | 9 | 55.6 (5) | 0.13 (39) |
| Asia | 53 | 94.3 (50) | 0.85 (1,449) |
| Europe | 112 | 83.9 (94) | 0.38 (1,369) |
| Africa | 186 | 87.6 (163) | 0.02 (645) |
| South and Central Africa | 62 | 93.5 (58) | 0.28 (587) |
| United States | 693 | 59.9 (415) | 0.25 (5,687) |
| Total | 1,115 | 70.4 (785) | 0.28 (9,776) |

[a] The *B. thuringiensis* index was calculated as a number of *B. thuringiensis* isolates recovered divided by the number of colonies of all bacteria examined.

## RESULTS

**Distribution of *B. thuringiensis*.** With the advent of a very selective procedure for separating *B. thuringiensis* spores from the spores of other soil microbes, we began a concentrated effort to recover *B. thuringiensis* from the environment. Since other efforts to isolate *B. thuringiensis* from soil have only met with minimal success, we were surprised that the first 25 soil samples subjected to acetate selection contained some *B. thuringiensis* isolates. Initially, we knew that we had different *B. thuringiensis* strains because of their different crystal morphologies.

This preliminary information led us to expand our effort to determine exactly what the worldwide distribution and abundance of *B. thuringiensis* were. In this expanded search we obtained 1,115 soil samples from across the United States and from 29 other countries around the world. The locations of these samples are summarized in Table 1.

Soil samples from Asia were extraordinarily rich in *B. thuringiensis*. Seventy-eight percent of all colonies examined formed crystals. On the other hand, in soil samples from

TABLE 2. Biochemical types of *B. thuringiensis*

| Biochemical type (described subspecies) | Biochemical test result[a] | | | |
|---|---|---|---|---|
| | Esculin | Salicin | Lecithinase | Sucrose |
| 1 (*thuringiensis*) | + | + | + | + |
| 2 (*kurstaki*) | + | + | + | − |
| 3 (*indiana*) | + | + | − | + |
| 4 (*galleriae*) | + | + | − | − |
| 5 (*sotto*) | + | − | + | + |
| 6 (*dendrolimus*) | + | − | + | − |
| 7 (*morrisoni*) | + | − | − | + |
| 8 (*darmstadiensis*) | + | − | − | − |
| 9 | − | + | + | + |
| 10 | − | + | + | − |
| 11 | − | + | − | + |
| 12 (*ostriniae*) | − | + | − | − |
| 13 | − | − | + | + |
| 14 (*israelensis*) | − | − | + | − |
| 15 | − | − | − | + |
| 16 | − | − | − | − |

[a] The + sign indicates a positive reaction, i.e., utilization of esculin, acid production from salicin and sucrose, and production of lecithinase.

the United States, and especially from the Washington, D.C., area, *B. thuringiensis* made up only 25% of the colonies examined. Although some of these areas were sprayed with the commercial *B. thuringiensis* subsp. *kurstaki*, we rarely recovered this variety from the Washington, D.C., area.

After acetate selection, 832 of 1,115 soil samples tested yielded colonies. Of these 832 samples, 785 (94%) contained at least one crystal-forming *B. thuringiensis* isolate. This suggests that *B. thuringiensis* is an ubiquitous microorganism.

**Identification.** Because of the number of samples (1,115 samples) and isolates (approximately 27,000 isolates) involved in this study, we found the usual method of identifying *B. thuringiensis* by the serotyping of flagellar antigens (2) to be impractical. However, we developed a series of rapid tests to identify different biochemical types of *B. thuringiensis* (11). This system was based on the biochemical tests that have been published for known varieties for which the serotypes have been identified (3). When several varieties were described as being identical, we used the varietal name that was first described or, in the case of *B. thuringiensis* subsp. *kurstaki*, the most familiar. Note that this use of preexisting varietal names is strictly an operational definition based on biochemical tests; i.e., classification as *B. thuringiensis* subsp. *israelensis* does not imply mosquito larvicidal activity.

We started with seven biochemical tests (starch hydrolysis; urease production; mannose, sucrose, and salicin fermentation; esculin utilization; and lecithinase production) which gave 128 possible biochemical types. By eliminating the three least discriminating tests (starch hydrolysis, urease production, and mannose fermentation), we were able to devise a simplified system of 16 biochemical types (Table 2) which paralleled the distribution in the more complex scheme. These biochemical types were based on esculin, salicin, lecithinase, and sucrose tests, which were the most variable among *B. thuringiensis* isolates. When the results from the biochemical tests yielded a combination that had not been described, we chose not to name the type until further characterization was done. We refer to these new types by the numbers given in Table 2.

Table 3 gives the worldwide distribution of *B. thuringiensis* by biochemical types. While some of these types differed by a single biochemical test, these differences were significant. For example, *B. thuringiensis* subsp. *kurstaki*, a relatively common biochemical type, differed from the least common biochemical type, *B. thuringiensis* subsp. *galleriae*, by a single biochemical test, lecithinase production (3). The least common type, *B. thuringiensis* subsp. *galleriae* ($Es^+$ $Sa^+$ $Le^-$ $Su^-$) was isolated only 51 times from a total of 8,916 *B. thuringiensis* isolates (0.6%). The most common biochemical type, *B. thuringiensis* subsp. *israelensis* ($Es^-$ $Sa^-$ $Le^+$ $Su^-$) occurred in 20.4% (1,817 isolates) of all *B. thuringiensis* isolates. The biochemical types *B. thuringiensis* subsp. *israelensis* and *dendrolimus* and biochemical types 13, 15, and 16, which differed from each other by a single biochemical test (Table 2), made up a cluster of strains which accounted for 60.5% of all environmental isolates.

Biochemical type was not indicative of location. Most biochemical types could be found everywhere. However, most locations had a distinct distribution of types. *B. thuringiensis* subsp. *israelensis*, the most common type overall, was the most prevalent type only in the United States (18.8%) and Europe (29.7%). In Asia, *B. thuringiensis* subsp.

TABLE 3. Distribution of *B. thuringiensis* types

| Biochemical type (described subspecies) | New Zealand | Asia | Europe | Africa | Central and South America | United States | Total (%) |
|---|---|---|---|---|---|---|---|
| 1 (*thuringiensis*) | 0 | 52 | 19 | 9 | 16 | 223 | 319 (3.6) |
| 2 (*kurstaki*) | 0 | 342 | 21 | 19 | 5 | 202 | 589 (6.6) |
| 3 (*indiana*) | 0 | 41 | 19 | 17 | 6 | 161 | 244 (2.8) |
| 4 (*galleriae*) | 0 | 3 | 8 | 0 | 2 | 38 | 51 (0.6) |
| 5 (*sotto*) | 0 | 45 | 18 | 13 | 11 | 155 | 242 (2.7) |
| 6 (*dendrolimus*) | 2 | 131 | 17 | 22 | 15 | 188 | 375 (4.2) |
| 7 (*morrisoni*) | 2 | 37 | 33 | 47 | 21 | 253 | 393 (4.4) |
| 8 (*darmstadiensis*) | 2 | 16 | 33 | 9 | 8 | 68 | 136 (1.5) |
| 9 | 0 | 52 | 155 | 45 | 32 | 290 | 574 (6.4) |
| 10 | 15 | 94 | 121 | 24 | 34 | 322 | 610 (6.8) |
| 11 | 2 | 11 | 26 | 26 | 15 | 155 | 235 (2.6) |
| 12 (*ostriniae*) | 2 | 10 | 11 | 12 | 11 | 81 | 127 (1.4) |
| 13 | 2 | 99 | 164 | 44 | 73 | 428 | 810 (9.2) |
| 14 (*israelensis*) | 6 | 297 | 395 | 120 | 86 | 913 | 1,817 (20.4) |
| 15 | 2 | 126 | 200 | 158 | 192 | 823 | 1,501 (16.8) |
| 16 | 4 | 93 | 90 | 80 | 60 | 566 | 893 (10.0) |
| Total | 39 | 1,449 | 1,330 | 645 | 587 | 4,866 | 8,916 |

TABLE 4. *B. thuringiensis*-insect association

| Location (infestation) | No. of samples | Total no. of *B. thuringiensis* | No. of isolates toxic to: | | | No. of nontoxic isolates |
|---|---|---|---|---|---|---|
| | | | Lepidoptera[a] | Diptera[b] | Both | |
| Jamaica (insect infested) | 4 | 28 | 8 | 2 | 4 | 14 |
| Nepal (few insects) | 6 | 203 | 103 | 6 | 4 | 90 |

[a] *T. ni* was used as the test insect.
[b] *C. pipiens* was used as the test insect.

*kurstaki* ($Es^+$ $Sa^+$ $Le^+$ $Su^-$) was the most common type. In Africa and South and Central America, biochemical type 15 ($Es^-$ $Sa^-$ $Le^-$ $Su^+$), which was similar to the biochemical type *B. thuringiensis* subsp. *israelensis*, was the most common biochemical type. Biochemical type 10 ($Es^-$ $Sa^+$ $Le^+$ $Su^-$), which was similar to *B. thuringiensis* subsp. *kurstaki*, was the most common biochemical type found in New Zealand.

**Toxicity and insect association.** Of the 1,052 isolates tested for toxicity against insects in the order Lepidoptera, 424 (40.3%) showed toxicity. We defined toxicity as 100% mortality of an undiluted spore-crystal preparation. Of the 502 isolates that were tested for toxicity against mosquitoes, 114 (22.7%) showed toxicity. Only 26 isolates were toxic to both mosquitoes and lepidopteran insects.

Toxicity could not be predicted from the crystal morphology. In a soil sample obtained from Jackson, Wyo., 39 *B. thuringiensis* isolates which formed bipyramidal crystals were tested for toxicity against cabbage loopers. Thirty-five were found to be toxic. In another sample, from a Silver Spring, Md., park, 32 *B. thuringiensis* isolates which formed bipyramidal crystals were nontoxic when tested against Lepidoptera. For *B. thuringiensis* isolates which formed amorphous or irregular crystals, the same pattern was found. In a sample from a South Korean bean field, all 32 isolates were found to be toxic against mosquitoes; but 16 amorphous crystal formers from three samples in Iceland were found to be toxic to mosquitoes (4 isolates), Lepidoptera (1 isolate), or both (1 isolate). Ten isolates from these samples were not toxic to the insects tested. Of the 224 isolates of the biochemical type *B. thuringiensis* var. *israelensis* tested for toxicity, 145 formed bipyramidal crystals, 68 formed amorphous or irregular crystals, and 11 formed other types of crystals. A total of 68 bipyramidal crystal-forming isolates were toxic to Lepidoptera, 9 were toxic to Diptera, 7 were toxic to both, and 49 were nontoxic. A total of 45 of the amorphous crystal-forming isolates were toxic to Diptera, 7 were toxic to Lepidoptera, and 6 were nontoxic. All of the 11 other crystal formers were nontoxic.

Because *B. thuringiensis* has been considered an insect pathogen and most strains have been isolated in association with insects, we initially concentrated our sampling efforts in two opposite areas: soils that had intense insect activity and, conversely, soils that had little to no insect activity. Table 4 shows a comparison of *B. thuringiensis* isolates obtained from soils from two such different areas. From mountains in Nepal, which had no detectable insects, we isolated a higher fraction of *B. thuringiensis* from the total number of bacterial colonies examined (0.92) than from the bacterial colonies from Jamaica (0.15). The caves in Jamaica were infested with fleas, and lice with other arthropods (mites and ticks) were also present.

From soil samples collected in Argentina, we were able to compare directly similar environments with and without a particular soil insect. Fifteen soil samples were taken in association with fire ant mounds. An additional 22 samples were collected from the same area but with no fire ants present. We recovered *B. thuringiensis* in 14 of 15 insect-associated samples and in 19 of 22 non-insect-associated samples. The *B. thuringiensis* index of the bacterial colonies examined from the fire ant mounds was 0.25 (120 of 480 isolates) and 0.17 (118 of 704 isolates) for the other samples with no detectable fire ants.

TABLE 5. *B. thuringiensis* isolation from plant communities

| Plant community | No. of samples examined | % of samples with at least one *B. thuringiensis* isolate (no. of samples) | *B. thuringiensis* index[a] (no. of *B. thuringiensis* isolates) |
|---|---|---|---|
| Beach | 20 | 25.0 (5) | 0.08 (53) |
| Subterranean | 200 | 29.0 (58) | 0.08 (536) |
| Desert | 180 | 92.2 (166) | 0.10 (594) |
| Savannah | 37 | 91.9 (34) | 0.20 (238) |
| Tropical rain forest | 15 | 66.7 (10) | 0.33 (161) |
| Urban[b] | 166 | 78.9 (131) | 0.35 (1,856) |
| Forest | 119 | 70.6 (84) | 0.37 (1,398) |
| Agricultural land | 170 | 87.1 (148) | 0.45 (2,437) |
| Steppe | 98 | 85.7 (84) | 0.46 (1,436) |
| Arctic tundra | 32 | 68.8 (22) | 0.51 (518) |
| Total | 1,037 | 71.6 (742) | 0.28 (9,227)[c] |

[a] See footnote *a* of Table 1 for a description of the *B. thuringiensis* index.
[b] An introduced rather than a native plant community.
[c] Other samples were collected, but they could not be placed into the indicated plant community categories.

**Environmental distribution.** Another way to group sample sites was by environments based on plant communities, independent of location (Table 5). Three communities, beach, subterranean (including caves and borings), and desert (Nile Valley), were poor (*B. thuringiensis* index, <0.11) in the percentage of *B. thuringiensis* isolates recovered per the number of colonies examined. Beaches and subterranean samples were also low in the number of samples from which *B. thuringiensis* was recovered. These samples were also low in the total number of bacteria recovered. Sixty-eight of the samples taken from construction site borings contained no bacteria which formed colonies after acetate selection.

Table 5 lists those environments in which *B. thuringiensis* is more likely to be found. However, there were exceptions. In one beach sample from Cedar Key Bay, Fla., 26 isolates of *B. thuringiensis* were recovered from a total of 26 colonies after acetate selection (*B. thuringiensis* index, 1.00). In a similar vein, although tropical rain forests are not typically rich in *B. thuringiensis*, one sample from the Amazon basin in Brazil had a *B. thuringiensis* index of 0.94 after acetate selection.

Of 30 soil samples collected in tundra plant communities, we recovered 33 or fewer colonies after acetate selection in 18 of these samples. We examined a total of 238 bacterial colonies after acetate selection and found 127 crystal-forming *B. thuringiensis* isolates. From two of these soil samples, no bacterial colonies were recovered after acetate selection.

When these plant community data were combined with location data, other observations could be made. Eight urban samples (introduced plant communities) from Vietnam yielded 124 *B. thuringiensis* isolates, or a *B. thuringiensis* index of 0.52. In contrast, from 72 urban samples obtained in

the United States we recovered 576 *B. thuringiensis* isolates for a *B. thuringiensis* index of only 0.25.

In 165 agricultural fields in the United States (potatoes, soybeans, collards, and corn), we recovered 1,662 *B. thuringiensis* isolates (*B. thuringiensis* index, 0.31). In seven agricultural fields (soybeans, peppers, and rice) in the Republic of Korea, we recovered 302 *B. thuringiensis* isolates (*B. thuringiensis* index, 0.67).

## DISCUSSION

The distribution of crystal formers around the world raised more questions than it answered. Were the *B. thuringiensis* isolates we found in the soil the same as previously described strains? Where is one most likely to find *B. thuringiensis*? Were the *B. thuringiensis* isolates toxic to insects? If *B. thuringiensis* is not associated with insects, then what is its role in the environment? We have answers to the first three questions, but the fourth question is still open to speculation.

With the exception of the biochemical type *B. thuringiensis* subsp. *israelensis*, many of the *B. thuringiensis* subspecies that were found in the soil were different from the previously described subspecies. Of our 16 biochemical types, 10 have already been described. These represented 4,293 (48.1%) of the *B. thuringiensis* isolates described, of which 20.4% were of the biochemical type *B. thuringiensis* subsp. *israelensis*. On the other hand, the six other biochemical types described in this study made up 51.9% (4,623 isolates) of the *B. thuringiensis* isolates we isolated (Table 3).

The study of DeLucca et al. (4) showed that new *B. thuringiensis* strains can be found in soil samples. We found *B. thuringiensis* almost everywhere we looked. However, the search for *B. thuringiensis* was more productive in some areas than others. Eastern Asia, especially the Republic of Korea, Nepal, and southern Vietnam, were rich in *B. thuringiensis*. Although we do not know why there was a high concentration of *B. thuringiensis* in samples from Asia, in other studies from Japan (13) and the Philippines (15), crystal formers have been found without the additional sensitivity of acetate selection.

For the purposes of this study, we only did toxicity screening to show that these new isolates were toxic to insects that are known to be susceptible to *B. thuringiensis*. We preselected crystal types that we thought would be toxic to these insects. As Ohbo and Aizawa (14) have also shown, however, no correlation could be made between crystal type and toxicity. Toxicity to certain types of insects appeared to be clustered in some samples. That is, some samples that consisted entirely of *B. thuringiensis* strains which formed bipyramidal crystals were toxic to Lepidoptera. In other soil samples, the *B. thuringiensis* isolates formed irregular crystals that were toxic to mosquitoes. Some soil samples also consisted of *B. thuringiensis* isolates that were not toxic to any insect tested. Other samples contained isolates toxic to both Lepidoptera and Diptera. Since we did not have extensive toxicity data, no firm conclusions could be drawn about the distribution of toxic *B. thuringiensis* isolates.

Even more interesting was the information that almost 40% of the crystal formers tested for toxicity were not toxic to any of the insects that were tested. Our first thought was that these crystals may be toxic to insects that are not normally susceptible to *B. thuringiensis*. Screens for activity against beetles did not support this hypothesis. Additional study is needed in order to predict the toxicity against insects of other orders.

Except for the fact that *B. thuringiensis* was previously found in association with insects, there is no reason to believe that this association is obligate. *B. thuringiensis* grows on minimal medium with few supplements (12; P. A. W. Martin, unpublished data), unlike other bacteria with obligate interactions. *B. thuringiensis* was found everywhere, and the nutrient requirements for growth suggest that turnover is possible. Soil samples with high levels of insect activity were found to be no more likely to have high numbers of *B. thuringiensis* than was a soil sample obtained at random. Insects or arthropods in these soil samples (fire ants, lice, and ticks) are not known to be affected by *B. thuringiensis*. We had more success in isolating high numbers of *B. thuringiensis* from environments with no detectable insects.

The normal role of *B. thuringiensis* in the environment remains an enigma. The ubiquity of *B. thuringiensis* in soil further supports the hypothesis of Dulmage and Aizawa (6) that this is the normal environment of *B. thuringiensis*. *B. thuringiensis* is not normally toxic to insect larvae that live in the soil, such as black cutworm, corn root worm, Japanese beetles, or wireworms; but it is toxic to insects that have aerial or water-borne larvae, such as cabbage loopers, gypsy moths, and mosquitoes. So one is left with the dilemma that either the bacteria make a crystal toxin for insects that it very rarely contacts or it makes the crystal for some other purpose than to kill these insects. Although at present we cannot demonstrate what this purpose might be, this hypothesis may lead to answers about the fundamental toxicity of the *B. thuringiensis* crystal.

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