

# Investigations of *Bacillus thuringiensis* Populations Associated with Animal Feces

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*Bacillus thuringiensis*, an aerobic Gram-positive bacterium, produces unique crystalline parasporal inclusions in sporulating cells. The inclusions often contain strong and specific insecticidal and nematocidal activities. This makes *B. thuringiensis* an environmentally safe agent for control of insect pests of agricultural, veterinary, and medical importance. For recent years, however, *B. thuringiensis* has been also often recovered from other natural environments: soils, phylloplanes, aquatic environments, activated sludges, and marine and intertidal brackish sediments. Relatively little work has been done, however, on the occurrence of *B. thuringiensis* in the feces of animals. It would be of value, for safety consideration in practical application of *B. thuringiensis*-based bioinsecticides, to assess the natural background levels of this organism in and on various animals.

The objectives of this study were: (1) to assess the natural frequency of *B. thuringiensis* in feces of animals, and (2) to characterize serological and biological properties of *B. thuringiensis* recovered from animal feces.

(1) A total of 71 fecal samples, collected from 56 animal species (47 mammals, 4 reptiles, and 5 avians), were examined for the occurrence of *Bacillus thuringiensis*. Most of the animals were the residents in Fukuoka Municipal Zoo, Fukuoka, Japan. The organism was detected in 32 (45%) samples. Among 2955 colonies of the *Bacillus cereus*/*B. thuringiensis* group examined, 531 (18%) were assigned to *B. thuringiensis* on the basis of the formation of parasporal inclusions. Fecal samples from animals feeding on vegetable matters contained *B. thuringiensis* at high frequencies. The results strongly suggest that the daily food intake of plant origin yields the feces containing *B. thuringiensis* at high levels.

(2) In total, 287 *B. thuringiensis* isolates, recovered from feces of 28 zoo-maintained animal

species, were examined for flagellar (H) antigenicity. Serologically, 209 isolates (72.8%) were allocated to the 8 H serogroups, 4 were untypable, and 74 were untestable. Among the 8 H serotypes detected, H3abc (serovar *kurstaki*) predominated at a high frequency of 88.0%, followed by H6 (serovar *entomocidus*) with a frequency of 7.7%.

A total of 62 isolates of *B. cereus*, randomly selected from populations occurring in feces of zoo-maintained animals, were examined for their H antigenicities with the reference H antisera against *B. thuringiensis* serotypes H1-H55. Of these, 37 (59.7%) were identified to 18 H serogroups of *B. thuringiensis*, and 25 were motile but untypable.

(3) Insecticidal activity was associated with 67.2% of the fecal populations: 188 isolates were toxic to both *Bombyx mori* (Lepidoptera: Bombycidae) and *Aedes aegypti* (Diptera: Culicidae), 2 isolates were specific for *B. mori*, and 3 isolates were toxic to *A. aegypti* only. Of the isolates with dual toxicity, 97.9% belonged to the serovar *kurstaki*, producing bipyramidal parasporal inclusions. All of the H7 (serovar *aizawai*) isolates were toxic to both insects.

Parasporal inclusion proteins extracted from 27 *B. thuringiensis* fecal isolates were screened for hemagglutination (HA) activities against erythrocytes of the four mammalian and one avian species. The HA activities were commonly associated with not only insecticidal fecal isolates but also non-insecticidal ones.

(4) All of the 13 *B. thuringiensis* fecal isolates belonging to four H serovars (*kurstaki*, *aizawai*, *galleriae*, and *morrisoni*) synthesized high-molecular-mass proteins of 120 to 130 kDa. It is very likely that these major proteins belong to the family Cry1 protein, whose toxicity is restricted to the order Lepidoptera. This was supported by the fact that the antibodies against parasporal proteins, including Cry1 proteins, of the *B. thuringiensis* reference strains bound to these proteins of the fecal isolates. In addition to the 120- to 130-kDa proteins, all of the *kurstaki* isolates produced proteins of 65 kDa which is assignable to the Cry2 protein. Two isolates of serovar *morrisoni* produced another major protein of 25 kDa.

(5) The *B. thuringiensis* serovar *sotto* isolate, 00-FZ46-12, was examined for characterization of mosquitocidal activity of parasporal inclusions. Microscopically, parasporal inclusions were round or irregular-shaped bodies. When examined with SDS-PAGE, parasporal inclusions consisted of multiple heterogeneous proteins with molecular masses of 72, 65, 56, and 27 kDa. Immunologically, 00-FZ46-12 proteins were unrelated to the inclusion proteins of the reference strain of *B. thuringiensis* serovar *israelensis*, while they were strongly sero-reactive to antibodies against proteins of the mosquitocidal *sotto* strain 96-OK-85-24. The data suggest that the strain 00-FZ46-12 synthesizes novel mosquitocidal Cry proteins with unique toxicity spectrum.

(6) A total of 34 fecal samples, collected from 14 species of wild mammals in Korea, were examined for the occurrence of *B. thuringiensis*. The organism was detected in 18 (53%) samples. Among the three food-habit groups, herbivorous animals yielded *B. thuringiensis*-positive samples at the highest frequency of 69%, followed by omnivorous animals with 50%. Of the six fecal samples from carnivorous animals, only one sample contained *B*

*thuringiensis*. Among 527 isolates belonging to the *B. cereus* / *B. thuringiensis* group, 43 (8%) were assigned to *B. thuringiensis* on the basis of the formation of parasporal inclusions. Of the 43 isolates, 13 were serologically allocated to the nine H-antigen serogroups: H3ad (serovar *sumiyoshiensis*), H15 (*dakota*), H17/27 (*tohokuensis*/ *mexicanensis*), H19 (*tochigiensis*), H21 (*colmeri*), H29 (*amagiensis*), H31/49 (*toguchni/muju*), H42 (*jinghongiensis*), and H44 (*higo*). Others were untypable by the H antisera available, or untestable. Insecticidal activity was associated with 23% of the fecal populations: three isolates killed larvae of the silkworm, *B. mori*, and seven were toxic to larvae of the mosquito, *A. aegypti*. There was no larvicidal activity against the three lepidopterans: *Plutella xylostella*, *Spodoptera exigua*, and *Spodoptera litura*. Overall results suggest that wild animals in Korea are in contact with naturally occurring *B. thuringiensis* at high frequencies through daily food intake of plants.

(7) Feces of the Kerama-jika (*Cervus nippon keramae*), a wild shika deer indigenous to the Ryukyus, Japan, were examined for the natural occurrence of *B. thuringiensis*. Of the ten fecal samples tested, seven contained this organism. The frequency of *B. thuringiensis* was 8.5% among 387 colonies of spore-forming bacteria belonging to the *B. cereus*/*B. thuringiensis* group. Of 33 *B. thuringiensis* isolates recovered, only one isolate, assigned to the serotype H3abc (serovar *kurstaki*), exhibited toxicity against larvae of the silkworm, *B. mori*, the diamondback moth, *P. xylostella*, the common cutworm, *S. litura*, and the mosquito, *A. aegypti*.