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Identification and bioactivity of native strains of *Bacillus thuringiensis* from grain-related habitats in Turkey

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Abstract

A native collection of *Bacillus thuringiensis* (Bt) strains originated from grain-related habitats in Turkey was characterized according to serotype, *cry1* gene content, and bioactivity against *Ephestia kuehniella* (Lepidoptera: Phycitidae) and *Spodoptera littoralis* (Lepidoptera: Noctuidae). Twenty-three different serotypes as well as 24 unknown serotypes were obtained from 56 positively agglutinated strains with previously characterized antisera. Most common serovars were *sotto*, *kim*, and *tochigiensis* with the percentages of 14, 14, and 13, respectively. Among the *cry1* gene-positive 36 strains, *cry1E* (100%), *cry1Aa* (94%), *cry1Ac* (92%), and *cry1D* (83%) genes were the most abundant. Bioactivity tests with 56 Bt strains carrying *cry1*, *cry2*, and/or *cry9* genes indicated that all of them resulted in growth retardation or inhibition of larvae of both *E. kuehniella* and *S. littoralis*; however, only one strain, 85PPb (serovar *morrisoni*), caused high mortality in both insects (84% and 100%, respectively). Different crystal morphology was observed for the strain 85PPb and the standard strain *B. thuringiensis* subsp. *morrisoni*. Finally, no correlation was found among serotype, *cry* gene content and biotoxicity of Bt strains in the collection.
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Keywords: *Bacillus thuringiensis*; Serotyping; *cry1* genes; Bioactivity; *Ephestia kuehniella*; *Spodoptera littoralis*

1. Introduction

The gram-positive, spore-forming bacterium *Bacillus thuringiensis* is the most widely used microbial biopesticide. It is also an alternative to synthetic insecticides that often have unintended harmful effects on non-target species. It produces parasporal crystals containing one or more Cry proteins that may be toxic for different insect orders including agriculturally important pests. Cry proteins are encoded by *cry* genes that are mostly carried on plasmids and to date nearly 300 *cry* genes have been identified and classified into 51 groups and subgroups on the basis of amino acid sequence similarity (Crickmore et al., 2007).

Worldwide, natural *B. thuringiensis* strains isolated from different habitats are established as *B. thuringiensis* culture collections (Martin and Travers, 1989; Ben-Dov et al., 1997; Bernhard et al., 1997; Bravo et al., 1998; Iriarte et al., 2000; Kim et al., 1998; Hongyu et al., 2000; Uribe et al., 2003). They are important for reflecting biodiversity through sampling sites and finding new strains with high toxicity against target insect species. Strains in the collections are characterized by a number of different methods to identify and to test and confirm their toxicity against different insect orders (Arango et al., 2002; Wang et al., 2003; Tamez-Guerra et al., 2004; Martinez et al., 2005). Identification of *cry* gene content by PCR is the most effective technique in screening large native collections to predict insecticidal activities of individual strains (Ben-Dov et al., 1997; Porcar and Juarez-Perez, 2002). In addition, serolog-

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ical analysis, biological activity tests, plasmid contents, chromosomal DNA, and protein profiling are used as complementary methods in the search for novel strains.

Based on flagellar H-antigens, Lecadet et al. (1999) reported 69 serotypes of Bt with 82 serovars. At present, this number has reached to 71 serotypes comprising 84 serovars, which can be obtained from the IEBC at the Pasteur Institute (France).

Previously, we reported the first *B. thuringiensis* collection from different grain-related habitats of the Central Anatolia and the Aegean Region in Turkey and characterized it based on crystal morphology, *cry* gene content by screening for *cry1*, *cry2*, *cry3*, *cry4*, and *cry9* genes using universal PCR primers, chromosomal, and plasmid DNA patterns (Apaydin et al., 2005). The results indicated a wide range of biodiversity among Bt strains in Anatolia. In this study, we further characterized our collection by serological analysis, *cry1* gene identification using gene-specific primers, and toxicity tests against the larvae of Mediterranean flour moth, *Ephestia kuehniella*, and the cotton leaf worm *Spodoptera littoralis*. In addition, crystal protein morphology of representative strains was investigated.

2. Materials and methods

2.1. Reference and native bacterial strains

Twelve reference strains were kindly supplied by the following sources: *B. thuringiensis* subsp. *kurstaki* (HD1), *B. thuringiensis* subsp. *aizawai* (HD133), *B. thuringiensis* subsp. *kumamotoensis* (HD876 (3–11)), *B. thuringiensis* biovar. *tenebrionis*, *B. thuringiensis* serovar *israelensis* (HD500) by Dr. Daniel R. Zeigler of the Bacillus Genetic Stock Center (Ohio, USA); *B. thuringiensis* subsp. *kyuhuensis* (NRRL H4553) and *B. thuringiensis* subsp. *darmstandiensis* (NRRL HD-146) by Dr. Alejandro P. Rooney of the ARS Culture Collection (USA); *B. thuringiensis* subsp. *morrisoni*, *B. thuringiensis* subsp. *alesti*, *B. thuringiensis* subsp. *finitimus*, *B. thuringiensis* subsp. *galleria*, and *B. thuringiensis* subsp. *thompsoni* by Ege University. One hundred and three native *B. thuringiensis* strains analyzed in this study were previously isolated from soil, grain, stored product dust, straw, insect cadavers and various residues collected from grain silos, caves, crop fields, farms, and haylofts (Apaydin et al., 2005).

2.2. Motility tests and serological analysis

Motility of the strains was tested using motility plates (1% (w/v) tryptone, 0.5% (w/v) NaCl, 0.3% (w/v) agar) as described by Guttmann and Ellar (2000). The strains were streak-inoculated onto the middle of the plate from top to bottom and incubated overnight. If a colony was observed to spread out from the inoculation site, the strain was scored as motile; otherwise it was scored as nonmotile. Serological analysis based on reactivity with flagellar antisera was performed according to a micromethod, using 96-well microtiter plates (U-bottom), as described by Laurent et al. (1996). The whole collection, total 84 antisera (71 serovar and 13 biovar), purchased from the Pasteur Institute (Paris, France) were assayed against the motile isolates to determine the serotypes of grain habitat-originated strains. Bacterial suspensions were prepared by fixation of 5–8 h cultures grown at 30 °C with 0.1% formalin. Initially, all motile strains were screened by two dilutions (1:10 and 1:20) of each antiserum. Bacterial suspensions without antiserum and 150 mM NaCl in sterile distilled water were used as negative controls. After incubation at 37 °C for 75 min, agglutination was observed under a white light. A clear supernatant with the floccular sediment at the bottom of the well was scored as positive. Depending on the results of the first screening, if a strain gave a positive reaction with more than one antiserum, it was titrated against lower dilutions (i.e., 1:40, 1:80, 1:160, ...) of these antisera until a positive reaction was obtained for the lowest dilution. Any *B. thuringiensis* strain not agglutinated by the available antisera in the updated collection was judged to be a potential new serotype (Lecadet et al., 1999).

2.3. DNA template and PCR analysis

PCR analysis was used to detect *cry1* genes encoding Lepidoptera-specific Cry1 proteins. As previously reported, 36 strains of our collection gave positive reaction with the *cry1* universal primer (Apaydin et al., 2005). In this study, they were screened using gene-specific primers designed to detect the presence of *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1Ad*, *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, and *cry1G* genes reported by Ceron et al. (1994, 1995). Genomic DNA was isolated according to the method described by Ausubel et al. (1994), and *B. thuringiensis* subsp. *kurstaki* (HD1) and *B. thuringiensis* subsp. *aizawai* (HD133) served as control strains. Five hundred microgram of genomic DNA was added to the reaction mix, which contained 200 µM dNTP, 0.2–0.4 µM each of the forward and reverse primer, 1.5 mM $MgCl_2$, and 2 U of Taq DNA polymerase in a volume of 50 µl. PCR conditions were as follows: a single denaturation step for 5 min at 95 °C, followed by a 35 amplification cycles including denaturation at 94 °C for 1 min, annealing at 50 °C for *cry1Ab*, *cry1Ac*, and *cry1C* genes; 51 °C for *cry1B* gene; 53 °C *cry1Ad* and *cry1D* genes; 54 °C for *cry1Aa* and *cry1G* genes; 58 °C for *cry1E* and *cry1F* genes for 1 min, elongation at 72 °C for 1 min and a final extension at 72 °C for 10 min. Amplifications were carried out in a thermal cycler (Techne Progene, England). After electrophoresis of 10 µl of each PCR product on 2.5% agarose-EtBr gel, DNA bands were visualized in a gel documentation system (Vilber Lourmat, France).

2.4. Bioassay

In order to evaluate the toxicity of native *B. thuringiensis* strains, first instar larvae of *E. kuehniella* were used. Spore–

crystal mixtures were prepared from 56 Bt strains producing Cry1, Cry2, and Cry9 proteins that were reported to be toxic to Lepidopteran insects (Bravo et al., 1998). Bt culture was grown in 100 ml of nutrient broth (NB) at 28 °C by shaking at 150 rpm for 3 days. Samples were centrifuged at 4 °C at 6000 rpm for 15 min and the pellets were washed twice with ice-cold 1 M NaCl and three times with cold sterile distilled water. The pellets were dried overnight at 37–40 °C, weighed and stored as powder at −20 °C until used. Neonates of *E. kuehniella* were kindly supplied by the Agricultural Research Institute, Plant Protection Department (İzmir, Turkey). The spore–crystal powders were suspended in distilled water containing 0.1% Tween 80. Suspensions were mixed with a diet that included wheat/corn powder (2:1) at the concentration of 20 ppm (i.e., 100 μg of spore–crystal mixture in 5 g compost) and dried. For some of the strains two higher doses (200 and 2000 ppm) were also applied to confirm their toxicity. Assays were performed using 25 larva per dose with three replicates. *B. thuringiensis* subsp. *kurstaki* (HD-1) or subsp. *aizawai* (HD-133) and the diet without toxin served as positive and negative controls, respectively. Toxicity tests were carried out at 25 °C, 70% RH with 16:8 h, L:D schedule; and larval growth and mortality was recorded after 14 days.

In addition, toxicity of Bt strains was tested against *S. littoralis* (Lepidoptera; Noctuidae), a species that causes considerable economic loss throughout Southeastern Europe, Africa, and Asia. Insects were reared on a premixed diet based on alfalfa powder and agar (Stonefly Industries, USA) at 20–23 °C and 16:8 h (light to dark) photoperiod for 10–15 generations. Bt toxins were added to the diet at the ratio of 100 μg toxin in 5 g compost and applied to the penultimate instars larvae of *S. littoralis* via food. Then, larval growth and mortality were monitored for 16 days.

2.5. Electron microscopy

Spore and crystal morphologies of representative *B. thuringiensis* strains were investigated by SEM. A loop of Bt culture, grown on NA plates for 72 h, was suspended in 1 ml of distilled water. After centrifugation at 5000 rpm for 15 min, the pellet was resuspended in 0.5 M NaCl to remove cell residues and incubated at 37 °C for 30 min. The suspension was centrifuged at 10,000 rpm for 10 min and the pellet was washed three times with distilled water. Then, a 10-μl of aliquot of the spore–crystal mixture dissolved in 1 ml of distilled water was placed on a microscope lid. After drying at 37 °C for 3 h, the sample was coated with gold and examined under the electron microscope (Philips XL 30 SFEG) at 5 kV.

3. Results

3.1. Motility evaluation and serological distribution of *B. thuringiensis* strains

All 103 native *B. thuringiensis* strains were subjected to motility test prior to serotyping. Motility was evaluated based on the expansion size of the colonies from the inoculation site. Eighty-four strains were judged to be motile to varying degrees. Of the 84 strains, 13 were very motile (++++), 26 were motile (+++), 17 were slightly motile (++), and 28 were poorly motile (+). The remaining 19 strains were scored as non-motile.

When the motile 84 *B. thuringiensis* strains were titrated against 71 antisera, 56 strains yielded positive agglutination, 4 strains showed autoagglutination, and 24 strains showed no agglutination (Table 1). Among the motile strains, 23 different serovars were identified. Even though the serovars *sotto* (14%), *kim* (14%), and *tochigiensis* (13%) were the most abundant in the collection, the serovars *canadensis*, *nigeriensis*, *toumanoffi*, *kyushuensis*, *indiana*, *yunnanensis*, *shandongiensis*, *mexicanensis*, *leesis*, *muju*, *xiaguengiensis*, and *jordanica* were represented only by a single strain (Table 1). The remaining 24 isolates gave no agglutination with the antisera used, and they are classified as 'unknown serotype'.

3.2. Identification of *cry1* gene composition

Thirty-six *B. thuringiensis* strains were initially identified as *cry1* gene-positive with a generic oligonucleotide primer. A more detailed characterization of these strains was carried out using gene-specific primers for 10 *cry1* genes: *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1Ad*, *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, and *cry1G*. All native *B. thuringiensis* strains were positive for more than one *cry1* gene ranging from two to nine, and 18 different *cry1* gene profiles were obtained (Table 2). The *cry1E* (100%) gene detected in all strains was the most frequent one followed by *cry1Aa* (95%), *cry1Ac* (91%), and *cry1D* (83%) genes (Fig. 1). The genes *cry1C* (67%), *cry1G* (58%), and *cry1F* (52%) were moderately frequent, whereas *cry1B* (3%), *cry1Ad* (6%), and *cry1Ab* (7%) were the least common genes (Fig. 1).

3.3. Biological activities of *B. thuringiensis* strains

The insecticidal toxicity of 56 native *B. thuringiensis* strains was investigated against first instar larva of *E. kuehniella*. Among the strains, 80% of the isolates showed varying degrees of toxicity, whereas 20% of the isolates were not toxic (Table 3). About half of the native strains (44.6%) caused mortality between 10% and 30%. In addition, the mortality range of 13 strains was lower than 10%; and that of 6 strains was moderate (30–50%). However, only one strain (85PPb) showed very high toxicity (84% mortality). In all tests, the mortality obtained from the control strain *B. thuringiensis* subsp. *kurstaki* varied from 91% to 100%. In addition to having variation in toxic activity at different ranges, nearly all strains retarded the growth rate of the larva and most of them prevented the larva from entering the pupae stage (data not shown).

Furthermore, *B. thuringiensis* strains exhibiting variable toxicity on *E. kuehniella* were also tested against *S. littoralis* using the single-dose assay. Similar to the results from *E.*

Table 1
Serotyping of *B. thuringiensis* isolates with flagellar based H antigens (H1–H71)

H antigen	H serovar	Number of isolates originated from			Total
		Soil	Stored product dust	Various residues	
4ab	*sotto*	7	1	0	8
5ac	*canadensis*	1	0	0	1
8ab	*morrisoni*	1	1	0	2
8bd	*nigeriensis*	1	0	0	1
10ab	*darmstandiensis*	2	0	0	2
11ab	*toumanoffi*	0	0	1	1
11ac	*kyushuensis*	0	1	0	1
14	*israelensis*	3	1	0	4
15	*dakota*	2	0	1	3
16	*indiana*	1	0	0	1
18ab	*kumamotoensis*	1	2	0	3
19	*tochigiensis*	4	3	0	7
20ab	*yunnanensis*	0	1	0	1
22	*shandongiensis*	0	0	1	1
27	*mexicanensis*	1	0	0	1
33	*leesis*	0	1	0	1
35	*seoulensis*	2	0	0	2
38	*oswaldocruzi*	2	1	0	3
40	*huazhongiensis*	2	0	0	2
49	*muju*	1	0	0	1
51	*xiaguangiensis*	1	0	0	1
52	*kim*	7	1	0	8
71	*jordanica*	0	1	0	1
Autoagglutinated		2	0	2	4
Unknown serotype[a]		15	6	3	24
Total		56	20	8	84

[a] Motile isolates but gave no agglutination with known reference antisera.

Table 2
cry1 gene profile of *B. thuringiensis* strains

Profile	*cry1* gene content	Name of isolate
1	*cry1C*, *cry1E*	57Hb
2	*cry1Aa*, *cry1Ac*, *cry1E*	24Fa
3	*cry1A*, *cry1D*, *cry1E*, *cry1G*	5Ca
4	*cry1Aa*, *cry1Ac*, *cry1E*, *cry1G*	7Bb
5	*cry1Aa*, *cry1Ac*, *cry1D*, *cry1E*	28Aa
6	*cry1Aa*, *cry1Ac*, *cry1E*, *cry1F*	28Ca
7	*cry1Aa*, *cry1Ac*, *cry1D*, *cry1E*, *cry1F*	11Ka, 13La, 35Kb
8	*cry1Aa*, *cry1Ac*, *cry1C*, *cry1D*, *cry1E*	25Pb, 33Yb, 55Ka, 60Na
9	*cry1Aa*, *cry1Ad*, *cry1D*, *cry1E*, *cry1F*	48Ra
10	*cry1Aa*, *cry1C*, *cry1E*, *cry1F*, *cry1G*	102Fb
11	*cry1Aa*, *cry1Ac*, *cry1C*, *cry1D*, *cry1E*, *cry1F*	1Aa, 28Lb
12	*cry1Aa*, *cry1Ac*, *cry1C*, *cry1D*, *cry1E*, *cry1G*	1Cb, 7Fa, 27Pb, 39Yb, 59Ya, 71Lb, 107Fa
13	*cry1Aa*, *cry1Ac*, *cry1D*, *cry1E*, *cry1F*, *cry1G*	25Fb, 71Na
14	*cry1Aa*, *cry1Ac*, *cry1Ad*, *cry1D*, *cry1E*, *cry1G*	35Pb
15	*cry1Aa*, *cry1Ac*, *cry1C*, *cry1E*, *cry1F*, *cry1G*	43Db
16	*cry1Aa*, *cry1Ac*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, *cry1G*	1Ab, 4Cb, 24La, 25Ua, 39Ya
17	*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, *cry1G*	58Kb, 93Fa
18	*cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, *cry1G*	85PPb

kuehniella, only one strain (85PPb) showed high toxicity against *S. littoralis* (Fig. 2). In the absence of toxin, larval weight of *S. littoralis* reached to 850 mg at day 6 and 7. However, toxin from 85PPb (serovar *morrisoni*) or *B. thruringiensis* subsp. *aizawai* prevented the larval growth 100% throughout the test period.

3.4. Crystal protein morphology

Two native strains, 85PPb and 43Fa, and a reference strain *B. thuringiensis* subsp. *morrisoni* were examined with SEM for crystal protein morphology. Spore–crystal preparations were made from 72-h *B. thuringiensis* cultures. As

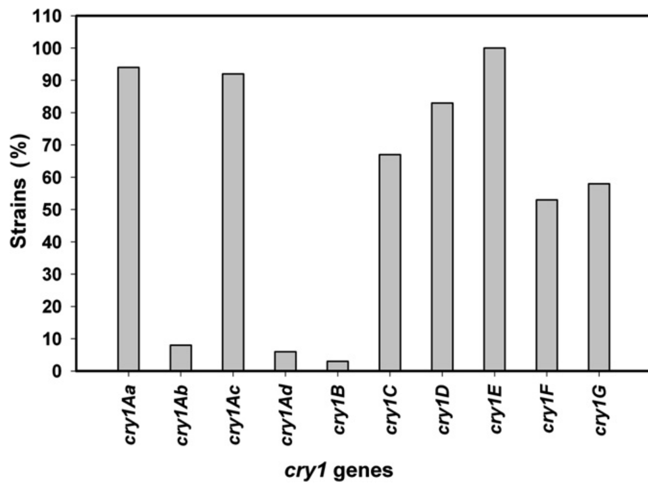

Fig. 1. *cry1* genotype distribution of *B. thuringiensis* strains.

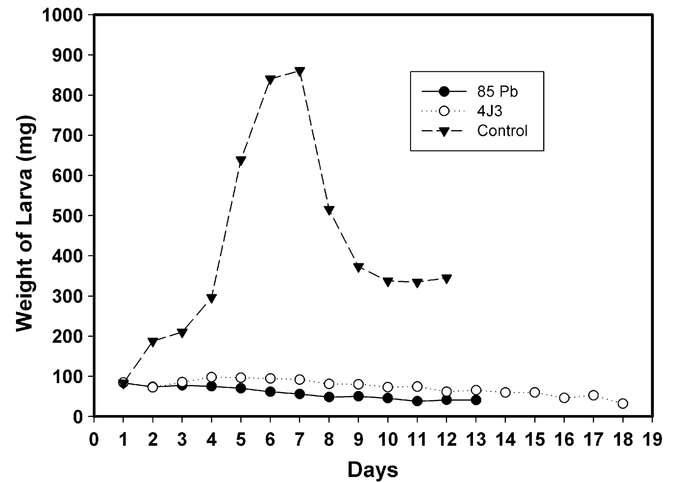

Fig. 2. Effects of *B. thuringiensis* strains on growth of *S. littoralis* (penultimate instar). The larvae were fed with diet including 20 ppm *B. thuringiensis* spore–crystal mixture and the larval growth was weighed every day during the test period. Figure indicates larval development in the control (without any toxins) and in the diet containing crystal–spore mixture of a reference strain *B. thuringiensis* subsp. *aizawai* (4J3) and a native strain 85PPb (serovar *morrisoni*).

shown clearly in Fig. 3, 85PPb produced bipyramidal crystal proteins, consistent with the presence of Cry1 protein. Similarly, *B. thuringiensis* subsp. *morrisoni* produced bipyramidal and/or rectangular proteins indicating the expression of Cry1 and Cry2 proteins (López-Meza and Ibarra, 1996).

4. Discussion

In this study, *B. thuringiensis* collection from grain-related habitats in Turkey was characterized with respect to serology, *cry1* specific gene content and insecticidal toxicity. In addition, protein profiles and crystal protein morphologies of the most toxic strain and related reference strains were analyzed.

A great deal of serological diversity was found among *B. thuringiensis* strains included in this study. In total, 23 different serovars were identified among 56 of the 84 motile strains (Table 1). Martinez et al. (2005) reported 19 serovars among 178 *B. thuringiensis* strains native to Spain. Interestingly, serotypes were unevenly distributed through the collection, and one type, *aizawai,* was present in nearly 50% of the strains. Unlike in their findings, we did not observe predominance of a particular type among our strains. In fact, we observed a regular distribution of serovars in our collection, where the number of strains varied from one to eight (Table 1). Moreover, Martin and Travers (1989) reported the serovar *kurstaki* as the most common type in Asia, whereas the serovar *israelensis* was most common in Europe and the United States. Similarly, Mizuki et al. (1999) found the serovar *kurstaki* to be the predominant on the phylloplanes in Japan. Another study from Asia also reported *israelensis* and *kurstaki* as the most frequent types in Jordanian habitats (Al-Momani et al., 2004). However, none of the strains in our collection was identified as *kurstaki* and the strains *sotto* (14%), *kim* (14%), *tochigiensis* (13%), and *israelensis* (7%) were the most abun-

Table 3
Toxicity of native *B. thuringiensis* strains against *E. Kuehniella*

Toxicity[*]	Name of Bt isolate	Percentage
Non-toxic[a]	25Fb, 93Fa, 28Lb, 71Lb, 4Cb, 28Ca, 34Bb, 18Fa, 8Ba, 62PPa, 24Fb	19.6 (11)[b]
⩽10%	48Ra, 71Na, 13La, 58Kb, 60Na, 19Rb, 87Fb, 43Fa, 18FFa, 1Bb, 35Kb, 27Cb, 82YYb	23.2 (13)[b]
10–30%	35Pb, 39Yb, 11Ka, 5Ca, 1Ab, 107Fa, 25Ua, 1Aa, 27Pb, 93Ha, 24La, 27Fb, 57Hb, 19Hb, 20Rb, 13Nb, 2Ib, 26Ba, 27Fa, 4Cb, 1Cb, 24Lb, 93FFa, 23Ba, 27Ka	44.6 (25)[b]
30–50%	25Pb, 43Db, 24Fa, 1CCb, 93Da, 7Bb	10.7 (6)[b]
⩾50%	85PPb	1.8 (1)[b]
Total		100.0 (56)[b]

[*] One dose (100 ppm) assay against first instar larvae of *E. kuehniella*.
[a] Isolates causing no mortality but slowing down the growth rate of larvae.
[b] Number of *B. thuringiensis* isolates.

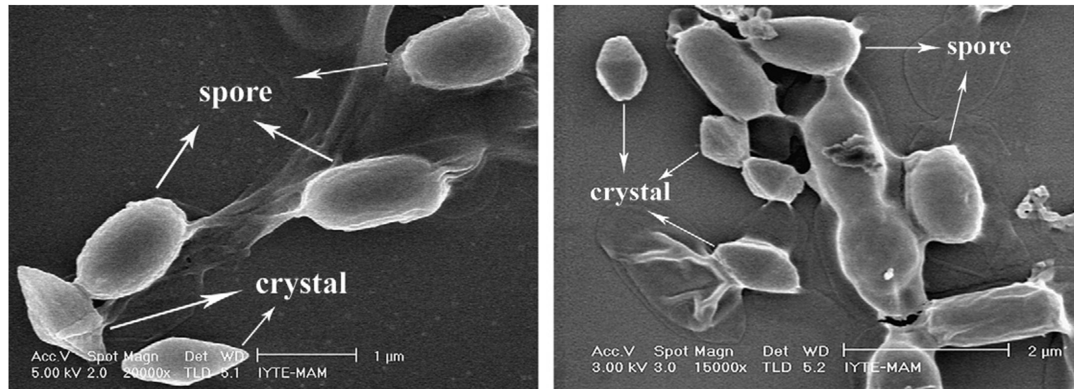

Fig. 3. Scanning electron micrographs of a sporulating culture of native strain 85PPb (serovar *morrisoni*) and the reference strain *Bacillus thuringiensis* subsp. *morrisoni*.

dant (Table 1). Twenty-four of 84 motile *B. thuringiensis* strains did not react with any of the 71 antisera tested and thus grouped as the 'unknown serotype'.

Toxic potency of *B. thuringiensis* strains depends on the type and subgroups of *cry* genes. Lambert et al. (1996) reported that Cry1Ab5 protein had greater toxicity against *H. armigera* (Lepidoptera) than Cry1Ac1 protein. Therefore, it is important to identify the subgroups of *cry* genes using gene-specific oligonucleotide primers. The PCR identification of *cry1*-type genes of 36 strains in the present study yielded 18 different profiles from the combinations of two to nine different *cry1* genes (Table 2). Martinez et al. (2005) analyzed 152 *B. thuringiensis* strains, native to Spain, based on 12 *cry1* and one *cry2* genes and they obtained 81 different profiles from combination of one to ten different genes. In another study from Colombia, Uribe et al. (2003) reported 30 different profiles of 137 strains screened for 8 *cry1*-type genes, each harboring one to six different genes.

In addition, we found that, *cry1E*, *cry1Aa*, *cry1Ac*, and *cry1D* were the most common genes, whereas *cry1B*, *cry1Ad*, and *cry1Ab* were the least common among the strains analyzed (Fig. 1). However, Bravo et al. (1998) found *cry1A*, *cry1B*, *cry1C*, and *cry1D* genes more common than *cry1E* and *cry1F* in Mexican Bt strains. Moreover, Martinez and Caballero (2002) reported that *cry1Aa* and *cry1Ab* were more abundant than *cry1Ad*, *cry1E*, *cry1F*, and *cry1G* genes in *B. thuringiensis* strains collected from aquatic and terrestrial habitats of Spain. Taken together, these results indicate that the distribution of *cry1* genes differs in collections depending on the origin of *B. thuringiensis* strains.

Martinez et al. (2005) and Martinez and Caballero (2002) reported that the distribution of *cry1* genes somewhat correlated with particular serovars. They carried out statistical analysis to see the relation between serotype and *cry1* gene frequency because the number of strains were relatively high for each type of *cry1* gene, ranging from 8 to 80. However, the lower number of our strains for each *cry1* gene, ranging from 1 to 8, did not allow us to do statistical analysis for distribution of *cry1* genotypes among serotypes. Nevertheless, it was noticed that the combination of *cry1* genes within the same serovar and/or among different serovars was very different (data not shown). This may be due to the plasmid transfer specificity of *cry1* genes among serovars or strains of the same serovar. Additionally, less frequent (1%) *cry1B* gene was only observed in the serovar *morrisoni*, the most toxic isolate.

Bioactivity tests revealed that most of the strains were toxic to *E. kuehniella* yielding different mortality levels (Table 3). Only one strain (85PPb) identified as serovar *morrisoni* caused a high level of mortality (84%) and was positive for *cry1* and *cry2* genes. It was also the only strain harboring nine *cry1* genes including *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry1B*, *cry1C*, *cry1D*, *cry1E*, *cry1F*, and *cry1G* among all *B. thuringiensis* strains tested. Similarly, a novel *B. thuringiensis* strain (serovar *kurstaki*) isolated from Tunisian soil was reported to be toxic to lepidopteran insects including *E. kuehniella* due to Cry1Aa, Cry1Ac, and Cry2Aa proteins (Tounsi et al., 1999, 2003; Tounsi and Jaoua, 2002, 2003). Similar to their results, *cry1Aa* and *cry1Ac* were the second and third most abundant genes, respectively, among the strains analyzed in the present study and some of the strains were positive for *cry2* gene as well. Even though there was a similar gene content, only one highly toxic strain was identified. This might have resulted from such factors as the *cry* gene combinations, expression of related genes at the protein level and possible synergy among *cry* genes. On the other hand, nearly all strains led to retarded larval growth, and some prevented the larva from entering the pupae stage. In terms of the protection of ecological balance in the environment, less toxic strains may become more important biological control agents in the future.

Similar toxic effect of *B. thuringiensis* isolates was also observed on the *S. littoralis* (data not shown) and the most toxic strain was found to be 85PPb. In addition to 8 *cry1* gene subgroups, presence of *cry1*C in the 85PPb is in agreement with the results of Escriche et al. (1998) who showed that some of the Cry1 insecticidal crystal proteins, especially Cry1Ca, that is active against *S. littoralis*, made brush border membrane vesicles permeable to KCl. More-

over, Polanczyk et al. (2000) examined the effects of different *B. thuringiensis* strains on the *S. frugiperda* larvae. In agreement with their results, we found that *B. thuringiensis aizawai* and 85PPb were the most toxic (100%) strains against *S. littoralis* compared to other serovars, and *B. thuringiensis kurstaki* exhibited the same effect with the control containing no toxin (data not shown).

In conclusion, 23 different serovars and 24 unknown serotypes were identified among 103 *B. thuringiensis* strains collected from grain-related habitats in Turkey. Furthermore, *cry1* gene content of 36 isolates was greatly variable within and/or among serovars. Moreover, most of the strains caused less than 50% mortality in *E. kuehniella*; however, one strain, 85PPb, was found to be highly toxic to *E. kuehniella* (84%) and *S. littoralis* (100%). Finally, there was no correlation among serotype, *cry* gene content, and toxicity of the isolates, indicating a great biodiversity of *B. thuringiensis* strains in Turkey. Future studies including detailed bioactivity assays on different pests, sequencing and transfection of toxin genes are likely to show the strain 85PPb as a potential biopesticide that originated from Central Anatolia.

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