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Isolation and Characterization of Native *Bacillus thuringiensis* Strains from Soil and Testing the Bioactivity of Isolates Against *Ephestia Kuehniella* Zeller (Lepidoptera: Pyralidae) Larvae

[Topraktan Doğal *Bacillus thuringiensis* Suşlarının İzolasyonu, Karakterizasyonu ve *Ephestia Kuehniella* Zeller (Lepidoptera: Pyralidae) Larvalarına Karşı Biyolojik Aktivitesi]

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ABSTRACT

Objective: This study aimed to find native strains of *Bacillus thuringiensis* that are toxic to some major insect pests harming the economically important stored products in Turkey.

Methods: Five *B. thuringiensis* strains were isolated from soil samples. These isolates were evaluated in terms of their novel activities according to the following criteria: parasporal inclusion morphology, SDS-PAGE, plasmid DNA patterns, toxicity against *Ephestia kuehniella,* and detection of *cry*1, *cry 2, cry3, cry4, cry5, cry7, cry8, cry12*, *cry*21 and *cyt* type genes with PCR.

Results: One strain, named as F21, gave positive results with both *cry*1 and *cry*2 general primers in PCR. However, two strains, named as F16 and F19, gave positive results only with *cry*2 general primers. The PCR amplified region of *cry*1 gene for F21 showed 97% similarity to *cry1Ac* and *cry*2 gene for F21, F16 and F19 showed 96% similarity to *cry2Ab.* The F21 and F19 isolates exhibited the highest toxic activity against *Ephestia kuehniella,* resulting in 83% and 80% mortality respectively. Three *B. thuringiensis* isolates produced typical inclusions, which were spherical, bipyramidal and cuboidal in shape, associated protein bands being approximately 130 and 65 kDa. The most larvaecidal isolates for *E. kuehniella* were F21, F19 and F16, with LC_{50} values of 1.08, 1.48, and 2.17, respectively.

Conclusion: The highly active *B. thuringiensis* isolates (F21 and F19) tested in this work appeared promising for new insecticide or biopesticides formulations and maybe even utilized to obtain genetically modified pest resistant plants.

Key Words: *Bacillus thuringiensis*, biological control, *Ephestia kuehniella,* bioassay, stored product

ÖZET

Amaç: Bu çalışmanın amacı Türkiye'de ekonomik olarak önemli olan depolanmış ürünlere zarar veren büyük böcek gruplarına karşı toksik etki gösteren doğal *Bacillus thuringiensis* izolatlarının bulunmasıdır.

Metod: Beş doğal *B. thuringiensis* izolatı toprak örneklerinden izole edilmiştir. Bu izolatlar yeni aktivitelerinin belirlenmesi için aşağıdaki kriterlere göre test edilmiştir: parasporal inklüzyon morfolojisi, SDS-PAGE, *Ephestia kuehniella*' ya karşı toksisitesi ve PCR ile *cry*1, *cry2, cry3, cry4, cry5, cry7, cry8, cry12*, *cry*21 ve *cyt* genlerinin analizi.

Bulgular: F21 olarak isimlendirilen bir izolat hem *cry*1 hem de *cry*2 genel primerleri ile yapılan PCR analizinde pozitif sonuç vermiştir. Bununla birlikte, F16 ve F19 olarak isimlendirilen iki suş ise yalnızca *cry*2 genel primeri ile pozitif sonuç vermiştir. F21 için PCR ile çoğaltılan *cry*1 gen bölgesi *cry1Ac* geni ile %97 oranında, F21, F19 ve F16 için *cry*2 gen bölgesi ise *cry2Ab* geni ile % 96 oranında benzer olarak bulunmuştur. F21 ve F19 izolatları sırasıyla %83 ve %80 ölüm oranları ile *E. kuehniella* larvalarına karşı oldukça yüksek bir toksik etki göstermiştir. Üç *B. thuringiensis* izolatı yaklaşık olarak 130 kDa ve 65 kDa ağırlığında protein bantları oluşturan küresel, baklava dilimli ve yuvarlak yapılı inklüzyonlar üretmektedirler. *E. kuehniella* larvaları için patojen olan F21, F16 ve F19 doğal izolatlarının LC_{50} değerleri sırasıyla 1.08, 1.48 ve 2.17 olarak belirlenmiştir.

Sonuç: Bu çalışmada test edilen oldukça yüksek toksik aktiviteye sahip *Bacillus thuringiensis* izolatları (F21 ve F19) yeni bir insektisit olarak oldukça ümit verici olabilir ve zararlı böceklere karşı dirençli bitki çeşitlerinin elde edilmesi yada biyopestisitlerin üretimi için kullanılabilir.

Anahtar Kelimeler: *Bacillus thuringiensis*, biyolojik kontrol, *Ephestia kuehniella,* biyoassay, depolanmış ürünler

Introduction

Bacillus thuringiensis is a member of a group of crystalliforeus spore forming aerobic, Gram-positive bacteria of the family *Bacillaece* (1). It produces parasporal crystals containing one or more Cry proteins that may be toxic for different insect orders including the ones damaging agricultural plants and products. It is also an alternative to synthetic insecticides that often have unintended harmful effects on non target species. The Cry proteins are encoded by *cry* genes that are frequently 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 (2).

Because crystal proteins are highly specific and environmentally safe (3), they have been successfully used as bioinsecticides against larvae of Lepidoptera, Diptera and Coleoptera (4-7). These crystal proteins are protoxins that are proteolytically converted into smaller toxic polypeptides in the insect midguts. The activated toxins interact with the midgut epithelium cells of the insects (8).

The growing public concern, stricter environmental regulations, and buildup of resistant biotypes to synthetics insecticides have led to an increased interest in alternative environment-friendly insect control strategies. Thus *B. thuringiensis* could offer an alternative to chemical insecticides. So far more than 50.000 *B. thuringiensis* strains have been isolated from several environments such as insects, plants, soil and marine environments (9). *Bacillus thuringiensis* strains are characterized by using a number of different methods to identify their toxicity against different insect orders (10, 11). Identification of *cry* gene content by PCR is the most effective technique in screening large native collections when predicting insecticidal activities of individual strains (12, 13). Biological activity tests, plasmid contents, 16S rDNA analysis, chromosomal DNA, crystal morphology and protein profiling are also used as complementary methods in the search for novel strains.

In this study, *B. thuringiensis* strains isolated from the soils sampled in Turkey, identification of cry (*cry*1, *cry*2, *cry*3, 10 *cry*4, *cry*5, *cry*7, *cry*8, *cry*12, *cry*14, *cry*21) and *cyt*1 genes was performed by using universal primers. The toxic activities of the gene products were tested against the larvae of Mediterranean flour moth, *Ephestia kuehniella*. Along with toxic activities, crystal protein morphology, Cry protein and plasmid DNA profiles of the representative strains were investigated.

Materials and Methods

Sample collection

Soil samples were taken from six different regions of Ankara Turkey. The samples were collected by scraping off surface material with a sterile spatula and about 10 g samples were obtained from 2–5 cm depth. All samples were placed in sterile plastic bags aseptically and stored at 4ºC until processed.

Isolation of strains

Isolation of *B. thuringiensis* strains was conducted according to the method described by Travers et al (14). One gram of each sample was suspended in 10 ml sterile distilled water and pasteurized at 80ºC for 30 min. For the selection of *B. thuringiensis* 1 ml of each suspension added to 10 ml of Luria-Bertani (Merck, Germany) broth buffered with 0.25 M sodium acetate pH 6.8. The suspensions were incubated at 30ºC for 4 h and then heated at 80ºC for 3 min. Suspensions were diluted and plated on T3 medium (per liter: 3 g tryptone, 2 g tryptose, 1.5 g yeast extract, 0.05 M sodium phosphate pH 6.8, and 0.005 g of $MnCl_2$). After incubation at 30ºC for 24 h, the colonies showing similar morphology were selected and examined under phase-contrast microscope to determine the presence of parasporal inclusions and spores. The reference strains *B. thuringiensis* subsp. *kurstaki* HD-1, *B. thuringiensis* subsp. *kurstaki* HD-73, *B. thuringiensis* subsp. *tolworthi* HD-125, *B. thuringiensis* subsp. *aizawai* HD-137, and *B. thuringiensis* 916, were kindly provided by Dr. Alejandra Bravo (Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico).

Determination of cry genes

Molecular characterization was performed to identify the toxin-encoding genes through PCR by using a variety oligonucleotide pairs specific for the following genes/gene families: *cry*1, *cry*2, *cry*3, *cry*4, *cry*5, *cry*7, *cry*8, *cry*12, *cry*14, *cry*21 and *cyt*1 (12, 15-19). The PCR mixtures were prepared using 0.2-0.4 µM each of the forward and reverse primers (Table 1), 2U of Taq DNA polymerase, 200 µM dNTP, 10 mM Tris, 50 mM KCl and 1.5mM $MgCl_2$. DNA sample obtained from plasmid in a final volume of 50 µl. PCR conditions were as the following: a single denaturation step for 2 min at 95ºC, followed by a 30 amplification cycles including denaturation at 95ºC for 1 min, annealing at 48ºC for *cry1* and *cry3* genes; 54ºC for *cry7* gene; 49ºC for *cry8* gene; 50ºC for *cry5, cry12, cry14* and *cry21 genes*; 51ºC for *cyt1Aa* and *cyt1Ab* genes for 1 min, elongation at 72ºC for 1 min and a final extension at 72ºC for 5 min. PCR conditions for *cry2* gene were a single denaturation step for 5 min at 94ºC, followed by a 25 amplification cycles including denaturation at 94ºC for 1 min, annealing at 45ºC for 45 s, elongation at 72ºC for 2 min and a final extension at 72ºC for 10 min. Amplifications were carried out in a thermal cycler (Biometra, Germany). After electrophoresis of 10 µl of each PCR product on 2.5% agarose-EtBr gel, DNA bands were visualized in a gel documentation system (Bio-Doc Analyzer, Germany). PCR product purification and sequencing analysis were performed by Macrogene Inc. (Seoul, Korea).

Table 1. General primers for *cry*1, *cry*2, *cry*3, *cry*5, *cry*7, *cry*8, *cry*12, *cry*14, *cry*21, and *cyt*1 genes

Primer pair	Sequence (5'-3')	Gene recognized	Product size (bp)	Annealing temperature(°C)	Reference
CJI-1 **CJI-2**	TGTAGAAGAGGAAGTCTATCCA TATCGGTTTCTGGGAAGTA	*cry1*	272-290	48	Ceron et al (1995)
II(+) **II(-)**	TAAAGAAAGTGGGGAGTCTT AACTCCATCGTTATTTGTAG	cry2	1556-1523	45	Sauka et al. (2005)
CJIII-20 **CJIII-21**	TTAACCGTTTTCGCAGAGA TCCGCACTTCTATGTGTCCAAG	*cry3*	652-733	48	Ceron et al (1995)
Un4(d) **Un4(r)**	GCATATGATGTAGCGAAACAAGCC GCGTGACATACCCATTTCCAGGTCC	*cry4*	439	60	Apaydin et al. (2005)
Un7,8(d) **Un7,8(r)**	AAGCAGTGAATGCCTTGTTTAC CTTCTAAACCTTGACTACTT	*cry7* *cry8*	420 423	54 49	Ben-Dov et al. (1997)
Gral-nem(d) **Gral-nem(r)**	TTACGTAAATTGGTCAATCAAGCAAA AAGACCAAATTCAATACCAGGGTT	*cry5* *cry12* *cry14* *cry21*	474 477 483 489	50	Bravo et al. (1998)
Gral-cyt(d) **Gral-cyt(r)**	AACCCCTCAATCAACAGCAAGG GGTACACAATACATAACGCCACC	*cyt1Aa* *cyt1Ab*	522 525	51	Bravo et al. (1998)

SDS-PAGE of δ-endotoxin

The δ – endotoxin analysis was performed by doing SDS-PAGE for characterization of *B. thuringiensis* using spore/crystal suspensions. *B. thuringiensis* strains were cultured in T3 liquid medium for sporulation. Particulates from 100 µl of sporulated culture were washed with 1 M NaCl containing 5 mM EDTA and then with 5 mM EDTA alone. Washed crystals and spores were extracted for 5 min at 100°C in 100 µl sample buffer (50 mM Tris-HCl, pH 7.5), 2% (w/v) SDS, 0.05 (w/v) bromophenol blue, 1 mM EDTA, 10% (v/v) glycerol, 15 mM DTT). Insoluble material was removed by centrifugation. The 20 µl aliquots were loaded onto 7.5% acrylamide gels. Following electrophoresis, the gels were stained in 0.1% Coomassie Brilliant Blue G-250. The molecular weights of proteins were determined by using protein standards (Fermentas SMO431) (20).

Scanning Electron Microscopy

B. thuringiensis isolates were incubated in T3 medium by shaking at 250 rpm at 30°C for 7 days. The cell cultures were centrifuged at 4000 rpm for 10 min. The pellets were resuspended in sterile distilled water for 3 times. The cells were fixed in 2.5% glutaraldehyde at 4°C for 12 h, and washed with sterile distilled water. They were dissolved in sterilized distilled water. One drop of the sample was transferred onto a microscope slide and air dried for 5 minutes. Samples were dehydrated serially with ethanol (50- 100%). The samples were taken into amyl acetate for 30 minutes. This process was repeated twice (21). The critical point drying was done in a Polaron CPD7501 critical point dryer, and then coated with gold on a Polaron SC502 Sputter Coater. The SEM micrographs were taken via a JEOL JSM 6060 LV digital scanning microscope.

Bioassays

Isolates with parasporal bodies were cultured in 100 m1 of T3 liquid medium and incubated for 7 days at 30°C with continuous shaking at 250 rpm. Samples were centrifuged at 5000 rpm for 15 min. Pellets (spores and parasporal protein crystals) were washed in 20 ml sterile distilled water and centrifuged at 5000 rpm for 5 minute. Washing procedure was repeated twice. The pellets were resuspended in 20 ml of sterile distilled water and kept at 4°C. The suspensions of *B. thuringiensis* strains were examined for their toxicity against third instar larvae of *Ephestia kuehniella*. The food for larvae was prepared by soaking one gram of peanut pieces in 10 ml of each bacterial suspension for 5 min using three fold serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}). The food was then dried and placed in a vial where 10 larvae were placed. The toxicity of each strain was assayed in triplicate for either the original toxin-spore suspension or the dilutions. The vials were incubated at 25 ± 2 °C, 70 ± 10% r.h, and a photoperiod of 16:8 (L:D) for 7 days (22). Mortality was scored in

comparison with parallel control in which peanut pieces soaked in sterile distilled water instead of bacterial suspension and used to correct the test mortality by using Abbot's Formula (23). The LC_{50} values were determined by probit analysis using SPSS for Windows (24).

Results and Discussion

The native strains were isolated according to acetate selection method from soil samples. Twelve isolates were analyzed by phase-contrast microscopy and were selected as *B. thuringiensis* depending on the presence of parasporal crystals. 16S rDNA fragments of the 12 spore forming strains were sequenced and analyzed. According to the results of 16S rDNA sequencing five strains were identified as *B. thuringiensis*.

For detection of crystal genes of *B. thuringiensis* strains, PCR analysis was performed using *cry* gene specific primers. PCR reactions for each isolate were carried out with universal primer for *cry*1, *cry*2, *cry*3, *cry*4, *cry5*, *cry*7, *cry*8, *cry*12, *cry*14, *cry*21, and *cyt*1 genes. Only *cry*1 and *cry*2 primers gave positive results. Among the tested native isolates, F21 was positive for *cry*1 (272 bp) and *cry*2 (1554 bp) primer (Figure 1-2), F16 and F19 were positive for only *cry*2 primer (Figure 2). The other two isolates were negative for the tested cry gene primers.

B. thuringiensis subsp. *kurstaki* HD-1, *B. thuringiensis* subsp. *kurstaki* HD-73, *B. thuringiensis* subsp. *tolworthi* HD-125, *B. thuringiensis* subsp. *aizawai* HD-137, and *B. thuringiensis* 916 were used as positive control for each primer (Figure1-2). While *B. thuringiensis* ssp. *kurstaki* HD-1, *B. thuringiensis* subsp. *kurstaki* HD–73, *B. thuringiensis* subsp. *aizawai* HD–137 and *B. thuringiensis* 916 showed products of size 272 bp while *B. thuringiensis* ssp. *tolworthi* HD–125 and *B. thuringiensis* ssp. *kurstaki* showed approximately 290 bp. *B. thuringiensis* subsp. *kurstaki* HD-1 and *B. thuringiensis* subsp. *tolworthi* HD-125 exhibited positive PCR product (1554 bp) for cry2 gene primer.

For the identification of different *cry*2 genes, a 10 µl of positive PCR product was digested with *Dde*I restriction enzyme according to manufacturer's instructions, analyzed by polyacrylamide (10%) gel electrophoresis and stained with ethidium bromide. The restriction analysis of *B. thuringiensis* reference strains (HD-1, HD-125, Btk) were performed as well. The expected restriction fragment sizes of the known *cry*2 genes were determined by doing *in silico* digestion of their available sequences in the Bt toxin nomenclature website with the software 'RestrictionMapper' (Table 2) (17). In an agreement with the predicted fragment sizes the polyacrylamide gels showed three main bands of 972, 450 and 134 bp for *cry*2Aa, and two main bands of 1386 and 134 bp for *cry*2Ab (Figure 3). The PCR amplified region of *cry*1 for native strain F21 showed 97% similarity to *cry*1Ac genes (GenBank Accession No. AF492767.1). The sequence of *cry*2 for native strains F16, F19 and F21 revealed 96% similarity to *cry*2Ab gene (GenBank Accession No. EF157306.1).

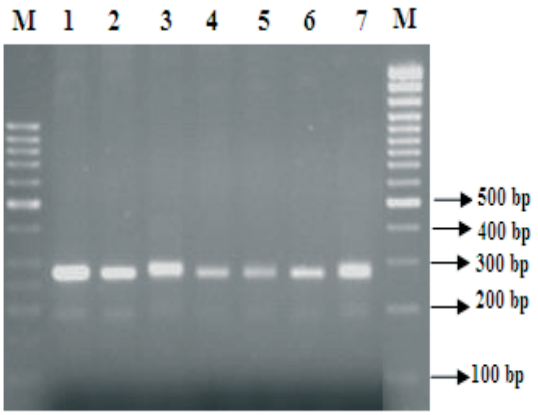

Figure 1. Detection of *Bacillus thuringiensis* strains' insecticidal genes with *cry1* general primers. Lane 1, *Bt* ssp. *kurstaki* HD-1; lane 2, *Bt* ssp. *kurstaki* HD-73; lane 3, *Bt* ssp. *tolworthi* HD-125; lane 4, *Bt* ssp. *aizawai* HD-137; lane 5, *Bt* 916; lane 6, F21; lane 7, *Bt* ssp. *kurstaki*; lane M- DNA ladder 100 bp.

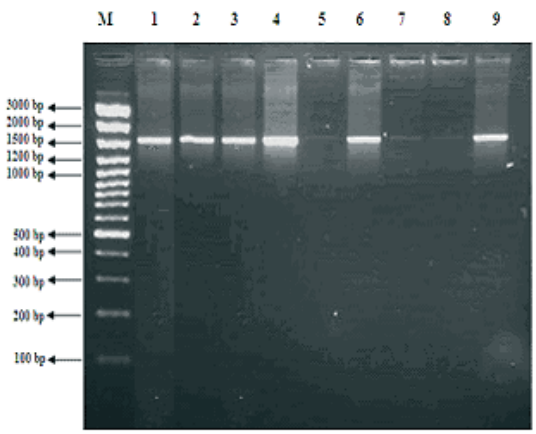

Figure 2. Detection of *Bacillus thuringiensis* strains' insecticidal genes with *cry2* general primers. Lane M, DNA ladder 100 bp; lane 1, F16; lane 2, F19; lane 3, F21; lane 4, *Bt* ssp. *kurstaki* HD-1; lane 5, *Bt* ssp. *kurstaki* HD-73; lane 6, *Bt* ssp. *tolworthi* HD-125; lane 7, *Bt* ssp. *aizawai* HD-137; lane 8, *Bt* 916; lane 9, *B t* ssp. *kurstaki*.

Table 2. Expected restriction fragment sizes of digested cry2 genes (17).

Gene	Fragment size (bp) with *Dde*I
cry2Aa	972, 450, 134
cry2Ab	1386, 134, 36
cry2Ac	915, 252, 162, 131, 36, 27
cry2Ad	663, 414, 309, 134, 36

Table 3. The profiles of *cry* genes and parasporal crystal morphology of *B. thuringiensis* strains.

Isolates	Crystal shape[a]	*cry* genes
F16	BP, S	*cry*2
F19	BP	*cry*2
F21	BP, S, C	*cry*1, *cry*2
Bt. ssp. kurstaki	BP, S, C	*cry*1, *cry*2
Bt .ssp. kurstaki HD-1	BP, S, C	*cry*1, *cry*2
Bt. .ssp. kurstaki HD-73	BP, S, C	*cry*1
Bt. ssp. kurstaki HD-125	BP, S, C	*cry*1, *cry*2
Bt. ssp. aizawai HD-137	S	*cry*1
***B. thuringiensis* 916**	**S**	***cry*1**

aC: Cuboidal, BP: Bipyramidal, S: Spherical.

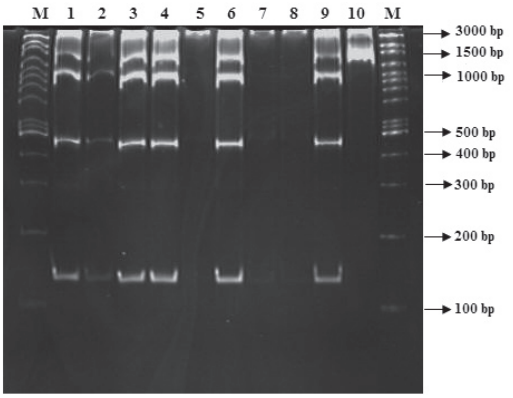

Figure 3. PCR-RFLP patterns of *cry*2 genes from *B. thuringiensis* native isolates and references strains. Lane M, DNA ladder 100 bp; lane 1, F16; lane 2, F19; lane 3, F21; lane 4, *Bt* ssp. *kurstaki* HD-1; lane 5, *Bt* ssp. *kurstaki* HD-73; lane 6, *Bt* ssp. *tolworthi* HD-125; lane 7, *Bt* ssp. *aizawai* HD-137; lane 8, *Bt* 916; lane 9, *Bt* ssp. *kurstaki*; lane 10, *Bt* ssp. *kurstaki* HD–1(uncut).

The spore-crystal mixture of the native isolates and the reference strains were analyzed by SDS-PAGE. F21, F16 and F19 isolates were produced major proteins of 130 and 65kDa (Figure 4) consistent with the *cry1 and cry2* genes detected by PCR. All reference strains showed a protein pattern similar to each other except HD137 and HD73. The results revealed that the strains synthesize a protein or a group of proteins with molecular weights between 130 and 140 kDa (consistent with the presence of a *cry1* gene), and a further protein of 65 kDa (consistent with the presence of a *cry2* gene).

The F19 and F21 isolates presented three different crystal inclusions (bipyramidal, cubical and spherical) but F16 exhibited two different crystal inclusions (bipyramidal and spherical) The reference strains HD-1, HD-73 and HD-125 exhibited three kinds of inclusions as in the F21 and F19. However, the other reference strains HD-137 and Bt-916 have only spherical crystals (Figure 5, Table 3).

Cry1 and Cry2 proteins are known to be active against lepidopteran insects (25). The toxicities of the crystal-spore mixtures of the native isolates and reference strains were assayed against third-instars of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae. *Ephestia kuehniella* is one of the major pests in industrial flour mills in temperate climates (26). It causes serious damage in amylaceous products. Apart from direct infestation, the faeces and webbings of larvae spoil the product. For the control of stored-cereal species, including *E. kuehniella*, the main categories of pesticides used are fumigants and residual grain protectants. However, the use of these substances is being reduced for health and environmental safety reasons (27, 28). Along with the consumers' demand for residue-free food, necessitates the evaluation of alternative, reduced-risk control methods. Insect pathogens, known also as microbials, are among the most promising alternatives to traditional pesticides in stored-product protection (29). The bacterium *B. thuringiensis* (Berliner) has been approved as a grain protectant in the United States (30), and is commercially available for the control of Indian meal moth larvae. Effective control using *B. thuringiensis* has been reported against lepidopteran larvae attacking stored products. In this study, a series of bioassays were performed by providing the larvae with food containing the spores and crystals. Spores and crystals were both included in suspensions because they produce higher level of mortality than either crystals or spores alone (31). The F21 isolate (positive for *cry1* and *cry2* genes) presented higher mortality rate (83%) than those of other isolates and the reference strain HD-1 (67%). The F16 and F19 isolates (positive for *cry*2 gene) were caused mortality rates 57% and 80%, respectively. From the mortality results, six statistically different groups could be seen among the isolates evaluated (ANOVA F= 29,837; df= 9 p=0.000) (Figure 6). Apaydın et al. (2005) examined the effects of different *B. thuringiensis* strains on the *E. kuehniella*. They found one strain (85PPb) identified as serovar morrisoni, caused a high level of mortality (84%) and was positive for *cry*1 and *cry*2 genes (18). Similarly, a novel *B. thuringiensis* strain (serovar *kurstaki*) isolated from Tunisian soils was reported to be toxic to lepidopteran insects including *E. kuehniella* due to Cry1Aa, Cry1Ac and Cry2Aa proteins (32-35).

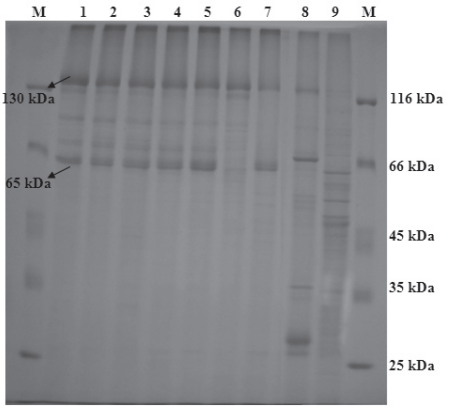

Figure 4. SDS-PAGE of spore-crystal from *B. thuringiensis* isolates. Lane M, Molecular Marker (Fermentas SMO431), lane 1, F16, lane 2, F19, lane 3, F21, lane 4, *Bt* ssp. *kurstaki*, lane 5, *Bt* ssp. *kurstaki* HD-1, lane 6, *Bt* subsp. *kurstaki* HD-73, lane 7, *Bt* subsp. *tolworthi* HD-125, lane 8, *Bt* ssp. *aizawai* HD-137, lane 9, *Bt* 916.

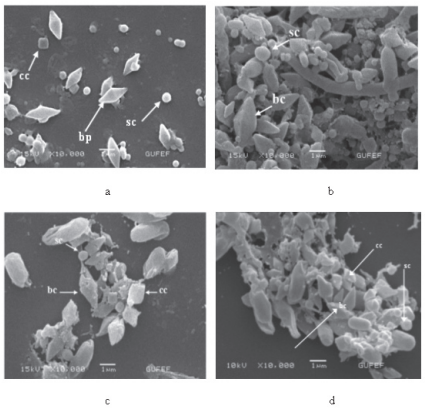

Figure 5. Scanning electron micrographs of *B. thuringiensis* Crystals. a- F21, b- F16, c- F19, d- *Bt* ssp. *kurstaki* HD-1 (bc: bipyramidal crystal; cc: cuboidal crystal; sc: spherical crystal).

Table 4. Probit analysis of *B. thuuringiensis* isolates and references strains

Isolates	[a]LC_{50}	[a]LC_{99}	X^2	df	p
B. t. subsp. *kurstaki* HD-1	1,83	5,78	25,182	2	,0001
B. t. subsp. *kurstaki* HD-73	3,14	7,21	22,726	2	,0001
B. t. subsp. *kurstaki* HD125	2,95	9,36	30,502	2	,0001
*B. t.*subsp. *kurstaki* HD-137	10,29	26,83	13,008	2	,001
B. thuringiensis 916	3,68	8,56	6,367	2	,001
B. t. subsp. *kurstaki*	3,71	9,89	3,705	2	,001
F16	2,17	6,56	38,354	2	,0001
F19	1,48	4,58	18,444	2	,0001
F21	1,08	4,06	18,088	2	,0001

[a]**LC_{50} and LC_{99}:** log (spore concentration ml^{-1}).
***X^2*:** Chi-square

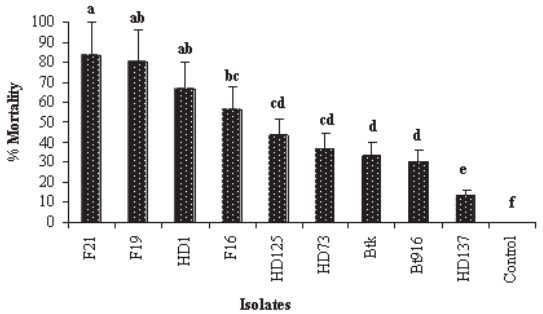

Figure 6. Mortality of Ephestia kuehniella larvae fed with diets containing spore-crystal mixture of different B. thuringiensis (Bt) strains.

The toxicity of the crystal-spore mixtures obtained from the isolates and reference strains indicated that the 50% lethal concentration (LC_{50}) and 99% lethal concentration (LC_{99}) of the crystal-spore mixtures for *E. kuehniella* larvae varied from 1.08 to 10.29 (spore concentration ml^{-1}), and from 4.06 to 26.83 (spore concentration ml^{-1}), respectively. The most pathogenic native isolates for *E. kuehniella* larvae were F21, F19 and F16, with LC_{50} values of 1.08, 1.48, and 2.17, respectively. Among the tested refernce strains, the most pathogenic one was *B. thuringiensis* ssp. *kurstaki* HD-1 (LC_{50}: 1.83). In the reference strain group, the LC_{50} values for *B. thuringiensis* ssp. *tolworthi* HD-125, *B. thuringiensis* ssp. *kurstaki* HD-73, *B. thuringiensis* ssp. *kurstaki, B. thuringiensis* 916, and *B. thuringiensis* ssp. *aizawai HD-137* were 2.95, 3.14, 3.68, 3.71 and 10.29, respectively (Table 4).

B. thuringiensis has been commercially used in the biological control of insect pests for the last four decades. The highly bioactive native *B. thuringiensis* isolates (F21 and F19) tested in this work appeared highly promising for new insecticide formulations and could be used for engineering pest resistant plants or in production of novel biopesticides. Our results indicate the presence of *B. thuringiensis* isolates showing insecticidal activity from soil samples in Ankara, Turkey. Future studies will deal with characterization of toxic agent(s) of our isolates, bioactivity assays against different pests and their probable use in industry.

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