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*Original article (Orijinal araştırma)*

# Bioactivities of *cry* gene positive *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) strains on *Ephestia kuehniella* Zeller, 1879 and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae)[1,2]

*cry* gene pozitif *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) izolatlarının *Ephestia kuehniella* Zeller, 1879 ve *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) üzerindeki biyoaktiviteleri

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## Summary

*Bacillus thuringiensis* is the bacterium most commonly used for biopesticide production due to parasporal crystal formation during its growth cycle. As a consequence of repeated use, *B. thuringiensis* biopesticides may cause the development of resistance in the pests. Therefore, it is necessary to explore new *B. thuringiensis* strains with a certain degree of bioactivity. In this study (2012-2013), the bioactivity of native *B. thuringiensis* strains from the Aegean Region of Turkey were tested against second instar larvae of *Ephestia kuehniella* and *Plodia interpunctella*. The bioactivity of 21 *B. thuringiensis* strains with *cry1*, *cry2* or *cry9* gene was determined as percent mortality according to Abbott's formula**.** The highest mortality rates were 42 and 63% in *E. kuehniella* and *P. interpunctella*, respectively. These mortality rates were equal to or 1.8 times greater than that of *B. thuringiensis* subsp. *kurstaki*. In addition, plasmid profiles of *B. thuringiensis* strains changed between 5-18 kb**.** Moreover, SDS-PAGE analysis of the most toxic strains indicated the presence of Cry1 and Cry2 proteins**.** Two different *cry2* gene profiles containing either *cry2Aa1* or combination of *cry2Aa1* and *cry2Ab2* genes were detected by PCR analysis**.** In addition, partial DNA sequence analysis of *cry2A* genes indicated phylogenetic differences among the toxic strains and *B. thuringiensis* subsp. *kurstaki*. As a result, these *B. thuringiensis* strains may be used to control both *E. kuehniella* and *P. interpunctella* as alternative biopesticides in cases of insect resistance to currently used *B. thuringiensis* preparations.

**Keywords:** *Bacillus thuringiensis*, bioactivity, *Ephestia kuehniella*, *Plodia interpunctella*

## Özet

*Bacillus thuringiensis* üreme döngüsü sırasında kristal oluşturması nedeniyle biyopestisit üretimi için en çok kullanılan bakteridir. *Bacillus thuringiensis* biyopestisitlerinin tekrarlayan kullanımları zararlılarda direnç gelişimine neden olabileceğinden, belirli düzeyde biyoakiviteye sahip yeni *B. thuringiensis* izolatlarının araştırılmasına ihtiyaç vardır. Bu çalışmada, Ege Bölgesin'den elde edilen doğal *B. thuringiensis* izolatlarının biyoaktivitesi *Ephestia kuehniella* ve *Plodia interpunctella*'nın ikinci dönem larvalarına karşı 2012-2013 yıllarında araştırılmıştır. *cry1*, *cry2* ya da *cry9* geni taşıyan 21 *B. thuringiensis* izolatının biyoaktivitesi Abbott formülüne göre yüzde ölüm olarak belirlenmiştir. En yüksek ölüm oranları, *E. kuehniella* ve *P. interpunctella*' ya karşı sırasıyla %42 ve %63 bulunmuştur. Bu ölüm oranları, *B. thuringiensis* subsp. *kurstaki*'ninkine eşit veya *B. thuringiensis* subsp. *kurstaki*'ninkinden 1.8 kat daha yüksektir. Buna ek olarak, *B. thuringiensis* izolatlarının plazmit profilleri 5-18 kb arasında değişmiştir. Ayrıca, en toksik izolatların SDS-PAGE analizi Cry1 ve Cry2 proteinlerinin varlığını göstermiştir. PCR analizi ile ya *cry2Aa1* veya *cry2Aa1* ve *cry2Ab2* genlerinin kombinasyonunu içeren iki farklı *cry2* gen profili belirlenmiştir. Ayrıca, *cry2A* genlerinin kısmi DNA sekans analizi, toksik izolatlar ve *B. thuringiensis* subsp. *kurstaki* arasındaki filogenetik farklılıkları göstermiştir. Sonuç olarak, bu *B. thuringiensis* izolatları bilinen *B. thuringiensis* preperasyonlarına karşı böcek direnci olması durumunda alternatif biyopestisitler olarak hem *E. kuehniella* hem de *P. interpunctella*'yı kontrol etmek için kullanılabilir.

**Anahtar sözcükler:** *Bacillus thuringiensis*, biyoaktivite, *Ephestia kuehniella*, *Plodia interpunctella*

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## Introduction

*Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) is a Gram positive, spore-forming bacterium that produces parasporal crystal inclusions during sporulation (Rowe et al., 1987; Schnepf et al., 1998). These parasporal crystals, also known as delta-endotoxins or insecticidal crystal proteins (ICPs), are mostly composed of one or more crystal (Cry) proteins and cytolytic proteins (Cyt) (Crickmore et al., 1998; Schnepf et al., 1998). *Bacillus thuringiensis* has being used for controlling insect pests from the different orders including Lepidoptera, Diptera, Coleoptera (Höfte & Whiteley, 1989), Hemiptera, Hymenoptera, Homoptera and Mallophaga (Schnepf et al., 1998). Also, it has been reported that some strains of *B. thuringiensis* exhibit activity against nematodes, acari and protozoa as well (Feitelson et al., 1992; Schnepf et al., 1998).

Given their high specificity and environmental safety, the spore-crystal mixtures of *B. thuringiensis* have been successfully used as bioinsecticides (Höfte & Whiteley, 1989; Schnepf et al., 1998) and approximately 2% of total insecticidal market consists of *B. thuringiensis* as a biological control agent (Bravo et al., 2011). Insecticidal Cry proteins have been used in preparations of novel insect formulations and transgenic plants have been constructed using genes encoding Cry proteins (Sanchis, 2011). Over 700 *cry* gene sequences have been identified and they are usually located on large plasmids (reviewed by Palma et al., 2014). As a result of the useful applications of insecticidal proteins there has been an intense search for new *B. thuringiensis* strains from the diverse habitats with different specificities.

*Ephestia kuehniella* Zeller, 1879 and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) are major lepidopteran pests of stored plant products (Sedlacek et al.,1995). These pests feeding on stored grain and crops can cause loss of weight and decreased quality. The aim of this study was to investigate the toxic effects of native *B. thuringiensis* strains on *E. kuehniella* and *P. interpunctella,* and to identify the plasmid profiles and *cry2*-type genes of the most toxic strains against both target pests.

## Materials and methods

### Bacterial strains

Twenty-one native *B. thuringiensis* strains from the *B. thuringiensis* collection of H. Gunes Molecular Biology Laboratory were selected based on their *cry1*, *cry2* or *cry9* gene content. The *B. thuringiensis* strains were originally obtained from fig-growing areas in Aegean Region of Turkey in an earlier study (Alper et al., 2014). The reference strain, *B. thuringiensis* subsp. *kurstaki* (BGSC 4D1), was kindly provided by Prof. Dr. Zeigler (*Bacillus* Genetic Stock Center, Columbus, Ohio, USA).

### Bioassay

Bioassays were carried out with spore-crystal mixtures of all native strains and the reference strain of *B. thuringiensis*. Each *B. thuringiensis* strain was cultured in 100 ml of nutrient broth medium for 3 days at 28°C in order to obtain the spore-crystal mixtures. After spinning the bacterial culture for 15 min at 4°C and 6000 rpm, the pellet was washed twice with ice-cold 1 M NaCl and three times with sterile distilled water. The pellet was dried overnight at 37-40°C and stored as powder at -20°C until used (Bravo et al., 1998).

Bioactivity of *B. thuringiensis* strains were investigated against second instar larvae of both *E. kuehniella* and *P. interpunctella* reared in 2012-2013 by the method of Ozkan (2006). Distilled water containing 0.1% Tween 80 was used to suspend the spore-crystal powder. A diet that included whole meal and wheat powder (3:1) was combined with suspension at 500 ppm (i.e., 500 µg spore-crystal mixture in 1 g compost) and dried. Diet containing spore-crystal mixture of *B. thuringiensis* subsp. *kurstaki* or diet without toxin was used as positive and negative control, respectively. Bioassays were performed using 20 larvae per toxin with three replicates at 25°C, 70% RH and a 16L:8D h photoperiod. Larval mortality was determined after 7 days. Corrected mortality data were calculated by using Abbott's formula (Abbott, 1925).

**Plasmid pattern**

After growing *B. thuringiensis* strains on nutrient agar overnight at 37°C, bacterial colonies were scraped gently with sterile distilled water and plasmid DNA was extracted according to the method of O'Sullivan & Klaenhammer (1993). Plasmid patterns were obtained by running undigested total plasmid DNA on an agarose gel at 80V and visualized in a gel documentation system (Vilber Lourmat, France). DNA ladder, SM1163 (Fermentas, St. Leon-Rot, Germany) was used as DNA weight marker.

**Determination of *cry2*-type gene**

Gene specific primers for the *cry2Aa1* and *cry2Ab2* genes as described by Salehi Jouzani et al. (2008) along with PCR were used to identify *cry2* gene content of *cry2* positive strains displaying bioactivity against the pests. Genomic DNA isolation was carried out according to the method of Ausebel et al. (2003). PCR mixture contained 500 ng of genomic DNA, 200 µM dNTP, 0.2-0.5 µM each of forward and reverse primer, 1.5 mM $MgCl_2$ and 2 U of *Taq* DNA polymerase (Fermentas) in a volume of 50 µl. The amplification reaction was carried out under following conditions: denaturation at 95°C for 1 min., annealing at 54°C for *cry2Aa1* and 60°C for *cry2Ab2* genes for 1 min., and extension at 72°C for 1 min. for total of 35 cycles and a final extension at 72°C for 10 min. Advanced Primus 96 Thermal Cycler (PeqLab, Erlangen, Germany) was used for amplifications. A total of 10 µl of each amplified PCR product was analyzed on 1% agarose-ethidium bromide gel in TAE buffer and DNA bands were visualized in a gel documentation system.

Table 1. The specific primers for identification of *cry2*-type genes

| Target gene | Sequence | Product size | Reference |
|---|---|---|---|
| *cry2Aa1* | F-5'- CGGATAAAATAATCTGGGAAATAGT -3' | 498 bp | Salehi Jouzani et al., 2008 |
| | R-5'- GAGATTAGTCGCCCCTATGAG -3' | | |
| *cry2Ab2* | F-5'- CGGATAAAATAATCTGGGAAATAGT -3' | 546 bp | Salehi Jouzani et al., 2008 |
| | R-5'- TGGCGTTAACAATGGGGGGAGAAAT -3' | | |
| *cry2* | F-5'-CGGATAAAATAATCTGGGAAATAGT- 3' | 1556 bp | Sauka et al., 2005 |
| | R-5'-TGGCGTTAACAATGGGGGGAGAAAT-3' | | |

**SDS-PAGE analysis**

In order to determine protein profiles of the six most toxic *B. thuringiensis* strains, they were inoculated in 5 ml nutrient broth and allowed to sporulate for 3-4 days at 30°C by shaking at 200 rpm. Preparation of spore-crystal mixtures and electrophoresis were done as described in Alper et al. (2014). For electrophoresis, 5 µg spore-crystal mixture determined by Bradford assay (Bradford, 1976) was loaded per lane along with protein weight marker, SM0661 (Fermentas). The gels were stained with Comassie Brilliant Blue R250 (Sigma-Aldrich, St. Louis, MS, USA).

**Partial DNA sequence analysis for *cry2* gene of toxic *Bacillus thuringiensis* strains**

In order to determine if toxic *B. thuringiensis* strains posses new *cry*-type genes, genomic DNA of the strains was amplified with *cry2A* forward and *cry2A* reverse universal primers as described by Sauka et al. (2005). This primer pair results in a PCR product at around 1556 bp. The PCR products were purified from the agarose gel using silica bead DNA gel extraction kit (Fermentas) and cloned in pJET1.2/blunt cloning vector (Fermentas). Transformation into competent *Escherichia coli* DH10B strain was carried out according to the manufacturer's protocol. The presence of inserts in recombinant colonies were verified using forward sequencing and reverse sequencing primers of the vector pJET1.2.

Afterwards, plasmid DNA was isolated using the GeneJET plasmid miniprep kit (Fermentas). Nucleotide sequence of cloned insert was performed by Refgen Inc. (Ankara, Turkey) and the sequences were combined with contig analysis of the BioEdit program 7.1 (http://bioedit.software.informer.com). After confirming the quality of the nucleotide peaks, DNA sequences were compared with known sequences using BLASTN database (http://www.ncbi.nlm.nih.gov). Sequence alignments were generated with the CLUSTAL W and analyzed using MEGA-4 program (Tamura et al., 2007). Phylogenetic tree was built with neighbor joining methods (Saitou & Nei, 1987).

### Statistical analysis

The data obtained from the bioassays were subjected to analysis of variance (one-way ANOVA) for comparing the toxicity of strains. After analysis, significance of differences between applications of different spore-crystal mixtures was determined by Duncan's multiple range test at significance levels of $P \leq 0.05$. All statistical analysis were carried out by SPSS Statistics (Version 10, 2003, IBM, Armonk, NY, USA).

## Results

### Profiles of *cry* genes of *Bacillus thuringiensis* strains

Given that Cry1, Cry2 and Cry9 proteins were toxic to lepidopteran insects (Bravo et al., 1998), we selected twenty-one native *B. thuringiensis* strains from our *B. thuringiensis* collection with *cry* genes encoding these Cry proteins. Profiles of *cry* genes of the strains were identified previously (Alper et al., 2014) and are presented in Table 2.

Table 2. Content of *cry* genes of *Bacillus thuringiensis* strains

| *cry* gene(s) | *Bacillus thuringiensis* strains and reference strain |
|---|---|
| *cry 1* | 1T, 2T, 7T, 113T, 5MY, 75MY, 76MY |
| *cry 9* | 3T |
| *cry 1, cry 2* | 5T, 8T, 9T, 11T, 176T, 43MY, 44MY |
| *cry 1, cry 2, cry 3* | 107T, 13MY, 42MY |
| *cry 1, cry 2, cry 9* | 6T, 10T |
| *cry 1, cry 2, cry 3, cry 9* | 4T |
| *cry1*, *cry2* | *B. thuringiensis* subsp. *kurstaki* (BGSC 4D1) |

### Bioactivity of *Bacillus thuringiensis* strains against *Ephestia kuehniella* and *Plodia interpunctella*

The bioassays against *E. kuehniella* and *P. interpunctella* revealed differences between the toxicities of the strains and their effect on the two pests. Against the larvae of *E. kuehniella*, three strains (2T, 3T 5MY) caused mortality less than 15%; 14 strains displayed mortality between 15 and 28%; and finally four strains (10T, 107T, 13MY and 42MY) exhibited mortality between 30 and 42% (Table 3). The highest mortality rates (41 and 42%) were obtained from the strains 42MY and 13MY, respectively. The reference strain *B. thuringiensis* subsp. *kurstaki* exhibited mortality similar to those strains. There was no significant difference among 13MY, 42MY and *B. thuringiensis* subsp. *kurstaki* in terms of toxicity on larvae of *E. kuehniella*. However, the toxic effects of these strains and the reference strain were statistically significant compared to negative control and the other strains (Table 3).

Table 3. Effects of native *Bacillus thuringiensis* strains on larvae of *Ephestia kuehniella* and *Plodia interpunctella* (Mean ± SE) with mortality range

| | *Ephestia kuehniella* | | *Plodia interpunctella* | |
|---|---|---|---|---|
| Strain | Alive larvae* | % Mortality | Alive larvae* | % Mortality |
| 1T | 17,57 ± 0,41 defghi | 19 | 15,04 ± 0,70 ef | 32 |
| 2T | 18,90 ± 0,22 ij | 10 | 16,66 ± 0,66 fg | 25 |
| 3T | 18,95 ± 0,26 ij | 9 | 17,85 ± 0,67 ghijk | 17 |
| 4T | 18,04 ± 0,40 fghi | 15 | 16,90 ± 0,73 fgh | 23 |
| 5T | 16,14 ± 0,57 bcde | 26 | 17,00 ± 0,61 gh | 22 |
| 6T | 18,04 ± 0,40 fghi | 15 | 16,90 ± 0,63 fgh | 23 |
| 7T | 16,14 ± 0,57 bcde | 26 | 16,95 ± 0,64 fgh | 23 |
| 8T | 17,09 ± 0,56 bcdefgh | 22 | 13,00 ± 0,70 cd | 41 |
| 9T | 16,52 ± 0,38 bcdef | 25 | 17,47 ± 0,55 ghi | 20 |
| 10T | 15,46 ± 0,45 bc | 30 | 17,66 ± 0,40 ghij | 18 |
| 11T | 18,04 ± 0,42 fghi | 15 | 16,52 ± 0,67 fg | 25 |
| 107T | 15,33 ± 0,60 b | 32 | 15,04 ± 0,70 ef | 32 |
| 113T | 16,23 ± 0,62 bcdef | 25 | 17,57 ± 0,46 ghij | 18 |
| 176T | 17,85 ± 0,57 efghi | 18 | 18,71 ± 0,26 hijkl | 12 |
| 5MY | 18,38 ± 0,27 ghij | 13 | 19,76 ± 0,09 kl | 3 |
| 13MY | 13,19 ± 0,90 a | 42 | 11,14 ± 1,08 b | 53 |
| 42MY | 13,33 ± 1,01 a | 41 | 9,04 ± 1,04 a | 63 |
| 43MY | 15,85 ± 0,64 bcd | 28 | 15,04 ± 0,67 ef | 32 |
| 44MY | 16,38 ± 0,49 bcdef | 25 | 11,38 ± 0,82 bc | 48 |
| 75MY | 16,80 ± 0,58 bcdefg | 23 | 19,28 ± 0,20 ijkl | 6 |
| 76MY | 17,28 ± 0,31 cdefghi | 20 | 19,47 ± 0,16 jkl | 5 |
| 4D1 | 13,14 ± 0,86 a | 42 | 14,33 ± 0,65 de | 34 |
| Negative control(NC) | 20,00 ± 0,00 j | 0 | 20,00 ± 0,00 l | 0 |

*Means with the same letter in the column are not different statistically (P>0.05).

When spore-crystal mixture of *B. thuringiensis* strains was applied to *P. interpunctella* larvae, different degree of mortality was observed. Mortality range of the most of the strains was between 20 and 50% (Table 3). Four strains 8T, 13MY, 42MY and 44MY were found to be the most toxic strains with their toxicity higher than that of the reference strain *B. thuringiensis* subsp. *kurstaki*. In other words, the mortality rates obtained from the native strains 42MY, 13MY and 44MY and 8T were respectively 1.8, 1.5, 1.4 and 1.2 times higher than that of the reference strain. In short, the toxic effects of 13MY, 42MY, 44MY and 8T were determined to be statistically significant compared to negative controls, *B. thuringiensis* subsp. *kurstaki* and the other strains (P< 0.05) (Table 3).

**Plasmid pattern**

Since *cry* genes are generally detected in several circular or linear plasmids in *B. thuringiensis* (Carlson et al., 1996), plasmid patterns of toxic strains were identified. Agarose gel electrophoresis of plasmid preparations indicated the presence of major plasmid band around 18 kb in all strains. Molecular weight of the plasmid bands for 13MY, 42 MY, 10T and 107T changed between 5 and 18 kb (Figure 1). In addition, the number of plasmid bands ranged from 1 to 6.

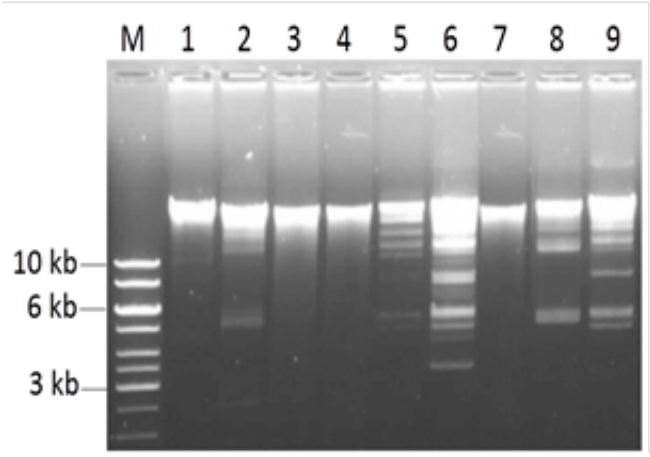


Figure 1. Plasmid profiles of *Bacillus thuringiensis* strains. M: DNA Marker, 1: 8T, 2: 10T, 3: 43MY, 4: 44MY, 5: 45MY, 6: 107T, 7: *B. thuringiensis* subsp. *kurstaki*, 8: 13MY, 9: 42MY.

**Identification of *cry2*-type gene composition by PCR**

A total of six strains, which are the most effective strains 10T, 107T, 13MY and 42MY against *E. kuehniella*; and 8T, 13MY, 42MY and 44MY against *P. interpunctella* were further characterized for their specific *cry2*-type genes using gene specific primers for *cry2Aa1* and *cry2Ab2* genes. PCR analysis indicated two different *cry2* gene profiles. The gene *cry2Aa1* was determined in all strains. Although two strains showed amplification with only *cry2Aa1*, four strains showed amplification with *cry2Aa1* and *cry2Ab2* genes together (Table 4).

Table 4. Profiles of the *cry2*-type gene of *Bacillus thuringiensis* strains

| Profile | *cry2* gene content | *Bacillus thuringiensis* strains |
|---|---|---|
| 1 | *cry2Aa1* | 8T, 10T |
| 2 | *cry2Aa1, cry2Ab2* | 107T, 13MY, 42MY, 44MY |

**Cry protein profiles**

The most toxic *B. thuringiensis* strains to *E. kuehniella* and *P. interpunctella* were subjected to SDS-PAGE analysis in order to determine their Cry protein contents (Figure 2). All profiles seem to be similar and density of two bands around 130 and 65 kDa were stronger than that the others. These results indicate that *cry1* and *cry2* genes were expressed in the toxic strains as well as in *B. thuringiensis* subsp. *kurstaki*. However, expression levels of Cry1 and Cry2 proteins from 8T and 10T seemed to be less than that of other *B. thuringiensis* strains.

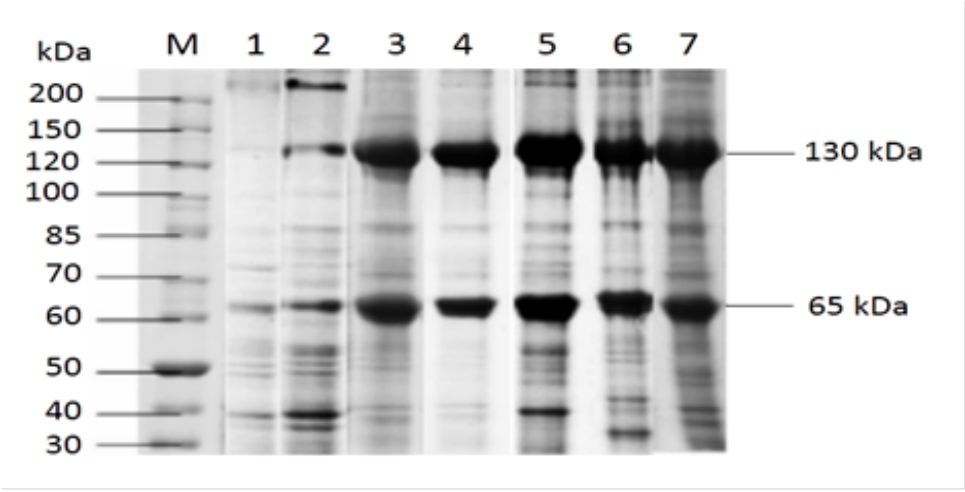


Figure 2. SDS-PAGE analysis of Cry Protein profiles from toxic *Bacillus thuringiensis* strains. M: Protein marker, 1: 8T, 2: 10T, 3: 13MY, 4: 42MY, 5: 44MY, 6: 107T, 7: *B. thuringiensis* subsp. *kurstaki*.

**Partial DNA sequence analysis of *cry2A* gene**

An amplicon of about 1556 bp was obtained for the *cry2A* gene (Figure 3). After cloning the PCR products of 13MY, 42MY, 44MY and 107T into pJET1.2/blunt vector, DH10B competent cells were transformed with the plasmid constructs. PCR products in recombinant colonies were verified and subjected to sequence analysis. DNA sequences of the *cry2A* genes from *B. thuringiensis* strains were aligned and compared with the reported sequences from GenBank. Analysis of the partial 471 bp *cry2* gene sequences of the strains revealed 94-95% matching to *cry2Aa* and *cry2Ab* genes. Given that 449 bp sequence belonged to endotoxin mid domain, more sequence differences may be present in domains I and III. The phylogenetic tree has two clusters with two and 15 sequences in each cluster (Figure 4). Based on this phylogenetic analysis, *cry2A* gene of strains 13MY, 107T, 42MY and 44MY appear to have diverged in different a lineage compared to *B. thuringiensis* subsp. *kurstaki,* which indicate that they could be distinct *cry2A* genes.

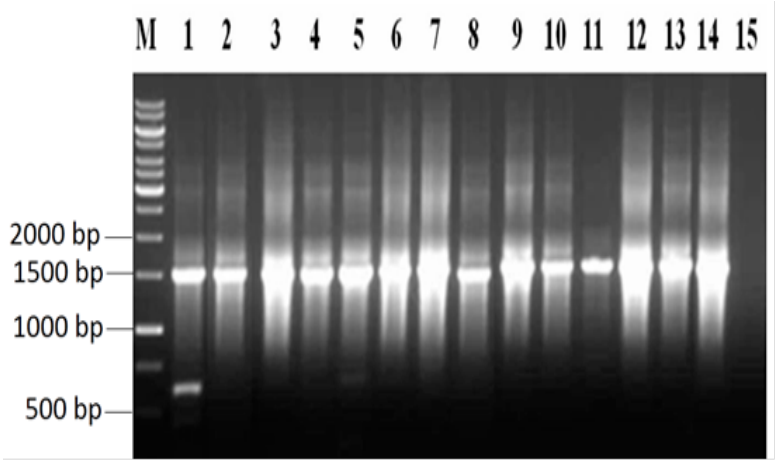


Figure 3. Amplification of *cry2A* gene in the *Bacillus thuringiensis* strains. M: Marker 1 kb DNA Ladder, 1: 8T, 2: 10T, 3: 23T, 4: 69T, 5: 71T, 6: 106T, 7: 107T, 8: 153T, 9: 13MY, 10: 42MY, 11: 44MY, 12: 45MY, 13: 51MY, 14: *B. thuringiensis* subsp. *kurstaki*, 15: negative control.

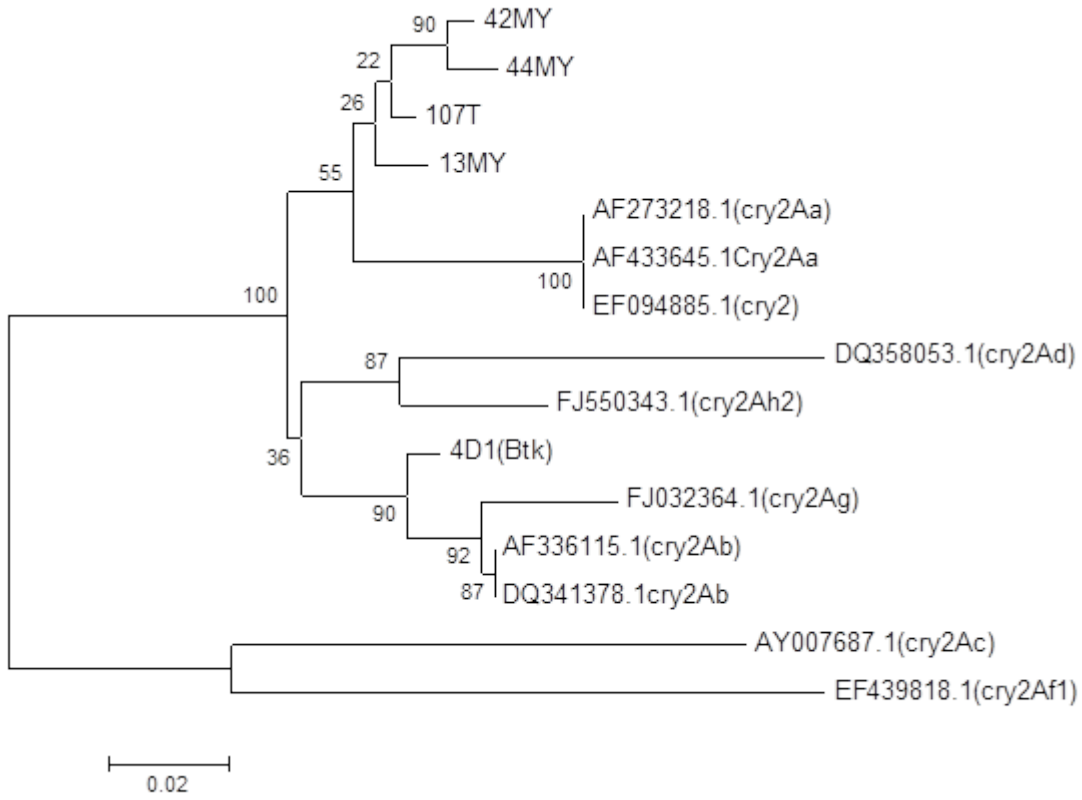


Figure 4. Phylogenetic tree obtained from the alignment of *cry2* gene sequences along with the *cry2A* genes of the native *Bacillus thuringiensis* strains as described in material and methods.

## Discussion

In this study, bioactivities of native *B. thuringiensis* strains against stored product pests *E. kuehniella* and *P. interpunctella* were investigated. In addition, plasmid patterns, protein profiles and *cry2*-type genes of the most toxic strains were identified. Substantial evidence indicates that *B. thuringiensis* from different regions produce genetically and functionally different toxins (Palma et al., 2014) and a single amino acid changes in insecticidal Cry proteins can significantly alter their toxicity (Udayasuriyan et al., 1994; Rajamohan et al., 1995). The fact that some insects develop resistance against *B. thuringiensis* toxins after repeated exposure (Tabashnik, 1994; Ferre et al., 1995) makes it necessary to detect and identify new *B. thuringiensis* strains for the management of resistant insect populations. Therefore, screening of *B. thuringiensis* strains with certain bioactivities against various pests is being undertaken worldwide.

Here, we applied spore and crystal mixtures to stored product pests during bioassays because the mixture is more effective on mortality compared to crystals or spores alone (Crickmore, 2006). The highest toxicity value of four strains changed between 30-42% for *E. kuehniella* larvae while two strains exhibited 53-63% toxicity to *P. interpunctella* larvae at a spore-crystal concentration of 500 ppm. The strains 13MY and 42MY were found to be the most toxic strains for both *E. kuehniella* and *P. interpunctella* larvae. The toxic effects of 13MY and 42MY on *E. kuehniella* larvae were similar to that of *B. thuringiensis* subsp. *kurstaki*. However, the toxic effects of 13MY and 42MY on *P. interpunctella* larvae were found to be 1.5 and 1.8 times higher than that of *B. thuringiensis* subsp. *kurstaki*, respectively.

These results are partially consistent with other studies. For example, Chilcott & Wigley (1993) reported that the mortality rates of *B. thuringiensis* strains in lepidopteran larvae ranged from 37 to 88%. Azizoglu et al. (2011) showed 40 and 47% mortality to *E. kuehniella* larvae while 50 and 54% mortality to *P. interpunctella* larvae with *B. thuringiensis* strains U14.1 and U6.6. In addition, Hongyu et al. (2000) reported more than 60% mortality to *P. interpunctella* larvae. Moreover, *B. thuringiensis* serovar *morrisoni* strain 85PPb with both *cry1* and *cry2* genes retarded larval growth of *E. kuehniella* by 84% (Apaydin et al., 2008). Furthermore, Bozlağan et al. (2010) showed that insecticidal activities of the most effective strains against *E. kuehniella* larvae and *P. interpunctella* larvae were 1.8 and 5.2 times higher than that of *B. thuringiensis* subsp*. kurstaki*, respectively. Finally, the mortality rates of spore-crystal mixture from a novel *B. thuringiensis* strain SY49-1 at concentration of 100 µg/g were 70% on *P. interpunctella* and 90% on *E. kuehniella* larvae (Azizoğlu et al., 2016). The discrepancy among these results might be due to *cry* gene content of the strains, expression of related genes at the protein level, susceptibility of target insects, and differences in methodology for bioassay or a combination of these factors.

One of the factors that affect the toxicity to pests is *cry* gene content of the strains. Therefore, identification of *cry* genes in a *B. thuringiensis* strain is important to estimate its insecticidal potential. Given that Cry1, Cry2 and Cry9 proteins are effective on lepidopteran larvae, we used *B. thuringiensis* strains positive for *cry1*, *cry2* and/or *cry9* genes. *Bacillus thuringiensis* subsp. *kurstaki* was used as a reference strain because it has both *cry1* and *cry2* genes. Bioassay results indicated that the native *B. thuringiensis* strains exhibited different level of toxicity to the same pest. This difference in toxicity may be most probably due to the difference in the level of expression of these proteins or type of *cry1* or *cry2* genes. In fact, we showed that the strains 13 MY and 42 MY contain *cry1* gene different from that of *B. thuringiensis* subsp. *kurstaki* in our previous study (Alper et al., 2014). Similarly, partial DNA sequence analysis of *cry2A* genes was different from that of the reference strain according to phylogenetic tree in this present study. Verification of sequence differences using Taq polymerase enzyme with proof reading activity will indicate that variations in nucleotide sequence of *cry2* gene among strains may be the reason for different bioactivity level of each Bt strain.

The *cry2* gene encode 65 kDa proteins in many *B. thuringiensis* strains (Höfte & Whiteley, 1989) and Cry2 group proteins were known to be toxic to both lepidopteran and dipteran insects (Bravo et al., 1998). Even though Cry2Aa protein is toxic to both lepidopteran and dipteran larvae, Cry2Ab protein is only toxic to lepidopteran insects. In the present study, we examined *cry2*-type gene of the most toxic strains using specific primers. We found two different profiles of *cry2* gene (Table 4). All of these strains carried *cry2Aa1*; however, two strains contained only *cry2Aa1* and other four strains had both *cry2Aa1* and *cry2Ab2* together. Similar to our result, Sauka et al. (2005) showed that among the native *B. thuringiensis* strains, 94.9% of them had *cry2Aa/cry2Ab* gene combination whereas 3.4% and 1.7% of them contained *cry2Ab* or *cry2Aa*, respectively. In addition, Liang et al. (2011) reported that *cry2Aa/cry2Ab* gene combination was the most frequent (90.4%), whereas percentages of *cry2Aa* and *cry2Ab* positive genes in their *B. thuringiensis* strains were 6.8 and 2.5, respectively. In other words, occurrence of *cry2Aa/cry2Ab* gene combination was the most frequent compared to that of individual cry2-type genes in *B. thuringiensis* strains. Given that the toxicity spectrum of Cry2A type proteins is wider than that of currently used Cry1A proteins, the toxic strains with *cry2*-type genes will be promising strains for management of the insect resistance.

In conclusion, the *B. thuringiensis* strains 13MY and 42MY were found to be the most toxic to the larvae of both *E. kuehniella* and of *P. interpunctella*. In addition, toxicity of the strains 42MY, 13MY, 44MY and 8T to *P. interpunctella* was 1.8, 1.5, 1.4 and 1.2 times higher than that of the reference strain of *B. thuringiensis* subsp. *kurstaki*. In the case of development of resistance due to repeated exposure to a certain kind of *B. thuringiensis* toxin, these *B. thuringiensis* strains will serve as an alternative resource of toxins and toxin genes for control of these pests. Future research arising from this study will be the cloning of *cry2*-type genes for recombinant production of the toxin.

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