Isolation of Iranian *Bacillus thuringiensis* strains and characterization of lepidopteran-active cry genes

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**Abstract**

**Background:** Insecticidal crystal proteins (encoded by cry genes) produced by *Bacillus thuringiensis* (*Bt*) are fatal for insects of different orders such as Lepidoptera. The genes that encoded these crystal proteins can be detected on plasmids and chromosomal DNA and show different types in various strains. Therefore, the objective of this study was to determine molecular characteristics of Iranian *Bt* strains as well as their toxicity against *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae).

**Results:** The collection sites included fields, gardens, and desert and semi-desert areas in 8 provinces of Iran. For crystal formation, each isolate was cultured in T3 medium. The results showed that the *Bt* isolates produced different types of crystals including spherical (73.33%), bipyramidal (53.33%), irregular (40%), cubical (33.33%), and elliptical (13.33%). Plasmid DNA extraction was performed and showed that most of the strains exhibited similar pattern in the number and the size of the plasmid bands to those of *Bt kurstaki* (*Btk*). Some specific primers were used for PCR amplification to distinguish different crystal genes including cry1A, cry1C, and cry1D and cry2Aa and cry2Ab. The primers related to cry1D and cry2Aa1 genes produced no amplicons. The results revealed that the most abundant gene was cry1-type. All strains analyzed for the cry2Ab2 gene presented unexpected bands. Electrophoretic profile of the protein crystals showed bands with different diversity in number, and size ranged from about 16 to 140 KDa. The bioassay result of some more toxic strains exhibited that the pathogenicity of 1019 was higher than the rest, even the reference strain, *Btk*. However, the toxicity of other strains was the same as *Btk*. According to the phylogenetic tree, 1019 was located in the same group with *Bt* subspecies *coreanensis*, *Bt* subspecies *indiana* and *Bt* subspecies *tolworthi*.

**Conclusions:** The investigated Iranian strains had the lepidopteran-active cry genes. The strains with the same toxicity to *E. kuehniella* had various cry genes, plasmid, and crystal protein profiles and vice versa. Therefore, characterization of cry genes of native strains could lead to access potent isolates as biocontrol agents against native insect pests.

**Keywords:** Bacteria, Bioassay, Cry gene, Crystal protein, Iranian isolates, Polymerase chain reaction, Plasmid DNA
**Background**

Insecticidal crystal proteins (encoded by cry genes) produced by a gram-positive bacterium, *Bacillus thuringiensis* (*Bt*) (Nazarian et al., 2009) are fatal for insects of different orders such as Lepidoptera, Coleoptera, and Diptera (Aronson and Shai, 2001). In addition, when the toxic genes are expressed in transgenic crops, they can control the pest populations such as Lepidoptera (Boukedi et al., 2016). The classification of crystal toxins of *Bt* has shown that there are 73 families and 6 groups of endotoxin proteins (Palma et al., 2014).

The genes that encoded these crystal proteins can be detected on plasmids and chromosomal DNA (Höfte and Whiteley 1989) and show different types in various strains. For example, cry1Aa, cry1Ac, cry2Aa, and cry2Ab were discovered in *Bt* subspecies *kurstaki* (Btk) (Baum and Malvar 1995). However, cry7A and cry1B were found in *Bt* subspecies *galleriae* (*Btg*) (Nazarian et al., 2009).

Some cases of resistance of insect pests to crystal toxins have been observed even in the commercial isolate of *Bt* (Tamez-Guerra et al., 2006). Therefore, many studies have been carried out to isolate and characterize new and native isolates. For instance, Yilmaz et al. (2012) introduced 6 cry genes including cry1C, cry1Aa/Ad, cry1B, cry5, cry9A, and cry9C belonging to a novel strain (SY49.1) isolated from Turkey. A new isolate of *Bt*, harboring cry30, cry40, and cry54 genes, was described by Boukedi et al. (2016) from Tunisia. The Iranian *Bt* strains containing lepidopteran-specific cry genes like cry1, cry2, and cry9 were presented (Seifinejad et al., 2008). *Bt* is able to insert through the peritrophic membrane and midgut epithelium and overcome the insect immune system (Raymond, 2017). In fact, the toxins of *Bt* are activated in the insect gut when they encounter the high pH, then bind to receptors located on midgut brush border membrane vesicles, and finally cause cell destruction and host death (Bravo et al., 2017).

Most of the bioassay investigation revealed that the natural strains could be efficient with high pathogenicity against insect pests. The pathogenesis of IS5053 strain was similar to the *Btk* against *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae) (Swiecicka et al., 2008). However, the SY49.1 strain showed high mortality for *Ephesia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) (Yilmaz et al., 2012) in comparison with *Btk*. Efficacy of BLB250 strain against 2 lepidopteran pests, *Spodoptera littoralis* (Boisdauval) (Lepidoptera: Noctuidae) and *E. kuehniella* was estimated, and the novel strain showed higher toxicity than the 2 *Bt* reference strains (BenFarhat-Touzri et al., 2016).

Despite of many preceding reports of *Bt* crystal protein genes, isolation and characterization of more indigenous isolates are essential. The objective of this study was to determine molecular characteristics of *Bt* strains as well as their toxicity against *E. kuehniella*. These isolates were obtained from soil samples in Iran.

**Methods**

**Soil collection**

Soil was collected in 50-ml sterile Falcon tubes from the depth of 5–10 cm. The samples were labeled and transported to the laboratory and were incubated at 4 °C. The collection sites included fields, gardens, and desert and semi-desert areas in 8 provinces of Iran.

**Bacterial isolation and identification**

*Bt* isolates were obtained from the soil samples according to the method of Travers et al. (1987). Sodium acetate (0.25 M) was added to Luria Bertani (LB) broth (20 ml), and then, 1 g of soil sample was poured into the buffer. The mixture was shaken at 200 rpm for 4 h at 30 °C. Then, each sample (1 ml) was heated at 80 °C for 10 min. A 50-ml suspension was transferred and cultured onto a nutrient agar (NA) (containing 0.5% peptone, 0.3% beef and yeast extracts, 1.5% agar, and 0.5% sodium chloride) Petri dish (8-cm diameter). The cultures were incubated at 35 °C, and *Bt*-like colonies were sub-cultured. The bacteria were observed under a light microscope after Gram staining. Three reference strains were used including *Bt* subsp. *kurstaki* (*Btk*), *Bt* subsp. *thuringiensis* (*Bt*), and *Bt* subsp. *galleriae* (*Btg*) (Persian Type Culture Collection in Iranian Research Organization for Science and Technology, Tehran, Iran). All *Bt* isolates were stored at −80 °C in 15% glycerol stock.

**Crystal morphology**

For parasporal crystal formation, each isolate was cultured in T3 medium (3 g triptone, 2 g triptose, 1.5 g yeast extract, 0.005 g MnCl2, 6 g NaH2PO4, and 7.1 g Na2HPO4) (Travers et al., 1987). For sporulation, the culture was incubated at 30 °C, 200 rpm for 7 days. Pellet of the culture was obtained (15,000 × g for 10 min) and was washed twice and centrifuged again in sterile distilled water. The pellet containing spore—crystal mixture was freeze dried and used for observation under phase contrast microscope by coomassie blue staining to examine the presence of crystals. The spore—crystal mixture of the high pathogenic isolate was evaluated by electron microscopy.

**Isolation of plasmid DNA**

Plasmid DNA extraction was performed by using the method of Sambrook et al. (1989). The GeneAll® Exprep™ Plasmid DNA kit (South Korea, GeneAll Biotechnology) was used according to the manufacturer’s instructions. Each isolate of *Bt* was grown in 10 ml LB for 16 h at 37 °C and shaken at 160 rpm. The bacterial
culture was centrifuged at 10,000 × g for 5 min, and the supernatant was removed. The pellet was resuspended in 250 μl of buffer 1 in the presence of RNase to release the plasmid DNA into supernatant.

To completely disrupt base pairing, 250 μl of buffer 2 was added and mixed by inverting. After adding 350 μl of buffer 3, the solution was centrifuged for 10 min. The cleared lysate was transferred to a column and centrifuged for 30 s. Buffers 4 (500 μl) and 5 (700 μl) were separately applied into the column and centrifuged for 30 s. Finally, 50 μl of 10 mM Tris (pH 8.5) was applied and incubated for 1 min. Following elimination, the residual wash buffer, the optimal elution of DNA, was obtained and subjected to electrophoresis in 0.5% agarose gel.

Polymerase chain reaction (PCR)
Plasmid DNA of each isolate and the reference strain was used for PCR as DNA template. PCR analysis was carried out according to Juarez-Perez et al. (1997), using the 6 primers to distinguish the pertaining cry genes (Table 1). Each PCR mixture (25 μl) was composed of Super PCR MasterMix (12.5 μl), primer (2 μl of both forward and reverse), 2 μl DNA template (μl) and 8.5 μl of distilled water.

The PCR program for the primers was as follows: initial denaturation at 95 °C for 3 min, followed by 35 cycles for denaturation at 94 °C for 1 min, 35 cycles for annealing (different for each primer pairs) for 1 min and extension step at 72 °C for 1 min, and last extension at 72 °C for 5 min. At the annealing part, the first cycle with a decrease of 5 °C was only conducted to detect cry1Aa, cry1C, and cry2Ab.

16S ITS rDNA gene analysis
Phylogenetic analysis was conducted, using the highest pathogenic isolate, 1019 (NCBI (National Center for Biotechnology Information) Accession No. MW485925), and the universal primer (Table 1). Other type strains with more than 99% sequence identity and B. subtilis subspecies natto as an out-group were used for the analysis. By the BLAST tool at the NCBI website (http://www.ncbi.nlm.com), the nucleotide sequence was analyzed to identify the bacterium based on sequence similarity.

The selected sequences from NCBI and 1019, were aligned using MAFFTv.7 (Katoh and Standley, 2013) with “Auto” settings; and then inspected manually by Mesquite v. 3.10 (Maddison and Maddison, 2015). The Linux version of IQ-tree (Nguyen et al. 2015) was used to build a Maximum likelihood tree to determine the phylogenetic relationship among aligned sequences. The created tree was visualized, using Figtree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

Protein electrophoresis
The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) was executed to determine the protein profiles of crystal components as described by Laemmli (1970). Hence, each spore–crystal mixture was added in a sample buffer and heated at 75 °C for 15–20 min. The buffer contents were 4 ml 10% SDS, 2 ml glycerol, 1.2 ml of 1M Tris (pH 6.8), 0.01% bromophenol blue, 10 ml β-mercaptoethanol, and 2.8 ml dH2O.

The protein samples of Bt isolates were loaded onto the acrylamide gel containing a 12% separating gel with a 5% stacking gel. Comassie brilliant blue R250 (0.1%) was used to stain the gel, and then the gel was destained.

| Primer name | Melting temperature (°C) | Sequence (5’ → 3’) | Reference |
|-------------|---------------------|------------------|-----------|
| 16S ITS rDNA(F) | 60 | AGAGTTTGATCCTGGCTCAG | Yilmaz et al., (2012) |
| 16S ITS rDNA(R) | 53 | CAAGGCATCCACCGT | Seifinejad et al. (2008) |
| Spcry1Aa (F) | 54 | TTCCCTTTATTTGGGAATGC | Seifinejad et al. (2008) |
| UNcry1 (R) | 51 | MDATYTCTAKRTCTTGACTA | Lep2 (F) |
| Lep2 (F) | 58 | CCGAGAAAGTCAAACATGCG | Boukedi et al. (2016) |
| Lep2 (R) | 58 | TACATGCCCTTACGTTCC | Lep2 (R) |
| St2C (F) | 56 | GGGACATTCCTGGTTCC | BenFarhat-Touzri et al. (2016) |
| St2Cinv(R) | 54 | GGTTGCGAGACTTTGAAC | UNcry2 (F) |
| St1D (F) | 49 | ATGGAATAATAATCAGAAAACC | BenFarhat-Touzri et al. (2016) |
| St1Dinv (R) | 51 | ACTAGATGGATACCTGTAC | Salehi Jouzani et al. (2008) |
| UNcry2 (F) | 55 | CGGATAAAATAAATCTGGGAATAGT | SPcry2Aa (R) |
| SPcry2Aa (R) | 57 | GAGATTAGTCGCCCTATGAG | Salehi Jouzani et al. (2008) |
| UNcry2 (F) | 55 | CGGATAAAATAAATCTGGGAATAGT | SPcry2Ab (R) |
| SPcry2Ab (R) | 65 | TGGCGITAACAATGGGGGAGAAAT | |
**Insect culture and bioassays**

The Mediterranean flour moth, *E. kuehniella*, was reared on a mix of flour and yeast (10:1) diet under laboratory conditions (27 ± 1 °C, 60 ± 5% RH, 16L (Light): 8D (Dark)), and the insecticidal activity of 9 high pathogenic isolates of *Bt* was examined against 12-day-old larvae. Accordingly, the bacterial isolates were grown on nutrient agar Petri dishes (8 cm diameter) at 35 °C. After 7 days, the spore–crystal mixtures were harvested by a loop tool and added to 10 ml of sterile distilled water with Tween 80 (0.02%).

Different concentrations of each *Bt* isolate and control (Tween 80 0.02%) were prepared. The reference strain, *Btk*, was used as a positive control. Experimental unit was a Petri dish (5.8 cm diameter) containing fifteen 12-day-old larvae fed on 1 g of the described diet. For bioassays, the diet described above was mixed with 1 ml of each concentration. Each treatment and the control were repeated three times at 27 ± 1 °C, 60 ± 5% RH and a photoperiod of 16L: 8D. The larvae were fed for 14 days, and during the period, dead larvae were checked and recorded daily.

**Statistical analysis**

The bioassay analysis was conducted using POLO-PC software (2002), and consequently, the value of LC50 (median lethal concentration) for each isolate was estimated.

**Results**

**Bacterial isolation and identification**

After checking the bacterial cultures, some *Bt*-like colonies were selected according their appearances. The colonies were cream colored or white, almost circular, and smooth or rough. All selected bacterial samples were gram positive.

**Crystal morphology**

The crystal-forming *Bt* strains were identified under a phase contrast microscope. The results showed that the *Bt* isolates produced different types of crystals including spherical (73.33%), bipyramidal (53.33%), irregular (40%), cubical (33.33%), and elliptical (13.33%). All isolates structured more than one type of crystal protein except 1036 (spherical type). The high pathogenic isolate, 1019, was examined by scanning electron microscopy. It was detected that the isolate produced the bipyramidal and spherical crystals (Fig. 1).

**Isolation of plasmid DNA**

Agarose gel electrophoresis showed different plasmid profiles for the native and reference isolates (Fig. 2). Nevertheless, most of the isolates exhibited similar pattern in the number and size of the plasmid bands to those of *Btk*. However, in the present study, the 1020 strain was similar to *Btg*, there was a small band in the native strain that was not appeared in the reference strain. Each of the 1032, 1033, 1036 and 1037 isolates presented variations in the plasmid profiles and the patterns were also different from the reference isolates. Moreover, all isolates showed the chromosomal DNA band, and there was only an obvious band for the 1032 isolate above the chromosomal band in terms of megaplasmid.

**Polymerase chain reaction (PCR)**

Specific primers were used for PCR amplification to distinguish different crystal genes including cry1 (A, C, and
D) and cry2A (a and b). The primers related to cry1D and cry2Aa1 genes produced no amplicons. All isolates exhibited to contain the cry IA with primer pairs Lep2 (Fig. 3a) and produced bands of the expected size. Some isolates including 1020 (986 bp) (NCBI Accession No. MW526365), 1022 (986 bp) (NCBI Accession No. MW567501), and 1032 (986 bp) (NCBI Accession No. MW567502) were randomly selected. The sequencing results declared their homology with cry1Ac.

In the present study, PCR unexpected band with a size of approximately 600 bp, corresponding to cry1Aa, were achieved for the 1032 isolate (Fig. 3b). Nevertheless, the 1002 and 1033 isolates produced bands of expected sizes, and the rest of isolates did not harbor any gene. All isolates with the exception of 1002 and 1046 were positive for the presence of cry1C (Fig. 3c). The 1003 isolate was only sequenced (895 bp) (NCBI Accession No. MW526366) and the gene had high identity to cry1Cb. All Bt isolates analyzed for the cry2Ab2 gene presented unexpected bands (Fig. 3d). However, the 1013, 1019, 1032, 1036, and 1046 isolates did not show any band.

**16S ITS rDNA gene analysis**

The 16S ITS rDNA gene analysis of the 1019 isolate was conducted based on the gene sequence. Based on the NCBI database, the BLAST results of the sequence showed more than 99% identity to all Bt strains used. According to the phylogenetic tree, 1019 was located in the same group with Bt subspecies coreanensis, Bt subspecies indiana, and Bt subspecies tolvorthi (Fig. 4).

**Protein electrophoresis**

Electrophoretic profile of the Iranian isolates was characterized by SDS–PAGE analysis and compared (Fig. 5). The protein patterns of tested isolates showed bands with different diversity in number and size ranged from about 16 to 140 KDa. Likewise, the results clarified that the most of shared bands produced above 45 KDa. The 2 isolates, 1013 and 1032 individually had unique protein pattern. Among the tested isolates, 1022 and 1046 nearly produced the same protein profiles that differed from the rest. The patterns created by the other isolates were almost similar and especially shared major protein bands with molecular weights of about 16, 17, 28, and 50 KDa, and a group of bands between about 45 and 140 KDa.

**Bioassays**

The results in Table 2 exhibited that the pathogenicity of 1019 strain was higher (with the lowest value of LC50) than the rest, even the reference strain, Btk. However, the toxicity of other strains was the same as Btk.
1032, 1037, and 1039 isolates had similar median lethal concentrations.

**Discussion**

The present study determined the characterization of some *Bt* strains collected from various biomes and sites of Iran. The results showed that 73.33 and 53.33% of the isolates produced spherical and bipyramidal crystals, respectively, compared to those of Seifinejad et al. (2008) who reported that most of Iranian isolates created bipyramidal ones. Similar to the obtained results, extraordinary variations of crystals (like spherical, irregular, bipyramidal) were observed among native *Bt* isolates collected from soil and other materials by El-kersh et al. (2016).

Among different characteristics of *Bt* strains, the plasmid profiles could help to identify strains (Saadaoui et al., 2010). Our result was in accordance with that of BenFarhat-Touzri et al. (2016), who demonstrated that BLB250 strain was identical to *Bt* strain *aizawai* and concluded that the strain might be a serotype of the reference isolate. Present study indicated also that some isolates from various regions and provinces had different plasmid profile even in comparison with the reference strains.

Likewise, Yilmaz et al. (2012) found several forms of plasmid patterns in the SY49.1 strain compared with *Btk* like Seifinejad et al. (2008) for Iranian isolates. Although, the 1013 and 1019 isolates were collected from different provinces, they showed the same pattern and produced one band.
Due to genetic diversity observed in the Bt strains and containing more than one cry gene located on the plasmids or chromosomes, polymerase chain reaction (PCR) can be a precise procedure to discover and characterize the genes (Salama et al., 2015). In the present study, all isolates exhibited bands of the expected size for cry1A, and few ones showed the expected bands for cry1Aa. Similar bands of the expected size for cry1A have been reported for new strains isolated from Tunisian soil samples with the primer pairs (BenFarhat-Touzri et al., 2016). On the contrary, Seifinejad et al. (2008) manifested that cry1Aa was the most abundant gene belonging to the strains isolated from different regions of Iran. Most of the strains harbored cry1C that was consistent with Seifinejad et al. (2008). However, previous study
showed that cry genes isolated from Iran exhibited unexpected size bands for cry1C (Nazarian et al., 2009). Totally, the findings of the present study are similar to those presented by Bravo et al. (1998) who reported that the most abundant gene was cry1 among the Mexican strains collected from soil samples. Compatible to our results, characterization of the strains isolated from Egyptian soil samples clarified that 77.77% of them contained the cry1C genes (Salama et al., 2015). Similar to the present study, Yilmaz et al. (2012) found that cry1A along with cry1C from the most pathogenic Bt strain (SY49.1) collected from Turkey. By comparison, Salehi Jouzani et al. (2008) elucidated that the most abundant gene detected from Iranian isolates was cry2-type such as cry2Ah (55%) and cry2Aa1 (37.5%). Compatible to the obtained results, all Iranian Bt strains isolated from soil and larvae harbored the cry2 gene, and some of them showed amplification products for cry1Ac, cry1Aa, and cry1C (Khorramnejad et al., 2018).

The results of the sequence analyzing of the highest pathogenic strain, 1019, showed more than (99%) identity to all Bt strains used. Yilmaz et al. (2012) analyzed the 16S rDNA gene of the SY49.1 strain and indicated that the similarity of the tested strain was (98%) with Bt subspecies andalouisiensis BGSC 4AW1 and Bt subspecies monterrey BGSC 4A1J. Moreover, the 16S rDNA gene analysis of native Bt isolates reported by Elkersh et al. (2016) showed that the high pathogenic isolate, BT63, had a strict relationship to Bt subspecies israelensis, and all isolates were homologous together. Consistent with our output, analysis of an Indian strain by the gene sequencing revealed high identity with B. cereus and Bt (Banik et al., 2019).

A previous investigation revealed that molecular mass of Cry1Aa, Cry1Ac, and Cry1C proteins were 133.2, 133.3, and 134.8 kDa analyzed through the SDS–PAGE, respectively (Kalman et al., 1995). All strains studied by Alper et al. (2016), harboring the cry1 and cry2 genes, and the reference strain, Btk, almost revealed similar crystal protein contents. Similar results have been achieved for the most Iranian strains studied in the present research. It was shown that all protein profiles of Iranian strains were almost similar to the reference strains, Btk and Btt, except those of 1013, 1022, 1032, and 1046 that were almost similar to the Btg protein pattern.

According to the present results, the 1013 and 1032 strains had different protein profiles. Both of them carried cry1Ac and cry1C; however, 1032 only showed an unexpected band for cry1Aa. Both of the 1022 and 1046 strains, with similar protein patterns, had cry1Ac; however, the 1022 strain harbored 2 extra genes including cry1C and cry2Ab2. Khorramnejad et al. (2018) clarified that the protein patterns of tested strains were similar to Btk with molecular mass between 60 and 130 kDa comprising Cry1Aa, Cry1Ab, Cry1Ac, Cry1Ca, Cry1Da, and Cry2Aa proteins. Also, these profiles were observed for the tested crystals in the present study.

Based on previous study, more than 50% of Iranian isolates structured proteins of 130–140 kDa, and there were also strains that produced proteins with a molecular mass between 28 and 140 kDa or even lower than 28 kDa (Seifinejad et al., 2008), in which the first range was active against lepidopteran pests (Herandez et al., 2005) that was in accordance with our findings. Additionally, the most of Iranian strains which harbored cry2-type genes showed protein bands of 21–140 kDa (Salehi Jouzani et al., 2008). Boukedi et al. (2016) reported that Bt HD1 and BUPM95 carried Cry proteins of 65–70 and 130–135 kDa related to the cry2 and cry1 genes, respectively. All investigations mentioned above for protein profiles of parasporal inclusions were in accordance with our results and confirmed that the Iranian strains were able to produce effective crystal protein toxin against lepidopteran pests, and the toxins were associated to their PCR profiles.

It was obvious that the cry1 and cry2A genes are active against lepidopteran insects (Park et al., 2011). Although, all tested strains especially 1019 were analyzed for the cry1A and cry1C genes and presented expected bands, they manifested different median lethal concentrations. Similarly, the high toxic Turkish strain, SY49.1, against E. kuehniella harbored the two latter genes (Yilmaz et al., 2012). Furthermore, E. kuehniella was so sensitive to the novel strain, BLB250, as it contained cry1A (BenFarhat-Touzri et al., 2016). Jalapathi et al. (2020) indicated that Cry1Ac protein toxin from Bt was highly pathogenic to the larvae of Tuta absoluta (Meyrick) (Gelechiidae: Lepidoptera) which was in accordance with our results. As it was obvious, our findings were consistent with those of previous outcomes, and the important role of cry1, as a determinant of pathogenicity against lepidopteran pests, was supported by the current results.

Consistent with this research, 2 Iranian strains, KON4 and YD5, harboring cry1A, cry1C, and cry2A, were more
pathogenic against *Helicoverpa armigera* (Hübner) larvae than *Btk* (Seifinejad et al., 2008). The highest mortality for *E. kuehniella* happened by the Tunisia isolate, BLB1, harboring cry1 and cry2 in comparison with *Btk* HD1 (Saadaoui et al., 2010). In comparison with the current bioassay result, some Turkish *Bt* strains harboring cry2Ab and cry2Aa1 led to 42% mortality in *E. kuehniella* (higher than *Btk*) (Alper et al., 2016).

The investigated Iranian strains had the lepidopteran-active cry genes; however, their toxicity potential against the Mediterranean flour moth varied. This finding was in close agreement with Ferrandis et al. (1999) that exhibited the inactivation possibility of the specific genes.

**Conclusions**

The present study found out that cry1 was the most abundant gene. Nonetheless, the strains that presented unexpected size bands might harbor putative novel cry genes against a new spectrum of insect pests. Likewise, it was concluded that the strains with the same toxicity to the Mediterranean flour moth had various cry genes, plasmid and crystal protein profiles, and vice versa. Therefore, characterization of cry genes of native strains could lead to access potent isolates as biocontrol agents against native insect pests.

**Abbreviations**

*Bt*: Bacillus thuringiensis; *Btg*: Bacillus thuringiensis subspecies galleriae; *Btk*: Bacillus thuringiensis subspecies kurstaki; *Bts*: Bacillus thuringiensis subspecies thuringiensis; *C*: Crystal; *CB*: Bipyrimal crystal; *CS*: Spherical crystal; *D*: Dark; *L*: Light; *LC*: Median lethal concentration; *NA*: Nutrient agar; *NCBI*: National Center for Biotechnology Information; *PCR*: Polymerase chain reaction; *RH*: Relative humidity; *SDS–PAGE*: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis; *Sp*: Spore

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**Authors’ contributions**

MR created the research plan, analyzed the data, and wrote the paper. MM, MT, and SS were advisors, and PSN performed the experiments. The authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable

**Competing interests**

The authors declare that there are no conflicts of interest in this publication.

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