Isolation and characterization of the local entomopathogenic bacterium, *Bacillus thuringiensis* isolates from different Egyptian soils

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Abstract

The local entomopathogenic bacterium, *Bacillus thuringiensis* (*Bt*) was isolated and characterized from 16 soil samples collected from different governorates in Egypt. Among 56 bacterial colonies obtained, only 16 colonies were characterized by traits of *Bacillus*. All the 16 isolates were toxic to the neonates of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Damietta and El-Sharkyia bacterial isolates showed appreciable mortality rates (100 and 96.6%), respectively, which were higher than that caused by the standard isolates of *Bt entomocidus* (that produce *Cry*1 C toxin) for which they were selected for further characterization. Scanning electron microscope of Damietta bacterial isolate showed the presence of a bipyramidal crystal protein; consistent with the presence of *Cry*1 toxin class, however, El-Sharkyia bacterial isolate produced spherical-shaped crystals consistent of *Cry*2 toxin class. Electrophoretic patterns of different isolates and standards revealed different molecular weight bands, ranged from 195 to 8KDa. Damietta and El-Sharkyia bacterial isolates produced major protein bands with molecular weights of 130 KDa, which was also present in *Bt entomocidus*. Both isolates also shared protein bands with *Bt entomocidus* with molecular weights of 80, 70, 65, 51, and 22 KDa. The 16S rRNA sequences of both isolates were submitted to the NCBI Gene Bank database, with accession numbers of LC070660 for Damietta isolate and LC070661 for El-Sharkiya isolate. The existence of different *Cry* genes in the 2 isolates was studied by PCR, using general primers of 5 *Cry* genes. *Cry1* gene was detected in both isolates; however, *Cry* 2 gene was detected only in Damietta isolate.

Keywords: *Bacillus thuringiensis*, Egyptian isolates, Crystal proteins, Bioassay, 16SrRNA, *Cry* genes

Background

Insect pests have adverse and damaging impacts on agricultural production and market access. Up to 28% of the world food production is damaged by insects, either in the field or during storage (Pimentel 2005). Current pest control strategies rely greatly on chemical insecticides, which lead to numerous harmful effects such as pesticide residues, development of insect resistance, and destruction of natural balance with beneficial insects. Such undesirable side effects prompted scientists to search for alternative, environmentally friendly control agents.

The entomopathogenic bacterium, *Bacillus thuringiensis* (*Bt*) is a rod-shaped, positive-gram, and spore-forming bacterium well-known for its insecticidal properties associated with its ability to produce crystal inclusions during sporulation. These inclusions are proteins encoded by *Cry* genes and have shown to be toxic to a variety of insects and other groups such as nematodes and protozoa (Sauka

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et al. 2010). *Cry* proteins primary function is to lyse midgut epithelial cells of the insects through insertion into the target membrane and eventually causing pore formation. Crystals are then solubilized in the alkaline environment of the midgut lumen and get activated by host proteases (Bravo et al. 2007). The characterization of *Cry* genes is critical in distinguishing the basic toxicity of *Cry* proteins active against certain insect orders (Schünemann et al. 2014). Identification of *Cry* genes by PCR technique has been exploited to predict the insecticidal activity of the *Bt* strains and to determine the distribution of *Cry* genes with a *Bt* strains (Ammouneh et al. 2010).

Although many *Bt* commercial products have been commonly used and various *Cry* proteins have been identified, identification of *Bt* strain is still ongoing. Because many insect species cannot be controlled by existing of *Bt* toxins and new *Bt* isolates are needed as alternatives when insect resistance to certain *Bt* appear or developed. Thousands of *Bt* isolates have already been collected worldwide by different authors (Ahmed et al. 2015, Hamedo 2016, Rabha et al. 2017, and Nair et al. 2018) in attempts to obtain new crystal proteins.

In the present study, soil samples, collected from different Egyptian Governorates, were screened for the presence of novel local *Bt* isolates.

Materials and methods
Samples collection
Quite numbers of Egyptian soil samples were collected from different governorates, covering most of Egypt from the north to the south, i.e., Matrouh, Alexandria, Domietta, Kafr-Elsheikh, El-Sharkyia, Dakahlyia, Gharbyia, Qalubyia, Beheira, Ismailia, North Saini (Rafah), Giza, Aswan, Luxor, Qena, and Elwadi Elgadeed. The soil samples were collected from areas that have never been exposed to *Bt* compounds (non-planted soils). Samples were collected by scraping off the surface material, using a sterile spatula. An aliquot of 50-100 g soil was obtained from the 5 cm below the surface, using a sampling soil cylinder (sample collector), as described by Ammouneh et al. (2010). The samples were placed in sterile plastic bags and stored at 4 °C until used.

Bacillus thuringiensis isolations
For isolating of *Bt* strains, the acetate selection method described by Travers et al. (1987) and modified by Ammouneh et al. (2010) was used. Half gram of each soil samples was suspended in 10 ml nutrient broth medium containing 0.25 M sodium acetate in a sterilized conical flask, and the mixtures were shacked at 180-200 rpm for 4 h at 30 °C. Heat treatment was then applied for 3 min at 80 °C to kill vegetative cells. The samples were then plated on nutrient agar plates and allowed to grow by incubation at 30 °C for 72 h. *Bt*-like colonies,

which are usually cream-colored and have the appearance of a fried egg on the plates were labeled and sub-cultured. Pure cultures resulted from sub-culturing were examined for parasporal crystal protein production and stored at − 80 °C in 20% glycerol stock for further use.

Bioassay against *Spodoptera littoralis*
All isolates, morphologically confirmed as *Bt* isolates, were tested for their insecticidal virulences against the newly hatched neonates of the cotton leaf worm, *Spodoptera littoralis* (Boisd.)(Lepidoptera: Noctuidae). *Cry*1C toxin produced from *Bt entomocidus* (Moussa et al. 2016) was used as a positive control.

Insect culture
The mother colony of *S. littoralis* was collected from a cotton field at Kafer-Elsheikh district. It was established under the lab conditions of 27 ± 1 °C, with a photoperiod of 14:10 (L: D) hours and 65-70% RH, using an artificial diet, described by Kranthi (2005) for 2 further generations before conducting the bioassay experiments.

Preparation of spore-crystal mixture of Bacillus thuringiensis
The spore-crystal mixture was prepared as described by Dulmage et al. (1970) with some modifications. The pure cultures of *Bt* isolates were shacked in nutrient broth for 72 h at 180-200 rpm and 30 °C. The growth was pelleted down for 10 min, and the pellet was re-suspended in 0.5 M sodium chloride for 10 min to avoid exoprotease activity. The suspension was centrifuged for 10 min at 10,000 rpm at 4 °C, and the pellets were washed thrice by sterile distilled water under cooling conditions. The pellet was suspended in 1/10-1/20 volume (based on original broth) of 6% lactose. The suspension was stirred for 30 min at room temperature and then centrifuged for 10 min at 10,000 rpm and 4 °C. Finally, 4 volumes of ice-cold acetone were added slowly, while stirring and the mixture was allowed to settle down, and then filtered through Whatman filter paper (no. 1) using a suction pump. Finally, the residue containing spore-crystal mixture was dried overnight in desiccators and then powdered, weighed, and stored in airtight sterile glass vials at 4 °C for further use.

Solubilization of crystal proteins
The solubilization process was done as described by Saravanan and Gujar (2005). The acetone powders of the spore-crystal complex of each *Bt* isolate was dissolved in solubilizing buffer (50 mM Na_2Co3, 10 Mm DTT). The samples were sonicated for 2 to 4 s, and the cell suspensions were incubated at 37 °C for 4 h. The supernatants containing solubilized crystal proteins were stored in

autoclaved Eppendorf tubes at − 20 °C for further bioassay.

Bioassay

The toxicity of the crystal protein (prepared as mentioned above) of 15 Bt isolates was evaluated against the newly hatched neonates of S. littoralis. The crystal protein was mixed with the artificial diets with a concentration of 4 μg/g diet and offered to the neonates. For each Bt isolate, 3 replicates (10 neonates each) were tested. Positive control, using the Cry1C toxin produced from Bt entomocidus (4 μg/g diet) was established. Control treatment was conducted, using the artificial diet only. Mortality observations were recorded every 24 h for 3 days. In order to obtain accurate results, each bioassay experiment was repeated thrice. The experiments were performed under laboratory conditions of 27 ± 1 °C and 65–70% RH. The percentages of larval mortality rates were corrected using Abbott's formula (Abbott 1925) and the most toxic Bt isolates with a mortality percentage above 90% were selected for further morphological, biochemical, and molecular characterization.

Characterizations of selected Bt isolates
Morphological characterizations
Gram staining test

The selected isolates were stained to observe Gram reaction. Cultures grown for 3 days in Luria-Broth media were microscopically examined to show the spore formation after staining according to the method described by Bartholomew and Mittwer (1952).

Scanning electron microscopy

An amount of 5 ml overnight freshly grown bacterial culture was harvested, fixed, dehydrated, and embedded essentially as described by Ammoumeh et al. (2010). The presence/absence of the spores and crystals shape of each isolate was examined, using the scanning electron microscope (Model JEOL.6390LA).

Biochemical characterizations
API E20 test

The Bt isolates were examined by API E20 system for relevant biochemical reactions to help in determining possible biochemical types. A single colony from each isolate was selected and emulsified into inoculating fluid for subsequent inoculation onto the micro plate test (MPT). The inoculum prepared to a specified transmittance, using a turbidity meter, as specified in the user guide described by Logan and Berkeley (1984).

Temperature and Na Cl tolerance

The growth rate response of the selected Bt isolates to different temperatures and Na Cl concentrations was tested. An amount of 5 ml of Luria-Broth (LB) was inoculated by 50 μl of freshly grown (16 ± 2 h) native Bt isolates and was shacked at different temperatures (25, 30, 40, and 50 °C) for 24 h. The tubes were observed for growth, which was indicated by turbidity change, following the method reported by Hamedo (2016) as the optical density was observed at 590 nm. Tolerance to Na Cl for the selected Bt isolates was tested by inoculation of 50 μl of freshly grown Bt isolates in 5 ml of Luria-Broth (LB) with different Na Cl concentrations (1, 2, 4, 6, and 8%), and the turbidity change was observed as mentioned above.

Molecular characterization
Protein characterization of Bt isolates

Protein profile of the selected Bt isolates and Bt entomocidus Cry 1C toxin was studied, using SDS-PAGE. The purified crystal protein of the selected Bt isolates was mixed by the sample buffer (0.5 M Tris–HCl pH 6.8, 25% glycerol, 1.0% blue of bromophenol, 10% SDS, and 1% 2-mercaptoethanol) in the ratio of 1:1. This mixture was then boiled at 100 °C for 10 min along with the protein molecular marker. Samples were loaded on SDS-PAGE (4% stacking gel and 10% separating gel). Electrophoresis was performed in a vertical system (Bio-Rad system) filled with 1 × run buffer (25 mMTris-base, 35 mM SDS, and 1.92 mM glycine) and charged at 150 V. After the run, the gel was stained by Comassie Brilliant Blue solution (50% methanol, 10% acetic acid, and 0.1% Comassie Brilliant BlueR) for 1 h at room temperature, and then destained in a 4:1 methanol:acetic acid solution for 24 h, until visualization of the protein bands corresponding to the toxins. The gel was observed for the presence of proteins of interest-based on the published data.

16S rRNA gene sequencing

The genomic DNA of the selected Bt isolates was isolated, using Gene JET genomic DNA Purification Kit (cat. #K0721), following the protocol provided by the manufacture. 16SrRNA gene was amplified by the genomic DNA of

Table 1 Oligonucleotide primers used for screening of partial Cry type genes

Cry gene	F	R
CryI	5′CCGGTGCTGGATTTGTGTTA 3′	5′AATCCCGTATTGTACC AGCG 3′
CryII	5′GTTATTCTTAATGCAGATGA ATGGG 3′	5′CGGATAAAATAATC TGGGAAATAGT3′
CryIII	5′AGGTGCCAACTAACCATGTT3′	5′GATCCTATGCTTGGTC TAGT 3′
CryIV	5′CAAGCCGCAAATCTTGTGGA 3′	5′ATGGCTTGTTTCGCTA CATC 3′
CryV	5′TTACGTAAATTGGTCAATCAAG CAAA3′	5′AAGACCAAATTCAA TACCAGGGTT3′

Bt isolates as described by Weisberg et al. (1991), using the universal primer pair of F (5′AGAGTTTGATCCTGGCT-CAG′3) and R (5′TACGGYTACCTTGTTACGACT′3). The PCR conditions were as follows; 95 °C for 5 min, 95 °C for 30 s, 55 °C for 30 s, 37 cycles, and 72 °C for final extension step. The amplified PCR product was resolved on 0.8% agarose gel and PCR products were then sent for sequencing. The 16S rRNA sequences were submitted to NCBI (National Center for Biotechnological Information) and the sequence was compared by the published sequenced of NCBI database.

Detection of crystal protein gene

The PCR technique was used to detect the presence of crystal protein genes. Five pairs of primers specified for *Cry*1, *Cry* 2, *Cry* 3, *Cry* 4, and *Cry* 5 genes were used and amplified as described by Theoduioz et al. (1997) and Jain et al. (2012) (Table 1). The PCR products were resolved in 0.8% agarose gel and 1 kb DNA Ladder (Promega) was used as a marker of molecular weight. The amplification products were visualized and photographed under UV light.

Results and discussion

Isolations of local *Bacillus thuringiensis*

In the present study, 16 soil samples were collected from different Egyptian governorates. Following observations under a light microscope, a total of 56 *Bacillus*-like colonies were identified of which only 16 isolates (one colony per sample) were putatively identified as *Bt* with a *Bt* index (number of identified *Bt* colonies divided by the total number of *Bacillus*-like colonies) of 0.286.

Screening of soil for novel and potent strain of *Bt* is one of the world strategies for pest management. Many studies were conducted to establish a worldwide collection of *Bt* isolates and proving the ubiquity of *Bt* that are found in any type of soil (Apaydin et al. 2005, Ammouneh et al. 2010, Hamedo 2016, and Nair et al. 2018). *Bt* has a circular shape, rough, and smooth surface of colony, slightly glossy, glossy, white, and yellowish-white

Table 2 Toxicity of *Bacillus thuringensis* isolates against the neonates of the cotton leaf worm, *Spodoptera littoralis*

Isolate	Soil type	Mortality %
Matrouh	Clay	30.0
Elwadi- Elgded	Sandy	66.6
Dakhlyia	Clay	66.6
Qena	Clay	40.0
Giza	Clay	63.3
Aswan	Clay	23.3
Beheira	Sandy	40.0
Luxor	Clay	30.0
Damietta	Clay	100
Kafr-Elsheikh	Clay	66.6
Ismalia	Clay	16.6
Alexandria	Sandy	26.6
Qalubyia	Clay	66.6
Rafah	Clay	13.3
Elsharkyia	Clay	96.6
Gharbyia	Sandy	53.3
Positive control	-	89.66

colonies (Salaki 2010). A total of 2671 colonies from 93 Egyptian soil samples were previously examined (Salama et al. 2012), and the total number of *Bt* positive soil samples was 40/93, i.e., 43.01%. The results indicated that the percentage of the occurrence of *Bt* in these samples was 3.818%. Ahmed et al. (2015) isolated 334 colonies from 59 soil samples in 13 local areas in Egypt of which only 16 isolates were identified as *Bt.*

Toxicity of *Bacillus thuringiensis* against *Spodoptera littoralis* neonates

A single bioassay concentration (4%) of the tested *Bt* isolates and the positive *Cry*1C toxin (produced from *Bt entomocidus*) against the neonates of *S. littoralis* was conducted by providing the larvae with a diet containing the crystal protein of each isolate. All the tested 16 *Bt*
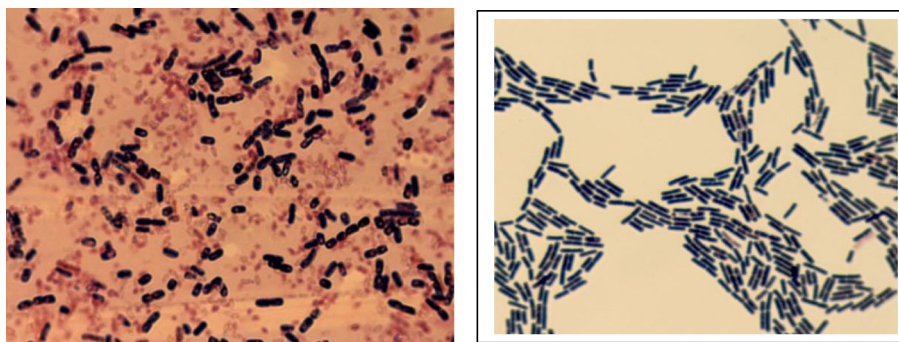
Fig. 1 Light microscope photomicrograph of Gram stained *Bacillus thuringensis* isolates, Damietta isolate (left) and El-Sharkyia isolate (right)

isolates caused mortality in the *S. littoralis* neonates after 96 h of treatment (Table 2). The mortality percentages ranged from 13.3% in the case of *Bt* isolated from Gharbyia to 100% mortality in the case of *Bt* isolated from Damietta region compared to the mortality of 89.66% obtained by the positive *Cry*1C toxin. High toxicity was observed in the case of *Bt* isolated from El-Sharkyia with a mortality percentage of 96.6%. Moderate toxicity rate (66.6%) was recorded in the case of the *Bt* isolated from Elwadi-Elgdeed, Dakahlyia, Kafr-Elsheikh, and Qalubyia. This result was consistent with that of Ammouneh et al. (2010) who reported that all the local *Bt* isolates were toxic to the tested lepidopteran larvae. Aly et al. (2009) isolated 8 *Bt* isolates from 7 Egyptian governorates. Two isolates caused 90 and 100% mortality rate against *S. littoralis*, using bacterial spores. Among the 16 *Bt* isolates, HD-1 isolate produced 86% mortality against *S. littoralis* (Ahmed et al. 2015). The highest mortality percentage range (80-96%) was obtained against *Helicoverpa armigera* (H.) by *Bt* isolates from Egyptian soils (Salama et al. 2015). As Damietta and El-Sharkyia bacterial isolates showed the highest toxicity, they were selected for further morphological, biological, and molecular characterization.

Morphological characterizations
Gram stain test
Gram stainings of Damietta and EL-Sharkyia bacterial isolates revealed Motil and Gram-positive rods with refractile spores that do not swell the cells (Fig. 1).

Scanning electron microscopy
Damietta and EL-Sharkyia bacterial isolates were investigated by an electron microscope for crystal protein morphology. As shown clearly in Fig. 2, Damietta bacterial isolate produced bipyramidal crystal proteins. On the other hand, El-Sharkyia bacterial isolate produced spherical-shaped crystals. It can be observed that some
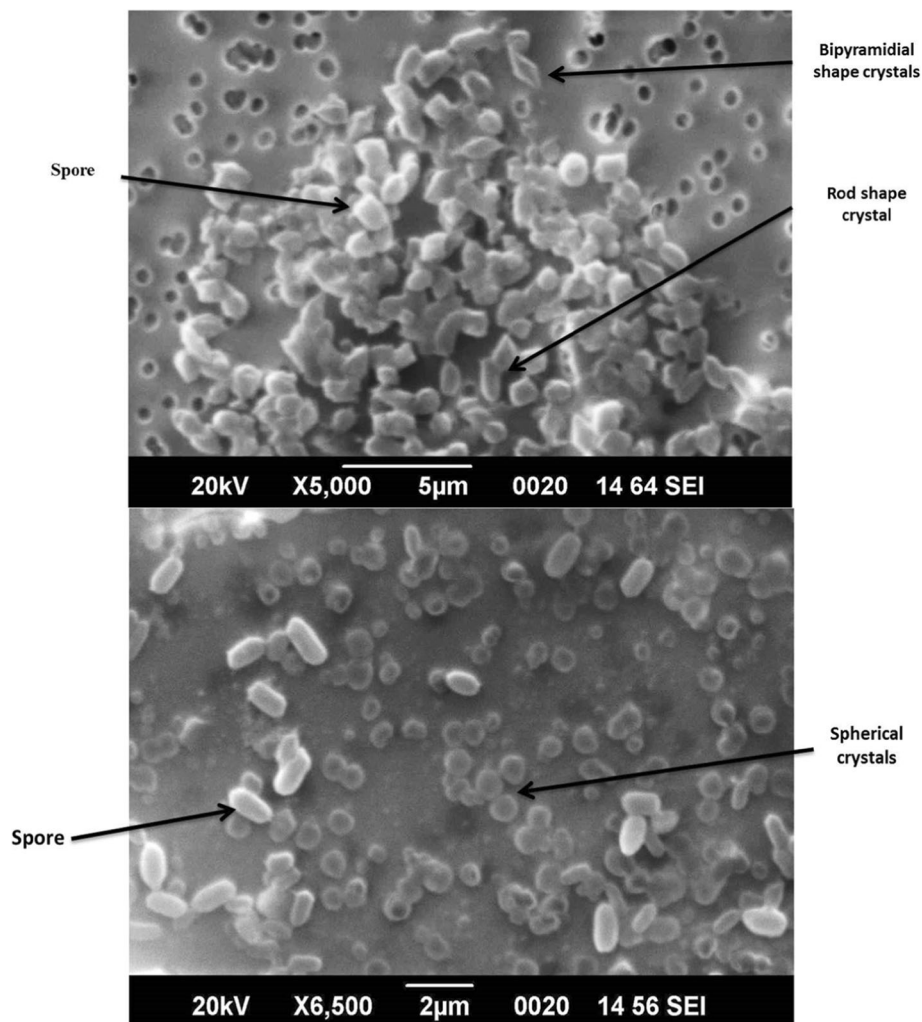
Fig. 2 Scanning electron micrograph of a sporulating culture of *Bacillus thuringiensis* isolates, (**a**) Damietta isolate and (**b**) EL-Sharkyia isolate

cells were lysed and spores and crystals released into the medium whereas the others were intact. Crystal morphology of Bt can give information about target insect spectra (Maeda et al. 2000). Federici et al. (2006) reported that the Bt strain produced bipyramidal Crystal proteins exhibited toxicity only to lepidopteran pests and were associated with Cry1, whereas cuboidal crystal proteins exhibited toxicity to Lepidoptera and Diptera and were associated with Cry2 toxin.

Biochemical characteristics of the bacterial isolates

API E20 test

Obtained data of sugar utilization, using the API 20E system for Damietta and El-Sharkyia bacterial isolates (Table 3) revealed that both isolates showed positive reactions in API 20E to ADH (decarboxylation of the amino acid arginine by arginine dihydrolase), TDA (tryptophan deaminase) and VP (the Voges-Proskauer test for the detection of acetone (acetyl methylcarbinol) produced by fermentation of glucose by bacteria utilizing the butylene glycol pathway). Contrastingly ONPG, LDC, ODC, CIT, H_2S, URE, IND, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, and ARA tests were negative in both isolates. Fakruddin et al. (2012) stated that 47 strains of Bt fermented glucose, maltose, and

Table 3 Sugar utilization of *Bacillus thuringensis* isolates using the API 20E system

Traits	Damietta isolate	El-Sharkyia isolates
ONPG	−	−
ADH	+	+
LDC	−	−
ODC	−	−
CIT	−	−
H_2S	−	−
URE	−	−
TDA	+	+
IND	−	−
VP	+	+
GEL	−	−
GLU	−	−
MAN	−	−
INO	−	−
SOR	−	−
RHA	−	−
SAC	−	−
MEL	−	−
AMY	−	−
ARA	−	−

trehalose, whereas they could not grow in the presence of arabinose, mannitol, rhamnose, sorbitol, lactose, or xylose.

Temperature and NaCl tolerance

El-Sharkyia bacterial isolate was able to tolerate up to 50 °C; however, Damietta were unable to tolerate 50 °C. Both isolates represented high growth rates at 35 °C. Damietta and El-Sharkyia bacterial isolates were tolerant to different concentrations of NaCl (1-8%), and could grow at the lowest concentration of NaCl (1%) (Table 4). The results revealed that the rates of bacterial growth decreased with increasing the salinity concentrations, this was in consonant with the report of Venosa and Zhu (2003).

Molecular characterizations

Taxonomic study of Bt isolates, using microbiological and physiological characters, is difficult as many Bt isolates are microbiologically indistinguishable, so identification by using molecular techniques seems to be a good solution (Ammouneh et al. 2010).

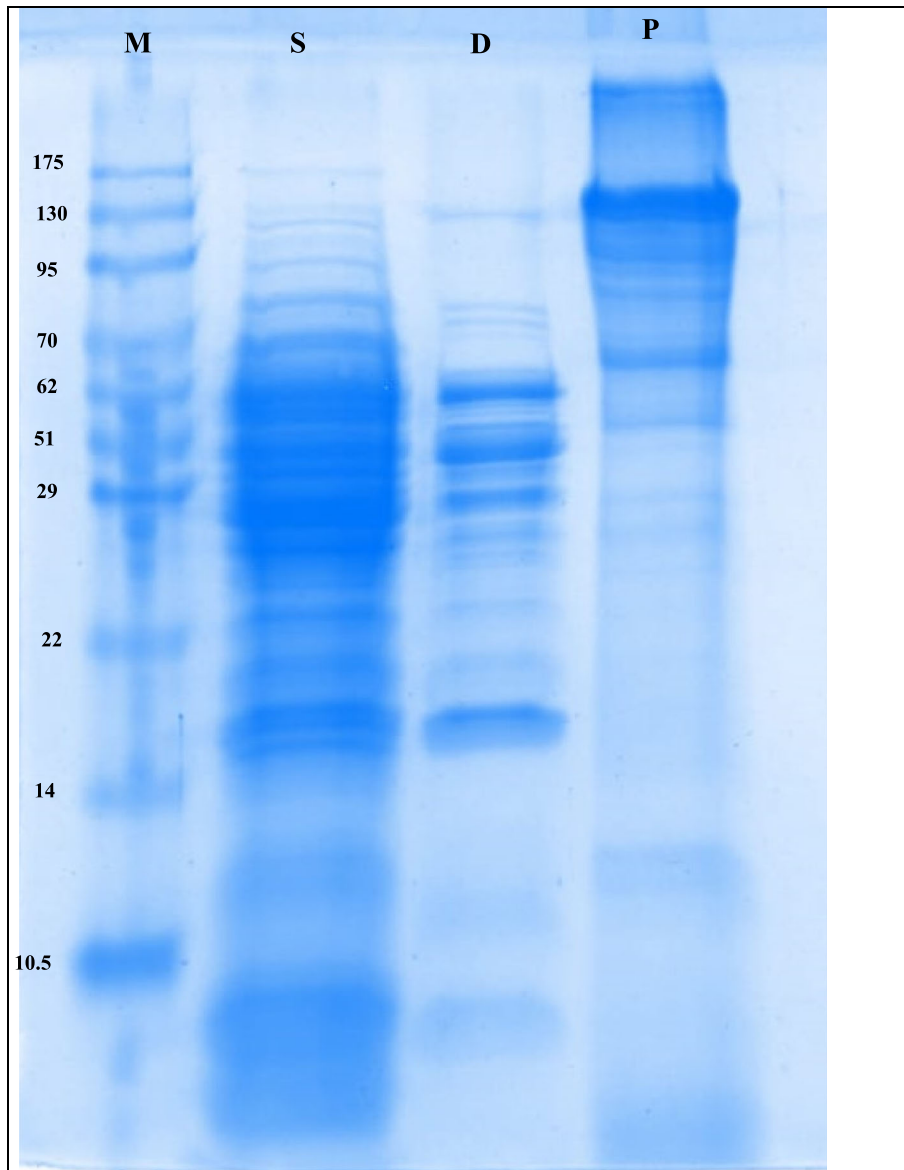### Protein characterization of Bt isolates

SDS-PAGE profile of the acetone powder of spore-crystal mixture of Damietta and El-Sharkyia bacterial isolates along with *Bt entomocidus* is illustrated in Fig. 3. Electrophoretic patterns of different isolates and standards revealed bands with different molecular weights, ranged from 195 to 8KDa. Damietta and El-Sharkyia bacterial isolates produced a major protein band with molecular weights of 130 KDa, which was also present in *Bt entomocidus*. Both isolates shared protein bands with *Bt entomocidus* with molecular weights of 80, 70, 65, 51, and 22 KDa. Damietta and El-Sharkyia bacterial

Table 4 Growth of *Bacillus thuringensis* isolates in different Na Cl concentrations and temperatures

Growth rate	Damietta isolate	El-Sharkyia isolate
Na Cl concentration (%)		
1	++++	++++
2	+++	+++
4	++	+++
6	−	++
8	−	−
Temperature (°C)		
25	++	++
30	+++	+++
35	++++	++++
40	++	+++
50	−	++

Fig. 3 SDS-PAGE of spore-crystal mixture from *Bacillus thuringensis* isolates. M, protein marker, S, El-Sharkyia isolate, D, Damietta isolate, and P, positive control from *B. thuringiensis entomocidus*

isolates shared a protein band of 17 KDa, which was undetected in *Bt entomocidus*.

Chilcott and Wighley (1993) reported that protein crystals of *Bt* isolates, the toxic against lepidopteran larvae, contained 130-65-kDa proteins at varying amounts. However, isolates that were toxic to dipterans contained proteins with molecular weights of 130, 68, and 28-kDa. Isolates that were toxic to coleopterans contained a 68-kDa protein. Donovan et al. (1988) reported that *Bt* isolates were toxic to lepidopteran/dipteran, contained proteins of 130 and 65-kDa. However, nontoxic isolates were found to synthesize proteins of 45 and 40 kDa. Ammouneh et al. (2010) reported that the presence of

protein bands of 130, and 65 kDa supported the suggestion that *Cry* 1 and *Cry*2 genes were expressed in the tested isolates. Salama et al. (2015) revealed that *Bt* isolates from Egyptian soils have protein bands with different molecular weights, ranged from 197 to 21 KDa, and a shared protein band of 130 KDa was detected in the most potent *Bt* isolates. Protein profiles of purified crystals of *Bt* isolates from Brazil showed polypeptides of ~ 70 and 140 kDa (Cerqueira et al. 2016).

16S rRNA gene sequencing
16S rRNA gene is one of the most reliable methods for the identification of bacteria at the species level. 16S
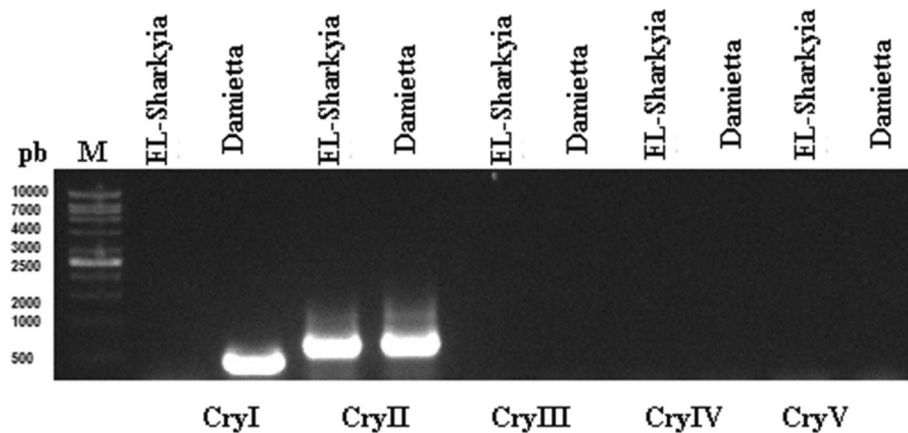
Fig. 4 Screening of selected *Bacillus thuringiensis* isolates using *Cry*I, *Cry*II, *Cry*III, *Cry*IV, *Cry*V specific oligonucleotide primers. M, represents page roller marker

rRNA gene from the selected *Bt* isolates was used. DNA from each isolates was amplified by the presence of primers for variable regions of 16S rRNA gene. Each isolate gave only one band at the expected size. Results of sequence alignment of Domietta bacterial isolate had (99%) similarity with the published sequence of *Bt* 407. El-Sharkyia bacterial isolate had (98%) similarity with the published sequence of ATCC 10792 *Bt* isolate. This confirms that Damietta and El-Sharkyia bacterial isolates were identified and characterized below the *Bt* family. The sequences of both isolates were submitted to the NCBI Gene Bank for 16S rRNA and accession number of LC070660 for Damietta bacterial isolate and LC070661 for El-Sharkyia bacterial isolates.

Detection of crystal protein gene

The *Cry* gene of content of a *Bt* isolate correlates to some extent to its insecticidal virulence (Ammouneh et al. 2010). The total DNA prepared from the selected isolates (El-Sharkyia and Damietta) was subjected to PCR analysis, using 5 pairs of oligonucleotide primers specific to *Cry*1, *Cry* 2, *Cry*3, *Cry*4, and *Cry*5. On the basis of the size of the PCR products, Damietta isolate amplified a single fragment of about 490 bp indicating that this isolate would belong to *Cry1* gene family. Damietta and El-Sharkiya bacterial isolates amplified a single fragment of about 698 bp indicating that both isolates would belong to the *Cry2* gene family (Fig. 4). Damietta and El-Sharkiya bacterial isolates did not yield any product with the *Cry*3, *Cry*4, and *Cry*5 primers. It is well known that the proteins toxic for lepidopteran insects belong to *Cry*1, *Cry*2, and *Cry*9 groups (Crickmore et al. 1998 and Xavier et al. 2007). *Cry*1 gene was detected in HD-1 *Bt* isolate from Egyptian soil that was effective against *S. littoralis* (Ahmed et al. 2015). Among

18 *Bt* isolates from the Egyptian soils were toxic to *S. littoralis* and *H. armigera*, *Cry*1 gene was detected in 15 isolates, while *Cry*2 gene was detected in 17 isolates (Salama et al. 2015).

Conclusion

The present study demonstrated how the *Bt* from diverse habitats differs in their protein and DNA profiles, which is reflected in varying levels of insecticidal virulence. Two potent *Bt* isolates from Damietta and El-Sharkyia were identified and characterized, and could play a crucial role in insect management programs. Continuous efforts to isolate novel *Bt* isolates from different environments and genetic manipulations of such isolates may be helpful in solving the problems of insect resistance.

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