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SHORT COMMUNICATION

# Characterization of Insecticidal Genes of *Bacillus thuringiensis* Strains Isolated from Arid Environments

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**Abstract** This study aimed at characterizing the insecticidal genes of eight *Bacillus thuringiensis* isolates that were recovered from the local environment of western Saudi Arabia. The screening for the presence of lepidopteran-specific *cry1A* family and *vip3A* genes, dipteran-specific *cry4* family and coleopteran-specific *cry3A*, *vip1A* and *vip2A* genes, was carried out by PCR. All eight isolates produced PCR products that confirmed the presence of *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* genes, but not *cry3A*, *vip1A* and *vip2A* genes. However, three isolates only were found to carry *vip3A* genes as revealed by PCR. The observation of *cry1* and *cry4* genes suggests that these eight isolates may have dual activity against Lepidoptera and Diptera species, while three isolates possessed *vip3* genes in addition to *cry1* and *cry4* which suggests that these three isolates have toxic crystals and vegetative proteins. The results of this study are interesting in the sense that they may help developing new strategies for controlling insects of economic and medical importance in Saudi Arabia, using *B. thuringiensis* strains that naturally exist in the local environment instead of the current control strategies that are based solely on chemical insecticides.

**Keywords:** *Bacillus thuringiensis* · *cry* Genes · PCR · *vip* Genes

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The insecticidal activities of *Bacillus thuringiensis* are mainly related to the production of parasporal inclusions, which are formed by polypeptides known as Cry proteins. These proteins showed entomopathogenic activities against wide spectrum of insect orders [1]. Other virulence factors that may play a role in the toxic activities of *B. thuringiensis* include vegetative insecticidal protein (vip), phospholipases, proteases, and chitinases [2]. Because of their specificity and safety to the environment, the crystal and vegetative proteins have been widely used for many years as biopesticides for the control of insect pests in agriculture, forestry and in the home [3, 4].

Despite the wide spectrum of *B. thuringiensis* toxicity against invertebrate, there were a number of crystal-bearing *B. thuringiensis* strains that exhibited no toxic activities [5], or their activities may threaten by the possible evolution of resistance from susceptible insect host [6]. Thus, there is a great interest for screening *B. thuringiensis* collection strains [7–9], and/or the isolation of novel strains/toxins [10–12], to discover insecticidal genes with broader insect host range [13].

We managed to isolate eight strains of *B. thuringiensis* for the first time from the environment of western Saudi Arabia. These isolates were found to be very toxic to Lepidoptera as revealed by toxicity assay [14]. We were unable to investigate the toxicity of these isolates against insects belonging to orders other than Lepidoptera by bioassay. Thus the aim of this study was to predict the insecticidal activity of our eight *B. thuringiensis* isolates by PCR.

The *B. thuringiensis* strains analyzed in this work were previously isolated from soil samples and dead larvae of *Spodoptera littoralis* collected from the environment of Makkah Province, Saudi Arabia. These eight isolates were confirmed by morphological and molecular methods, and

**Table 1** Sequence of primers used in PCR screening

| Primer | Sequence | Product size (bp) | Gene(s) recognized | Reference |
|---|---|---|---|---|
| Lep1A | 5′-CCGGTGCTGGATTTGTGTTA-3′ | 490 | *cry1Aa*, *cry1Ab*, *cry1Ac* | [15] |
| Lep1B | 5′-AATCCCGTATTGTACCAGCG-3′ | | | |
| Dip1A | 5′-CAAGCCGCAAATCTTGTGGA3-′ | 797 | *cry4A*, *cry4B* | [15] |
| Dip1B | 5′-ATGGCTTGTTTCGCTACATC-3′ | | | |
| Col1A | 5′-GTCCGCTGTATATTCAGGTG-3′ | 649 | *cry3A* | [15] |
| Col1B | 5′-CACTTAATCCTGTGACGCCT-3′ | | | |
| 5-vip1A | 5′-GGATCCGATGAAAAATATGAAGAA-3′ | 2300 | *vip1A* | [16] |
| 3-vip1A | 5′-GTCGACTTATCTAGATTTGTTAGGT-3′ | | | |
| 5-vip2A | 5′-GGATCCGATGAAAAGAATGGAGGG-3′ | 1300 | *vip2A* | [16] |
| 3-vip2A | 5′-GTCGACTTAATTTGTTAATAATGTTG-3′ | | | |
| vip3A | 5′-ATGAACAAGAATAATACTAAA-3′ | 2300 | *vip3A* | [17] |
| vip3A | 5′-GCGGCCGCTTACTTAATAGAGAC-3′ | | | |

finally by toxicity assay against *S. littoralis*. These strains were identified as *B. thuringiesis* IBL 200 (GenBank accession number: NK01000211) [14].

Screening of toxicity genes was carried out by PCR. Primers used for the amplification of crystal toxic genes were Lep1A/Lep1B Lepidopteran-specific, Dip1A/Dip1B Dipteran-specific and Col1A/Col1B Coleopteran-specific crystal genes [15]. For amplification of vegetative toxic genes, primers 5-vip1A/3-vip1A, 5-vip2A/3-vip2A [16] and vip3A [17] were used. The sequence of each primer, the target genes and the expected product size are listed in Table 1.

PCR mixtures were prepared as described by Carozzi et al. [15], briefly, a loopful of cells from an overnight culture, was suspended in 100 µl sterile water and boiled for 10 min. One microliter of this suspension was used as template DNA and was added to 50 µl PCR mix containing 0.25 mmol $l^{-1}$ dNTPs, 1 mmol $l^{-1}$ $MgCl_2$, 0.6 mmol $l^{-1}$ of each primer (Bioneer, Alameda, USA), and 1 unit *Taq* DNA polymerase (ABgene, Surry, UK). Amplification was done under the following conditions: a 45 s denaturation step at 95°C, anneal for 45 s at 45°C, and extended at 72°C for 1 min, for total of 35 cycles. A total of 20 µl of PCR reaction mix was analyzed by gel electrophoresis on a 0.8% agarose gel (Bioline, London, UK) in Tris–borate buffer, and made visible by ethidium bromide (0.5 µg $ml^{-1}$) staining and UV transillumination.

The results showed that all eight isolates were found to produce PCR products with 490 bp when primers Lep1A and Lep1B were used. Similarly, when primers Dip1A and Dip1B were used, the amplicon size of PCR products from all eight isolates was 797 bp. However when primers Col1A and Col1B were used, no PCR products were observed. With regards to vegetative toxic genes, PCR products of 2,300 bp were observed when primers vip3 from three out of eight isolates (Fig. 1), while no PCR products from all eight isolates were observed when primers vip1 and vip2 primers were used.

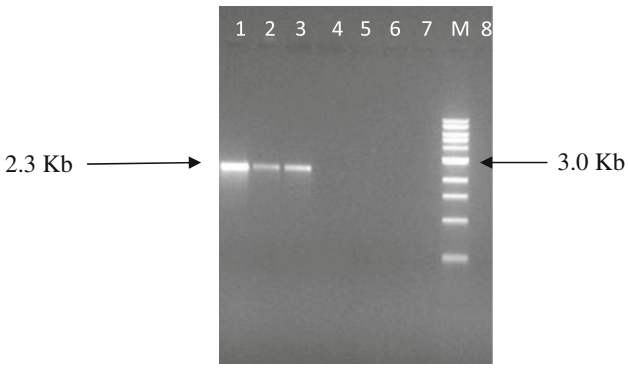


**Fig. 1** Agarose gel electrophoresis of PCR products amplified from native *B. thuringiensis* isolates with vip3A forward and reverse primers. *Lane M* 1 kb DNA ladder, *Lanes 1–8 B. thuringiensis* isolates Bt1 to Bt8

The PCR method we have used was suggested to be a useful rapid screening test to predict the insecticidal activities of new *B. thuringiensis* isolates [15]. The use of these primers had provided valuable data about the content and the activity of toxic genes in a large number of *B. thuringiensis* strains worldwide [8–12, 15, 18–21].

The content of *cry* genes in our isolates appears to be in concordance with the toxicity of these strains against *S. littoralis* previously reported by Assaeedi et al. [14]. The observation of a 490 bp band with primers Lep1A/Lep1B, confirms the presence of *cry1Aa*, *cry1Ab* and *cry1Ac* genes (Table 2). The *cry1* genes family is widely known for their activity against Lepidoptera [12]. The *cry1Aa*, *cry1Ab* and *cry1Ac* are probably the most common profiles of *cry1* gene family that are carried by lepidopteran-toxic *B. thuringienisis* strains [7, 10, 22, 23].

When primers Dip1A/Dip1B were used, PCR profiles suggest that these isolates carry *cry4A* and *cry4B* genes,

**Table 2** Crystal and vegetative toxic genes of eight *Bacillus thurngiensis* isolates as determined by PCR

| Bt isolate no. | Source | Crystal genes present | Vegetative genes present |
|---|---|---|---|
| Bt1 | Urban soil | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | *vip3A* |
| Bt2 | Urban soil | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | *vip3A* |
| Bt3 | Agricultural soil | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | *vip3A* |
| Bt4 | Agricultural soil | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | – |
| Bt5 | Agricultural soil | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | – |
| Bt6 | Dead larvae | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | – |
| Bt7 | Dead larvae | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | – |
| Bt8 | Dead larvae | *cry1Aa*, *cry1Ab*, *cry1Ac*, *cry4A*, *cry4B* | – |

and may have activity against Dipteran species (Table 2). Similar results were reported elsewhere using various sets of primers, and it was observed that insecticidal activity predicted by PCR corresponded with the insecticidal activity of insect bioassay [15, 19]. In this respect we suggest that our eight *B. thuringiensis* isolates may have dual insecticidal activities against Lepidoptera and Diptera.

PCR analysis of all eight isolates yielded no detectable *cry3A* gene products when primers Col1A/Col1B were used (Table 2). This result may suggest that these eight isolates do not carry *cry3A* gene. Similarly, Chak et al. [22] reported the absence of *cry3* genes in 225 isolates of *B. thuringiensis* in Taiwan. The observation of no PCR products, may also suggests that the genes are gone undetected [15].

The *vip3A* genes are toxic to Lepidoptera and other species, with different mode of action from *cry* genes [24]. We found that (37.5%) of our eight isolates carried *vip3A* genes (Table 2). Similarly, the low frequency of *vip3A* genes was reported elsewhere [25]. The coleopteran-specific *vip1* and *vip2* genes were not detected in any of the eight isolates (Table 2). Altogether the absence of *cry3A*, *vip1A* and *vip2A* genes further suggests that these isolates may have no insecticidal activity against coleopteran species.

In conclusion, the findings of this study are very interesting and need further investigation since dengue fever is widely common in Makkah region [26], thus, if the *cry4* genes of these eight isolates are active against *Aedes aegypti*, it would be best developing control strategies of this pest in particular, or other insects of economic importance in general, using locally derived *B. thuringiensis* strains instead of the current control strategies that are based solely on chemical insecticides.

## References


1. van Frankenhuyzen K (2009) Insecticidal activity of *Bacillus thuringiensis* crystal proteins. J Invertebr Pathol 101:1–16
2. Chattopadhyay A, Bhatnagar NB, Bhatnagar R (2004) Bacterial insecticidal toxins. Crit Rev Microbiol 30:33–54
3. Crickmore N (2006) Beyond the spore—past and future developments of *Bacillus thuringiensis* as a biopesticide. J Appl Microbiol 101:616–619
4. Roh JY, Jae YC, Ming SL, Byung RJ, Yeon HJ (2007) *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. J Microbiol Biotechnol 17:547–559
5. Yasutake K, Binh ND, Kagoshima K, Uemori A, Ohgushi A, Maeda M, Mizuki E, Yu YM, Ohba M (2006) Occurrence of parasporin-producing *Bacillus thuringiensis* in Vietnam. Can J Microbiol 52:272–365
6. Herrero S, Oppert B, Ferré J (2001) Different mechanisms of resistance to *Bacillus thuringiensis* toxins in the Indian meal moth. Appl Environ Microbiol 67:1085–1089
7. Wang J, Boets A, Van Rie J, Ren G (2003) Characterization of *cry1*, *cry2*, and *cry9* genes in *Bacillus thuringiensis* isolates from China. J Invertebr Pathol 82:63–71
8. Seifinejad A, Salehi Jouzani GR, Hosseinzadeh A, Abdmishani C (2008) Characterization of Lepidoptera-active cry and vip genes in Iranian *Bacillus thuringiensis* strain collection. Biol Control 44:216–226
9. Hernández-Rodríguez CS, Boets A, Van Rie J, Ferré J (2009) Screening and identification of *vip* genes in *Bacillus thuringiensis* strains. J Appl Microbiol 107:219–225
10. Armengol G, Escobar MC, Maldonado ME, Orduz S (2007) Diversity of Colombian strains of *Bacillus thuringiensis* with insecticidal activity against dipteran and lepidopteran insects. J Appl Microbiol 102:77–88
11. Gao M, Li R, Dai S, Wu Y, Yi D (2008) Diversity of *Bacillus thuringiensis* strains from soil in China and their pesticidal activities. Biol Control 44:380–388
12. Saadaoui I, Rouis S, Jaoua S (2009) A new Tunisian strain of *Bacillus thuringiesis kurstaki* having high insecticidal activity and δ-endotoxin yield. Arch Microbiol 191:341–348
13. Franco-Rivera A, Benintende G, Cozzi J, Baizabal-Aguirre VM, Valdez-Alarcon JJ, Lopez-Meza JE (2004) Molecular characterization of *Bacillus thuringiensis* strains from Argentina. Antonie van Leeuwenhoek 86:87–92
14. Assaeedi ASA, Osman GEH, Abulreesh HH (2011) The occurrence of *Bacillus thuringiensis* in the arid environments. Aust J Crop Sci 5:1185–1190
15. Carozzi NB, Kramer VC, Warren GW, Evola S, Koziel MC (1991) Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product profiles. Appl Environ Microbiol 57:3057–3061
16. Shi Y, Xu W, Yuan M, Tang M, Chen J, Pang Y (2004) Expression of *vip1/vip2* genes in *Escherichia coli* and *Bacillus thuringiensis* and analysis of their signal peptides. J Appl Microbiol 97:757–765
17. Estruch JJ, Warren GW, Mullins MA, Nye GJ, Craig JA, Koziel MG (1996) vip3A, a novel *Bacillus thuringiensis* vegetative

insecticidal protein with a wide spectrum activities against lepidopteran insects. Proc Natl Acad Sci USA 93:5389–5394
18. Procar M, Caballero P (2000) Molecular and insecticidal characterization of a *Bacillus thuringiensis* strain isolated during a natural epizootic. J Appl Microbiol 89:309–316
19. Martínez C, Caballero P (2002) Contents of cry genes and insecticidal toxicity of *Bacillus thuringiensis* strains from terrestrial and aquatic habitats. J Appl Microbiol 92:745–752
20. Beard CE, Court L, Boets A, Mourant R, van Rie J, Akhurst RJ (2008) Unusually high frequency of genes encoding vegetative insecticidal proteins in an Australian *Bacillus thuringiensis* collection. Curr Microbiol 57:195–199
21. Song L, Gao M, Dai S, Wu Y, Yi D, Li R (2008) Specific activity of *Bacillus thuringiensis* strain against *Locusta migratoria manilensis*. J Invertebr Pathol 98:176–196
22. Chak KF, Chao DC, Tseng MY, Kao SS, Tuan SJ, Feng TY (1994) Determination and distribution of cry-type genes of *Bacillus thuringiensis* isolates from Taiwan. Appl Environ Microbiol 60:2415–2420
23. Bravo A, Sarabia S, Lopez L, Ontiveros H, Abarca C, Ortiz A, Ortiz M, Lina L, Villalobos F, Pena G, Nunes M, Soberón M, Quintero R (1998) Characterization of cry genes in a Mexican *Bacillus thuringiensis* strain collection. Appl Environ Microbiol 64:4965–4972
24. Lee MK, Walters FS, Hart H, Palekar N, Chen J-S (2003) The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein vip3A differs from that of Cry1AB $\delta$-endotoxin. Appl Environ Microbiol 69:4648–4657
25. Arrieta G, Hernandez AG, Espinoza AM (2004) Diversity of *Bacillus thuringiensis* strains isolated from coffee plantations infested with the coffee berry borer *Hypothenemus hampei*. Rev Biol Trop 52:757–764
26. MOH (2008) Health statistical year book. Ministry of Health Press, Riyadh