Indian Journal of Agricultural Sciences **91** (8): 1230–5, August 2021/Article
https://doi.org/10.56093/ijas.v91i8.115882

Characterization of native *Bacillus thuringiensis* strains against storage pest *Tribolium castaneum* (Coleoptera: *Tenebrionidae*)

DARAVATH VEERANNA[1,2], ANUBHAV MITTAL[1,3], MANDLA RAJASHEKHAR[1,4] and VINAY K KALIA[1]*

ICAR-Indian Agricultural Research Institute, New Delhi 110 012

Received: 20 July 2020; Accepted: 20 July 2021

ABSTRACT

Fifty one *Bacillus thuringiensis* (*Bt*) like bacteria were isolated from diverse sources, viz. soil, silo dusts, insect cadaver, and phyllosphere and evaluated by feeding bioassays against larvae and adults of coleopteran pest red flour beetle, *Tribolium castaneum* (Herbst) in Division of Entomology, ICAR-IARI, New Delhi during 2014–15. It is a common pest known for attacking and infesting stored grains or products. Fifteen potential *Bt* strains were shortlisted on the basis of preliminary screening by single dose assays at 100 µg/g of diet against larvae and adults of *T. castaneum*. The virulence (LC_{50}) of potential isolates was further assessed. The most effective *Bt* isolate was VKK-GJ4 (LC_{50}=7.02 µg/g of diet) against neonates followed by VKK-GA6 (LC_{50}=19.03 µg/g of diet) against adults of *T. castaneum*. Gene profiling of potential *Bt* strains revealed the presence of *cry1*, *cry3*, *cry7*, *cry8*, *cry9, cry11, cry24* and *cry28* gene. However, in three *Bt* strains, viz. JK-5, Tri5-5 and JKII3-2 no cry gene was found but they showed insecticidal activity against *T. castaneum.* Thus, quantification of the toxicity by insect bioassay is the only way to assess the potential of a strain for the pest control.

Keywords: *Bt* strains, *Cry* gene, Insecticidal activity, *Tribolium castaneum*

Storage pests cause economic losses to stored grain and grain products globally. Grain production has been steadily increasing due to advances in production technology, but improper storage results in high losses in production**.** During storage, significant quantitative as well as qualitative losses occur due to insect pests. Therefore, to increase the effective food grain production is to protect and sustain the harvest by effective pest management. At present pest control is largely dependent on synthetic insecticides, but overdependence, indiscriminate, uncontrolled and continuous use of chemical insecticides has led to the resistance in insect pests (Georghiou 1990). More than 500 species of insects and related arthropods have evolved resistance to one or more insecticides globally (Whalon *et al*. 2013). Safety and environmental issues surrounding the use of chemical insecticides has led to the emphasis on development of alternative control measures. A major viable alternative to chemical control of insects is bio-pesticides. *Bacillus thuringiensis* (*Bt*) represent the most successful and widely used bio-pesticide. *Bt* is an aerobic, gram positive, spore-forming, facultative bacterial pathogen that produces parasporal crystals containing one or more insecticidal crystal proteins (Cry), which are selectively toxic to insects and widely distributed in the environment (Schnepf *et al.* 1998, Bravo *et al*. 2005). Crickmore *et al*. (1998) classified the Cry toxins into five major groups based on their specific insecticidal activity, namely, Cry1 (Lepidoptera), Cry2 (Lepidoptera and Diptera), Cry3 (Coleoptera), Cry4 (Diptera), and Cry5 (Lepidoptera and Coleoptera). To date 731 Cry toxins are reported (Crickmore *et al*. 2020).

Bt strains are being screened worldwide in search of new strains having novel insecticidal *cry* genes. Diversity and activity of *Bt* strains have a relationship with geographical origin of samples (Armengol *et al*. 2007). In the present paper, insecticidal activity of native *Bt* isolates has been evaluated against the coleopteran pest red flour beetle, *Tribolium castaneum* (Herbst). It is a common pest known for attacking and infesting stored flour and grains in silos, warehouses, grocery stores, and the household. Further, molecular characterization was carried out of potential native *Bt* isolates showing insecticidal activity.

MATERIALS AND METHODS

Bacterial strains: Standard strain *Bt* subsp. *kurstaki* HD1 (*Btk*-HD1) having *cry1Aa, cry1Ab, cry1Ac, cry2Aa*

Present address: [1]Indian Agricultural Research Institute, New Delhi; [2]Professor Jayashankar Telangana State Agricultural University, Hyderabad; [3]Animal Quarantine and Certification Service, Chennai; [4] Krishi Vigyan Kendra, Palem, PJTSAU, Hyderabad. *Corresponding author e-mail: vkalia@iari.res.in.

and *cry2Ab* genes and *Bt subsp. tolwarthi (Btt)* having *cry3Ba* and *cry9Ca* were obtained from Pasteur Institute, Paris. Fifty-one native *Bt* isolates, from four different sources, viz. warehouses (29 isolates), soil samples (14 isolates), insect cadavers (2 isolates), and phyllosphere (6 isolates) were retrieved from bacterial stock of Division of Entomology, IARI, New Delhi. Acetone precipitated spore-crystal mixture of all the *Bt* isolates was prepared as described by Dulmage *et al.* (1970).

Test insect and screening of bacterial strains against Tribolium castaneum: The adults as well as neonates of red flour beetle, *T. castaneum* were obtained from laboratory colony and reared as per Daravath *et al*. (2015). The neonates and one day old adults were used for bioassays. As per our preliminary studies and earlier reports coleopteran are less susceptible to *Bt* and required much higher concentrations than Lepidoptera. Based on that 51 native *Bt* isolates along with standard *Btk*HD1 and *Btt* were screened separately against neonates and adults of *T. castaneum* at 100 µg/g concentration by diet overlay method using wheat flakes as per Daravath *et al.* (2015). Each container having one flake served as one replicate and ten neonates (neonate assay) and 10 one day old adults (adult assays) were released on each treated flake and replicated thrice. All bioassays were carried out at 30±1°C. The data of mortality on the 7th day of treatment were corrected for control mortality using Abbott's formula (1925).

Virulence bioassays with shortlisted Bt isolates: Five different concentrations, viz. 0.1, 1.0, 10, 100 and 500 µg g^{-1} flake were tested by diet overlay method as mentioned above against both neonates and adults separately. Each treatment was replicated thrice. A minimum of 180 neonates/ adults were used for each bioassay independently. Mortality was recorded on 7th day, and LC_{50} was calculated.

Statistical analysis: The data of mortality on the 7th day of treatment were subjected to Statistical Analysis

Table 1 Characteristics of the primer sets used to identify *cry* genes in native Bt isolates by PCR

Cry gene	Primer sequence	AT*	Expected product size (bp)	References
Cry1	F: 5'-CCGGTGCTGGATTTGTGTTA-3' R: 5'-AATCCCGTATTGTACCAGCG-3'	50	276	Bravo *et al*. (1998)
Cry 8	F: 5'- ATGAGTCCAAATAATCTAAATG-3' R: 5'- TTTGATTAATGAGTTCTTCCACTCG-3'	48.5	373- 376	
Cry 11	F: 5'- TTAGAAGATACGCCAGATCAAGC-3' R: 5'- CATTTGTACTTGAAGTTGTAATCCC-3'	50	305	
Cry 2	F: 5'-GTTATTCTTAATGCAGATGAATGGG-3' R: 5'- CGGATAAAATAATCTGGGAAATAGT-3'	47	689- 701	Ben-Dov *et al*. (1997)
Cry 9	F: 5'-CGGTGTTACTATTAGCGAGGGCGG-3' R: 5'- GTTTGAGCCGCTTCACAGCAATCC-3'	55.5	351-354	
Cry 3	F: 5'- CGTTATCGCAGAGAGATGACATTAAC-3' R: 5'- CATCTGTTGTTTCTGGAGGCAAT-3'	50	589	
Cry 4	F: 5'-CAAGCCGCAAATCTTGTGGA-3' R: 5'- ATGGCTTGTTTCGCTACATC-3'	45.5	797	Carozzi *et al*. (1991)
Cry 6	F: 5'-TAYGGTTTTAAAKKTGCTGG-3' R: 5'- TRAATYCTATTRAACAATCCTA-3'	42	587	Porcar and Juarez-Perez (2003)
Cry 7	F: 5'-AGTGGAGAGTTTACGGTAGCC-3' R: 5'- CAATCCCAGTGTTTACTTGGAC-3'	50	211	
Cry 12	F: 5'- CTCCCCCAACATTCCATCC-3' R: 5'- AATTACTTACACGTGCCATACCTG-3'	49	363	Ejiofor and Johnson (2002)
Cry 18	F: 5'- CCGAGGCGATTTGGATAGAT-3' R: 5'- TGCCGGTGTAAACAAAGAAGG-3'	47.5	419	
Cry 19	F: 5'-AGGGGAGTCCAGGTTATGAGTTAC-3' R: 5'- ATTTCCCTAGTTAGTTCGGTTTTT-3'	51	355	
Cry 20	F: 5'- CAATCCCTGGCTTCACTCGT-3' R: 5'- CCGCGGGCATTAGGATT-3'	49	490	
Cry 24	F: 5'- AGGGGGCGATGGATACGAC-3' R: 5'- GGCCCTGCTACAACCGAAACTA-3'	50	355	
Cry 28	F: 5'- GTATTGGACCGAGGAGATGAAAGT-3' R: 5'- GTACGGCAAAGCGACAGAACA-3'	50	466	
Cry 23	F: 5'-GAAAGAGGTATATGGTGCAACAACT-3' R: 5'-CGAGCGACCTTTATTATCATCTAGT-3'	50	687	Ashwini (2006)

*AT: Annealing temperature

System (SAS) version 4.2 (SAS Institute Inc., Cary, USA) to analyse significant differences with one-way analysis of variance (ANOVA). The significantly different means (<0.05) were separated using Tukey's Studentized Range (HSD) Test. LC_{50} values for bioassays were calculated using maximum likelihood programme (MLP) 3.01 (Ross 1987). The significance of difference between two LC_{50} was determined on the basis of overlap of 95% fiducial limits.

Polymerase chain reaction (PCR) based characterization: Potential isolates showing efficacy against neonates and adults of *T. castaneum* were further characterized by *cry* gene profiling. Sample of *Bt* isolates for PCR was prepared as per Bravo *et al*. (1998). The extracted DNA was subjected to PCR amplification by using 16 oligonucleotide pairs specific for *cry* genes (Table 1) in GenPro (Biover) Thermal Cycler. All PCR reactions were carried out in 25 µl reaction volumes. DNA template, 10 µl was mixed with reaction 4.3 µl Taq assay buffer (10X) with $MgCl_2$ (15 mM),1 µl dNTPs (10 mM), 1 µl of each primer (10 pM), 0.2 µl Taq DNA polymerase (5 U/µl) and 7.5 µl nuclease free water. The PCR amplified products were resolved on 1.2% agarose gel stained with ethidium bromide and visualized in gel documentation system. (Alphaimager^{TM}) and analysed with AlphaEase FC.

RESULTS AND DISCUSSION

Ten isolates, viz. VKK-FCI3, VKK-GA6, VKK-GA7, VKK-GJ4, VKK-MGA3, Tri5-5, JK-5, JKII3-2, MPII5-1, MPII5-2 against neonates and nine isolates, viz. VKK-GA2, VKK-GA3, VKK-GA5, VKK-GA6, VKK-GA7, VKK-GJ1, VKK-MGA1, JK-5 and MPII5-1 against adults, showed mortality more than standard *Btt* (50% and 60% neonates and adults respectively) at 100 µg/g of diet on 7th day (Fig 1 and 2). Of these, 10 potential *Bt* isolates from warehouses, one from insect cadaver and four from soil. Based on screening bioassays studies, 15 isolates along with one standard *Btt* were selected for virulence and molecular studies.

Further, quantified toxicity (LC_{50} estimates) of Bt isolates showed that VKK-GJ4 (LC_{50}=7.02 µg/g of diet) was found to be the most virulent and significantly different from standard *Btt* (LC_{50}=101.03 µg/g of diet), against neonates of *T. castaneum*. In rest of the seven *Bt* isolates LC_{50} values varied from 15.42 µg/g of diet to 56.06 µg/g of diet (MPII-5-1) but they were found to be at par as the fiducial limits were overlapping (Table 2). Similarly, LC_{50} values varied from 19.03 µg/gm of diet (VKK-GA6) to 87.98 µg/g of diet (VKK-GA2) against adults of *T. castaneum* (Table 3). But none of *Bt* isolates were found to be significantly different as the fiducial limits were overlapping. Four isolates, viz. VKK-GA6, VKK-GA7, JK-5 and MPII-5-1 were found to be effective against both neonates and adults. Out of these four isolates JK-5 was found to be most effective. Correspondingly, it was reported that bioassays with *Bt* isolates from soil and warehouses resulted in high mortality rates against Coleopteran and Lepidopteran larvae (Kaelin *et al*. 1999, Yılmaz *et al*. 2012, Gorashi *et al*. 2014). The

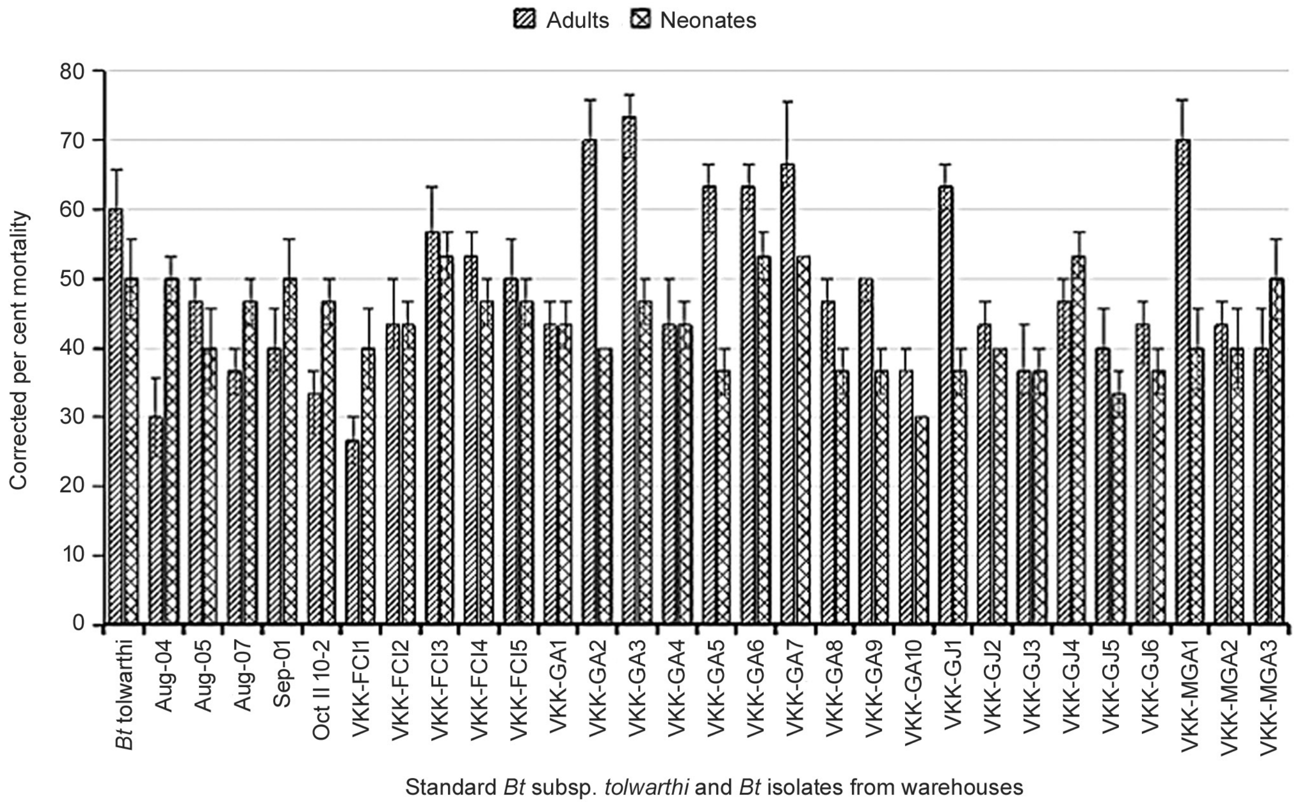

Fig 1 Insecticidal activity of *Bt* isolates isolated from warehouses and standard *Bacillus thutingiensis* subsp. *tolwarthi* against the adults and neonates of *Tribolium castaneum*.

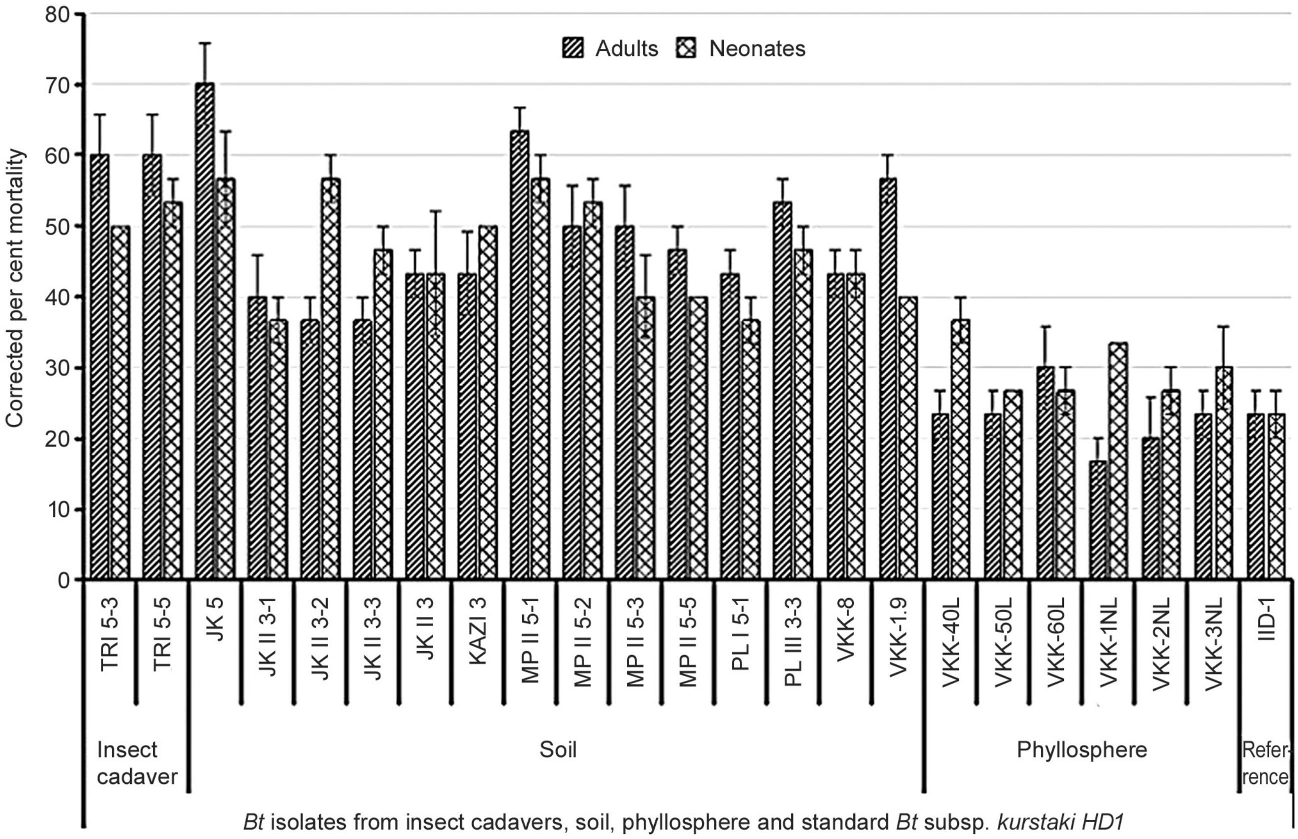

Fig 2 Insecticidal activity of *Bt* isolates isolated from insect cadavers, soil, phyllosphere and standard *Bacillus thutingiensis* subsp. *kurstaki* HD1 against the adults and neonates of *Tribolium castaneum.*

Table 2 Comparative toxicity of selected native *Bacillus thuringiensis* isolates against neonates of *Tribolium castaneum*

Bt isolates	LC$_{5}$ μg/gm of diet (7th day)	95% Fiducial Limit		Slope ± SE	χ^2	Df	p_c
		Lower	Upper				
Standard							
Bt tolwarthi	101.03	20.83	558.03	0.33 ± 0.139	0.32	3	0.956
Ware house							
VKK-FCI3	57.72	18.18	280.59	0.48± 0.10	3.60	3	0.308
VKK-GA6	62.13	18.51	350.92	0.45± 0.10	1.76	3	0.623
VKK-GA7	41.40	14.54	146.74	0.54 ± 0.106	2.44	3	0.486
VKK-GJ4	7.02	2.66	16.42	0.69 ± 0.114	1.13	3	0.769
VKK-MGA3	16.97	6.14	45.46	0.62±0.11	1.01	3	0.798
Insect cadaver							
Tri5-5	28.75	9.82	97.47	0.53± 0.10	1.44	3	0.696
Soil							
JK-5	31.74	12.05	90.59	0.62±0.11	0.74	3	0.863
JKII3-2	10.36	4.05	26.66	0.66± 0.11	0.42	3	0.936
MPII5-1	56.06	13.69	504.27	0.38± 0.09	0.74	3	0.863
MPII5-2	15.42	5.82	39.30	0.64± 0.11	0.77	3	0.856

SE, Standard error; χ^2, Pearson χ^2 of the slope; df, degree of freedom for χ^2; pc, critical probability of the slope.

higher mortality with native *Bt* isolates may be either due to its Cry protein types or due to higher toxin yield.

PCR-based approach has been extensively utilized for the identification of known and novel *cry* genes in *Bt* since its introduction as a tool to predict insecticidal activity by Carozzi *et al.* (1991). Five out of 15 *Bt* isolates showed the

Table 3 Comparative toxicity of selected native *Bacillus thuringiensis* isolates against adults of *Tribolium castaneum*

Bt strains	LC µg/gm of diet 5 (7th day)	95% Fiducial Limit		Slope ± SE	χ^2	Df	p_c
		Lower	Upper				
Standard							
Bt. tolwarthi	54.46	19.28	194.34	0.58±0.12	0.64	3	0.887
Ware house							
VKK-GA2	87.98	29.51	411.86	0.55±0.12	0.09	3	0.993
VKK-GA3	56.28	19.37	216.15	0.56± 0.11	1.88	3	0.597
VKK-GA5	54.53	17.91	228.36	0.53± 0.11	0.23	3	0.693
VKK-GA6	19.03	6.58	55.94	0.57± 0.10	1.06	3	0.786
VKK-GA7	20.01	7.70	48.33	0.72± 0.13	1.11	3	0.774
VKK-GJ1	35.22	13.05	104.17	0.62 ± 0.11	0.77	3	0.856
VKK-MGA1	56.58	22.37	150.16	0.74± 0.15	1.45	3	0.693
Soil							
JK-5	26.27	10.03	66.51	0.70± 0.13	0.71	3	0.870
MPII5-1	45.63	17.25	135.12	0.65 ±0.12	0.74	3	0.863

SE = Standard error. χ^2 = Pearson χ^2 of the slope. df = degree of freedom for χ^2 . pc = critical probability of the slope.

presence of *cry*1 gene and out of them three strains showed the presence of either *cry2*, *cry9* or *cry24* along with *cry1* gene. Three *Bt* strains showed presence of more than two *cry* genes, viz. VKK-GJ1 (*cry1, cry8, cry24*), VKK-MGA3 (*cry1, cry3, cry28*) and VKK-GA2 (*cry3, cry11, cry28*). Gene *cry3* was identified in two strains isolated from warehouse (VKK-GA2 and VKK-MGA2) besides reference strain *Bt tolwarthi*. Three *Bt* strains isolated from warehouse showed *cry9* gene (VKK-GA3, VKK-GA5 and VKK-GA6). Two strains isolated from soil showed the presence of *cry7* gene. Among the genes identified in this study *cry3*, *cry*7, *cry*8, *cry*9 and *cry*28 encode coleopteran-specific toxins (Palma *et al*. 2014, Fernández-Chapa *et al*. 2019).

Present study showed that the red flour beetle is susceptible to Cry3, Cry8 and Cry9 toxins, as was shown previously for other Coleopteran pests (Pereyra-Alferez *et al*. 1999, Arrieta *et al*. 2004, Tamez-Guerra *et al*. 2004, Yu *et al*. 2006, Gorashi *et al*. 2014). The PCR analysis of four *Bt* strains which were found to be effective against both larvae and adults of *T. castaneum* amplified *cry9* and *cry24* genes respectively in VKK-GA-6 and VKK-GA-7. No specific PCR product of any of targeted *cry* gene was observed in three strains, viz. JKII3-2, JK-5 and Tri5-5. But in JK-5 unexpected PCR product (220 bp) was amplified for *cry8* gene while in Tri5-5 with *cry7* (350 bp) and *cry28* gene (250 bp). Ceron *et al.* (1995) reported that an unexpected amplified fragment might correspond to a new *cry* gene. Porcar and Juarez-Parez (2003) also supplemented this explanation.

Though, no specific *cry* genes amplicons were present in three isolates (JKII3-2, JK-5 and Tri 5-5) but they showed insecticidal activity against *T. castaneum.* Thus, quantification of the toxicity by insect bioassay is the only way to assess the potential of a strain for the pest control as also reported by Martìnez *et al.* (2004). However, molecular characterization gives impetus to fish out the gene of interest for developing novel strain for broad-spectrum insecticidal activity but unable to assess its potential in pest management. Further, evaluation of these isolates can be carried out for the insecticidal activity against other storage as well as agricultural field pests besides coleopteran, as they showed diverse group of *cry* gene.

ACKNOWLEDGMENTS

First author is grateful to Indian Agricultural Research Institute for the award of Junior Research Fellowship. The authors are grateful to Dr G T Gujar, Ex-Head, Division of Entomology, ICAR-IARI, New Delhi for providing Standard *Bt* strains.

REFERENCES

Abbott W S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18** : 265–67.

Armengol G, Escobar M C, Maldonado M E and Orduz S. 2007. Diversity of Colombian strains of *Bacillus thuringiensis* with insecticidal activity against dipteran and lepidopteran insects. *Journal of Applied Microbiology* **102**:77–88.

Arrieta G, Hernandez A and Espinoza A M. 2004. Diversity of *Bacillus thuringiensis* strains isolated from coffee plantations infested with the coffee borer *Hypothenemus hampei*. *Revista de Biología Tropical* **52**: 757.

Ashwini B K. 2006. 'Molecular characterization of insecticidal genes in *Bacillus thuringiensis* isolates from Western ghats of Chikmagalur & Goa. M Sc thesis, University of Agricultural Sciences, Dharwad, Karnataka.

Ben-Dov E, Zaritsky A, Dahan E, Barak Z and Sinai R. 1997. Extended screening by PCR for seven *cry*-group genes from field collected strains of *Bacillus thuringiensis*. *Applied Environmental Microbiology* **63**: 4883–90.

Bravo A, Gill S S and Soberon M. 2005. *Bacillus thuringiensis* mechanisms and use. *Comprehensive Molecular Insect Science* **6**: 175–205.

Bravo A, Sarabia S, Lopez L, Ontiveros H and Abarca C. 1998. Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. *Applied and Environmental Microbiology* **64**: 4965–72.

Carozzi N B, Kramer V C, Warren G W, Evola S and Koziel M G. 1991. Prediction of insecticidal activity of *Bacillus thuringiensis* strains by polymerase chain reaction product proøles. *Applied and Environmental Microbiology* **57**: 3057–61.

Ceron J, Ortiz A, Quintero R and Bravo A. 1995. Specific primers directed to identify *cry*1 and *cry*3 genes in a *Bacillus thuringiensis* strain collection. *Applied and Environmental Microbiology* **61**: 3826–31.

Crickmore N, Berry C, Panneerselvam S, Mishra R and Connor T R. 2020. Bacterial Pesticidal Protein Resource Center, viewed (21.01.2021 accessed the site), https://www.bpprc.org.

Crickmore N, Zeigler D R, Feitelson J, Schnef E and Van Rie J. 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. *Microbiology and Molecular Biology Reviews* **62**: 807-813.

Daravath V, Mittal A, Mandla R and Kalia V. 2015. Distribution and insecticidal activity of *Bacillus thuringiensis* strains isolated from warehouses in India. *Biopesticide International* **11**(2): 96–107.

Dulmage, H T, Correa J A and Martinex A J. 1970. Co-precipitation with lactose as a means of recovering the spore-crystal complex of *Bacillus thuringiensis*. *Journal of Invertebrate Pathology* **15**: 15–20.

Ejiofor A O and Johnson T. 2002. Physiological and molecular detection of crystalliferous *Bacillus thuringiensis* strains from habitats in the South Central United States. *Journal Industrial Microbiology and Biotechnology* **28**: 284.

Fernández-Chapa D, Jesica Ramírez-Villalobos and Luis Galán-Wong. 2019. Toxic potential of *Bacillus thuringiensis*: An overview. DOI: 10.5772/intechopen.85756

Georghiou G P. 1990. Overview of insecticide resistance. (In) *ACS Symposium series-American Chemical Society,* USA, pp. 18–41.

Gorashi N E, Tripathi M, Kalia V and Gujar G T. 2014. Identification and characterization of the Sudanese *Bacillus thuringiensis* and related bacterial strains for their efficacy against *Helicoverpa armigera* and *Tribolium castaneum*. *Indian Journal of Experimental Biology* **52**: 637–49.

Kaelin P, Zaugg L, Albertini A M and Gadani F. 1999. Activity of *Bacillus thuringiensis* isolates on *Lasioderma serricorne* (F) (Coleoptera:Anobiidae). *Journal of Stored Product Research* **35**: 145.

Martìnez C, Porcar M, Lopez A, de Escudero I R and Perez-L larena F J. 2004. Characterization of a *Bacillus thuringiensis* strain with a broad spectrum of activity against Lepidopteran insects. *Entomologia Experimentalis et Applicata* **111**: 71–77.

Palma L, Muñoz D, Berry C, Murillo J and Caballero P. 2014. Bacillus thuringiensis toxins: an overview of their biocidal activity. *Toxins* **6**: 3296–3325.https://doi.org/10.3390/toxins6123296

Pereyra-Alferez B, Sanchez-Alejo E, Flores-Guerra A C, Iracheta M M and Galan-Wong L J. 1999. Isolation and characterization of Mexican *Bacillus thuringiensis* strains toxic against *Tribolium castaneum* (Herbst) and *Oryzaephilus surinamensis* (L.). *Biotechnology of Bacillus huringiensis,* pp 21–25. Zinui Y, Ming S, Zidou L (Eds). Science Press, Beijing, NY,

Porcar M and Juarez-Perez V. 2003. PCR based identification of *Bacillus thuringiensis* pesticidal crystal genes. *FEMS Microbiology Reviews* **26**: 419–32.

Ross G J S. 1987. *MLP User manual*. Numerical Algorithms Group, Oxford.

Schnepf E, Crickmore N, Van Rie J, Lereclus D and Baum J.1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Journal of Microbiology Molecular Biology* **62**:775–806.

Tamez-Guerra P, Iracheta M M, Pereyra-Alferez B, Galan-Wong L J and Gomez-Flores R. 2004. Characterization of Mexican Bacillus thuringiensis strains toxic for lepidopteran and coleopteran larvae. *Journal of Invertebrate Pathology* **86**: 7–18.

Tripathi M, Kumar A, Kalia V, Saxena A K and Gujar G T. 2016. Isolation and characterization of lepidopteran specific *Bacillus thuringiensis* predominantly from North-Eastern region of India. *Indian Journal of Experimental Biology* **54** (7): 431–52.

Whalon M E, Mota-Sanchez D and Hollingworth R M. 2013. Arthropod Pesticide Resistance Database. (http://www.pesticideresistance.com/index.php)

Yılmaz S, Ayvaz A, Akbulut M, Azizoglu U and Karaborklu S. 2012. A novel *Bacillus thuringiensis* strain and its pathogenicity against three important pest insects. *Journal of Stored Products Research* **1**: 33–40.

Yu H, Zhang J, Huang D, Gao J and Song F. 2006. Characterization of *Bacillus thuringiensis* Strain Bt185 Toxic to the Asian Cockchafer: *Holotrichia parallela*. *Current Microbiology* **53**: 13–17.