

“Root Biotron”- Eco friendly and sustainable management of the Fall Army Worm and Brinjal Ash Weevil

Anusha, D¹., Nagesha S.N^{2*}., Mahadeva Swamy, H.M³, Asokan, R³., Nagesh, N¹, Nethra Nagarajappa⁴, Geetha Govind⁵
*Email ID nageshasn@uasbangalore.edu.in , clinonbio@gmail.com

Abstract

After its discovery in May 2018, FAW has emerged as India's most damaging maize pest. Its rapid expansion through India's maize-growing areas in diverse agro-ecologies in just 16 months poses a major threat to smallholder maize growers, the maize-based industry, and food and nutritional welfare. Other crops, such as sorghum and millets, have also been confirmed to have FAW, with varying degrees of economic loss [1].

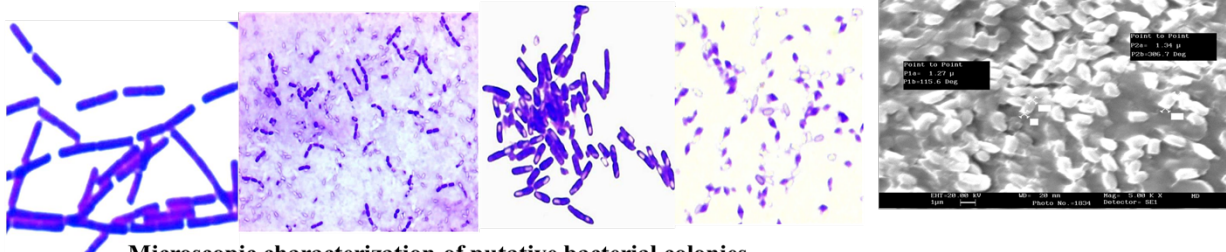
Mylokerus subfasciatus, the brinjal ash weevil, is a major pest of eggplant and other solanaceous crops in Asia and Southeast Asia. It causes damage to the brinjal throughout the year [2]. Unfortunately, considering a wide variety of commercialized chemicals, biopesticide use remains limited globally. Research and development of more effective and cheaper biopesticides is also vital in order to promote and encourage people to adopt safer IPM approaches. Toxins and virulence factors are constantly being screened and new species discoveries, guided evolution methods and genetic modification offer immense resources to include in the formulation of a biopesticide. In India, the majority of farmers are small-holder farmers who cannot afford to buy costly insecticides. Since FAW is a modern invasive species in India, there are no laboratory data on how to treat it. As a result, determining the effectiveness and potency of local natural enemies and native biopesticide strains is critical before deploying them in the IPM module. The aim of this study is to produce novel *Bt* strains that maintain the favourable characteristics of biopesticides while providing significantly improved control of insect populations that have established resistance to traditional insecticides and are not effectively handled by commercially available *Bt* strains.

Twelve *Bt* strains were isolated from root nodules using six approaches, viz., analysis of crystal protein production by microscopy; detection of *cry* gene content by PCR, Plasmid profiling, SDS-PAGE profiling; cloning and sequencing, phylogenetic analysis; and toxicity testing. Under a light microscope, all 12 *Bt* strains examined were gram-positive, endospore-forming, and had normal *Bt* crystal structures. A bipyrinidal inclusion was predominant in 42.2 %. Five distinct plasmids were observed in the present study. Universal primers and gene specific primers were used to detect Cry-type genes by the PCR. The nucleotide sequences of amplified new *cry* genes were BLASTed against NCBI database sequences, and they were labelled as native *Bt* strains with high homology (60–100%) to existing *Bt* strains. The nucleotide sequences of new *Bt cry* genes were deposited in the NCBI GenBank database. Three new *cry1*, *cry3A* and *cry26* complete *cry* genes were isolated, Cry genes were cloned and expressed in the pRSET vector. The inferred 3-D structural model of the novel *cry* gene, which was predicted using Phyre2 homology modeling, demonstrates that the gene has three domains that participate in the development of a pore and define the receptor's binding selectivity. The new *Bt* strains were highly pathogenic with pathogenicity ranging from 93 to 100 percent against *Spodoptera frugiperda* larvae and *Mylokerus subfasciatus* adults. In conclusion, native *Bt* strains from root nodules were shown to have bio-insecticidal activity on larvae of *S. frugiperda* and adults of *M. subfasciatus*.

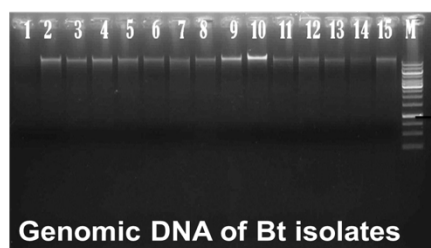


Isolation of novel Bt strains from root nodules

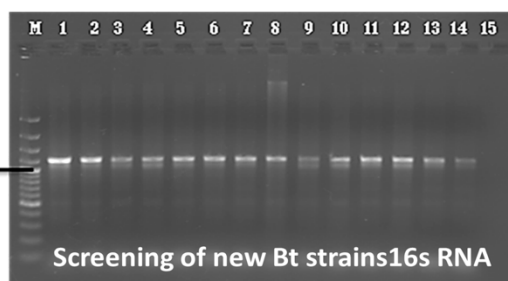
Characterization of Bt strains



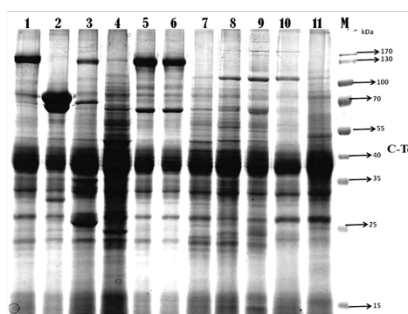
Microscopic characterization of putative bacterial colonies



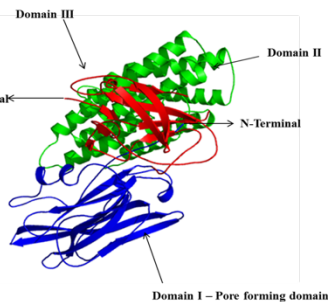
Genomic DNA of Bt isolates



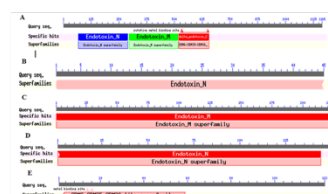
Screening of new Bt strains 16S rRNA



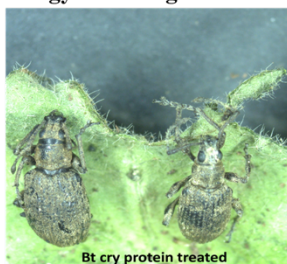
SDS PAGE Polyacrylamide gel electrophoretic pattern of total proteins of Sporulated Bt isolates



3D homology modeling of new Bt toxin



Conserved domain analysis of the deduced amino acid sequences of new Bt cry26 genes



References

[1] P S, Shanmugam & Sangeetha, M & Kabirdoss, Indhumathi. (2018). Management of ash weevil *Mylocherus subfasciatus* Guerin-Meneville (Coleoptera; Curculionidae) in Brinjal. *Journal of Entomology and Zoology Studies*. 6. 1230-1234.

[2] Suby, S. & PL, Soujanya & Yadava, Pranjal & Patil, Jagadeesh & Subaharan, K & Prasad, Shyam & Babu, K & Jat, Shankar & Yathish, K & Vadassery, Jyothilakshmi & Bakthavatsalam, N & Sekhar, J. & Rakshit, Sujay. (2020). Invasion of fall armyworm (*Spodoptera frugiperda*) in India: nature, distribution, management and potential impact. *Current science*. 119. 44-51.

