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Molecular identification of coleopteran specific Sri Lankan *Bacillus thuringiensis* isolates based on 16S rRNA and *gyrB* gene sequence analysis

R A W M R S U Alahakoon^{1, 2}, O V D S J Weerasena² and R R Samarasekera^{1*}

¹Herbal Technology Section, Industrial Technology Institute, Baudhaloka Mawatha,
Colombo 07

²Institute of Biochemistry, Molecular Biology and Biotechnology, University of Colombo,
Colombo 03

Bacillus thuringiensis Berliner (*Bt*) is a facultative anaerobic, spore forming and gram positive bacterium. It is best known for its production of proteinaceous parasporal inclusions, δ -endotoxins which are toxic to insect larvae of orders Lepidoptera, Diptera and Coleoptera. *Bacillus thuringiensis* strains *Bt*.AB58, *Bt*.AB107 and *Bt*.AB110 isolated from Sri Lanka was identified by morphological, biochemical and *cry*, *vip*, *cyt* gene based molecular studies. Furthermore, coleopteran insecticidal activity against white grubs (root feeding coleopteran pest damaging fruits and vegetables), at laboratory and field levels confirmed their coleopteran insecticidal activity. Therefore, the objective of this study was to identify the sub species of *Bt*.AB58, *Bt*.AB107 and *Bt*.AB110 isolates by 16S rRNA and *gyrB* gene sequencing as 16S rRNA gene and *gyrB* gene sequences have been widely used as molecular markers to distinguish bacterial species and subspecies. Amplification of 16S rRNA gene by PCR and *gyrB* gene by nested PCR was performed for each of these isolates and purified amplicon was sequenced using Big dye terminator cycle sequencing kit and Applied biosystem 3500 dx genetic analyzer. Sequences were searched in the GenBank database at NCBI using BLAST. According to the results Sri Lankan isolates *Bt*.AB58, *Bt*.AB107 and *Bt*.AB110 were identified as *Bt*. sub species *kurstaki*, *Bt*. sub species *israelensis* and *Bt*. sub species *aizawai* respectively. However, 16S rRNA region was not capable in distinguishing *Bacillus thuringiensis* at sub species level in this study.

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