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DETECTION OF CYT2 GENE IN BACILLUS THURINGIENSIS ISOLATES WITH ACTIVITY AGAINST WIREWORMS

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ABSTRACT

In the pursuit of biologically acceptable solutions for controlling pests that attack economically important crops and cause significant yield losses, it is essential to identify novel bacterial isolates that are both effective and environmentally sustainable. Such approaches contribute not only to pest management but also to the preservation of the environment and the advancement of sustainable agriculture. *Bacillus thuringiensis* (Bt) is a well-established entomopathogenic bacterium known for synthesizing a diverse range of insecticidal proteins, including Cyt toxins. These toxins become active in the insect midgut, although their precise mode of action has not yet been fully elucidated. To explore potential biocontrol strategies against wireworms (*Agriotes lineatus* larvae), this study aimed to detect the presence of the *cyt2* gene in soil-derived Bt isolates previously shown to exhibit insecticidal activity against wireworms in *in vitro* assays. The working hypothesis was that the presence of the *cyt2* gene may be associated with the observed larvicidal effects. Six Bt isolates were screened for the *cyt2* gene using conventional PCR with specific DNA primers: *cyt2*gral-F (ATTACAAATTGCAAATGGTATTCC) and *cyt2*gral-R (TTTCAACATCCACAGTAATTTCAAATGC). PCR amplification yielded products of the expected length (355 bp), which were confirmed via agarose gel electrophoresis. Among the tested isolates, only BHC2.4—previously shown to induce over 60% mortality in *A. lineatus* larvae—tested positive for the *cyt2* gene. These findings suggest a possible correlation between the presence of *cyt2* gene and insecticidal activity against wireworms. Therefore, the *cyt2* gene may serve as a useful molecular marker for the preliminary screening and selection of promising Bt isolates for further bioefficacy testing. **Funding:** This research was funded by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia, contract Nos. 451-03-136/2025-03/200011, 451-03-136/2025-03/200214 and by the Science Fund of the Republic of Serbia, GRANT No. 10815, The necessity of healthy crops: Development of a multifunctional bacterial inoculant for the biological protection of cereals - BioHealCrop.

Keywords: *Bacillus thuringiensis*, *cyt2* gene, wireworms, *Agriotes lineatus*, biological control, sustainable agriculture, soil bacteria, PCR screening