Comprehensive characterization of Cry9 insecticidal toxins – a vital insight into *Bacillus thuringiensis* biology and a key to progress in biological pest control

For centuries, agriculture has been facing an unrelenting challenge from lepidopteran pests, one of the most destructive threats to crops. While synthetic pesticides have been widely adopted to combat these invaders, their use comes at a steep cost, including risks to human health, environmental damage, and the unintended harm to beneficial species like bees.

Bioinsecticides derived from invertebrate-active toxins produced by the entomopathogenic bacterium *Bacillus thuringiensis* have revolutionized global pest management, by offering a safer and environmentally friendly alternative to chemical pesticides. These toxins effectively target lepidopteran pests while remaining harmless to humans and non-target organisms. However, the successful use of *B. thuringiensis*-based bioinsecticides is now under threat due to increasing reports of insect resistance, particularly to bacterial Cry1 and Cry2 proteins, which are central to efficacy of these biocontrol agents. Without innovative solutions, this vital technology may gradually fail, creating a critical void in the biological pest control market and significant challenges for global agriculture.

But where should we seek a solution? The answer likely lies close at hand. The vast yet unexplored arsenal of *B. thuringiensis* once more offers us a significant chance for advancing bioinsecticide development. Among its promising components are the Cry9 proteins – an enigmatic group of insecticidal proteins exhibiting unique properties and potential efficacy against a broader range of pests, including resistant species. However, unlocking their utility first requires a deep understanding of the mechanisms underlying these unique toxins.

Although the sequences of 15 different Cry9 toxins are currently known, our understanding of their biological roles remains limited, especially compared to the extensively studied Cry1 and Cry2 proteins. Our recent findings, however, reveal the remarkable activity of one of Cry9 pesticidal molecules towards lepidopteran pests of significant economic concern – this allows us to suspect high entomopathogenic potential "hidden" within the Cry9 group. Preliminary phylogenetic analyses further reveal intriguing domain architectures among Cry9 proteins, suggesting significant evolutionary diversification. These structural differences may underlie the atypical host range observed within this group, paving the way for future exploration of their pesticidal properties.

This project aims to conduct a comprehensive study of Bt Cry9-type pesticidal proteins, encompassing:

- Pangenome-wide search for novel Cry9 variants and related toxins,
- Inclusive *in silico* analyses of known and newly identified Cry9 proteins,
- Cry9 toxin expression, purification, and functional characterization,
- Detailed bioassays against lepidopteran pests and other invertebrates,
- Structure-function analyses to elucidate the molecular basis of their specificity.

This research will unravel the genomic, molecular, and insecticidal properties of Cry9 toxins, significantly advancing our understanding of *B. thuringiensis* biology and its diverse entomopathogenic mechanisms. Through inclusive characterization of these novel toxins we will uncover their evolutionary adaptations, structure-function relationships, and unique modes of action, providing critical insights into toxin-host interactions. Moreover, the results of this fundamental research can significantly advance current biological pest control methods, which are increasingly challenged by insect resistance. A thorough exploration of Cry9 toxins, alongside the evaluation of their biological activity, will establish a critical baseline for the deployment of these pesticidal proteins as novel bioinsecticides. By addressing the growing resistance issues, this study could help shape the next generation of pest control strategies in agriculture, horticulture, and forestry, providing more sustainable, effective solutions while reducing reliance on chemical pesticides.