

Bacillus thuringiensis: A Broader View of Its Biocidal Activity

Leopoldo Palma ^{1,2,*} , Diego Herman Sauka ^{2,3,*}  and Jorge E. Ibarra ^{4,*} ¹ Instituto de Biotecnología y Biomedicina (BIOTECMED), Universitat de València, 46100 Burjassot, Spain² Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Ciudad Autónoma de Buenos Aires 1425, Argentina³ Instituto de Microbiología y Zoología Agrícola (IMYZA), Instituto Nacional de Tecnología Agropecuaria (INTA), Hurlingham, Ciudad Autónoma de Buenos Aires 1686, Argentina⁴ Departamentos de Biotecnología y Bioquímica, Centro de Investigación y de Estudios Avanzados del IPN-Unidad Irapuato, Apartado Postal 629, Irapuato 36500, Guanajuato, Mexico

* Correspondence: leopoldo.palma@uv.es (L.P.); sauka.diego@inta.gob.ar (D.H.S.); jorge.ibarra@investav.mx (J.E.I.)

Bacillus thuringiensis (Bt) is a Gram-positive bacterium that forms spores and produces parasporal crystalline inclusions containing Cry and Cyt proteins [1]. These proteins exhibit toxicity against various insect orders, nematodes, and human cancer cells [2,3]. Widely utilized as bioinsecticides, Bt strains and their insecticidal proteins effectively control caterpillars, beetles, flies, mosquitoes, and blackflies. During vegetative growth, Bt can also secrete insecticidal proteins targeting lepidopterans (Vip3) and coleopterans (Vpab1/Vpab2). Another less-explored secretory protein, Mpp5Aa1 (formerly Sip1A), has also been described to exhibit activity against coleopteran pests [4]. These features have bestowed Bt as the most specific and effective tool for the control of insect pests for several years, either through insecticidal formulations (a mix of spore and parasporal crystals) or by the production of insecticidal proteins in transgenic plants (Bt plants) [5]. However, some species, such as *Plutella xylostella* (Lepidoptera), have developed field resistance to both formulated products and insecticidal proteins expressed in transgenic plants [2], making screenings for novel strains and pesticidal proteins highly essential in order to provide novel tools for the control of pests and the management of insect resistance.

The aim of this Special Issue, “*Bacillus thuringiensis*: A Broader View of Its Biocidal Activity”, was to gather information on novel Bt strains and proteins showing novel pesticidal properties to provide biotechnological tools with useful resources for pest control in agriculture and to incentivize researchers to perform such necessary research. This subject has been of great interest, allowing the publication of 12 research papers from top researchers working in the field worldwide, which have shed light on the diverse and multifunctional properties of novel (unreported) Bt strains and proteins. Beyond the conventional focus, these studies delve into various aspects, including structural insights, insecticidal proteins, toxin interactions, and the evaluation of novel strains.

Additionally, this editorial aims to provide an overview of key findings from these papers; for example, Unzue et al. showcased the broad spectrum of Bt applications and their potential implications, such as the multifunctional properties of Bt strain BST-122, encompassing the biocidal properties beyond those of the well-known pesticidal parasporal crystals (contribution 1). Li et al. presented a deeper understanding of the structure of Cry5B, unraveling the active form of Cry5B that could contribute to the development of more effective and targeted nematicidal products (contribution 2). The study by Best et al. showed the crystal structure of Bt Tpp80Aa1 protein (formerly Cry80Aa1) and its interaction with galactose-containing glycolipids, unveiling molecular details that may have implications for understanding the specificity and selectivity of Bt toxins (contribution 3). Two papers about binary Mpp23Aa/Xpp37Aa proteins and the novel Bt strain Bt_UNVM-84 were presented by de Oliveira et al. and Sauka et al., respectively, highlighting the potential application of these resources in pest control, particularly against *Anthonomus*



Citation: Palma, L.; Sauka, D.H.; Ibarra, J.E. *Bacillus thuringiensis*: A Broader View of Its Biocidal Activity. *Toxins* **2024**, *16*, 162. <https://doi.org/10.3390/toxins16030162>

Received: 13 March 2024
Accepted: 18 March 2024
Published: 20 March 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

grandis (Coleoptera), a harmful pest causing high economic losses in the cotton industry in the Americas (contributions 4 and 10). Covering proteins with activity against mosquitoes, Lai et al.'s paper examines the role of Cyt proteins in enhancing the activity of other Bt toxins against *Aedes albopictus*, emphasizing the cooperative interactions between different toxin classes. Understanding these synergies could contribute to the development of more effective mosquito control strategies (contribution 5). Yang's study described a Cry protein with activity against the rice leaffolder *Cnaphalocrocis medinalis*. This paper explored the processing properties and potency of Cry toxins in the context of rice leaffolder control. Insights into the interaction between a Cry protein and this target pest are crucial for optimizing their efficacy in agricultural settings (contribution 6). The study by Xue et al. covered novel synergistic interactions beyond those known among different insecticidal proteins. In this paper, a new synergistic interaction between the extracellular polysaccharide from Bt subsp. *kurstaki* HD-270 and the insecticidal protein Cry1Ac is described, examining this particular synergistic activity and providing valuable information on the interplay between different components in Bt formulations (contribution 7). Trisyono et al. presented a paper covering the baseline susceptibility of field populations of *Ostrinia furnacalis* in Indonesia to Cry1A.105 events and Cry2Ab2 proteins were also covered, assessing the susceptibility of this pest to specific Cry proteins. This research lays the groundwork for understanding the dynamics of resistance and informs us about the sustainable use of Bt technologies in agriculture (contribution 8). Interesting outputs about the field evaluation of transgenic cotton expressing Mpp51Aa2 (formerly Cry51Aa2) as a management tool for the cotton fleahopper *Pseudatomoscelis seriatus* were also published in this Special Issue, contributing valuable data for its implementation. Finally, two papers presented by Hou et al. and Shao et al. describe interesting aspects related to the yet poorly understood mode of action of Vip3A proteins, focusing on molecular details of Vip3A proteins and highlighting specific domains involved in receptor binding and liposomal membrane disruption, respectively (contributions 11 and 12).

In conclusion, the collection of papers featured in this editorial underscores the versatility and complexity of Bt as a bioinsecticide. From structural insights to practical field applications, researchers continue to unravel the potential of Bt strains in addressing agricultural and public health challenges. These findings collectively contribute to the ongoing efforts to harness the full potential of Bt-based technologies for sustainable pest management.

Funding: This research received no external funding.

Acknowledgments: The editors express their gratitude to the authors who made significant contributions to the Special Issue entitled "*Bacillus thuringiensis*: A Broader View of Its Biocidal Activity." A special acknowledgement goes also to peer reviewers for their expertise contributions in their evaluations, enhancing the overall quality of the research works and reviews featured in this Special Issue. Leopoldo Palma would like to express gratitude to the Spanish Government-Universities Ministry, the Next Generation EU and Recovery, Transformation, and Resilience plans for funding his awarded María Zambrano contract (ref. ZA21-003). Lastly, we extend our thanks to the MDPI management team and staff for their invaluable contributions, organization, and editorial support.

Conflicts of Interest: The authors declare no conflicts of interest.

List of Contributions:

1. Unzue, A.; Caballero, C.J.; Villanueva, M.; Fernández, A.B.; Caballero, P. Multifunctional Properties of a *Bacillus thuringiensis* Strain (BST-122): Beyond the Parasporal Crystal. *Toxins* **2022**, *14*, 768. <https://doi.org/10.3390/toxins14110768>.
2. Li, J.; Wang, L.; Kotaka, M.; Lee, M.M.; Chan, M.K. Insights from the Structure of an Active Form of *Bacillus thuringiensis* Cry5B. *Toxins* **2022**, *14*, 823. <https://doi.org/10.3390/toxins14120823>.
3. Best, H.L.; Williamson, L.J.; Lipka-Lloyd, M.; Waller-Evans, H.; Lloyd-Evans, E.; Rizkallah, P.J.; Berry, C. The Crystal Structure of *Bacillus thuringiensis* Tpp80Aa1 and Its Interaction with Galactose-Containing Glycolipids. *Toxins* **2022**, *14*, 863. <https://doi.org/10.3390/toxins14120863>.

4. de Oliveira, J.A.; Negri, B.F.; Hernández-Martínez, P.; Basso, M.F.; Escriche, B. Mpp23Aa/Xpp37Aa Insecticidal Proteins from *Bacillus thuringiensis* (Bacillales: Bacillaceae) Are Highly Toxic to *Anthonomus grandis* (Coleoptera: Curculionidae) Larvae. *Toxins* **2023**, *15*, 55. <https://doi.org/10.3390/toxins15010055>.
5. Lai, L.; Villanueva, M.; Muruzabal-Galarza, A.; Fernández, A.B.; Unzue, A.; Toledo-Arana, A.; Caballero, P.; Caballero, C.J. *Bacillus thuringiensis* Cyt Proteins as Enablers of Activity of Cry and Tpp Toxins against *Aedes albopictus*. *Toxins* **2023**, *15*, 211. <https://doi.org/10.3390/toxins15030211>.
6. Yang, Y.; Wu, Z.; He, X.; Xu, H.; Lu, Z. Processing Properties and Potency of *Bacillus thuringiensis* Cry Toxins in the Rice Leafhopper *Cnaphalocrocis medinalis* (Guenée). *Toxins* **2023**, *15*, 275. <https://doi.org/10.3390/toxins15040275>.
7. Xue, B.; Wang, M.; Wang, Z.; Shu, C.; Geng, L.; Zhang, J. Analysis of Synergism between Extracellular Polysaccharide from *Bacillus thuringiensis* subsp. *kurstaki* HD270 and Insecticidal Proteins. *Toxins* **2023**, *15*, 590. <https://doi.org/10.3390/toxins15100590>.
8. Trisyono, Y.A.; Aryuwandari, V.E.F.; Rahayu, T.; Martinelli, S.; Head, G.P.; Parimi, S.; Camacho, L.R. Baseline Susceptibility of the Field Populations of *Ostrinia furnacalis* in Indonesia to the Proteins Cry1A.105 and Cry2Ab2 of *Bacillus thuringiensis*. *Toxins* **2023**, *15*, 602. <https://doi.org/10.3390/toxins15100602>.
9. Arthur, B.P.; Suh, C.P.; McKnight, B.M.; Parajulee, M.N.; Yang, F.; Kerns, D.L. Field Evaluation of Cotton Expressing Mpp51Aa2 as a Management Tool for Cotton Fleahoppers, *Pseudatomoscelis seriatus* (Reuter). *Toxins* **2023**, *15*, 644. <https://doi.org/10.3390/toxins15110644>.
10. Sauka, D.H.; Peralta, C.; Pérez, M.P.; Molla, A.; Fernandez-Göbel, T.; Ocampo, F.; Palma, L. *Bacillus thuringiensis* Bt_UNVM-84, a Novel Strain Showing Insecticidal Activity against *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). *Toxins* **2024**, *16*, 4. <https://doi.org/10.3390/toxins16010004>.
11. Hou, X.; Li, M.; Mao, C.; Jiang, L.; Zhang, W.; Li, M.; Geng, X.; Li, X.; Liu, S.; Yang, G.; et al. Domain III β 4– β 5 Loop and β 14– β 15 Loop of *Bacillus thuringiensis* Vip3Aa Are Involved in Receptor Binding and Toxicity. *Toxins* **2024**, *16*, 23. <https://doi.org/10.3390/toxins16010023>.
12. Shao, E.; Huang, H.; Yuan, J.; Yan, Y.; Ou, L.; Chen, X.; Pan, X.; Guan, X.; Sha, L. N-Terminal α -Helices in Domain I of *Bacillus thuringiensis* Vip3Aa Play Crucial Roles in Disruption of Liposomal Membrane. *Toxins* **2024**, *16*, 88. <https://doi.org/10.3390/toxins16020088>.

References

1. Schnepf, E.; Crickmore, N.; Van Rie, J.; Lereclus, D.; Baum, J.; Feitelson, J.; Zeigler, D.R.; Dean, D.H. *Bacillus thuringiensis* and its pesticidal crystal proteins. *Microbiol. Mol. Biol. Rev.* **1998**, *62*, 775–806. [[CrossRef](#)]
2. Jouzani, G.S.; Valijanani, E.; Sharafi, R. *Bacillus thuringiensis*: A successful insecticide with new environmental features and tidings. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 2691–2711. [[CrossRef](#)] [[PubMed](#)]
3. Ohba, M.; Mizuki, E.; Uemori, A. Parasporin, a new anticancer protein group from *Bacillus thuringiensis*. *Anticancer Res.* **2009**, *29*, 427–433.
4. Chakroun, M.; Banyuls, N.; Bel, Y.; Escriche, B.; Ferré, J. Bacterial Vegetative Insecticidal Proteins (Vip) from Entomopathogenic Bacteria. *Microbiol. Mol. Biol. Rev.* **2016**, *80*, 329–350. [[CrossRef](#)]
5. Bravo, A.; Likitvivanavong, S.; Gill, S.S.; Soberon, M. *Bacillus thuringiensis*: A story of a successful bioinsecticide. *Insect Biochem. Mol. Biol.* **2011**, *41*, 423–431. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.