

Model-guided genome editing strategies, supported by CRISPR-Cas systems, allow for the rational design of metabolic pathways and the systematic testing of hypotheses (Jakočiūnas et al., 2017). Computational tools for the design of gRNAs and the prediction of off-target effects are also essential for the successful application of CRISPR technology in metabolic engineering (Jakočiūnas et al., 2016). These tools help to minimize unintended genetic modifications and to ensure the accuracy and efficiency of genome editing efforts.

The advances in CRISPR-Cas technology, high-throughput screening methods, and computational modeling have significantly enhanced the capabilities of metabolic engineering. These tools have enabled the precise and efficient modification of microbial genomes, facilitating the development of engineered strains with improved production of insecticidal proteins and other valuable compounds. The continued integration of these technologies promises to drive further innovations in the field of metabolic engineering.

8 Concluding Remarks

The systematic review of the metabolic engineering of *Bacillus thuringiensis* (Bt) for enhanced production of insecticidal proteins has highlighted several key findings. Bt proteins, particularly Cry and Vip toxins, have played a crucial role in pest management through their use in genetically engineered (GE) crops such as corn, cotton, and soybean. These proteins are highly specific to target pests while remaining safe for non-target organisms, including humans. However, the emergence of resistance in some pest populations presents a significant challenge, emphasizing the need for the development of new Bt proteins and strategies to manage resistance. Advances in protein engineering have led to the creation of chimeric and optimized Bt proteins with enhanced insecticidal activity and broader spectrum efficacy. Environmental and biosafety assessments of Bt crops generally support their safety, though ongoing monitoring and evaluation are essential.

The metabolic engineering of Bt for enhanced production of insecticidal proteins holds substantial potential for agriculture. Developing Bt proteins with higher potency and broader spectrum activity can improve pest control, reduce reliance on chemical insecticides, and promote sustainable agricultural practices. Enhanced Bt proteins can also help mitigate the issue of pest resistance, ensuring the long-term efficacy of Bt crops. Integrating Bt crops into integrated pest management (IPM) strategies can support the conservation of natural enemies and overall ecosystem health. The economic benefits for farmers, including increased crop yields and reduced pest management costs, further underscore the positive impact of metabolic engineering on agriculture.

Future research should focus on maximizing the benefits of metabolic engineering of Bt. Continued development and optimization of new Bt proteins are necessary to stay ahead of evolving pest resistance. Exploring novel protein engineering techniques and bioconjugation methods to enhance the efficacy and stability of Bt proteins is important. Comprehensive biosafety evaluations should be conducted to assess the environmental impact of new Bt proteins and ensure their safety for non-target organisms. Investigating the mechanisms of resistance in pests is essential for developing more effective resistance management strategies. Integrating Bt crops into broader IPM frameworks and promoting the use of refuges can help sustain the long-term effectiveness of Bt technology.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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