

4.3 Enhancing precursor availability

Enhancing the availability of precursors is another critical strategy in the metabolic engineering of Bt. By ensuring a steady supply of essential building blocks, the production of insecticidal proteins can be significantly increased. For instance, the use of molecular docking and bioinformatic analysis to engineer chimeric proteins like Cry1AcF has shown broad-spectrum efficacy against pests, indicating that optimizing precursor availability can lead to the development of more effective Bt strains (Rathinam et al., 2019). Moreover, the bioconjugation of Cry2Ab with AVM not only improved binding affinity but also suggested potential mechanisms for enhancing precursor availability through structural modifications (Pan et al., 2019).

The metabolic engineering of Bt for enhanced production of insecticidal proteins involves a multifaceted approach, including genetic modifications, optimization of metabolic flux, and enhancing precursor availability. These strategies collectively contribute to the development of more potent and efficient Bt strains, thereby improving their efficacy in pest management.

5 Case Studies of Successful Engineering in Bt

5.1 Enhanced Cry protein production

By employing various genetic engineering strategies, the production of Cry proteins in *Bacillus thuringiensis* (Bt) has been significantly enhanced. For example, the developed chimeric protein Cry1AcF exhibits broad-spectrum insecticidal effects. This chimeric protein is engineered to effectively interact with aminopeptidase 1 receptors from different insect species, thereby enhancing its insecticidal activity (Rathinam et al., 2019). Studies have shown that genetically engineered Bt proteins have higher stability and effectiveness, showing significant resistance enhancement against multiple pests, effectively addressing the issue of resistance to traditional Bt proteins (Figure 2). Furthermore, the seed industry is also optimizing the activity of Bt insecticidal proteins by modifying the protein structure to improve their effectiveness against target pests (Yamamoto et al., 2022).

The study by Rathinam et al. (2019) demonstrates that through genetic engineering strategies, the production of Cry proteins in *Bacillus thuringiensis* (Bt) has been significantly improved. Specifically, these strategies include protein structure optimization and gene combination modifications, resulting in engineered Bt with higher insecticidal efficiency and broad-spectrum resistance. This enhanced production capability effectively addresses the challenge of pest resistance, providing a more powerful tool for agricultural pest management.

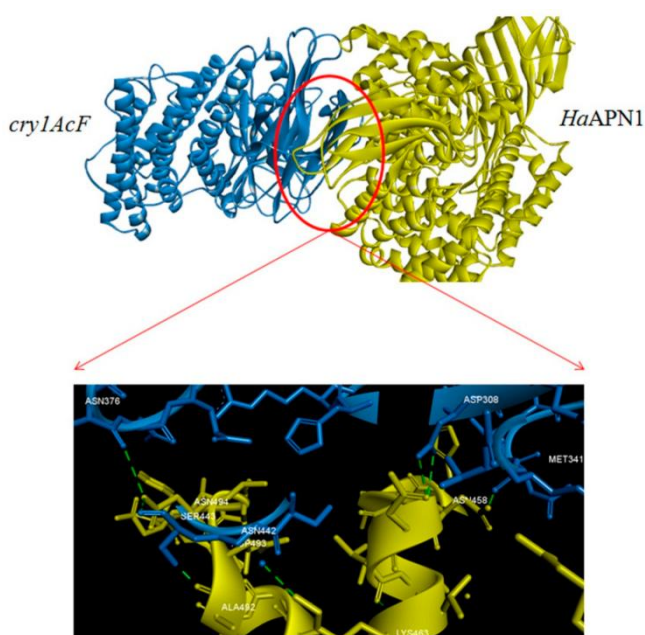


Figure 2 Molecular interaction between Cry1AcF and SIAPN1 (insect: specific region of interaction between the two proteins) (Adopted from Rathinam et al., 2019)