

5.2 Improved stability and yield

Improving the stability and yield of Bt insecticidal proteins is crucial for their effectiveness in agricultural applications. One innovative approach involves the use of magnesium hydroxide nanoparticles (MHNPs) to control the loss of Cry1Ac protein. These nanoparticles enhance the adhesion of Cry1Ac to plant surfaces, thereby increasing its stability and insecticidal activity. The Cry1Ac-loaded MHNPs showed a significant increase in pest mortality and remained stable during the adsorption process, making them a promising adjuvant for biopesticides (Rao et al., 2018). Furthermore, studies have shown that the environmental behavior of Bt proteins, including their adsorption, retention, and degradation in soils, can impact their stability and efficacy. Understanding these behaviors helps in designing strategies to maintain the activity of Bt proteins in field conditions (Li et al., 2022).

5.3 Integration of multiple engineering strategies

The integration of multiple engineering strategies has been pivotal in enhancing the production and effectiveness of Bt insecticidal proteins. For example, the combination of different Cry proteins in transgenic crops has been employed to delay resistance and broaden the spectrum of pests controlled. Second-generation Bt crops producing multiple toxins, such as Cry1Ac and Cry2Ab, have been developed to combat resistance in pests like *Helicoverpa armigera*. This approach has shown success in maintaining the efficacy of Bt crops over time (Tabashnik et al., 2015). Additionally, the creation of chimeric proteins by combining domains of different Cry proteins has led to enhanced insecticidal properties and broader pest control (Koch et al., 2015). These integrated strategies not only improve the effectiveness of Bt proteins but also contribute to sustainable pest management practices.

6 Challenges and Future Directions

6.1 Technical challenges in metabolic engineering

Metabolic engineering of *Bacillus thuringiensis* (Bt) for enhanced production of insecticidal proteins faces several technical challenges. One significant issue is the optimization of Bt insecticidal proteins to overcome resistance in target pests. For instance, pests such as *Heliothis virescens* and *Ostrinia nubilalis* have developed resistance to Cry1Ac and Cry1Ab proteins, necessitating the engineering of new Bt proteins with higher activity levels (Yamamoto et al., 2022). Additionally, the structural complexity of polyketide insecticidal agents poses challenges for chemical modification, which is essential for enhancing their efficacy and production (Yi et al., 2023). The metabolic pathways involved in the production of these proteins are intricate, and any genetic modifications can have unintended effects on bacterial growth, sporulation, and protein formation, as seen with the *gabD* and *sucA* gene knockouts.

6.2 Ecological and safety concerns

The ecological and safety concerns associated with Bt proteins are paramount. Bt insecticidal proteins, when released into the environment through transgenic plants or biopesticides, can persist in the soil and potentially affect non-target organisms. The structural and functional differences between naturally occurring Bt proteins and those expressed in genetically modified organisms (GMOs) necessitate thorough biosafety evaluations before field deployment (Li et al., 2022). Moreover, the evolution of resistance in pests not only reduces the efficacy of Bt crops but also raises concerns about the long-term sustainability of Bt technology (Jurat-Fuentes et al., 2021; Tabashnik et al., 2023). While extensive studies have shown that current Bt crops do not adversely affect non-target species, continuous monitoring and assessment are essential to ensure environmental safety (Koch et al., 2015; Romeis et al., 2019).

6.3 Scaling up production and commercialization

Scaling up the production of Bt insecticidal proteins for commercial use involves overcoming several hurdles. The production of stable isotope-labeled Cry proteins, for instance, requires precise recombinant expression protocols and purification processes, which can be technically demanding and costly (Wang et al., 2020). Additionally, the optimization of Bt proteins for higher insecticidal activity, as required for commercial viability, involves complex protein engineering and metabolic engineering strategies (Rathinam et al., 2019; Yamamoto et al., 2022). The development of high-yield strains through metabolic engineering and combinatorial biosynthesis is crucial for the