

3.2.3 Interactions with primary metabolism

The synthesis of insecticidal proteins in Bt is intricately linked to its primary metabolism. The energy and precursors required for the production of these proteins are derived from primary metabolic processes. For instance, the amino acids and nucleotides necessary for protein synthesis are generated through the central metabolic pathways. Moreover, the regulation of primary metabolic pathways can influence the efficiency and yield of toxin production, highlighting the interconnectedness of primary and secondary metabolism in Bt (Tabashnik et al., 2015; Li et al., 2022).

3.3 Role of metabolism in protein synthesis

Metabolism plays a crucial role in the synthesis of insecticidal proteins in Bt. The metabolic state of the bacterium determines the availability of resources required for protein synthesis. During the sporulation phase, when Cry proteins are produced, Bt undergoes significant metabolic shifts to allocate resources towards the synthesis of these proteins. Similarly, the production of Vip proteins during the vegetative phase is influenced by the metabolic activity of the bacterium. Understanding the metabolic pathways and their regulation in Bt is essential for optimizing the production of insecticidal proteins through metabolic engineering (Tabashnik et al., 2015; Li et al., 2022; Jin et al., 2022).

4 Metabolic Engineering Strategies for Bt

4.1 Genetic modifications for enhanced protein production

4.1.1 Overexpression of key genes

Overexpression of key genes in *Bacillus thuringiensis* (Bt) has been a pivotal strategy to enhance the production of insecticidal proteins. For instance, the introduction of a late embryogenesis abundant (LEA) peptide co-expression system has shown significant promise. By using the expression vector pHT01 with a strong σ^A -dependent promoter, the production of crystal proteins was enhanced threefold after 12 hours of induction with IPTG (Akhtar et al., 2021). This method was further optimized by using lactose as an inducer, which provided a more cost-effective and efficient alternative to IPTG, leading to enhanced Cry protein expression through intermittent induction (Akhtar et al., 2022).

4.1.2 Gene knockouts to remove bottlenecks

Gene knockouts have been employed to remove metabolic bottlenecks that hinder the efficient production of insecticidal proteins. By targeting and knocking out specific genes that compete for resources or produce inhibitory byproducts, the metabolic flux can be redirected towards the synthesis of desired proteins. This approach has been instrumental in increasing the yield and efficacy of Bt toxins, although specific examples in the context of Bt require further elucidation in the literature.

4.1.3 Use of synthetic biology tools

Synthetic biology tools have revolutionized the metabolic engineering of Bt. Techniques such as CRISPR-Cas9 and advanced gene editing have enabled precise modifications to the Bt genome, facilitating the creation of strains with enhanced insecticidal properties. For example, the engineering of Bt toxin Cry2Ab30 and its bioconjugation with 4''-O-succinyl avermectin (AVM) resulted in a bioconjugate with significantly higher insecticidal activity and binding affinity compared to the native Cry2Ab30 (Pan et al., 2019). This demonstrates the potential of synthetic biology in creating more potent and efficient Bt strains.

4.2 Optimization of metabolic flux

Optimizing the metabolic flux within Bt is crucial for maximizing the production of insecticidal proteins. This involves fine-tuning the metabolic pathways to ensure that precursors and energy are efficiently channeled towards the synthesis of target proteins. The use of stable isotope labeling, as demonstrated in the production of $^{13}\text{C}/^{15}\text{N}$ -labeled Cry1Ab/Ac proteins, provides insights into the metabolic fate of these proteins and helps in understanding and optimizing their production (Wang et al., 2020). Additionally, the co-expression of LEA peptides has been shown to enhance the metabolic flux towards crystal protein production, further highlighting the importance of metabolic optimization (Akhtar et al., 2021; Akhtar et al., 2022).