

economical production of these proteins (Yi et al., 2023). Furthermore, the commercialization of Bt crops and biopesticides must navigate regulatory frameworks that ensure their safety and efficacy, adding another layer of complexity to the scaling-up process (Koch et al., 2015).

While the metabolic engineering of Bt for enhanced production of insecticidal proteins holds great promise, it is fraught with technical, ecological, and commercial challenges. Addressing these challenges through innovative research and stringent safety evaluations will be key to the successful application and sustainability of Bt technology in pest management.

7 Advances in Biotechnology and Tools

7.1 CRISPR-Cas and genome editing

The advent of CRISPR-Cas technology has revolutionized the field of metabolic engineering, providing a precise and efficient method for genome editing. This technology allows for targeted DNA cleavage, enabling various genome engineering modes such as insertions, deletions, and chromosomal rearrangements (Nishida and Kondo, 2020). The versatility of CRISPR systems has led to the development of derivative technologies, such as deaminase-mediated base editing, which introduces point mutations with reduced cytotoxicity. Additionally, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) enable temporary control of gene expression without altering the genomic sequence, further expanding the toolkit available for metabolic engineering (Nishida and Kondo, 2020).

CRISPR-Cas systems have been successfully applied to a wide range of organisms, including industrially relevant microbes like *Escherichia coli* and *Saccharomyces cerevisiae* (Mougiakos et al., 2016). These systems have facilitated the rapid development of microbial cell factories by enabling high-efficiency genome editing and transcriptional regulation (Jakočiūnas et al., 2016). For instance, CRISPR-Cas9 has been used to introduce multiple mutations simultaneously in *E. coli*, optimizing metabolic pathways for the overproduction of valuable compounds such as β -carotene (Li et al., 2015). The discovery of novel Cas9-like systems from diverse microbial environments continues to broaden the range of organisms that can be engineered using CRISPR technology (Mougiakos et al., 2018).

7.2 High-throughput screening methods

High-throughput screening methods are essential for the efficient evaluation of genetic modifications and the identification of optimal metabolic pathways. CRISPR-Cas technology has significantly enhanced the capabilities of high-throughput screening by enabling multiplexed genome editing and the construction of guide RNA (gRNA) libraries (Ding et al., 2020). These advancements allow for the comprehensive discovery and evaluation of metabolic pathways, accelerating the development of engineered strains with desired traits (Nishida and Kondo, 2020).

The integration of CRISPR-Cas systems with high-throughput screening has been particularly impactful in the field of microbial biotechnology. For example, CRISPR-based tools have been used to perform genome-wide perturbations and model-guided genome editing strategies, enabling the systematic exploration of metabolic networks (Jakočiūnas et al., 2017). This approach has been applied to both model organisms and non-model microbes, facilitating the development of novel production hosts for biotechnologically relevant products (Mougiakos et al., 2018).

7.3 Computational modeling and simulation

Computational modeling and simulation play a crucial role in the design and optimization of metabolic engineering strategies. These tools enable researchers to predict the effects of genetic modifications on metabolic pathways and to identify potential bottlenecks and targets for further engineering. The integration of CRISPR-Cas technology with computational modeling has further enhanced the precision and efficiency of metabolic engineering efforts (Jakočiūnas et al., 2017).