

4.3 Horizontal gene transfer

Horizontal gene transfer (HGT) plays a crucial role in the genomic evolution of *Bacillus* species, including Bt. HGT events have been identified as a significant source of genomic novelty, contributing to the acquisition of virulence factors and antibiotic resistance (Sevillya et al., 2020). In Bt, the presence of multiple pesticidal protein genes on megaplasmsids suggests that these genes may have been acquired through HGT. Additionally, a study on the CRISPR-Cas system in *Mycobacterium tuberculosis* highlighted the role of HGT in the evolution of this system, providing a parallel to similar mechanisms that may occur in *Bacillus* species (Singh et al., 2021). The distribution of virulence genes in Bt does not always correlate with phylogenetic positions, further supporting the role of HGT in shaping the genomic landscape of these bacteria (Shikov et al., 2021).

In summary, the comparative genomics of Bt and related *Bacillus* species reveal a complex interplay of conserved and unique genomic regions, shaped significantly by phylogenetic relationships and horizontal gene transfer. These findings underscore the dynamic nature of bacterial genomes and the evolutionary processes that drive their diversity and adaptation.

5 Functional Genomics

5.1 Gene expression profiling

Gene expression profiling in *Bacillus thuringiensis* (Bt) and related *Bacillus* species has been extensively studied to understand the regulation of insecticidal protein production and other metabolic processes. For instance, the comparative analysis of the Bt X022 strain revealed that the presence of Cu^{2+} significantly influences the metabolic regulation of carbon flux, leading to increased production of insecticidal crystal proteins (ICPs) during the spore-release period (Quan et al., 2016). Similarly, in Bt strain 4.0718, gene expression profiling indicated that certain genes, such as *cry2Ab* and *cryIIa*, were either silenced or expressed at very low levels due to the lack of functional promoters or the presence of transposons (Rang et al., 2015). These findings underscore the importance of gene expression profiling in identifying the regulatory mechanisms that control the production of key proteins in *Bacillus* species.

5.2 Proteomics and metabolomics

Proteomics and metabolomics provide a comprehensive understanding of the protein expression and metabolic pathways in *Bacillus* species. In Bt X022, proteomic analysis during the spore-release period identified several ICPs, including Cry1Ca, Cry1Ac, and Cry1Da, which were not predicted by genomic data alone (Quan et al., 2016). This highlights the complementary nature of proteomics in validating genomic predictions. Additionally, the proteomic analysis of Bt strain 4.0718 revealed the presence of sporulation-related proteins such as Spo0A~P, SigF, SigE, SigK, and SigG, which play crucial roles in the spore formation regulatory network (Rang et al., 2015). Metabolomic studies have also been instrumental in elucidating the metabolic pathways involved in the production of secondary metabolites. For example, the comparative genomic and functional analyses of *Bacillus cereus* strains identified conserved genes related to plant-growth-promoting traits, which are crucial for their ecological adaptation (Zeng et al., 2018). These studies demonstrate the power of proteomics and metabolomics in uncovering the functional aspects of *Bacillus* species.

5.3 Functional studies of specific genes

Functional studies of specific genes in *Bacillus* species have provided insights into their roles in various biological processes. In the *Bacillus cereus* group, genes under positive selection were found to be involved in antibiotic resistance, DNA repair, nutrient uptake, metabolism, cell wall assembly, and spore structure, indicating their importance in ecological adaptation (Rasigade et al., 2018). Additionally, the large-scale bioinformatics analysis of *Bacillus* genomes uncovered conserved roles of natural products in bacterial physiology, with certain specialized metabolites acting as developmental signals that inhibit sporulation (Grubbs et al., 2017). These functional studies are crucial for understanding the genetic basis of the diverse phenotypic traits observed in *Bacillus* species and for developing strategies for their genetic modification and application in various fields.