

3.2.2 Gene expression and regulation

The expression of Bt toxin genes is tightly regulated and can be influenced by various factors. For instance, proteomic analysis of the parasporal crystals of the GR007 strain revealed the presence of eight Cry proteins, with differential toxicity against different insect larvae (Pacheco et al., 2021). In the case of Bt X022, the presence of Cu²⁺ was found to increase the production of insecticidal crystal proteins, indicating a regulatory mechanism linked to environmental factors (Quan et al., 2016).

3.2.3 Evolution of toxin genes

The evolution of Bt toxin genes is marked by horizontal gene transfer and recombination events. For example, the coexistence of *cry9A* and *vip3A* genes in the same plasmid in certain Bt strains suggests a recombination mechanism responsible for their association (Wang et al., 2020). Phylogenetic analysis of toxin genes in the mosquitocidal Bt strain S2160-1 also indicates evolutionary relationships among various Cry and toxin proteins (Liu et al., 2018).

3.3 Plasmids and mobile genetic elements

Plasmids play a crucial role in the genetic diversity and adaptability of Bt. In the Bt GR007 strain, plasmids pGR340 and pGR157 contain multiple insecticidal protein genes, including *cry* and *vip* genes. These findings help understand the evolutionary mechanisms of Bt and its adaptability in different environments (Figure 1) (Pacheco et al., 2021). Similarly, the plasmid in the T414 strain carries various insecticidal genes and additional toxic factors, such as chitinase and protease (Reyaz et al., 2019). Mobile genetic elements, including insertion sequences and prophages, significantly contribute to the plasticity of the Bt genome. For example, the presence of IS3 and *bcr1* elements in *B. cytotoxicus* highlights the role of mobile genetic elements in genomic diversity and horizontal gene transfer (Fayad et al., 2021).

Figure 1 shows the genomic structure of the Bt GR007 strain, including one circular chromosome and three plasmids (pGR340, pGR157, and pGR55). These plasmids carry various insecticidal protein gene clusters, particularly the toxin gene clusters on plasmids pGR340 and pGR157, demonstrating the crucial role of Bt in genetic diversity and environmental adaptation. The pGR340 plasmid contains *cry* and *vip* gene clusters, which are exchanged and recombined among different Bt strains through horizontal gene transfer, thereby enhancing Bt's adaptability and insecticidal efficacy. Figure 1 provides a detailed depiction of these plasmids and gene clusters, offering important references for studying the genetic structure and function of Bt insecticidal protein genes.

4 Comparative Genomics with Related *Bacillus* Species

4.1 Phylogenetic relationships

The phylogenetic relationships within the *Bacillus* genus, particularly between *Bacillus thuringiensis* (Bt) and other *Bacillus* species, have been extensively studied using whole-genome sequencing and phylogenomic analyses. A study involving nearly 900 whole genome sequences of *Bacillus cereus* and Bt strains revealed that these species form a well-supported monophyletic clade, distinct from other *Bacillus* species (Baek et al., 2019). This clade is further divided into two genomovars: *B. thuringiensis* gv. *thuringiensis* and *B. thuringiensis* gv. *cytolyticus*, indicating significant genomic diversity within Bt itself (Baek et al., 2019). Additionally, the Genome BLAST Distance Phylogeny (GBDP) approach has been used to classify *Bacillus cereus* group strains into distinct clusters, further elucidating their phylogenetic relationships (Liu et al., 2015).

4.2 Conserved and unique genomic regions

Comparative genomic analyses have identified both conserved and unique genomic regions within *Bacillus* species. A large-scale bioinformatics analysis of 1 566 *Bacillus* genomes uncovered that the majority of specialized metabolites produced by *Bacillus* species are highly conserved, with significant roles in bacterial physiology and development (Grubbs et al., 2017). However, unique biosynthetic gene clusters (BGCs) were also identified, which are scattered across the genus and may encode unknown natural products (Grubbs et al., 2017). In Bt, specific pesticidal protein genes, such as *cry* and *vip* genes, are often located on megaplasmids, which are not uniformly distributed across all strains, indicating a level of genomic uniqueness (Pacheco et al., 2021).