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Phylogenetic Analysis of Bt Strains: Insights into Genetic Relationships and Divergence

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Abstract This study reviews the current phylogenetic analysis of *Bacillus thuringiensis* (Bt) strains, focusing on their genetic relationships and divergence. By analyzing various genetic markers, such as the 16S rRNA gene and multi-locus sequence typing (MLST), we reveal significant genetic diversity and complex evolutionary relationships among Bt strains. Horizontal gene transfer (HGT) and recombination events play crucial roles in the genetic adaptability and diversity of Bt, particularly through the plasmid-mediated transfer of insecticidal toxin genes. The paper further explores the applications of Bt in biopesticide development, highlighting its role in agricultural pest control and environmental management. Utilizing whole-genome sequencing and comparative genomics, we provide detailed insights into novel genes and toxins found in Bt strains. Additionally, the study outlines the potential applications of Bt in medicine, plant growth promotion, and bioremediation. I discuss future research directions, emphasizing the importance of further exploring the evolutionary mechanisms of Bt strains, genetic engineering enhancements, and the long-term impacts of Bt applications in various ecosystems.
Keywords *Bacillus thuringiensis*; Phylogenetic analysis; Genetic diversity; Horizontal gene transfer

1 Introduction

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium widely recognized for its insecticidal properties. This microorganism produces crystalline inclusions during sporulation, which contain delta-endotoxins (Cry and Cyt proteins) that are toxic to various insect larvae upon ingestion. Bt has been extensively utilized in agriculture as a biological pesticide due to its specificity to target pests, reducing the need for chemical pesticides and minimizing environmental impact (Reyaz et al., 2019; Peralta et al., 2021). Bt strains exhibit a diverse array of insecticidal proteins, which are effective against Lepidoptera, Diptera, and Coleoptera, among other insect orders (Barbosa et al., 2015; Quan et al., 2016).

Phylogenetic analysis is crucial for understanding the genetic relationships and evolutionary history of Bt strains. By examining the genetic markers and sequences, researchers can classify Bt strains, identify new strains with unique insecticidal properties, and track the genetic divergence and adaptation mechanisms within the species (Wang et al., 2018; Shikov et al., 2021). This analysis helps in maintaining the efficacy of Bt as a biopesticide and also aids in the discovery of novel genes and proteins that can be leveraged for pest control (Lechuga et al., 2020). Phylogenetic insights contribute to the understanding of the horizontal gene transfer events that shape the genome architecture of Bt, facilitating the development of effective biocontrol agents (Baek et al., 2019).

This study provides a comprehensive summary of the current understanding of the genetic diversity and systematic relationships of *Bacillus thuringiensis* (Bt) strains. It emphasizes the importance of systematic analyses in identifying and classifying Bt strains with unique insecticidal properties. By exploring the latest advancements in systematic methods, this study highlights the application of these methods in Bt research, helping to develop and deploy more effective Bt-based biopesticides. The study will discuss the impact of systematic findings on the ongoing struggle against pest resistance, providing insights into how these findings can promote sustainable agricultural practices. Ultimately, this study provides valuable resources for researchers and practitioners in the field of biological control, promoting a deeper understanding of the genetic landscape of Bt and enhancing the strategic use of this important bacterium in pest management programs.

2 Overview of Bt Strains

2.1 Diversity of Bt strains

Bacillus thuringiensis (Bt) strains display a remarkable degree of genetic diversity, which significantly contributes to their efficacy as biopesticides. This diversity arises from the extensive variety of Cry and Cyt toxins produced by different Bt strains, which target specific insect orders. Studies employing techniques such as random amplified polymorphic DNA (RAPD) analysis have shown significant genetic variation among Bt strains. For instance, research on Bt isolates from Kuwait revealed the existence of multiple genetically distinct groups within the Bt population, underscoring the complexity and adaptability of this species (Qasem et al., 2015).

The genome sequencing of Bt strains such as Bt-UNVM_94 from Argentina has highlighted their ability to produce a broad spectrum of insecticidal proteins, demonstrating dual activity against lepidopteran and coleopteran pests (Peralta et al., 2021). This genetic diversity not only enhances the effectiveness of Bt as a biopesticide but also aids in overcoming insect resistance, a growing challenge in pest management. The broad genetic variation found in Bt strains ensures a continuous supply of novel toxins that can be leveraged to develop new biopesticides, thereby maintaining the long-term efficacy of Bt-based pest control strategies.

2.2 Key characteristics and applications

Bacillus thuringiensis is characterized by its ability to produce crystalline inclusions during sporulation. These inclusions contain delta-endotoxins, specifically Cry and Cyt proteins, which are toxic to a wide range of insect larvae upon ingestion. The insecticidal properties of Bt make it an invaluable tool in integrated pest management (IPM). For example, the Brazilian strain Bt 147 has been noted for its high insecticidal activity against various insect larvae, providing an environmentally friendly alternative to traditional chemical pesticides (Barbosa et al., 2015).

Bt's specificity to target pests minimizes collateral damage to non-target organisms, making it a safer option for pest control. Another notable strain is Bt-UNVM-84, which shows significant insecticidal activity against the cotton boll weevil, a major pest in the cotton industry, thereby contributing to sustainable agricultural practices (Sauka et al., 2023). The wide array of toxins produced by different Bt strains allows for targeted pest control, reducing the likelihood of pest resistance development. Bt has been employed in genetically modified crops, such as Bt corn and Bt cotton, which express Bt toxins and provide built-in pest resistance, further reducing the need for chemical pesticides. The versatility and effectiveness of Bt underscore its pivotal role in modern agriculture.

2.3 Historical perspective on Bt research

The history of *Bacillus thuringiensis* research dates back to its discovery in 1901 by Japanese bacteriologist Ishiwata Shigetane. Initially identified as a pathogen in silkworms, Bt's potential as a biological control agent was recognized in the mid-20th century. The first commercial Bt-based biopesticide was developed in the 1950s, marking the beginning of extensive research into its insecticidal properties and applications. Over the decades, advancements in molecular biology and genomics have significantly enhanced our understanding of Bt. The sequencing of various Bt strains, such as BM-BT15426 and Bt-UNVM_94, has revealed insights into their genetic makeup and pathogenic mechanisms (Liu et al., 2017; Peralta et al., 2021).

These genomic studies have facilitated the identification of numerous Cry and Cyt toxins, broadening the scope of Bt's applications. Research has explored Bt's role in integrated pest management, highlighting its environmental benefits compared to chemical pesticides. Historical research has also focused on the mechanisms of insect resistance to Bt, leading to the development of strategies to mitigate this challenge. Overall, the historical evolution of Bt research reflects its growing importance in sustainable agriculture and its potential to address future pest management challenges.

3 Methods for Phylogenetic Analysis

3.1 Sample collection and identification

Sample collection is a crucial first step in the phylogenetic analysis of *Bacillus thuringiensis* (Bt) strains. The process typically involves isolating Bt from various environmental sources such as soil, plants, and insect larvae.

For example, in a study conducted in Kuwait, researchers isolated 109 Bt strains from soil samples using culture and serological methods, ultimately identifying 15 subspecies of Bt thuringiensis (Qasem et al., 2015).

The isolation process often includes selective media and biochemical tests to differentiate Bt from other *Bacillus* species. Once isolated, the strains are identified using a combination of morphological characteristics and molecular techniques. Techniques such as 16S rRNA sequencing are commonly employed to confirm the identity of Bt strains. In addition to morphological and biochemical identification, advanced methods like serotyping and analysis of flagellin proteins are used to classify the strains further (Shikov et al., 2021). This comprehensive approach ensures accurate identification and categorization of Bt strains, which is essential for subsequent phylogenetic analysis.

3.2 DNA extraction and sequencing

DNA extraction and sequencing are fundamental to phylogenetic studies. The extraction process involves breaking down the cell walls of Bt strains to release DNA, which is then purified. Various kits and methods, such as phenol-chloroform extraction and commercial DNA extraction kits, are used to obtain high-quality DNA. Once extracted, the DNA undergoes sequencing to analyze the genetic material. Whole-genome sequencing and targeted sequencing of specific genes, such as those encoding for Cry and Cyt proteins, are commonly used.

For instance, Wang et al. (2018) utilized multi-locus sequence typing (MLST) to analyze seven housekeeping genes (*glpF*, *gmK*, *ilvD*, *pta*, *pur*, *pycA*, and *tpi*) in 233 Bt strains. This method allowed the researchers to establish genetic relationships and identify new sequence types (STs). Another study employed the random amplified polymorphic DNA (RAPD) technique to assess genetic diversity among Bt strains, demonstrating the variability in DNA patterns (Qasem et al., 2015). High-throughput sequencing technologies, such as Illumina and PacBio, provide detailed genomic data, facilitating in-depth phylogenetic analysis and evolutionary studies.

3.3 Computational tools and software

Computational tools and software are essential for analyzing sequencing data and constructing phylogenetic trees. These tools help in aligning sequences, detecting genetic variations, and visualizing evolutionary relationships. One commonly used software is MEGA (Molecular Evolutionary Genetics Analysis), which facilitates sequence alignment, model testing, and phylogenetic tree construction. In their study, Rabha et al. (2018) used MEGA for phylogenetic analysis of Bt isolates from Assam, identifying unique sequence types and analyzing vegetative insecticidal protein (vip) genes.

Another tool, the Composition Vector Tree (CVTree) method, was employed by Wang et al. (2023) for high-resolution typing of Bt strains, proving effective for genomic variability analysis. Software like BioEdit and ClustalW are used for multiple sequence alignments, while RAxML and BEAST provide robust platforms for phylogenetic inference and evolutionary analysis. Phylogenetic trees can be visualized using tools like FigTree and Dendroscope. These computational tools enable researchers to interpret complex genomic data and understand the genetic relationships and evolutionary history of Bt strains comprehensively.

4 Genetic Markers and Loci

4.1 Selection of genetic markers

The selection of appropriate genetic markers is critical for the effective phylogenetic analysis of *Bacillus thuringiensis* (Bt) strains. Genetic markers are specific sequences of DNA that can be used to identify and differentiate between various strains. These markers are chosen based on their ability to provide clear, reproducible, and polymorphic data that reflect the genetic diversity and evolutionary relationships among Bt strains. Commonly selected markers include housekeeping genes, which are essential for basic cellular functions and are highly conserved across different strains. For instance, multi-locus sequence typing (MLST) often employs housekeeping genes such as *glpF*, *gmK*, *ilvD*, *pta, pur*, *pycA*, and *tpi* to assess genetic variability and establish phylogenetic relationships (Wang et al., 2018).

Random amplified polymorphic DNA (RAPD) markers are used to analyze genetic diversity without prior knowledge of the genome, offering a quick and effective means of strain differentiation (Qasem et al., 2015). The

selection process also considers the ease of amplification and the level of polymorphism provided by the markers, ensuring that the chosen markers can effectively distinguish between closely related strains and reveal evolutionary patterns.

4.2 Commonly used loci in Bt phylogenetics

In Bt phylogenetics, several loci are frequently utilized to construct phylogenetic trees and study the genetic relationships among strains. The 16S rRNA gene is a widely used marker due to its presence in all bacteria and its slow evolutionary rate, making it suitable for distinguishing broad taxonomic groups (Rahman et al., 2022). However, for higher resolution, additional loci such as housekeeping genes are employed. MLST schemes often use genes like *glpF*, *gmK*, *ilvD*, *pta*, *pu*r, *pycA*, and *tpi*, which provide detailed insights into the genetic makeup of Bt strains (Wang et al., 2018).

The Cry and Cyt toxin genes, responsible for the insecticidal properties of Bt, are also crucial markers. These genes not only help in identifying specific Bt strains but also in understanding their pathogenic potential (Reyaz et al., 2019). Other loci include the vip genes, which encode vegetative insecticidal proteins and are used to study the diversity and phylogenetic relationships of Bt strains with biopesticide potential (Rabha et al., 2018). These commonly used loci provide a comprehensive understanding of the genetic diversity, evolutionary history, and functional capabilities of Bt strains.

4.3 Comparative analysis of marker effectiveness

Comparative analysis of different genetic markers is essential to determine their effectiveness in resolving phylogenetic relationships among Bt strains. Housekeeping genes used in MLST, such as those mentioned earlier, offer high-resolution data and are effective in distinguishing closely related strains (Wang et al., 2018). They provide robust phylogenetic trees that can reveal subtle genetic differences and evolutionary patterns. The 16S rRNA gene, while useful for broad classification, often lacks the resolution needed for finer phylogenetic distinctions among closely related strains (Rahman et al., 2022).

In contrast, RAPD markers are highly polymorphic and can differentiate strains without prior genomic information, making them useful for initial screening and diversity studies (Qasem et al., 2015). However, RAPD results can sometimes be less reproducible compared to MLST due to their random nature. The Cry and Cyt toxin genes are highly specific and effective in identifying Bt strains with particular insecticidal properties, but they may not provide a complete picture of the overall genetic diversity (Reyaz et al., 2019). Therefore, a combination of different markers is often employed to leverage the strengths of each and provide a comprehensive phylogenetic analysis. This integrative approach ensures accurate and detailed insights into the genetic relationships and evolutionary history of Bt strains.

5 Phylogenetic Tree Construction

5.1 Methods for tree construction

Constructing phylogenetic trees is essential for understanding the evolutionary relationships among *Bacillus thuringiensis* (Bt) strains. Several methodologies are employed to achieve accurate phylogenetic analysis. One of the most commonly used methods is Multi-Locus Sequence Typing (MLST), which involves sequencing multiple housekeeping genes and analyzing their combined data. For instance, Wang et al. (2018) performed MLST using seven housekeeping genes (*glpF*, *gmK*, *ilvD*, *pta*, *pur*, *pycA*, and *tpi*) to analyze 233 Bt strains, revealing significant genetic relationships and evolutionary patterns.

Another effective method is Whole-Genome Sequencing (WGS), which provides comprehensive genomic data allowing for detailed phylogenetic analysis. Wang and Ash (2015) demonstrated the effectiveness of WGS in constructing phylogenetic trees by comparing 50 complete Bacillus genome sequences using the Feature Frequency Profile (FFP) method, which supported the monophyletic status of Bt. The use of high-throughput sequencing technologies, such as Illumina and PacBio, has facilitated the assembly of complete genomes and the resolution of complex phylogenetic relationships (Lechuga et al., 2020). Tools like MEGA, RAxML, and BEAST

are commonly used for sequence alignment, model testing, and tree construction, providing robust platforms for phylogenetic inference.

5.2 Interpretation of phylogenetic trees

Interpreting phylogenetic trees involves understanding the evolutionary relationships and genetic distances between different Bt strains. A phylogenetic tree visually represents these relationships, with branches indicating divergence points from common ancestors. Nodes on the tree represent hypothetical common ancestors, while the length of branches corresponds to genetic distances or evolutionary time. For example, the phylogenetic analysis of Bt strains by Wang et al. (2018) using MLST revealed two major clusters containing 21 sub-groups, highlighting the genetic diversity and evolutionary lineage within the Bt species. Similarly, Lechuga et al. (2020) constructed a phylogenetic tree based on whole-genome sequences, which placed Bt HER1410 within a clade comprising members from the *B. thuringiensis* serovar thuringiensis and other serovars, illustrating the intermingled taxonomy within the *B. cereus* group.

By comparing phylogenetic trees constructed using different markers, researchers can validate the consistency of evolutionary relationships and identify potential discrepancies. For instance, the use of both 16S rRNA and housekeeping genes helps ensure a more accurate depiction of phylogenetic relationships (Shikov et al., 2021). Phylogenetic trees also provide insights into horizontal gene transfer events, gene loss, and the evolutionary pressures shaping the genetic diversity of Bt strains.

5.3 Case studies of phylogenetic trees

Case studies of phylogenetic tree construction in Bt research provide practical examples of how these methodologies are applied. One notable study by Rabha et al. (2018) utilized MLST to analyze Bt isolates from Assam soil, identifying 14 unique sequence types (STs) and demonstrating the phylogenetic diversity of these isolates. The phylogenetic tree constructed in this study revealed three major lineages, with most isolates belonging to Bt, and a few clustering with B. cereus, highlighting the genetic relationships and diversity within the Bt strains.

Another case study by Shikov et al. (2021) used proteomic and genomic data to assess the phylogenetic relationships of Bt strains, finding that the distribution of several genomic virulence determinants did not align with traditional serotyping classification, suggesting the need for phylogenomics approaches for more accurate classification (Figure 1).

Wang and Ash (2015) employed the FFP method to construct a whole-genome phylogeny of *Bacillus* species, confirming the placement of Bt within a single clade and demonstrating the effectiveness of genome-wide analyses in resolving phylogenetic relationships. These case studies illustrate the practical applications and importance of phylogenetic tree construction in understanding the genetic and evolutionary dynamics of Bt strains.

6 Genetic Relationships and Divergence

6.1 Identification of genetic relationships

Identifying genetic relationships among *Bacillus thuringiensis* (Bt) strains is crucial for understanding their evolutionary dynamics and potential applications. The genetic relationships are typically determined using phylogenetic analysis based on various genetic markers, such as housekeeping genes, 16S rRNA, and toxin genes. For example, Multi-Locus Sequence Typing (MLST) has been widely used to assess the genetic diversity and relationships among Bt strains. In a study by Wang et al. (2018), MLST analysis of 233 Bt strains revealed significant genetic relationships, identifying two major clusters containing 21 sub-groups. This method allows for the classification of Bt strains based on their genetic sequences, providing a detailed picture of their evolutionary lineage.

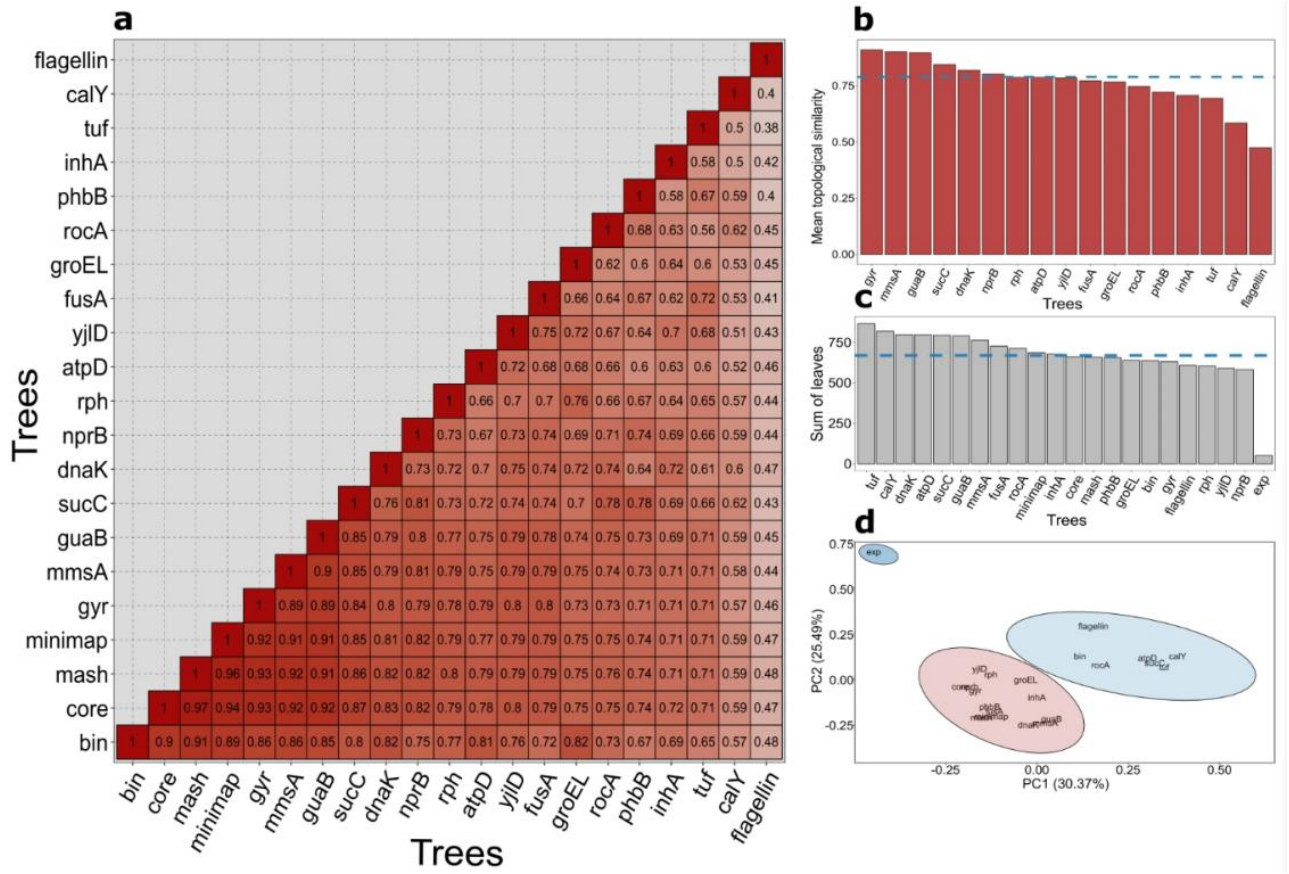

Figure 1 Topological comparison between trees and their relevance to serological classification (Adopted from Shikov et al., 2021)
Image caption: Red, blue, and green represent the proteins of different Bt strains, with red for strain 800/3, blue for strain 109/25, and green for strain 800/15. The overlapping of proteins labeled with different fluorescent colors on the 2D-DIGE image reveals the presence of protein differences among the strains during the sporulation phase. Although all strains form crystal inclusions, three-domain Cry toxins were not detected in any of the strains. Analyzing these differences allows for a better understanding of the virulence characteristics and classification relationships of different Bt serotypes (Adapted from Shikov et al., 2021)

Whole-genome sequencing (WGS) is another powerful tool that offers comprehensive insights into the genetic relationships of Bt strains. Lechuga et al. (2020) utilized WGS to analyze the complete genome of Bt HER1410, revealing its close genetic relationship with *B. thuringiensis* serovar thuringiensis and other serovars, thus highlighting the intermingled taxonomy within the *B. cereus* group. By employing these advanced genomic techniques, researchers can accurately identify genetic relationships, trace evolutionary histories, and classify Bt strains more effectively (Figure 2).

6.2 Analysis of genetic divergence

Genetic divergence among Bt strains is analyzed to understand how genetic variations contribute to the evolution and adaptation of these bacteria. Genetic divergence can be quantified by comparing genetic sequences and identifying differences that have accumulated over time. Techniques such as MLST, WGS, and comparative genomics are used to study genetic divergence. For instance, a study on Bt strains from Kuwait using random amplified polymorphic DNA (RAPD) analysis showed significant genetic divergence among the isolates, which were grouped into distinct clusters based on their genetic patterns (Qasem et al., 2015).

Comparative genomic studies also provide insights into genetic divergence by identifying unique genomic features and variations among strains. In their study, Rabha et al. (2018) conducted a comparative analysis of Bt isolates from Assam soil, revealing substantial genetic divergence and the presence of unique vegetative insecticidal protein (vip) genes in different strains. The transfer of plasmids carrying toxin genes contributes to genetic divergence and adaptation to new hosts. Zheng et al. (2017) demonstrated that the acquisition of plasmids

with cry genes plays a significant role in the genetic divergence of Bt strains, enabling them to adapt to various invertebrate hosts. Analyzing genetic divergence helps in understanding the mechanisms driving the evolution of Bt and its adaptation to different ecological niches.

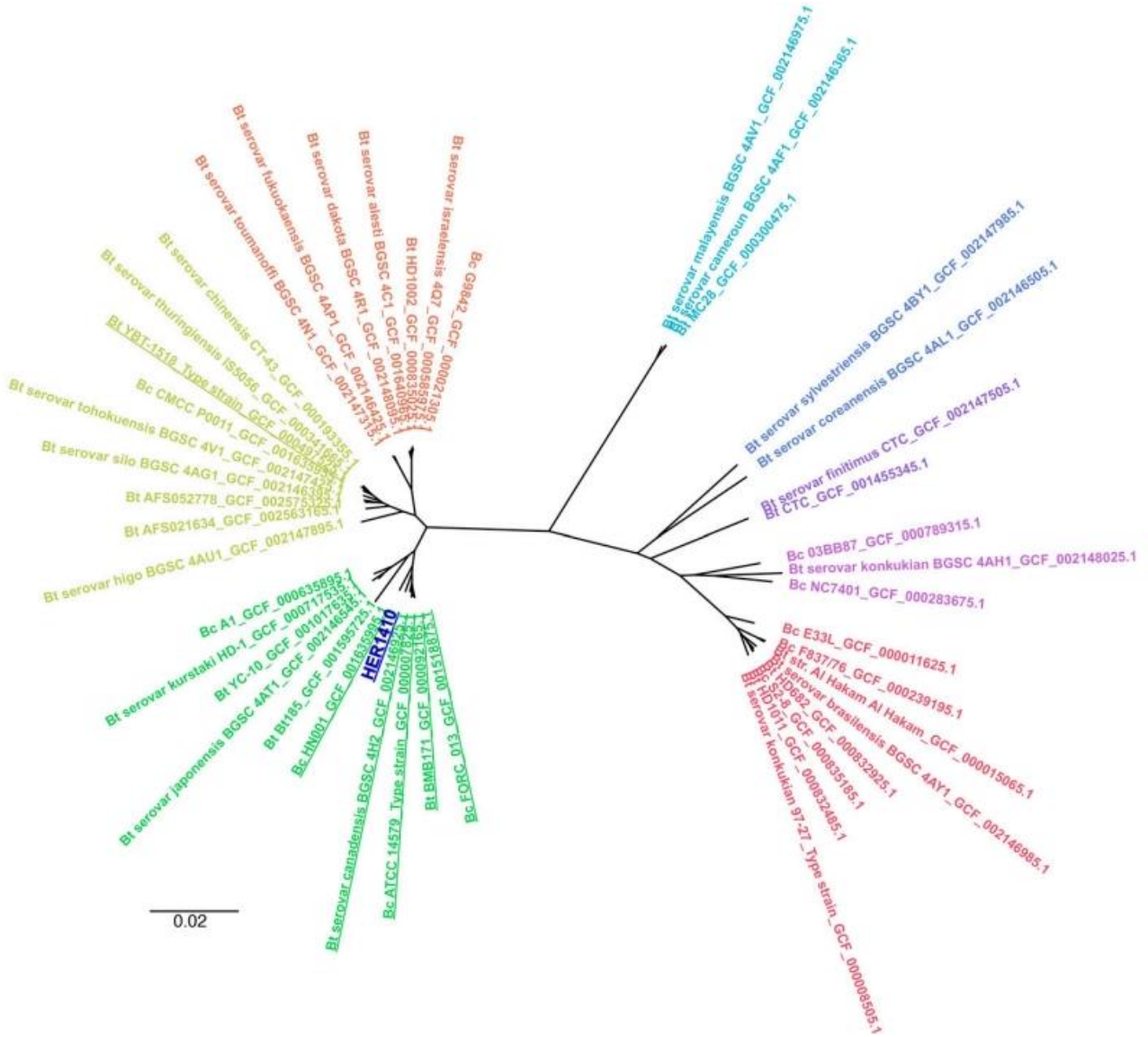

Figure 2 Overall position of *B. thuringiensis* HER1410 in the *Bacillus cereus-thuringiensis* phylogeny (Adopted from Lechuga et al., 2020)

Image caption: This figure is an unrooted tree showing the phylogenetic position of HER1410 (dark blue) among the selected *B. thuringiensis* (Bt) and *B. cereus* (Bc) strains. A maximum likelihood tree was generated using core genes from multiple sequence alignments, and different strains were grouped into distinct clades. The clade containing the HER1410 strain is underlined. This analysis shows a high degree of genomic similarity between HER1410, *B. cereus*, and other *B. thuringiensis* strains (Adopted from Lechuga et al., 2020)

6.3 Implications for Bt evolution

The genetic relationships and divergence among Bt strains have profound implications for understanding their evolution and potential applications. The ability of Bt to acquire and exchange genetic material through horizontal gene transfer, particularly via plasmids, is a key factor in its evolutionary success. The presence of diverse cry and vip genes on plasmids allows Bt strains to develop new insecticidal properties and adapt to various hosts (Zheng et al., 2017). This genetic plasticity is essential for the evolution of Bt as an effective biocontrol agent.

The study of genetic divergence and relationships can inform the development of more effective Bt-based biopesticides by identifying strains with unique and potent insecticidal properties. The evolutionary insights gained from phylogenetic and genomic analyses also help in predicting the emergence of resistance in target pests and developing strategies to mitigate this issue. Understanding the genetic basis of host specialization and adaptation in Bt can lead to the discovery of novel biocontrol agents with broader or more specific target ranges, enhancing the sustainability of pest management programs. Overall, the genetic study of Bt strains provides valuable knowledge that drives the evolution of more efficient and adaptable biocontrol solutions.

7 Horizontal Gene Transfer and Recombination

7.1 Role of horizontal gene transfer

horizontal gene transfer (hgt) plays a pivotal role in the evolution and diversification of *Bacillus thuringiensis* (Bt). It facilitates the rapid acquisition of new genes, including those encoding insecticidal toxins, which enhance the bacterium's ability to adapt to different environmental niches and hosts. HGT occurs through several mechanisms, including conjugation, transformation, and transduction. For instance, the large conjugative plasmid pXO16 from Bt serovar israelensis demonstrates the ability to transfer genes efficiently between different Bt strains and even other Bacillus species. This plasmid can mobilize and retro-mobilize non-conjugative plasmids, enhancing genetic diversity and adaptability (Makart et al., 2017).

The transfer of plasmids carrying mosquitocidal toxin genes, such as the 144-kb plasmid pTAND672-2, underscores the importance of HGT in spreading beneficial traits among bacterial populations (Geng et al., 2023). These plasmids often carry genes for toxins like Cry and Cyt proteins, which are crucial for Bt's insecticidal properties, thereby significantly contributing to the bacterium's ecological success.

7.2 Evidence of recombination events

Recombination events are integral to the genetic evolution of Bt, allowing for the reshuffling of genetic material and the creation of novel gene combinations. Evidence of recombination is often observed in the genetic structure of Bt strains, where segments of DNA are exchanged between different plasmids or between plasmids and the chromosome. For example, the plasmid pXO16 has been shown to transfer chromosomal markers at significant frequencies without the need for integration into the chromosome, indicating a unique mobilization mechanism (Makart et al., 2017). Studies have demonstrated the integration and circularization of plasmids like pTAND672-2 into the chromosome of recipient bacteria, highlighting the role of site-specific recombination in gene transfer (Geng et al., 2023).

Another study by Wang et al. (2016) described the use of the Mob/oriT recombination system for markerless genetic manipulation in Bt, demonstrating the practical applications of recombination events in genetic engineering. These recombination events contribute to the genetic plasticity of Bt, enabling it to acquire and maintain a diverse array of genes that enhance its survival and ecological versatility (Figure 3).

7.3 Impact on genetic diversity

The impact of horizontal gene transfer and recombination on genetic diversity in Bt is profound. These processes introduce new genetic material into bacterial populations, increasing genetic variation and enabling rapid adaptation to changing environments. The exchange of plasmids carrying toxin genes, for example, enhances the insecticidal capabilities of Bt strains, allowing them to target a wider range of insect pests. This genetic diversity is crucial for the long-term effectiveness of Bt as a biocontrol agent, as it helps prevent the development of resistance in target insect populations.

The study by Hinnekens et al. (2019) on the extended host spectrum of pXO16 demonstrates how plasmid transfer can broaden the ecological niches that Bt can occupy. The genetic manipulation techniques developed by Wang et al. (2016) and others leverage recombination to create genetically enhanced Bt strains with improved biocontrol properties. The continuous influx of new genes through HGT and recombination ensures that Bt remains a dynamic and adaptable organism, capable of evolving in response to environmental pressures and maintaining its role as a key player in biological pest control.

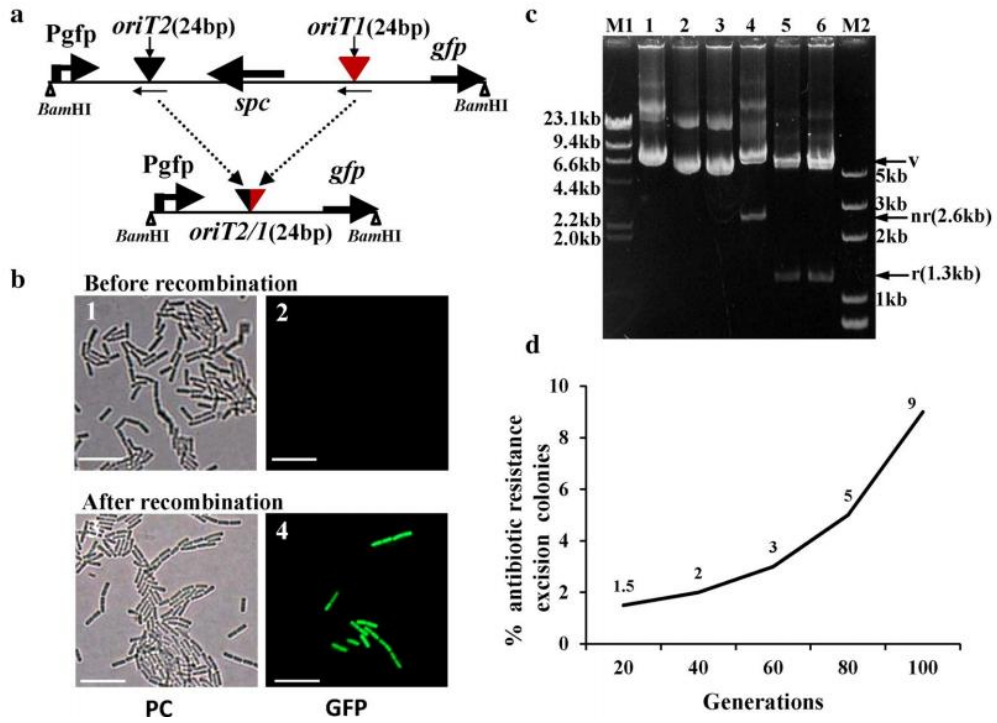

Figure 3 Recombination frequency of pBMBTmini (Adopted from Wang et al., 2016)

Image caption: (a) Illustrates the structure of the recombination cassette and the resulting product after recombination. (b) Presents the observations of BMB171 (pBMBTmini + pBMBmob1) under phase-contrast microscopy and fluorescence microscopy before and after recombination. (c) Displays the restriction enzyme digestion analysis of the substrate plasmid before and after recombination. The results indicate that the Mob02281/mini-oriT system can mediate the deletion of the target gene, with the recombination frequency increasing with the number of generations (Adapted from Wang et al., 2016)

8 Applications and Implications

8.1 Biopesticide development

Bacillus thuringiensis (Bt) is one of the most successful and widely used microbial biopesticides. The primary application of Bt in pest management is due to its ability to produce insecticidal proteins, such as Cry and Cyt toxins, which target specific insect pests while being safe for humans, animals, and the environment. Bt-based biopesticides have been extensively used in agriculture to control a variety of insect pests, thereby reducing the reliance on chemical pesticides (Kumar et al., 2021).

These biopesticides are highly specific, targeting particular insect orders like Lepidoptera, Coleoptera, and Diptera, which minimizes the impact on non-target organisms. Recent advances in molecular biology have enabled the development of genetically modified (GM) crops expressing Bt toxins, such as Bt cotton, Bt maize, and Bt potatoes. These GM crops have inherent pest resistance, significantly reducing crop damage and increasing yield (Jouzani et al., 2017). Novel encapsulation strategies, such as microencapsulation and nanoencapsulation, have been developed to improve the stability and efficacy of Bt formulations, protecting the insecticidal proteins from environmental degradation and enhancing their delivery to target pests (de Oliveira et al., 2021).

A specific example of the ongoing innovation in Bt biopesticides is the characterization of the novel mosquitocidal toxin Cry50Ba. This toxin, identified by Zhang et al. (2017), shows significant potential in mosquito control. The study highlighted not only the efficacy of Cry50Ba but also its synergistic effects when used in combination with other mosquitocidal toxins, offering a promising strategy for enhancing the effectiveness of Bt-based mosquito control products. This synergy can potentially reduce the doses required for effective pest management, thereby minimizing environmental impact and slowing the development of resistance in mosquito populations.

8.2 Agricultural and environmental applications

Beyond pest control, Bt has several other applications in agriculture and environmental management. Bt strains have been shown to possess plant growth-promoting properties, acting as biofertilizers. These strains can solubilize phosphate, produce siderophores, and produce phytohormones, which enhance plant growth and nutrient uptake (Gomis-Cebolla and Berry, 2023). Bt also exhibits antagonistic activity against various plant pathogens, including fungi and bacteria, making it a valuable agent in integrated pest management (IPM) systems (Subbanna et al., 2019).

Bt has been used in bioremediation efforts to degrade environmental pollutants such as heavy metals and pesticides. For instance, Bt Berliner has demonstrated the ability to biodegrade the pyrethroid insecticide cypermethrin, highlighting its potential in reducing environmental contamination (Birolli et al., 2021). The versatility of Bt extends to the biosynthesis of metal nanoparticles and production of biopolymers like polyhydroxyalkanoates, which have applications in biotechnology and environmental sustainability (Jouzani et al., 2017).

8.3 Future research directions

Future research on Bt should focus on several key areas to enhance its applications and address emerging challenges. One promising direction is the exploration of Bt's potential in medical applications, particularly in cancer treatment. Parasporins, a class of proteins produced by Bt, have shown cytotoxic effects against cancer cells, indicating their potential as anticancer agents (Santos et al., 2021). Further research is needed to elucidate their mechanisms of action and evaluate their efficacy in vivo. Another critical area is the development of new Bt strains with improved insecticidal properties and broader pest spectra. Advances in genome editing technologies, such as CRISPR/Cas9, could be employed to engineer Bt strains with enhanced toxin production and resistance to environmental stresses (Patel et al., 2015).

Additionally, understanding the ecological interactions and long-term impacts of Bt applications in natural ecosystems is essential to ensure environmental safety and sustainability (Belousova et al., 2021). Continued monitoring and assessment of resistance development in target pest populations will be crucial for maintaining the efficacy of Bt-based biopesticides. Lastly, integrating Bt applications with other biological control agents and IPM strategies will further enhance its role in sustainable agriculture.

9 Concluding Remarks

This study has provided a comprehensive overview of the phylogenetic analysis of *Bacillus thuringiensis* (Bt) strains, highlighting the genetic relationships and divergence among these important biocontrol agents. Key findings include the significant genetic diversity among Bt strains, which is largely influenced by horizontal gene transfer (HGT) and recombination events. These processes contribute to the adaptability and ecological success of Bt by enabling the acquisition of new insecticidal toxin genes and other beneficial traits. Phylogenetic analyses using various genetic markers, such as MLST and whole-genome sequencing, have revealed complex evolutionary relationships within Bt strains, often showing close genetic ties with other *Bacillus* species like *B. cereus*. The role of plasmids in gene transfer and the impact of recombination on genetic diversity have been underscored, illustrating the dynamic genomic landscape of Bt. Furthermore, the study has highlighted the applications of Bt in agriculture and environmental management, emphasizing its use as a biopesticide and its potential in bioremediation and plant growth promotion.

Continued phylogenetic research on Bt is crucial for several reasons. It enhances our understanding of the evolutionary mechanisms that drive the diversification and adaptability of Bt strains. This knowledge is essential for developing new and more effective Bt-based biopesticides that can overcome pest resistance and expand the range of target pests. Phylogenetic studies also provide insights into the genetic basis of toxin production and other beneficial traits, which can be harnessed to improve the efficacy and safety of Bt applications. Phylogenetic research helps in the accurate classification and identification of Bt strains, which is vital for regulatory purposes and for ensuring the consistency and reliability of biopesticide products. Understanding the genetic relationships

and evolutionary history of Bt also aids in predicting the potential environmental impacts of Bt applications and in developing strategies to mitigate any adverse effects.

Future research on Bt should focus on several key areas to further advance our understanding and application of this important bacterium. There is a need for more extensive genome sequencing and comparative genomic analyses to identify novel genes and genetic elements that contribute to the insecticidal and environmental properties of Bt. These studies should include a diverse range of Bt strains from different ecological niches to capture the full spectrum of genetic diversity. The mechanisms of HGT and recombination in Bt should be studied in greater detail to understand how these processes facilitate the rapid adaptation and evolution of Bt strains. This could involve the use of advanced molecular techniques such as CRISPR/Cas9 to investigate the genetic regulation of HGT and recombination events. Research should explore the potential of Bt beyond pest control, including its use in medical applications, such as cancer treatment, and its role in promoting plant growth and bioremediation. Interdisciplinary studies that integrate phylogenetic research with ecological, environmental, and agricultural sciences will be crucial for developing sustainable and environmentally friendly biopesticide strategies. Such studies will help ensure that Bt remains a valuable tool in integrated pest management and other biotechnological applications.

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Conflict of Interest Disclosure

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