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Comparative Analysis of Plasmid Prfiles in Bt Islates from Different Habitats

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Abstract The study investigates the genetic diversity and plasmid profiles of *Bacillus thuringiensis* (Bt) isolates collected from various ecological niches. Bt is a widely used biopesticide due to its insecticidal properties, which are largely attributed to the presence of plasmid-borne genes encoding Cry and Vip proteins. This research aims to compare the plasmid profiles of Bt isolates from different habitats to understand their genetic diversity and potential for biocontrol applications. The study utilized techniques such as whole genome sequencing, PCR amplification, and bioassays to characterize the isolates. Results revealed significant genetic diversity among the isolates, with multiple unique plasmid profiles identified. The findings suggest that different habitats harbor distinct Bt strains with varying plasmid compositions, which could be exploited for developing novel biopesticides. This comparative analysis enhances our understanding of Bt genetic diversity and its implications for sustainable pest management.

Keywords *Bacillus thuringiensis*; Plasmid profiles; Genetic diversity; Biopesticides; Insecticidal proteinse

1 Introduction

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium that is widely recognized for its insecticidal properties. It produces parasporal crystal inclusions containing Cry proteins, which are toxic to a variety of insect larvae, particularly those of the orders Lepidoptera, Coleoptera, and Diptera (Dorsch et al., 2002; Singh et al., 2021). Bt has been extensively used as a biological control agent in agriculture to manage pest populations, thereby reducing the reliance on chemical pesticides (Paeket al., 2022). The bacterium's ability to produce a diverse array of Cry toxins, each targeting specific insect pests, makes it a versatile tool in integrated pest management programs (Wang et al., 2020).

Plasmids play a crucial role in the genetic diversity and adaptability of Bt strains. They often carry genes encoding for insecticidal proteins, such as Cry and Vip toxins, which contribute to the bacterium's pathogenicity against insect pests (Wang et al., 2020). Understanding the plasmid profiles of Bt isolates can provide insights into the genetic mechanisms underlying their insecticidal properties and potential for resistance management. For instance, the coexistence of cry and vip genes on the same plasmid has been shown to enhance synergistic insecticidal activity, thereby delaying the development of resistance in target insect populations (Wang et al., 2020). Additionally, plasmid studies can aid in the identification and development of novel Bt strains with enhanced insecticidal properties, as demonstrated by the isolation and characterization of highly toxic Bt strains against specific pests (Park et al., 2022).

The study aim to conduct a comparative analysis of plasmid profiles in Bt isolates from different habitats. By examining the diversity and distribution of plasmid-encoded insecticidal genes, we aim to elucidate the genetic factors contributing to the efficacy and adaptability of Bt strains. This study will also explore the potential applications of these findings in the development of new bio-insecticides and strategies for resistance management. Through a comprehensive analysis of existing literature, provide a deeper understanding of the role of plasmids in shaping the insecticidal capabilities of Bt and their implications for sustainable pest control practices.

2 Overview of Bt Isolates and Habitats

2.1 Common habitats of Bt isolates

Bacillus thuringiensis (Bt) is a ubiquitous bacterium found in a variety of habitats worldwide. Commonly, Bt isolates are oBtained from soil, plant surfaces (phylloplanes), and insect guts. For instance, a study conducted in Bangladesh identified Bt isolates from vegetable and crop-cultivated soils, phylloplanes, and insect guts, with the highest prevalence in soil samples (Shishir et al., 2012). Similarly, research in Qatar revealed a diverse collection of Bt isolates from soil, highlighting the soil as a rich source of Bt diversity (Figure 1) (Nair et al., 2018). In Iran, Bt strains were isolated from fields, gardens, and desert and semi-desert areas, further emphasizing the adaptability of Bt to different environmental conditions (Rashki et al., 2021).

2.2 Environmental factors influencing Bt distribution

The distribution of Bt isolates is influenced by various environmental factors, including soil composition, climate, and the presence of host insects. For example, the diversity of Bt strains in Qatar was attributed to the unique soil ecology of the region, which supports a wide range of Bt isolates with different crystal morphologies and endotoxin profiles (Nair et al., 2018). In Bangladesh, the prevalence of Bt in soil samples compared to leaf and insect samples suggests that soil properties and agricultural practices may play a significant role in Bt distribution (Shishir et al., 2012). Additionally, the presence of specific insect hosts can influence the distribution and diversity of Bt isolates, as seen in the study of Brazilian Bt isolates, where genetic diversity was linked to the ability to target Aedes aegypti larvae (Fernandes et al., 2021).

2.3 Collection and isolation techniques

The collection and isolation of Bt isolates involve several techniques to ensure the recovery of diverse and representative samples. Common methods include selective culturing, molecular characterization, and bioassays. In Bangladesh, selective methods were used to oBtain Bacillus cereus-like isolates, which were then identified as Bt based on hemolytic activity, parasporal crystal proteins, and plasmid profiles. In Qatar, scanning electron microscopy was employed to analyze the crystal forms of Bt isolates, revealing a high abundance of spherical crystals (Shishir et al., 2012). Molecular techniques such as 16S rDNA gene sequencing and PCR amplification are also used to confirm the identity of Bt strains and characterize their genetic profiles (Shishir et al., 2012; Rashki et al., 2021).

Advanced techniques like pulsed field gel electrophoresis (PFGE) are utilized to separate and identify plasmid profiles in Bt strains. PFGE is particularly effective for separating high molecular weight plasmid DNAs, which are difficult to resolve using conventional gel electrophoresis. This method was successfully applied to analyze plasmid profiles in 10 Bt strains, providing detailed information on the number and size of plasmids (Zhou et al., 2014). Additionally, techniques like amplified fragment length polymorphism (AFLP) and repetitive element polymorphism (Rep-PCR) are used to assess genetic variability and molecular markers among Bt isolates (Valicente and Silva, 2017).

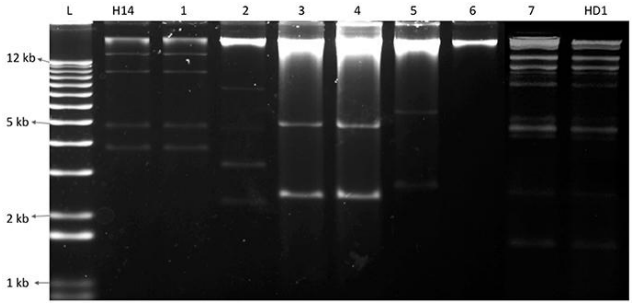

Figure 1 Electrophoresis gel showing seven different plasmid patterns observed among the Bt collection (1–7) (Adopted from Nair et al., 2018)

Image caption: L represents a 1 kb plus ladder; H14 is the reference strain *Bacillus thuringiensis* israelensis, HD1 is the reference strain *Bacillus thuringiensis* kurstaki; 1, QBt229; 2, QBt6; 3, QBt43; 4, QBt212; 5, QBt99; 6, QBt3; 7, QBt375 (Adopted from Nair et al., 2018)

In conclusion, the study of Bt isolates from different habitats provides valuable insights into the diversity and distribution of this bacterium. Environmental factors such as soil composition, climate, and host insects significantly influence Bt distribution. The use of various collection and isolation techniques, including selective culturing, molecular characterization, and advanced electrophoresis methods, ensures the recovery of diverse Bt isolates with potential applications in biopesticide development and insect pest contro (Nair et al., 2018).

3 Plasmid Composition and Structure in Bt

3.1 Types of plasmids in Bt

Bacillus thuringiensis (Bt) is known for its diverse plasmid content, which plays a crucial role in its adaptability and pathogenicity. Plasmids in Bt can be categorized based on their incompatibility groups and the functions they encode. For instance, IncF plasmids are prevalent and often carry multiple resistance genes, as seen in various studies (Doumith et al., 2012; Ajayi et al., 2021; Douarre et al., 2020). These plasmids are not only limited to antibiotic resistance but also include genes that confer resistance to heavy metals and other environmental stressors (Falgenhauer et al., 2017; Dolejská et al., 2018). Additionally, plasmids from different environments, such as wastewater treatment plants and livestock farms, show a wide range of resistance and virulence genes, indicating their adaptability to diverse habitats (Falgenhauer et al., 2017; Ajayi et al., 2021).

3.2 Plasmid structure and genetic elements

The structure of Bt plasmids is highly complex, often comprising multiple replicons and a variety of genetic elements such as transposons, integrons, and insertion sequences. For example, the IncF/MOBF12 plasmid pFEMG (209 357 bp) isolated from wastewater treatment plants harbors a cluster of resistance genes interspersed with transposons and insertion sequences, which facilitate horizontal gene transfer (Ajayi et al., 2021). Similarly, the IncHI2 plasmids found in livestock farms carry a mosaic of resistance genes and heavy metal resistance determinants, indicating a high level of genetic recombination and evolution (Falgenhauer et al., 2017). The presence of multiple addiction systems, such as toxin-antitoxin modules, ensures the stable maintenance of these plasmids within their bacterial hosts (Doumith et al., 2012).

3.3 Functions of plasmid-encoded genes

Plasmid-encoded genes in Bt serve a variety of functions that enhance the bacterium's survival and pathogenicity. These functions can be broadly categorized into antibiotic resistance, heavy metal resistance, and virulence factors. For instance, plasmids carrying the *blaCMY-42*, *blaTEM-1β*, and *blaNDM-5* genes confer resistance to beta-lactam antibiotics, while genes like mphA-mrx-mphR provide resistance to macrolides (Ajayi et al., 2021). Heavy metal resistance genes such as ter, mer, and sil are also commonly found on Bt plasmids, enabling the bacteria to thrive in contaminated environments (Falgenhauer et al., 2017; Dolejská et al., 2018). Additionally, plasmids often carry virulence genes that contribute to the pathogenicity of Bt, such as those encoding for toxins and other virulence factors (García et al., 2018).

In summary, the plasmid composition and structure in Bt are highly diverse and complex, reflecting the bacterium's ability to adapt to various environmental conditions. The presence of multiple resistance and virulence genes on these plasmids underscores their importance in the survival and pathogenicity of Bt. Further research into the genetic elements and functions of these plasmids will provide deeper insights into the mechanisms of horizontal gene transfer and the evolution of antibiotic resistance and virulence in Bt.

4 Methods for Plasmid Profiling

4.1 Extraction and purification techniques

The extraction and purification of plasmids from *Bacillus thuringiensis* (Bt) isolates are critical steps in plasmid profiling. Traditional methods often struggle with separating plasmid DNAs with molecular masses greater than 25 Kb. Pulsed Field Gel Electrophoresis (PFGE) has emerged as an ideal method for separating and identifying plasmid profiles, especially for large plasmids. PFGE leverages regular changes in the direction and size of the electric field to enable the separation of high molecular weight DNAs, making it suitable for Bt strains that commonly contain multiple plasmids ranging from 10 Kb to over 600 Kb (Zhou et al., 2014).

In a study focusing on Bt isolates from Indian soils, distinct plasmid bands of different sizes were observed, with some isolates showing plasmids above 33 500 bp. This indicates the diversity and complexity of plasmid profiles in Bt strains (Rangeshwaran et al., 2014). Another study utilized selective methods to isolate Bt from various habitats, confirming the presence of plasmids through hemolytic activity and parasporal crystal protein profiles. The extracted plasmids were comparable to reference strains, demonstrating the effectiveness of these methods in recovering plasmid DNA (Shishir et al., 2012).

4.2 Molecular characterization methods

Molecular characterization of plasmids involves several techniques to understand the genetic variability and properties of Bt isolates. Polymerase Chain Reaction (PCR) is commonly used for genetic profiling and toxicity prediction. Repetitive element polymorphism (Rep-PCR) using ERIC, REP, and BOX primers helps in understanding genetic diversity. Amplified Fragment Length Polymorphism (AFLP) is another technique used to detect molecular markers and assess genetic variability (Valicente and Silva, 2017).

In addition to PCR and AFLP, plasmid characterization is crucial for detecting the number and patterns of plasmids. For instance, a study on Bt isolates from Bangladesh used 16S rDNA gene sequencing for strain identification and SDS-PAGE for analyzing crystal proteins. The plasmid analysis revealed at least one 15 Kb DNA band, which was comparable to the reference strain, indicating the presence of Cry1, Cry2, and Cry9 type proteins (Shishir et al., 2012).

4.3 Bioinformatics tools for plasmid analysis

Bioinformatics tools play a significant role in the comparative analysis of plasmid content, especially with the advent of whole genome sequencing (WGS). Plasmid Profiler is a pipeline designed to perform comparative plasmid content analysis without the need for de novo assembly. It rapidly identifies plasmid sequences by mapping reads to a plasmid reference sequence database and annotates predicted plasmid sequences with their incompatibility group. The results can be visualized as an interactive heat map, facilitating the analysis of plasmid genes or regions of interest (Zetner et al., 2017).

PlasFlow is another tool that uses a neural network approach to identify bacterial plasmid sequences in environmental samples. It can recover plasmid sequences from assembled metagenomes with high accuracy, making it suitable for analyzing plasmidomes in diverse environments (Krawczyk et al., 2018). Similarly, Platon uses protein sequence-based replicon distribution scores to distinguish plasmid-borne from chromosome-borne contigs. It achieves high accuracy and is suitable for high-throughput taxon-independent analyses (Figure 2) (Schwengers et al., 2020).

The MOB-suite is a set of modular tools for the reconstruction and typing of plasmids from draft assembly data. It provides high sensitivity and specificity in identifying plasmid contigs and offers replicon typing, relaxase typing, and prediction of conjugation potential. The MOB-suite reduces errors in plasmid reconstruction and is available as an open-source tool (Robertson and Nash, 2018).

In summary, the combination of advanced extraction techniques, molecular characterization methods, and sophisticated bioinformatics tools provides a comprehensive approach to plasmid profiling in Bt isolates. These methods enable researchers to uncover the diversity and functional roles of plasmids in different habitats, contributing to our understanding of microbial adaptation and resistance mechanisms.

5 Comparative Analysis of Plasmid Profiles

5.1 Plasmid diversity in different habitats

Plasmids are crucial genetic elements that facilitate the rapid adaptation and evolution of bacterial populations by transferring genes that confer selective advantages, such as antibiotic resistance and metabolic capabilities. The diversity of plasmids varies significantly across different habitats, influenced by the environmental conditions and the bacterial communities present. For instance, F-type plasmids, which are known for carrying antimicrobial resistance (AMR) genes, exhibit substantial diversity in both environmental and livestock settings. These plasmids adapt to their specific niches, with unique combinations of core and accessory genes that reflect their environmental origins (Matlock et al., 2021).

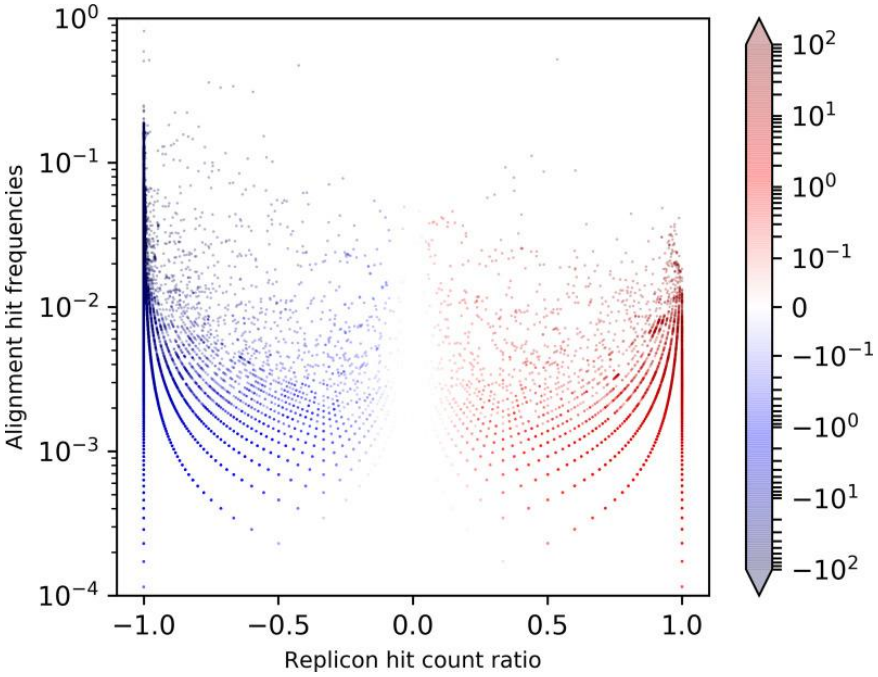

Figure 2 Replicon distribution and alignment hit frequencies of marker protein sequences (Adopted from Schwengers et al., 2020)

Image caption: Shown here are summed plasmid and chromosome alignment hit frequencies per marker protein sequence plotted against plasmid/chromosome hit count ratios scaled to [-1, 1]; Hue:normalized replicon distribution score values (min=-100, max=100), hit count outliers below 10-4 and above 1 are discarded for the sake of readability (Adopted from Schwengers et al., 2020)

In environmental samples, plasmids play a pivotal role in the adaptation of microorganisms to various stressors, such as heavy metal contamination. Tools like PlasFlow have been developed to predict plasmid sequences in metagenomic data, revealing that plasmids constitute a significant fraction of microbial communities in contaminated environments and carry genes involved in heavy-metal homeostasis (Figure 3) (Krawczyk et al., 2018). This highlights the importance of plasmids in enabling microorganisms to thrive under adverse conditions.

Moreover, large-scale analyses of plasmid relationships through gene-sharing networks have shown that plasmids from different habitats often cluster together based on their genetic content rather than their environmental origin. This suggests that horizontal gene transfer between different environments is a common occurrence, further contributing to the genetic diversity of plasmids (Tamminen et sl., 2012).

5.2 Common and unique plasmids

The comparison of plasmid profiles between different bacterial isolates reveals both common and unique plasmids. For example, in a study comparing the plasmid content of clinical and commensal strains of Escherichia coli and Klebsiella pneumoniae, it was found that while some replicons were common to both populations, others were unique to either clinical or commensal strains. Specifically, replicons L, M, A/C, and N were detected only in clinical strains, whereas HI1 was found exclusively in commensal strains. Despite these differences, certain replicons, such as I1 and F, were prevalent in both populations, indicating some level of shared plasmid content across different habitats (Rodríguez-Navarro et al., 2020).

The COMPASS database, which compiles a vast collection of plasmid sequences from various bacterial species, further illustrates the prevalence of multireplicon plasmids and the extensive diversity of IncF plasmids. This database has revealed that many plasmids carry multiple replicons, an adaptive mechanism that extends their host range and enhances their survival and dissemination. Notably, IncF alleles are frequently found in multireplicon plasmids, particularly in Enterobacteriaceae, underscoring their role in the widespread dissemination of these genetic elements (Douarre et al., 2020).

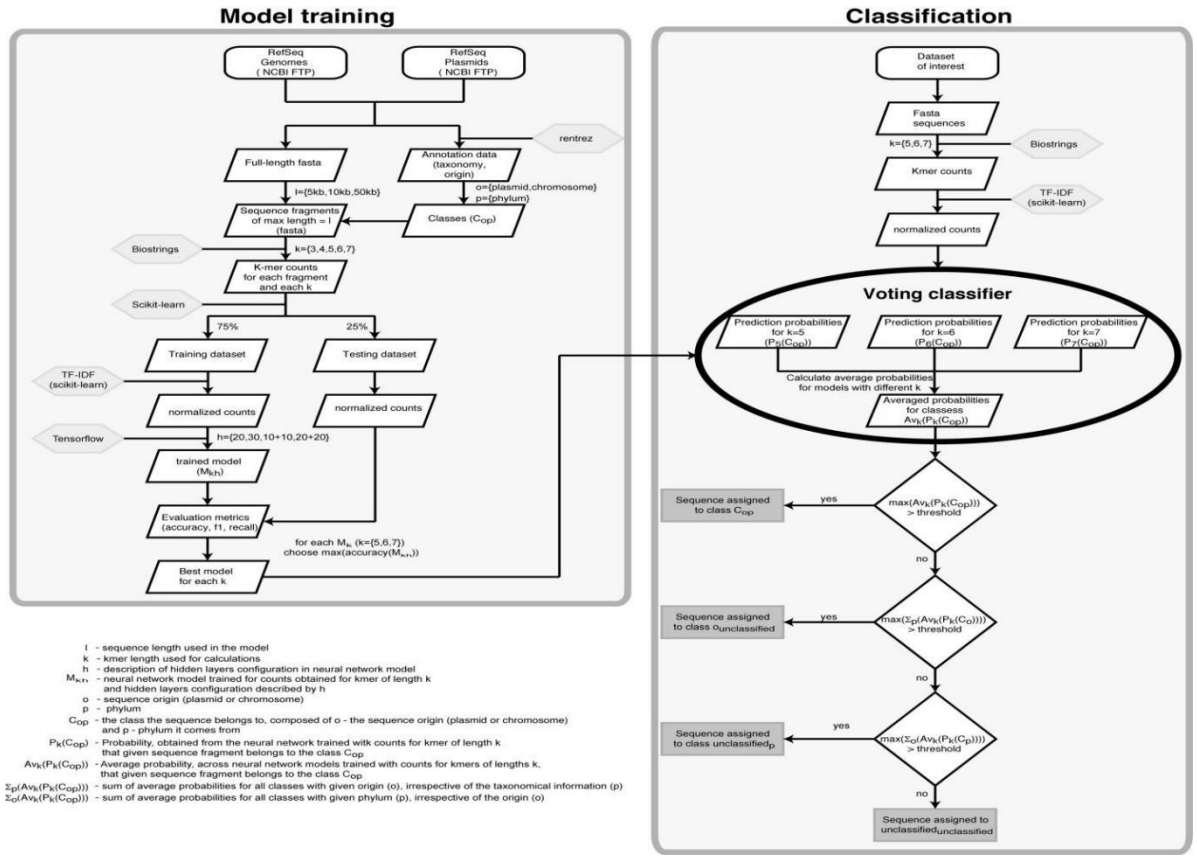

Figure 3 Flowchart describing the training and classification procedures implemented in the PlasFlow (Adopted from Krawczyk et al., 2018)

Image caption: This diagram illustrates the process of model training and classification for plasmid and chromosome sequences. The left part describes the model training process, using RefSeq genomes and plasmid data. Sequence fragments are generated with K-mer counts, which are then used to create training and testing datasets. TF-IDF and neural networks are applied for training and evaluation. The right part shows the classification process, where sequences from the dataset of interest are input into the model. A voting classifier calculates the average probabilities from models with different K-mer lengths and classifies sequences based on set thresholds. Finally, sequences are assigned to plasmid, chromosome, or unclassified categories (Adopted from Krawczyk et al., 2018)

5.3 Correlation with environmental factors

The distribution and diversity of plasmids are closely linked to environmental factors, which shape the genetic landscape of bacterial communities. Plasmids provide a means for bacteria to rapidly acquire and disseminate genes that confer advantages under specific environmental conditions. For instance, the presence of heavy metals in the environment can select for plasmids carrying genes involved in heavy-metal resistance, as demonstrated by the analysis of microbial mats in contaminated sites (Krawczyk et al., 2018).

Furthermore, the ecological dynamics of plasmids are influenced by their host range and mobility. Plasmids with a broad host range and high mobility are more likely to spread across different environments, facilitating the exchange of genetic material between diverse bacterial communities. This is supported by the observation that mobile plasmids are central to gene-sharing networks, playing a crucial role in the dissemination of antibiotic resistance genes and other adaptive traits (Tamminen et sl., 2012).

In summary, the comparative analysis of plasmid profiles in bacterial isolates from different habitats reveals a complex interplay between genetic diversity, environmental factors, and bacterial adaptation. Plasmids serve as key vehicles for the horizontal transfer of genes, enabling bacteria to rapidly respond to changing environmental conditions and maintain their ecological fitness. Understanding the diversity and distribution of plasmids across various habitats is essential for developing strategies to mitigate the spread of antibiotic resistance and other plasmid-mediated traits.

6 Functional Implications of Plasmid Variation

6.1 Role in Bt adaptation and survival

Plasmids play a crucial role in the adaptation and survival of *Bacillus thuringiensis* (Bt) in various environments. These mobile genetic elements enable rapid genetic changes that can confer selective advantages to their bacterial hosts. For instance, plasmids can carry genes that help Bt adapt to different ecological niches by encoding traits such as toxin production, stress tolerance, and nutrient acquisition). The presence of multiple replicons within plasmids, as observed in the COMPASS database, extends the host range and enhances the survival of Bt by allowing it to thrive in diverse environments (Douarre et al., 2020). Additionally, plasmids can harbor genes involved in heavy metal homeostasis, which is essential for Bt to survive in contaminated environments (Krawczyk et al., 2018). This adaptability is further supported by the ability of plasmids to carry genes that facilitate horizontal gene transfer, thereby spreading advantageous traits within bacterial communities (Smalla et al., 2015).

6.2 Impact on Bt virulence and toxin production

The virulence of Bt is significantly influenced by the plasmids it harbors. Plasmids can carry virulence-associated genes, including those encoding Cry toxins, which are pivotal for Bt's pathogenicity against insect hosts. Studies have shown that the presence of specific plasmids can enhance the virulence of Bt by increasing the copy number of toxin genes, thereby boosting toxin production (Masri et al., 2015). For example, the co-introduction of plasmids harboring different carbapenemase genes in bacterial hosts has been shown to increase fitness and virulence, suggesting a similar mechanism could be at play in Bt. Furthermore, plasmids can also carry genes that enhance Bt's ability to form biofilms, resist host immune responses, and survive within host organisms, all of which contribute to its virulence (Lee et al., 2020). The genetic diversity of virulence plasmids, as seen in various Escherichia coli strains, underscores the potential for plasmids to drive the evolution of virulence traits in Bt (Hazen et al., 2015).

6.3 Contribution to genetic diversity

Plasmids are a major source of genetic diversity in bacterial populations, including Bt. They facilitate the horizontal transfer of genes between different strains and species, leading to the rapid dissemination of beneficial traits. This genetic exchange is crucial for the evolution of new virulent strains and the adaptation to changing environmental conditions (Smalla et al., 2015). The diversity of plasmid-encoded genes, such as those involved in antibiotic resistance, virulence, and metabolic functions, contributes to the overall genetic variability within Bt populations (Wawire et al., 2021). Comparative genomic analyses have revealed that plasmids from different ecological niches exhibit distinct genetic signatures, reflecting the adaptation of Bt to specific environments (Davray et al., 2020). The dynamic nature of plasmid acquisition and loss, as observed in Shiga toxin-producing E. coli, highlights the role of plasmids in shaping the pangenome and virulence factor repertoires of bacterial populations (Nakamura et al., 2020). This genetic plasticity, driven by plasmid variation, is essential for the long-term survival and evolutionary success of Bt in diverse habitats.

In summary, plasmid variation in Bt isolates from different habitats has profound functional implications. Plasmids enhance Bt's adaptability and survival by encoding niche-specific traits, increase its virulence through the amplification of toxin genes, and contribute to genetic diversity by facilitating horizontal gene transfer. These factors collectively underscore the importance of plasmids in the ecological and evolutionary dynamics of Bt.

7 Case Studies of Plasmid Profiles in Specific Habitats

7.1 Soil-derived Bt isolates

Soil is a rich reservoir for *Bacillus thuringiensis* (Bt) isolates, which exhibit a diverse range of plasmid profiles and insecticidal properties. For instance, a study conducted in Qatar identified seven distinct plasmid profiles among 700 Bt isolates from soil samples. These isolates displayed a variety of crystal morphologies and endotoxin protein profiles, indicating a high level of genetic diversity. The study highlighted the potential of these isolates for developing novel bio-insecticides targeting different insect orders, such as Dipteran, Lepidopteran, and Coleopteran insects (Nair et al., 2018).

Similarly, Bt X022, a novel strain isolated from Chinese soil, was found to possess seven plasmids. Genomic and proteomic analyses revealed the presence of multiple Cry proteins and a vegetative insecticidal protein (Vip3A), which are crucial for its insecticidal activity. The study also uncovered a metabolic regulation mechanism influenced by Cu^{2+} treatment, which enhances the production of insecticidal crystal proteins (ICPs) (Quan et al., 2016).

In Brazil, Bt isolates from various ecosystems, including the Amazon, Caatinga, and Cerrado biomes, were characterized for their pathogenicity against mosquito larvae. The study identified 400 Bt strains, with a significant number showing larvicidal activity. Plasmid analysis and gene profiling revealed the presence of multiple *cry* and *cyt* genes, which are essential for the insecticidal properties of these isolates (Soares-da-Silva et al., 2017).

7.2 Aquatic-derived Bt isolates

Aquatic environments also serve as a source of diverse Bt isolates with unique plasmid profiles. Although specific studies focusing solely on aquatic-derived Bt isolates are limited, the genetic diversity observed in soil and insect-derived isolates suggests that aquatic habitats could harbor equally diverse Bt strains. The presence of various *cry* and *cyt* genes in these isolates indicates their potential for controlling aquatic insect pests, such as mosquito larvae, which are vectors for diseases like malaria and dengue (Soares-da-Silva et al., 2017).

7.3 Insect-derived Bt isolates

Insect-derived Bt isolates exhibit distinct plasmid profiles and insecticidal properties, making them valuable for biopesticide development. In Bangladesh, Bt isolates were oBtained from insect guts, phylloplanes, and soil. The study identified 57 Bt isolates with diverse parasporal crystal proteins and plasmid profiles. These isolates showed significant insecticidal activity against pulse beetles, demonstrating their potential for biopesticide production (Shishir et al., 2012).

In Turkey, Bt isolates from soil, fig leaves, and fruits were characterized for their insecticidal activity and Cry protein composition. The study identified several isolates with high toxicity against lepidopteran species, surpassing the reference strain HD1. Plasmid analysis revealed the presence of *vip3* genes, which encode for Vip3 proteins with significant insecticidal properties (Şahin et al., 2012).

In the Andaman and Nicobar Islands, Bt isolates from soil were characterized for their protein and cry gene profiles. The study identified a diverse range of Cry proteins, including novel cry genes potentially active against Coleoptera insects. This genetic diversity is attributed to the unique ecological factors and spatial separation of the islands, which influence the evolution of Bt strains (Swamy et al., 2011).

The comparative analysis of plasmid profiles in Bt isolates from different habitats reveals significant genetic diversity and potential for biopesticide development. Soil-derived isolates exhibit a wide range of plasmid profiles and insecticidal properties, while insect-derived isolates show unique plasmid compositions and high toxicity against specific insect pests. Although studies on aquatic-derived isolates are limited, the genetic diversity observed in other habitats suggests that aquatic environments could also be a valuable source of novel Bt strains.

8 Technological Advances in Plasmid Research

8.1 High-throughput sequencing technologies

High-throughput sequencing technologies have revolutionized plasmid research by enabling the rapid and comprehensive analysis of plasmid genomes. These technologies allow for the sequencing of entire plasmidomes, providing insights into the diversity, structure, and function of plasmids within various bacterial communities. For instance, the use of high-throughput sequencing in a biopurification system (BPS) facilitated the identification of high-molecular-weight plasmids, revealing a large diversity of plasmid replicons and horizontal gene transfer events within the habitat (Martini et al., 2016). Similarly, the metaplasmidSPAdes tool has been developed to assemble plasmids from metagenomic data sets, significantly reducing the false positive rate of plasmid detection and uncovering thousands of previously undetected plasmids (Antipov et al., 2019). These advancements

underscore the importance of high-throughput sequencing in uncovering the hidden diversity of plasmids and their roles in bacterial adaptation and evolution.

8.2 CRISPR and gene editing techniques

CRISPR and other gene editing techniques have opened new avenues for plasmid research by enabling precise manipulation of plasmid DNA. These technologies allow researchers to edit plasmid sequences, insert or delete genes, and study the effects of these modifications on bacterial physiology and plasmid function. For example, CRISPR-based tools can be used to investigate the role of specific genes in plasmid replication, stability, and transfer. Additionally, these techniques can be employed to engineer plasmids with desired traits, such as enhanced antibiotic resistance or improved biodegradation capabilities. The ability to manipulate plasmid DNA with high precision has significant implications for understanding plasmid biology and developing novel biotechnological applications.

8.3 Future directions in plasmid studies

The future of plasmid research lies in the integration of advanced sequencing technologies, bioinformatics tools, and gene editing techniques to achieve a comprehensive understanding of plasmid biology. One promising direction is the development of more sophisticated bioinformatics pipelines for plasmid detection and characterization. Tools like PlasFlow, which uses genomic signatures and neural networks to identify plasmid sequences in environmental samples, represent a significant step forward in this regard (Krawczyk et al., 2024). Another important area of research is the study of plasmid ecology and the factors influencing plasmid-host interactions. Understanding the ecological dynamics of plasmids, including their distribution, abundance, and host range, is crucial for addressing issues such as the spread of antibiotic resistance genes (Smalla et al., 2015).

Moreover, the creation of comprehensive plasmid databases, such as COMPASS, which compiles thousands of complete plasmid sequences with associated metadata, provides a valuable resource for comparative analysis and evolutionary studies (Douarre et al., 2016). These databases can help researchers identify patterns of plasmid dissemination, horizontal gene transfer, and the co-integration of replicon types from different incompatibility groups. Additionally, the use of advanced analytical techniques, such as pulsed field gel electrophoresis (PFGE), continues to play a vital role in the separation and identification of large plasmids, facilitating the study of plasmid profiles in various bacterial strains (Zhou et al., 2018).

The integration of high-throughput sequencing, CRISPR-based gene editing, and advanced bioinformatics tools is poised to drive significant advancements in plasmid research. These technological innovations will enhance our understanding of plasmid diversity, function, and ecology, ultimately contributing to the development of novel strategies for combating antibiotic resistance and harnessing plasmids for biotechnological applications. The continued exploration of plasmidomes in diverse environments and the refinement of analytical methods will be key to unlocking the full potential of plasmid research in the coming years.

9 Concluding Remarks

The comparative analysis of plasmid profiles in *Bacillus thuringiensis* (Bt) isolates from different habitats has revealed significant diversity and complexity. The study identified seven distinct plasmid profiles among 700 Bt isolates from Qatari soil, with a notable variety in crystal morphology and (\delta)-endotoxin content. This diversity underscores the adaptive mechanisms of plasmids, which facilitate the rapid dissemination of advantageous genetic traits, such as antibiotic resistance and insecticidal properties, across different bacterial communities. Additionally, the study highlighted the prevalence of multireplicon plasmids, particularly those carrying IncF alleles, which play a crucial role in extending the host range and enhancing plasmid survival.

Comparative plasmid studies are essential for understanding the evolutionary dynamics and ecological roles of plasmids in microbial communities. These studies provide insights into how plasmids contribute to the rapid adaptation and evolution of bacteria by transferring genes that confer selective advantages, such as antibiotic resistance and insecticidal properties. The identification of diverse plasmid profiles and their associated genetic

elements can inform the development of novel biotechnological applications, including the creation of new bio-insecticides and strategies to combat antibiotic resistance. Furthermore, understanding the distribution and diversity of plasmids across different habitats can help predict and mitigate the spread of resistance genes in both clinical and environmental settings.

Future research should focus on expanding the scope of comparative plasmid studies to include a wider range of habitats and bacterial species. This will help uncover novel plasmids and genetic elements that have yet to be discovered. Additionally, there is a need for the development of more advanced tools and methodologies for plasmid detection and assembly, particularly in metagenomic data sets, to reduce false positives and improve the accuracy of plasmid identification. Researchers should also investigate the functional roles of different plasmid types and their interactions with host bacteria to better understand the mechanisms underlying plasmid-mediated gene transfer and adaptation. Longitudinal studies tracking the evolution and dissemination of plasmids over time in various environments could provide valuable insights into the long-term impacts of plasmid diversity on microbial ecology and public health.

By addressing these research gaps, scientists can enhance our understanding of plasmid biology and leverage this knowledge to develop innovative solutions for pressing global challenges, such as antibiotic resistance and sustainable agriculture.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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