

Exploring the Evolutionary History of Different Strains of *Bacillus thuringiensis* (B.t.) in Combating the *Phenacoccus solenopsis*

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Bt Research, 2024, Vol.15, No.1 doi: [10.5376/bt.2024.15.0001](https://doi.org/10.5376/bt.2024.15.0001)

Received: 05 Nov., 2023

Accepted: 18 Dec., 2023

Published: 01 Jan., 2024

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Preferred citation for this article:

Wang F.F., 2024 , Exploring the evolutionary history of different strains of *Bacillus thuringiensis* (B.t.) in combating the *Phenacoccus solenopsis*, Bt Research, 15(1): 1-9 (doi: [10.5376/bt.2024.15.0001](https://doi.org/10.5376/bt.2024.15.0001))

Abstract This study aims to investigate the gene evolution process of different strains of *Bacillus thuringiensis* (B.t.) in combating *Phenacoccus solenopsis*, commonly known as *Phenacoccus solenopsis*. The research findings highlight that B.t. strains undergo genetic changes through selective pressure and gene mutations during their long-term interaction with cotton mealybugs. By collecting B.t. strains from different geographic regions and conducting morphological and physiological characterization, significant differences in their resistance performances were observed. Further experimental research reveals the functionality of these candidate genes and their association with resistance. Gene knockout and functional analysis demonstrate that some of these genes play a critical role within the mealybug, significantly correlating with the virulence of B.t. strains. This suggests that these genes are closely linked to the development of resistance in B.t. strains and may be one of the key factors in resistance evolution. Through selective pressure and gene mutations, B.t. strains gradually adapt to the resistance of *Phenacoccus solenopsis*, leading to the development of novel resistance-related gene variations. These research findings are crucial for understanding the adaptive evolution of B.t. strains, resistance development, and the formulation of pest control strategies. Future studies can further explore the mechanisms of action of these resistance-related genes to enhance the efficacy of insecticides and minimize resistance development.

Keywords B.t. strains; *Phenacoccus solenopsis*; Gene evolution; Esistance; Toxin

In recent years, *Phenacoccus solenopsis*, a harmful insect that seriously endangers the cotton industry, has attracted widespread attention. This pest has high reproductive capacity, strong adaptability, and a wide range of host species, and spreads rapidly, causing serious economic losses to cotton production. Globally, the spread of *Phenacoccus solenopsis* has spread to many countries and regions, becoming one of the important factors restricting agricultural sustainable development (Hamza et al., 2022).

To combat the serious pest *Phenacoccus solenopsis*, there are many problems in the use of traditional chemical pesticides, such as environmental pollution, impact on non-target organisms, and the emergence of resistance of pests to pesticides. Therefore, it is imperative to seek safe, efficient, and environmentally friendly control strategies. As a widely used biopesticide for pest control, *Bacillus thuringiensis* (B.t.) has high potential (Chen et al., 2021). This study aims to explore the gene evolution of B.t. in the process of combating *Phenacoccus solenopsis*, in order to reveal the adaptive evolution mechanism of B.t. strains in the process of long-term interaction with *Phenacoccus solenopsis*. By collecting and studying B.t. strains from different geographical regions, their morphological and physiological characteristics will be analyzed to understand the differences in resistance performance of different strains to *Phenacoccus solenopsis*. At the same time, genome sequencing and alignment analysis will be carried out to identify candidate sequences of genes related to resistance, and functional annotation and verification will be conducted (Waqas et al., 2021).

Through the implementation of this study, it is expected to gain a deeper understanding of the gene evolution process of B.t. strains in combating *Phenacoccus solenopsis*, Reveals the mechanism of resistance development in the interaction between B.t. strains and *Phenacoccus solenopsis*. This will help better understand the adaptive evolution process of B.t. strains and provide theoretical guidance and practical basis for the development of pest control strategies. Through in-depth research on the mechanism of resistance evolution of B.t. strains, it will also

enhance understanding of pest resistance and response strategies, making contributions to sustainable agricultural development.

1 Biological Characteristics and Damage Degree of *Phenacoccus solenopsis*

1.1 The classification and distribution of *Phenacoccus solenopsis*

Phenacoccus solenopsis is a widely distributed pest in the world, belonging to the Coccidae family in the Gastropoda class. It is an insect with an extra-long slender stylet mouthpart, and its body length is about 1-2 mm. *Phenacoccus solenopsis* is red or orange in the adult stage, and has a strong sucking effect, parasitizing on plants, leading to yellowing, distortion, and even death of leaves, causing serious harm to crops such as cotton, fruit trees, vegetables, etc. (Figure 1) (Jin et al., 2018).

The distribution of *Phenacoccus solenopsis* is widespread, with the earliest origin in southern America, and then rapidly spread to Europe, Africa, Asia, and Australia. Especially in Asia, *Phenacoccus solenopsis* has become an important agricultural pest, causing serious damage to the yield and quality of crops such as cotton, tomato, pepper, etc. Its transmission routes are diverse, and can be rapidly transmitted to new geographical regions through wind, insects, human activities, etc., forming new pest populations.

In China, *Phenacoccus solenopsis* has rapidly spread since its first appearance in 2008, becoming one of the main threats to cotton cultivation. It has a wide distribution in China, mainly distributed in the southern coastal provinces of the main cotton-producing regions, such as Guangdong, Hainan, Fujian, etc. Due to its high reproduction and strong adaptability, coupled with the lack of effective prevention and control measures, *Phenacoccus solenopsis* has caused huge losses to China's cotton industry. *Phenacoccus solenopsis*, as an agricultural pest with a wide distribution, its harmfulness affects crop yields and farmers' incomes globally. With the intensification of global trade and human mobility, the spread of *Phenacoccus solenopsis* has become a global challenge (Omar et al., 2019).

1.2 The extent of damage caused by *Phenacoccus solenopsis* to cotton and other crops

The damage caused by *Phenacoccus solenopsis* to cotton and other crops is severe, posing a significant economic loss and risk to agricultural production. *Phenacoccus solenopsis* is one of the major pests of cotton, feeding on plant juices and parasitizing on cotton leaves and tender branches. It satisfies its nutritional needs by absorbing large amounts of plant juice, leading to serious damage to cotton, yellowing and desiccation of leaves, affecting photosynthesis and nutrient supply, and further reducing the growth and yield of cotton bolls. At the same time, the feeding of *Phenacoccus solenopsis* can also cause mechanical damage to plants and transmit pathogens, further increasing the risk of plant diseases (Heckel, 2020).

Besides cotton, *Phenacoccus solenopsis* can also attack other crops, causing similar damage. It prefers to parasitize on various fruit trees, vegetables and other cash crops, such as tomato, pepper, eggplant, and mango. When *Phenacoccus solenopsis* appears on these plants, it will also suck plant juice, leading to limited plant growth, leaf wilting and deformation, and causing fruit yield reduction, quality decline or even death (Figure 1). This poses a great threat to farmers' income and food security.

The worrying thing is that due to the high reproduction and adaptability of *Phenacoccus solenopsis*, as well as the lack of effective prevention and control measures, the degree of its harm is gradually increasing. Not only that, *Phenacoccus solenopsis* also has the potential for rapid adaptive evolution and resistance to pesticides, making targeted prevention and control more difficult. The serious harm caused by *Phenacoccus solenopsis* highlights the importance of strengthening research on its ecological characteristics, biological characteristics, and prevention and control methods to develop more effective prevention and control strategies to ensure crop production and farmers' interests.



Figure 1 The wax moth sucks crop juice (Prevention and Control of Magnolia Wax Moth, pp.1)

1.3 The control measures and challenges of combating *Phenacoccus solenopsis*

The control measures taken to combat *Phenacoccus solenopsis* include both management and chemical approaches. Management measures include removing host plants and taking reasonable farmland management measures. The removal of host vegetation is one of the important measures, as eliminating the host plants of *Phenacoccus solenopsis* can reduce the number of insect sources. In addition, reasonable farmland management measures include promoting the healthy growth of plants and enhancing their resistance to insects, such as controlling the application of nitrogen fertilizer and maintaining sufficient irrigation. These measures can reduce the reproduction and spread of *Phenacoccus solenopsis*.

Chemical control is another major means of combating *Phenacoccus solenopsis*. It mainly includes spraying pesticides to control the population of *Phenacoccus solenopsis*. The choice of pesticides should be decided based on specific circumstances, selecting appropriate pesticides according to different growth stages and severity, while paying attention to the rational use of pesticides to prevent pesticide damage and excessive residues. In addition, the rotation and combination of pesticide use can reduce the development of pesticide resistance and improve control effectiveness.

The prevention and control of *Phenacoccus solenopsis* also faces some challenges. For example, the high reproduction rate and rapid adaptive evolution of *Phenacoccus solenopsis* make it increasingly resistant to pesticides, and the traditional pesticide control effect is gradually weakening. Improper use of pesticides may have negative impacts on the environment and human health, requiring the scientific and rational selection of pesticides and strict adherence to usage guidelines. In addition, *Phenacoccus solenopsis* has a wide range of host plants and diverse transmission routes, requiring more research on its ecological characteristics and transmission patterns to develop more targeted prevention and control strategies.

2 The Application Effect of Different B.t. Strains in the Control of *Phenacoccus solenopsis*

2.1 The screening and selection criteria for B.t. strains

B.t. strains are a type of biopesticide that is commonly used for pest control. They also have a certain effect on *Phenacoccus solenopsis* (Azizoglu, 2019). In the process of screening and selecting B.t. strains for *Phenacoccus solenopsis*, several criteria are usually considered:

Toxicity refers to the pathogenic ability of B.t. strains against *Phenacoccus solenopsis*. It is evaluated through laboratory or field experiments to assess the lethal effect and toxicity of B.t. strains against *Phenacoccus solenopsis*. Selecting B.t. strains with strong toxicity and a high mortality rate against *Phenacoccus solenopsis* is crucial for effectively suppressing its reproduction and spread.

Resistance spectrum refers to the range of pest resistance of B.t. strains. Since B.t. strains can be effective against multiple pests, it is important to select B.t. strains that have high virulence against *Phenacoccus solenopsis* and low resistance to other important pests of agricultural crops to ensure no adverse impact on the ecosystem.

Specificity and selectivity refer to the minimal impact of B.t. strains on non-target organisms. Selecting B.t. strains that are targeted specifically against *Phenacoccus solenopsis* can minimize the impact on beneficial insects, other organisms, and even humans.

Stability, that is, the stability and persistence of B.t. strains. It is necessary to select B.t. strains that can maintain activity and stable efficacy under different environmental conditions to ensure long-term control of the *Phenacoccus solenopsis* (Heckel, 2020).

During the screening and selection process of B.t. strains, a large number of laboratory and field experiments are required. By comprehensively considering the above criteria and combining with practical application situations, the most suitable B.t. strain for *Phenacoccus solenopsis* can be selected to improve the control effect and reduce environmental impact.

2.2 Evaluation of the toxicity and efficacy of different B.t. strains against the *Phenacoccus solenopsis*

The evaluation of the toxicity and efficacy of different B.t. strains against *Phenacoccus solenopsis* is conducted in both laboratory and field settings. In the laboratory, the toxicity tests are mainly carried out using artificial diets. A certain number of *Phenacoccus solenopsis* colonies are established, and the culture medium or extracts of different B.t. strains are added to the diets for exposure. The mortality and growth and development of the insects are then observed and recorded. The toxicity levels of different B.t. strains against *Phenacoccus solenopsis* are determined based on the mortality rates, growth inhibition rates, and growth abnormalities after treatment (Aalaoui and Sbaghi, 2022).

Field trials are used to validate the feasibility of laboratory results and to verify the effectiveness of B.t. strains in the field environment. Treatments with different B.t. strains and control groups are set up in actual cotton fields, and the density of *Phenacoccus solenopsis*, population size, and crop damage are observed and recorded. By comparing the differences between different B.t. strain treatment groups and control groups, the control effect of B.t. strains on *Phenacoccus solenopsis* is evaluated and verified.

During these assessment processes, by comparing the toxicity and efficacy of different B.t. strains, and combining the results of artificial diet and field trials, those B.t. strains with higher mortality rates, growth inhibition rates, and better control effects on *Phenacoccus solenopsis* can be identified. These assessment results provide a basis for selecting suitable B.t. strains for the control of *Phenacoccus solenopsis*. However, due to differences in environmental conditions and *Phenacoccus solenopsis* populations, the assessment results have certain limitations and require further practical verification and optimization to ensure the effectiveness and stability of *Phenacoccus solenopsis*.

2.3 The practical application effect of B.t. strains in the real environment

In the real environment, B.t. strains are widely used in agricultural production and have good control effects on *Phenacoccus solenopsis*. They have high toxicity to pests, and have high safety and environmental friendliness. However, in practical applications, factors such as application methods, dosage selection, application time, and environmental conditions should be comprehensively considered to ensure the effective application and control effect of B.t. strains.

For example, B.t. strains have broad-spectrum insecticidal activity and are effective against multiple pests. Therefore, in agricultural production, B.t. strains are widely used to control various pests, including *Phenacoccus solenopsis*. By spraying or applying B.t. strain preparations, the density of pest populations can be effectively reduced, reducing the harm caused by pests to crops. As biological pesticides, B.t. strains have high safety and environmental friendliness. Compared with synthetic pesticides, B.t. strains grow and exist in the natural environment on the same day, without causing long-term pollution to the environment and ecosystem. B.t. strains have low toxicity to non-target organisms, minimal impact on humans, natural predators, and other beneficial insects, which is more conducive to maintaining ecological balance (Rajesh et al., 2018).

However, the application effect of B.t. strains is affected by various factors. Among them, application methods, treatment doses, application time, and environmental conditions are important influencing factors. The appropriate application time and dose are key to ensuring effective control of pests. At the same time, environmental factors such as temperature, humidity, light, etc. can also affect the growth and effect of B.t. strains. In practical applications, these factors need to be comprehensively considered, and the application time and dose need to be reasonably selected to maximize the control effect of B.t. strains.

2.4 The application and research results of B.t. strains in the control of *Phenacoccus solenopsis*

B.t. strains, as a kind of Gram-positive bacteria, can produce insecticidal crystal proteins called δ -endotoxins, which are toxic to multiple Lepidoptera, Diptera, and Coleoptera pests. Among them, B.t. kurstaki and B.t. texensis are the two most widely studied strains (Heckel, 2020).

Taking B.t. kurstaki as an example, its control effect on *Phenacoccus solenopsis* has been widely studied. Studies have shown that the δ -endotoxin produced by this strain has high toxicity to the larvae of *Phenacoccus solenopsis* and can effectively control its population. Further studies have shown that during the process of combating *Phenacoccus solenopsis*, genes related to the synthesis of insecticidal crystal proteins in the genome of B.t. kurstaki have evolved to better adapt to the biological characteristics of *Phenacoccus solenopsis*. Additionally, B.t. texensis has also been used as a biopesticide to combat *Phenacoccus solenopsis*. Unlike B.t. kurstaki, the δ -endotoxin produced by B.t. texensis is also toxic to adults of *Phenacoccus solenopsis*. Studies have shown that some strains of B.t. texensis produce a new insecticidal crystal protein that is highly toxic to both adults and larvae of *Phenacoccus solenopsis*.

Although B.t. has shown great potential in combating *Phenacoccus solenopsis*, as resistance to B.t. increases, its control effect may decline. Therefore, further research on the genetic evolution of B.t. is needed to develop more effective biopesticides to combat *Phenacoccus solenopsis*.

3 Genomics Analysis and Gene Evolution Research of Different B.t. strains

3.1 The relationship between gene evolution and resistance to scale insects

There is a close relationship between the gene evolution of different B.t. strains and their resistance to scale insects. The resistance of B.t. strains to scale insects is determined by the specific genes or anti-insect factors carried by them. These anti-insect factors encode toxin proteins that target the pests, such as δ -endotoxins. They affect the normal physiological functions of the pests by acting on their digestive systems, leading to their death (Jin et al., 2018).

The gene evolution of B.t. strains is related to their resistance to scale insects because it directly affects their virulence and resistance levels. In the natural environment, pest populations continuously interact and evolve with B.t. strains. Pests gradually develop resistance to the toxins of B.t. strains through genetic variation and selection adaptation strategies. This leads to the emergence of resistant pest populations and challenges the anti-insect effect of B.t. strains.

To cope with the development of pest resistance, researchers are constantly working to select and modify B.t. strains with new anti-insect mechanisms. By using genetic engineering techniques, people can introduce anti-insect-related genes into B.t. strains or adjust the expression levels of existing genes to enhance their virulence and resistance. These modified B.t. strains can produce stronger toxic effects on newly emerged resistant pest populations, helping to continuously and efficiently control pests.

3.2 The genetic mechanisms and rates of gene evolution

The gene evolution of B.t. strains is mainly driven by mutation and gene recombination. Mutation refers to random changes in gene sequences, including point mutations, insertion mutations, and deletion mutations, which may lead to changes in gene expression, protein structure, or function. Gene recombination, on the other hand, refers to the exchange and recombination of different genes, which usually occurs during the process of gene transfer between strains.

The rate of gene evolution may vary among different B.t. strains. The rate of gene evolution is influenced by multiple factors, including mutation rate, selection pressure, and genetic drift. A higher mutation rate results in a faster rate of gene evolution. Selection pressure refers to the selection of different mutant types. Mutations that are disadvantageous to survival and reproduction will be eliminated, while mutations that are beneficial for adaptation to the environment will be retained. Genetic drift refers to random changes in gene frequencies due to random genetic drift.

The rate of gene evolution is also influenced by the reproductive mode and environmental factors of B.t. strains. B.t. strains usually reproduce asexually, through the production and dissemination of spores. This asexual reproductive mode can accelerate the rate of gene evolution because each spore has the potential for genetic variation. Additionally, environmental factors such as climate, nutrition, and competition can also affect the rate of gene evolution in B.t. strains.

3.3 Analysis and comparison of genetic diversity among strains

The genetic diversity of different B.t. strains refers to the differences and variations among them at the genetic level. This diversity originates from genotype and phenotype differences among strains, including variations in genome structure, gene sequences, protein composition, and physiological characteristics. The analysis and comparison of genetic diversity among B.t. strains is of great significance for understanding their evolution, distribution, and efficacy.

A commonly used method is to analyze the genotypes of different B.t. strains through molecular genetics techniques. This can include technologies such as PCR amplification, DNA sequencing, and genome alignment. By comparing their genotypes, at the level of DNA sequences, genetic differences and relationships among different strains can be identified (Figure 2). This provides important clues for studying the phylogenetic relationships and evolutionary history of B.t. strains.

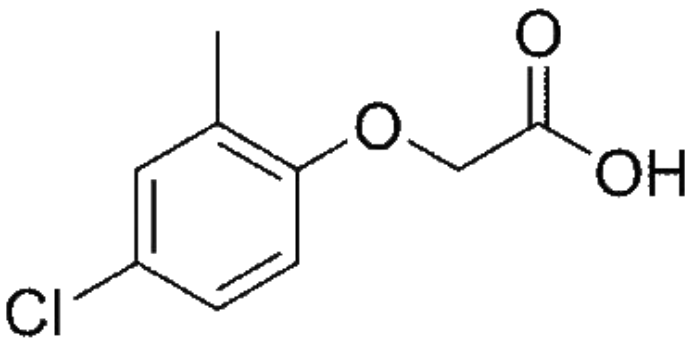


Figure 2 Chemical bond structure of Bt protein (Azizoglu, 2019)

Besides genotype comparison, the genetic diversity among B.t. strains can also be studied by comparing their phenotype characteristics. This includes growth rate, sporulation quantity, toxicity activity, and host range of the strains. By quantifying these phenotype characteristics and correlating them with the genotypes of the strains, further understanding of the impact and contribution of genetic diversity to phenotypic traits can be achieved.

Finally, the analysis and comparison of genetic diversity among B.t. strains can also provide guidance for the application and utilization of B.t. strains. Strains with different genetic backgrounds may have better insecticidal effects against different pest species or under different environmental conditions. By analyzing and comparing genetic diversity, B.t. strains with specific characteristics can be identified, allowing for more targeted applications in agricultural production and pest control strategies.

4 The Impact of Gene Evolution on The Control of *Phenacoccus solenopsis* By B.t.

4.1 The diversity and efficacy of insect resistance genes

B.t. is an important biological pesticide in the prevention and control of *Phenacoccus solenopsis*. The insecticide toxin produced by B.t. is encoded by its genome's insect resistance genes, and the diversity and potency of these

genes are crucial for the prevention and control of *Phenacoccus solenopsis* (Tawfik-Abbas, 2018). There are multiple different insect resistance genes present in the genome of B.t. strains. These insect resistance genes encode different toxin proteins that have insecticidal activity against *Phenacoccus solenopsis*. Different B.t. strains have different combinations of insect resistance genes and toxin expression levels, leading to diversity in their insecticidal potency. Some B.t. strains may be rich in multiple insect resistance genes, with broad-spectrum insecticidal activity, while others may only have certain specific insect resistance genes, targeting specific pests. The diversity of insect resistance genes in B.t. strains is closely related to their insecticidal potency. A variety of combinations of insect resistance genes can provide a broader spectrum of insecticidal activity, effectively controlling different genotypes of *Phenacoccus solenopsis* pests. Additionally, the expression levels of different genes and the characteristics of insecticidal toxins also affect the insecticidal potency. Some insect resistance genes have higher insecticidal activity and stable expression levels, resulting in better control effects on *Phenacoccus solenopsis*.

Understanding the diversity and potency of insect resistance genes in B.t. strains is of guiding significance for selecting and developing biologically active pesticides with efficient insect resistance capabilities. By analyzing the diversity of insect resistance genes, strains with higher insecticidal potency can be selected, and further research and improvement can be conducted to enhance their application in the prevention and control of cotton red wax scale. Additionally, understanding the diversity of insect resistance genes in different B.t. strains can also help reduce the occurrence of resistant strains and maintain the long-term effectiveness of B.t.

4.2 Gene evolution and the enhancement of insect resistance ability and its mechanism

Bacterial evolution of B.t. plays an important role in the enhancement of insect resistance in the prevention and control of *Phenacoccus solenopsis*. B.t. strains have high genetic plasticity and can enhance their insect resistance through gene evolution. They interact with pests in the natural environment over long periods of time, leading to genetic variation and accumulation. When B.t. strains are subject to selection pressure from pests, only genotypes with strong insecticidal activity can survive and reproduce. This evolutionary selection promotes the gradual increase in the frequency of insect resistance genes in B.t. strains, thereby enhancing their insect resistance against cotton red wax scale.

Gene evolution can also enhance the insect resistance of B.t. strains through the mechanism of horizontal gene transfer. B.t. strains can absorb new insect resistance genes from other strains or the environment through horizontal gene transfer and integrate them into their own genome. In this way, B.t. strains can acquire new insect resistance genes and may exhibit broader or more effective insecticidal activity. This mechanism of horizontal gene transfer can promote the enhancement and diversity of insect resistance in B.t. strains (Malkie et al., 2018). Bacterial evolution of B.t. in the prevention and control of *Phenacoccus solenopsis* can also enhance insect resistance through the mechanism of mutation and selection. Mutation refers to changes in the DNA sequence of the genome, which may lead to the production of new insect resistance genes or changes in existing genes. When mutant individuals with stronger insecticidal activity appear, they have a greater survival advantage under selection pressure for insect resistance. This allows B.t. strains to enhance their insect resistance through natural selection and adapt to the constantly changing pest environment.

4.3 The sustainability of gene evolution for the long-term application of B.t.

The gene evolution of B.t. in the prevention and control of *Phenacoccus solenopsis* is of great significance to its sustainability in long-term application. Gene evolution can enhance the insect resistance of B.t. strains and reduce the emergence of pest resistance to them, thus maintaining their effectiveness in long-term application. It can also promote the diversity of insect-resistant genes of B.t. strains. With the long-term interaction with *Phenacoccus solenopsis*, B.t. strains constantly undergo gene mutations and accumulations, generating diverse insect-resistant genes. This diversity can increase the flexibility and response ability of B.t. strains in insect resistance, help to deal with the risk of pests quickly adapting and forming resistance, thus enhancing their sustainability.

Gene evolution can also reduce pest resistance to B.t. In the long-term use process, pests may gradually develop resistance to B.t., reducing its insecticidal effect. However, gene evolution enables B.t. strains to constantly adjust

and enhance the expression of insect-resistant genes to adapt to pest resistance changes. This dynamic response mechanism can effectively delay pest resistance to B.t., maintaining its sustainability in long-term application.

Gene evolution improves the sustainability of B.t. by increasing its insecticidal activity against *Phenacoccus solenopsis*. With the progress of evolution, B.t. strains may form stronger insect-resistant genes with higher insecticidal activity. This enables B.t. to maintain efficient control of pests in long-term application and reduce reliance on other pesticides, thereby improving sustainability and environmental friendliness.

5 Summary and Outlook

Significant progress has been made in the genetic evolution of B.t. strains in their fight against *Phenacoccus solenopsis*. Gene evolution enables B.t. strains to adapt to pest selection pressure and enhances the frequency and expression of their insect-resistant genes. Through diverse insect-resistant genes, reduced resistance emergence, and enhanced insecticidal activity, the sustainability of B.t. in long-term applications has been improved (Tabashnik et al., 2003).

On the other hand, the development of B.t. genetic engineering has provided tremendous potential for enhancing insecticidal effectiveness. Genetic engineering techniques allow for the insertion or modification of genes in B.t. strains to enhance their insect-resistant capabilities. This includes acquiring new insect-resistant genes from other species, increasing the expression levels of insect-resistant genes, and designing more targeted insecticidal mechanisms. The advancement of genetic engineering has opened up new opportunities for the application of B.t. in cotton mealybug control and broadened the potential of its insecticidal role. In terms of genetic engineering, continuous development of new techniques and methods is crucial. With the continuous improvement of genetic engineering technology, more efficient and precise gene editing techniques can be explored to achieve the goal of targeted modification of B.t. strains. Additionally, researchers should pay further attention to the impact of genetic engineering on non-target organisms to ensure that the application of B.t. remains environmentally friendly and sustainable.

In the future, it is hoped that attention will continue to be paid to the genetic evolution of B.t. strains and the application of genetic engineering technology. For genetic evolution, researchers can deeply explore the interactions between B.t. strains and pests, further analyzing the origin and evolutionary mechanisms of insect-resistant genes. Additionally, areas such as reducing pest resistance to B.t., enhancing the insecticidal activity of B.t. strains, and others are also worthy of in-depth study. Strengthening research on the genetic evolution mechanism of B.t. strains, enhancing collaboration with related fields such as pest ecology and applied ecology to gain a more comprehensive understanding, and enhancing collaborative applied research with other pesticides to explore green and sustainable integrated pest management strategies can improve insecticidal effectiveness and reduce pest resistance to B.t. and other pesticides.

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