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Address:

11388 Stevenston Hwy,

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Review Article

Open Access

Risk Communication Strategies for Bt-based Public Health Interventions

Jin Wang, Lin Liu, Congbiao You ✉

Hainan Tropical Agricultural Resources Research Institute, Tropical Microbial Resources Research Center, Sanya, 572025, Hainan, China

✉ Corresponding author: congbiao.you@hitar.orgBt Research, 2024, Vol.15, No.4 doi: [10.5376/bt.2024.15.0016](https://doi.org/10.5376/bt.2024.15.0016)

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Abstract Bt-based public health interventions represent a significant advancement in disease control and vector management, leveraging the biological insecticidal properties of *Bacillus thuringiensis* (Bt). This study aims to analyze the risk communication strategies employed in Bt-based public health initiatives. We explore the principles and mechanisms of Bt interventions and set forth the objectives of this study to enhance public understanding and acceptance. Theoretical frameworks underpinning risk communication are discussed, encompassing key concepts, models, and ethical considerations. We detail strategies for identifying target audiences, crafting effective messages, and addressing public misconceptions. Through case studies of successful Bt interventions, we identify best practices and common challenges in risk communication. The review evaluates the effectiveness of these strategies using various metrics and emphasizes the need for continuous feedback and improvement. Finally, we discuss the implications for integrating risk communication into public health planning, offer policy recommendations, and propose future research directions. Effective risk communication is crucial for the success of Bt-based interventions, ensuring public trust and cooperation, ultimately contributing to improved public health outcomes.

Keywords Bt-based interventions; Risk communication; Public health; Vector management; *Bacillus thuringiensis*

1 Introduction

Bacillus thuringiensis (Bt) is a Gram-positive bacterium widely recognized for its insecticidal properties, particularly against dipteran insects, which include many vectors of human diseases such as mosquitoes (Zhou et al., 2020). The use of Bt-based interventions in public health has gained significant attention due to their effectiveness and environmental safety.

Bt-based interventions have been employed in various public health initiatives to control mosquito populations, which are vectors for diseases such as malaria, dengue, and Zika virus. The bacterium produces δ -endotoxins during its sporulation phase, which are highly toxic to insect larvae upon ingestion (Raymond et al., 2010), leading to their death (Nair et al., 2020). Bt subsp. israelensis (Bti) is particularly effective against mosquito larvae and has been used in community-based larval source management (LSM) programs to reduce mosquito populations and, consequently, the incidence of mosquito-borne diseases (Ingabire et al., 2017).

The primary mechanism of Bt's insecticidal action involves the production of Cry and Cyt proteins, which form crystalline inclusions during sporulation (Ralte et al., 2012). These proteins are ingested by insect larvae, where they bind to specific receptors in the gut, causing cell lysis and death (Sanchis and Bourguet, 2011; Zhang et al., 2021). Bt-based bioinsecticides are considered environmentally friendly as they specifically target insect larvae without harming non-target organisms, including humans and other animals (Sanchis, 2011; Mendoza-Almanza et al., 2020). The effectiveness of Bt interventions can be influenced by factors such as the strain of Bt used, the method of application, and environmental conditions (Patil et al., 2011).

The objectives of this study are to examine the current state of Bt-based public health interventions, identify the key challenges and barriers to their implementation, and propose effective risk communication strategies to enhance community acceptance and participation. By synthesizing findings from various studies, this study aims to provide a comprehensive understanding of the socio-economic and operational factors that influence the success of Bt interventions and offer recommendations for future scale-up and integration into public health policies.

2 Theoretical Frameworks of Risk Communication

2.1 Definition and key concepts of risk communication

Risk communication is the process of exchanging information about risks between decision-makers and the public (Savoia et al., 2017). It aims to inform and educate individuals about potential hazards, enabling them to make informed decisions and take appropriate actions to mitigate those risks. Effective risk communication involves understanding the audience's perceptions, beliefs, and attitudes towards risk, and tailoring messages to address these factors (Fitzpatrick-Lewis et al., 2010).

2.2 Theories and models in risk communication

Several theories and models underpin the practice of risk communication. One prominent model is the risk information seeking and processing (RISP) model, which integrates concepts from the heuristic-systematic model of information processing and the theory of planned behavior. The RISP model emphasizes the role of information sufficiency, channel beliefs, and perceived information-gathering capacity in shaping how individuals seek and process risk information (Griffin, 2013).

Another important framework is the conceptual model for evaluating emergency risk communication, developed in collaboration with the centers for disease control and prevention (CDC). This model outlines key constructs for assessing internal processes and outcomes of emergency risk communication, such as changes in knowledge, attitudes, beliefs, and behaviors (Seeger et al., 2018).

Additionally, the landscape of risk communication research highlights the interdisciplinary nature of the field, with roots in psychology, social sciences, medicine, and environmental sciences (Schmälzle et al., 2017). This research domain has evolved to address various applied contexts, including public health, environmental hazards, and emergency response (Goerlandt et al., 2020).

2.3 Ethical considerations in risk communication

Ethical considerations are paramount in risk communication, as the process often involves conveying information that can significantly impact individuals' lives and well-being. Ethical failures in risk communication can arise from inadequate theoretical understanding of the ethical assumptions embedded in risk discourses or from conflicts between ethical purposes, such as improving decision-making effectiveness, empowering recipients, or ensuring informed consent (Thompson, 2012).

The ethical motivation for risk communication may vary, but it generally aims to ensure that individuals are adequately informed about risks and can make decisions consistent with ethical criteria (Coyle and Gillies, 2020). Philosophical theories of ethics provide a useful framework for understanding these differences and guiding ethical risk communication practices (Thompson, 2012). Moreover, the fairness of risk distribution across social groups and the use of persuasion in risk communication are critical ethical issues (Dickmann, 2013). Communicators must strive to present information transparently and equitably, avoiding manipulation or coercion (Trakoli, 2015).

In summary, theoretical frameworks in risk communication encompass a range of models and ethical considerations that guide the effective and responsible dissemination of risk information. These frameworks help practitioners understand the complex dynamics of risk perception and communication, ultimately supporting informed decision-making and public health interventions.

3 Risk Communication Strategies and Methods

3.1 Identifying and understanding target audiences

Understanding the target audience is the first step in effective risk communication. It involves recognizing the audience's knowledge, perceptions, concerns, and beliefs about the health risks and interventions being communicated. Studies have shown that risk perception is influenced by personal experiences, cultural and social factors, and trust in information sources (Fitzpatrick-Lewis et al., 2010). For instance, the public's response to COVID-19 risk communication was shaped by factors such as immediacy, uncertainty, and trust in institutions

(Malecki et al., 2020). Similarly, engaging communities in the development of messaging and understanding their literacy levels can significantly improve the effectiveness of communication (Ghio et al., 2021; Lindsey et al., 2022).

3.2 Crafting clear and relevant messages

Crafting messages that are clear, relevant, and tailored to the audience's needs is crucial. Effective messages should be fact-based, transparent, and empathetic (Lurer et al., 2022). They should also be delivered by credible sources and framed to increase understanding, social responsibility, and personal control (Ghio et al., 2021). Multi-media approaches, combining text and diagrams, have been found to be more effective than single media approaches. Additionally, messages should address both the hazard and the outrage components of risk perception, as these shape public acceptance and adherence to health interventions (Figure 1).

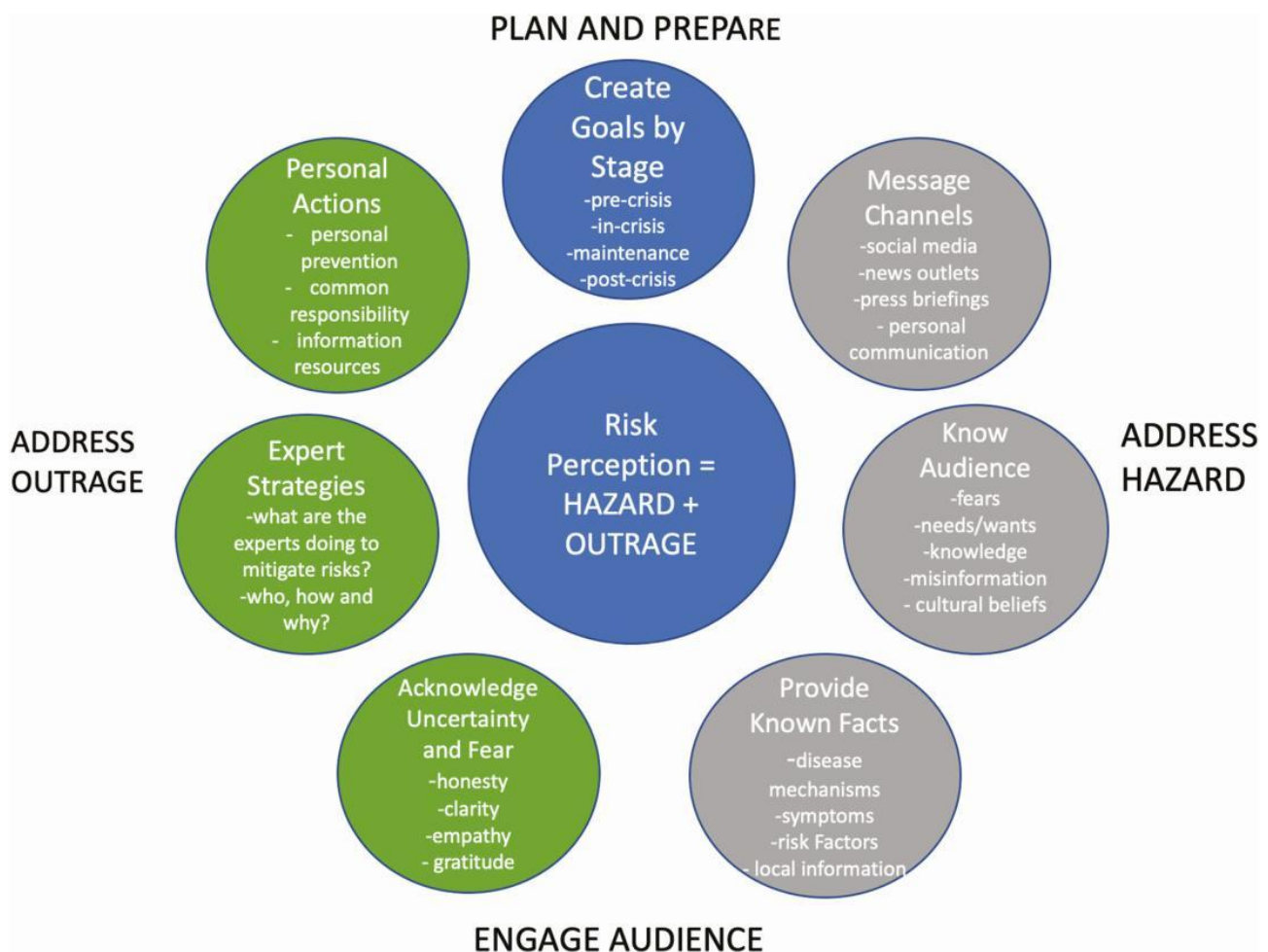


Figure 1 Crisis communication: addressing hazard+outrage during the COVID 19 pandemic (Adopted from Malecki et al., 2020)

The research of Malecki et al. (2020) outlines an effective framework for crisis communication during the COVID-19 pandemic, emphasizing the need to address both hazard and public outrage to manage risk perception. Central to the framework is the formula: Risk Perception = Hazard + Outrage. The model advocates for a comprehensive approach divided into four main strategies: Plan and Prepare, Address Outrage, Address Hazard, and Engage Audience. In the Plan and Prepare phase, it is crucial to set goals tailored to different crisis stages (pre-crisis, in-crisis, maintenance, post-crisis) and select appropriate message channels like social media, news outlets, and personal communication. Addressing outrage involves acknowledging public fears and uncertainties with honesty, clarity, empathy, and gratitude, while communicating expert strategies to mitigate risks effectively. Addressing the hazard requires providing accurate information about the crisis, including disease mechanisms, symptoms, and risk factors. Engaging the audience involves understanding their fears, needs, and cultural beliefs,

and combating misinformation through clear, accurate information. This holistic approach builds trust and encourages proactive behaviors during a crisis.

3.3 Addressing misconceptions and public concerns

Addressing misconceptions and public concerns is a critical component of risk communication. Misinformation can significantly hinder public health efforts, as seen in historical and contemporary health campaigns. Effective strategies include aggressively addressing misinformation, ensuring consistent messaging, and leveraging social media to quickly convey accurate information. Moreover, understanding and addressing the public's misperceptions by using plain language and providing actionable solutions can improve comprehension and response to health risks (Shrivastava et al., 2016).

In conclusion, effective risk communication for Bt-based public health interventions requires a comprehensive understanding of the target audience, the development of clear and relevant messages, and proactive addressing of misconceptions and public concerns (Su et al., 2022). By incorporating these strategies, public health officials can enhance the uptake and success of health interventions.

4 Case Studies in Bt-based Public Health Interventions

4.1 Successful Bt interventions and their communication strategies

Successful Bt-based public health interventions have demonstrated the importance of effective risk communication strategies. For instance, the community water fluoridation campaign in Saskatoon, Canada, during 1953/54, utilized extensive community outreach, involvement of local experts, and aggressive addressing of misinformation to gain public support (Leurer et al., 2022). Similarly, the Romanian Orthodox Church's involvement in COVID-19 mitigation efforts in Romania highlights the positive outcomes of productive consultations between public health authorities and religious institutions, emphasizing the importance of engaging faith-based communities in public health initiatives (Dascalu et al., 2021). These examples underscore the necessity of tailored communication strategies that involve local stakeholders and address community-specific concerns.

4.2 Challenges faced in risk communication

Despite the successes, several challenges have been encountered in risk communication for Bt-based public health interventions. The COVID-19 pandemic has revealed significant issues, such as delayed decision-making and limited information disclosure, which hindered effective communication in Wuhan, China (Figure 2) (Zhang et al., 2020). Additionally, the analysis of government communication in the United States during the COVID-19 pandemic highlighted the consequences of ineffective communication, including public confusion and misunderstanding, which prolonged the health crisis (Kim and Kreps, 2020). These challenges illustrate the critical need for timely, transparent (Rooney et al., 2020), and accurate communication to build public trust and ensure compliance with health interventions.

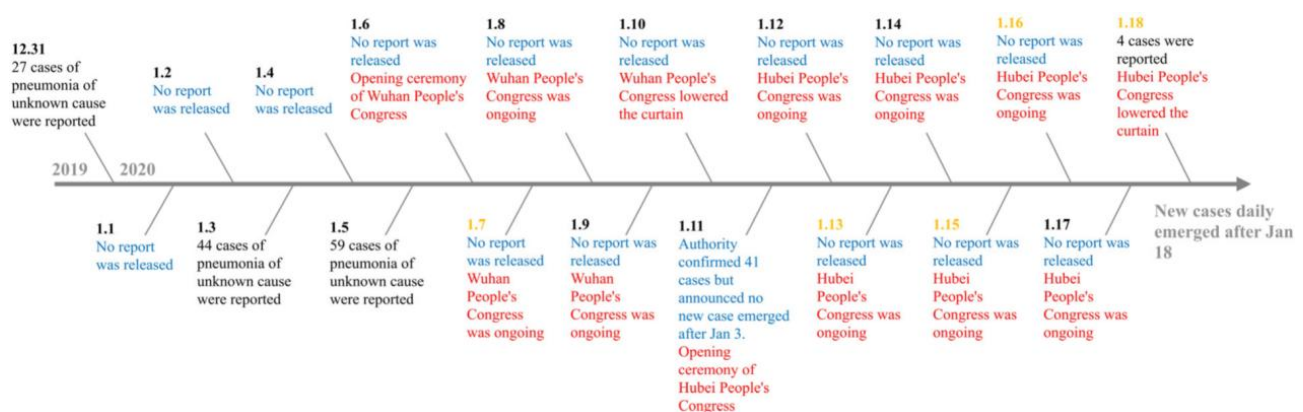


Figure 2 Timeline of official reports about infected cases by the Wuhan Health Commission (Adopted from Zhang et al., 2020)

Image caption: Blue represents "No report"; Red represents Wuhan and Hubei's Congress; Date in yellow represents confirmed infected case of medical worker (Adopted from Zhang et al., 2020)

The timeline in the research of Zhang et al. (2020) presents the sequence of events and official reports related to pneumonia cases of unknown cause in Wuhan, China, at the onset of the COVID-19 pandemic. Starting on December 31, 2019, with the initial report of 27 cases, the timeline highlights significant gaps in reporting, especially during the period when the Wuhan and Hubei People's Congress sessions were ongoing. Notably, from January 1 to January 17, 2020, multiple days passed without any reports being released, even as the number of pneumonia cases increased to 59 by January 5. On January 11, authorities confirmed 41 cases but stated that no new cases had emerged since January 3. It wasn't until January 18, when the People's Congress sessions concluded, that new cases were reported daily. This timeline underscores potential delays and inconsistencies in public health reporting, possibly influenced by political events, which may have impacted early responses to the outbreak. The emphasis on the lack of reporting during the People's Congress suggests a critical period where transparency and timely communication were crucial yet lacking.

4.3 Lessons learned from case studies

From these case studies, several key lessons can be drawn to improve future Bt-based public health interventions. First, the importance of community engagement and the involvement of local experts cannot be overstated, as demonstrated by the successful fluoridation campaign in Saskatoon. Second, the need for a collaborative approach that includes diverse stakeholders, such as religious institutions, can enhance the effectiveness of public health measures, as seen in Romania (Dascalu et al., 2021). Third, the experiences from the COVID-19 pandemic highlight the necessity of timely and transparent communication to prevent public confusion and ensure effective response to health crises (Warren and Lofstedt, 2021). Lastly, the use of social media and other modern communication tools should be integrated with traditional methods to reach a broader audience and address misinformation effectively (Toppenberg-Pejcic et al., 2019; Arije et al., 2023).

By incorporating these lessons, future Bt-based public health interventions can be better equipped to navigate the complexities of risk communication and achieve greater public support and compliance.

5 Evaluating the Effectiveness of Risk Communication

Evaluating the effectiveness of risk communication strategies for Bt-based public health interventions is crucial to ensure that the intended messages are accurately received and acted upon by the target audience. This evaluation can be broken down into three main components: metrics and indicators of success, feedback mechanisms, and continuous improvement.

5.1 Metrics and indicators of success

To measure the success of risk communication strategies, it is essential to establish clear metrics and indicators. These can include changes in awareness, knowledge, attitudes, and behaviors among the target population. For instance, a systematic review highlighted that multi-media approaches and printed materials combining text and diagrams are more effective in enhancing public understanding and response to environmental health risks (Fitzpatrick-Lewis et al., 2010). Additionally, the effectiveness of health-risk assessments with feedback has been demonstrated in worksite health promotion programs, where improvements in health behaviors and physiological estimates were observed (Soler et al., 2010). Metrics such as the rate of early detection, response times, and coordination efficiency during public health emergencies can also serve as indicators of successful risk communication (Dickmann et al., 2015).

5.2 Feedback mechanisms

Feedback mechanisms are vital for assessing the impact of risk communication and making necessary adjustments. These mechanisms can include surveys, focus groups, and direct feedback from the community (Brehaut et al., 2016). For example, patient-mediated interventions, such as patient-reported health information and patient education, have been shown to improve healthcare professionals' adherence to clinical practice guidelines, indicating the importance of incorporating patient feedback into communication strategies (Fønhus et al., 2016). Moreover, tailored risk feedback and message framing have been found to correct risk perceptions and increase behavioral intentions, underscoring the need for personalized feedback mechanisms (Goh et al., 2021).

5.3 Continuous improvement

Continuous improvement of risk communication strategies involves regularly updating and refining messages based on feedback and new evidence. This iterative process ensures that communication remains relevant and effective. A systematic review of personalized disease risk communication found that presenting risk information alone does not produce strong behavioral changes, suggesting the need for integrating additional behavior change techniques and theoretical frameworks (French et al., 2017). Furthermore, the development of high-quality communication tools that match patients' numerical abilities and provide clear, understandable information is essential for improving decision-making processes (Zipkin et al., 2014). By continuously evaluating and improving risk communication strategies, public health interventions can achieve better outcomes and foster greater trust and engagement within the community (Valle et al., 2018).

In conclusion, evaluating the effectiveness of risk communication for Bt-based public health interventions requires a comprehensive approach that includes defining clear metrics, implementing robust feedback mechanisms, and committing to continuous improvement. By leveraging these strategies, public health practitioners can enhance the impact of their communication efforts and better protect public health.

6 Implications for Future Bt-based Public Health Programs

6.1 Integrating risk communication into public health planning

Effective risk communication is essential for the success of Bt-based public health interventions. Research indicates that a multi-media approach, which combines various types of information delivery such as text, diagrams, and visual aids, is more effective than single-method approaches (Fitzpatrick-Lewis et al., 2010). Additionally, integrating emergency risk communication (ERC) into public health systems can enhance preparedness and response activities by reforming leadership structures, modifying organizational factors, and removing regulatory obstacles (Jha et al., 2018). For Bt-based interventions, it is crucial to incorporate these strategies into public health planning to ensure that the target audience receives and understands the risk messages, thereby improving compliance and trust in public health measures (Warren and Lofstedt, 2021).

6.2 Policy recommendations

To optimize the effectiveness of Bt-based public health programs, several policy recommendations can be made. First, policies should mandate the use of multi-faceted risk communication strategies that address the diverse needs of the target population (Fitzpatrick-Lewis et al., 2010). Second, it is important to establish networks, task forces, and committees that facilitate information sharing and coordination across different organizations and levels of response (Jha et al., 2018). Third, policies should encourage the engagement of local stakeholders to ensure the flow of information and build trust within communities (Scholz et al., 2021). Finally, integrating social science intelligence into epidemiologic risk assessments can provide a more comprehensive understanding of public perceptions and improve the design of risk communication strategies (Dickmann et al., 2016).

6.3 Future research directions

Future research should focus on several key areas to enhance the effectiveness of Bt-based public health interventions. First, there is a need for more empirical studies, particularly in low- and middle-income countries, to understand the specific challenges and opportunities in these settings (Jha et al., 2018). Second, research should explore the impact of personalized risk communication on behavior change, as current evidence suggests that personalized risk information alone does not produce strong or consistent effects (French et al., 2017). Third, studies should investigate the effectiveness of community-based and participatory interventions, especially in low-resource settings, to determine their potential for improving public health outcomes (Hale et al., 2014; Schiavo et al., 2014). Finally, research should address the gaps in evaluating the cost-effectiveness and health equity implications of risk communication strategies to inform better policy and practice (Meng et al., 2016).

By addressing these areas, future Bt-based public health programs can be better equipped to communicate risks effectively, engage communities, and achieve desired health outcomes.

7 Concluding Remarks

The review of literature on risk communication strategies for Bt-based public health interventions highlights several critical elements for effective communication. Effective public health messaging is characterized by delivery from credible sources, community engagement, and increasing awareness and knowledge, which are essential for managing risks and preventing infectious diseases. Multi-media approaches and printed materials combining text and diagrams are more effective than single media approaches. Additionally, integrating social science intelligence into epidemiologic risk assessments and strengthening multisectoral collaboration are crucial for improving risk communication. Trust in information sources, such as the WHO, significantly influences public adherence to preventive measures.

Effective risk communication is paramount in public health interventions as it fosters public trust, compliance, and support for non-pharmaceutical interventions (NPIs). It is essential for managing public perceptions and behaviors during health crises, such as the COVID-19 pandemic. Effective communication strategies can mitigate misinformation, enhance public understanding of health threats, and promote social responsibility and personal control. Moreover, integrating emergency risk communication (ERC) into public health systems ensures timely and accurate information dissemination, which is vital for preparedness and response activities.

Stakeholders should prioritize engaging communities in the development of public health messages to ensure they are acceptable and increase understanding and perceived susceptibility to health threats. It is recommended to address uncertainty immediately and with transparency to build and maintain public trust. Utilizing a multi-media approach and combining different types of information in printed materials can enhance the effectiveness of communication strategies. Strengthening multisectoral collaboration, including with local organizations, is essential for a cohesive and comprehensive risk communication strategy. Regular feedback from the public on their familiarity and compliance with preventive measures should be sought to adapt and improve communication strategies as the situation evolves. Finally, stakeholders should ensure that risk communication strategies are proactive, participatory, and multisectoral to facilitate better governance and collaboration.

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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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Review Article

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Bt in Organic Farming: Benefits and Limitations

Chunxiang Ma ✉

Modern Agricultural Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China

✉ Corresponding email: chunxiang.ma@cuixi.orgBt Research, 2024, Vol.15, No.4 doi: [10.5376/bt.2024.15.0017](https://doi.org/10.5376/bt.2024.15.0017)

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Abstract Bt (*Bacillus thuringiensis*) is a widely used biological pesticide known for its high specificity and safety in pest control. In organic agriculture, the application of Bt provides an effective and environmentally friendly pest control method that aligns with the principles and requirements of organic farming. However, the use of Bt in organic agriculture also faces challenges such as the risk of resistance development, environmental constraints, and regulatory issues. This study systematically reviews the mechanisms of action of Bt, its benefits and limitations in organic agriculture, and successful case studies and lessons learned. The comprehensive analysis of the current application and future prospects of Bt in organic agriculture demonstrates its significant effectiveness in pest control and environmental friendliness. Nonetheless, continuous monitoring of resistance development and the implementation of effective management strategies are essential. Additionally, innovations in Bt formulations and their integration with other organic practices are key areas for future research. This study aims to provide a scientific basis for promoting the application of Bt in organic agriculture and to guide future research and development.

Keywords Bt; Biological pesticide; Organic farming; Resistance management; Environmental benefits

1 Introduction

Bacillus thuringiensis (Bt) is a gram-positive, spore-forming bacterium that has gained prominence due to its potent insecticidal properties. During sporulation, Bt produces crystal proteins (Cry proteins) that are toxic to a wide range of insect pests, particularly those belonging to the orders Coleoptera, Diptera, and Lepidoptera (Xiao and Wu, 2019; Sánchez-Yáñez et al., 2022). These Cry proteins have been extensively utilized in both microbial insecticides and genetically modified crops to enhance pest resistance (Jouzani et al., 2017; Rajadurai et al., 2023). Bt's ability to target specific pests while being safe for humans, animals, and non-target organisms has made it the most widely used biopesticide globally (Gutiérrez et al., 2019; Rajadurai et al., 2023). Additionally, Bt has shown potential in other applications such as bioremediation, plant growth promotion, and even cancer prevention (Jouzani et al., 2017; Sánchez-Yáñez et al., 2022).

Organic farming is an agricultural practice that emphasizes the use of natural inputs and processes to enhance soil fertility, biodiversity, and ecological balance. It avoids synthetic chemicals, such as pesticides and fertilizers, to produce food in a sustainable and environmentally friendly manner. The increasing consumer demand for organic products is driven by concerns over food safety, environmental sustainability, and health benefits. Organic farming practices contribute to soil health, reduce pollution, and promote biodiversity, making it a crucial component of sustainable agriculture (Loguercio and Argôlo-Filho, 2015; Rajadurai et al., 2023). Integrating biopesticides like Bt into organic farming aligns with these principles, offering an effective and eco-friendly solution for pest management (Gomis-Cebolla and Berry, 2023; Rajadurai et al., 2023).

To evaluate the benefits and limitations of using *Bacillus thuringiensis* (Bt) in organic farming, this study assesses the efficacy of Bt as a biopesticide in controlling various insect pests in organic farming systems. It explores the potential non-insecticidal benefits of Bt, such as plant growth promotion and bioremediation. It identifies the challenges and limitations associated with the use of Bt in organic farming, including the development of pest resistance and ecological impacts. Additionally, it provides recommendations for future research and practical applications to optimize the use of Bt in organic farming. By synthesizing current knowledge and research findings, this study aims to provide a comprehensive understanding of the role of Bt in organic farming and its potential to contribute to sustainable agricultural practices.

2 Mechanisms of Bt Action

2.1 Bt toxins and their targets

Bacillus thuringiensis (Bt) produces a variety of insecticidal proteins, including Cry and Vip toxins, which target specific insect pests. These toxins are highly selective, affecting only certain insect species while being safe for humans and other non-target organisms (Gassmann and Reisig, 2022). The Cry toxins, in particular, are produced as protoxins that require activation within the insect gut to become toxic. The specificity of these toxins is due to their ability to bind to specific receptors in the insect midgut, leading to cell lysis and death (Tabashnik, 2015; Chattopadhyay and Banerjee, 2018; Heckel, 2020).

2.2 Mode of action in insect pests

The mode of action of Bt toxins involves several steps. Initially, the protoxins are ingested by the insect and activated by gut proteases, converting them into their toxic form. The activated toxins then bind to specific receptors on the midgut epithelial cells, such as cadherins and ABC transporters. This binding facilitates the formation of pores in the cell membrane, leading to cell lysis and ultimately the death of the insect (Chattopadhyay and Banerjee, 2018). Recent studies have highlighted the role of ABC transporters in the mechanism of action, suggesting that these proteins are crucial for the binding and pore formation of Cry toxins (Tabashnik, 2015; Heckel, 2020).

2.3 Specificity and safety of Bt toxins

Bt toxins are highly specific to their target pests, which makes them an attractive alternative to broad-spectrum chemical insecticides. This specificity is due to the unique receptors present in the midgut of susceptible insects, which are absent in non-target organisms (Tabashnik, 2015; Chattopadhyay and Banerjee, 2018; Gassmann and Reisig, 2022). For instance, Cry1Ac and Cry2Ab toxins are effective against lepidopteran pests but have little to no effect on other insects or mammals. The safety of Bt toxins is further supported by their long history of use in agriculture without adverse effects on human health or the environment. However, the evolution of resistance in target pests poses a significant challenge, necessitating ongoing research and the development of new strategies to manage resistance (Tabashnik, 2015; Gassmann and Reisig, 2022).

Bt toxins offer a highly specific and safe method for pest control, with their mode of action involving the activation of protoxins, binding to specific midgut receptors, and subsequent cell lysis. The specificity and safety of these toxins make them a valuable tool in integrated pest management, although the evolution of resistance remains a critical issue that requires continuous monitoring and innovation.

3 Benefits of Bt in Organic Farming

Bt crops provide numerous benefits in organic farming, including effective pest control, environmental sustainability, and alignment with organic farming principles. These advantages make Bt crops a promising option for enhancing the productivity and sustainability of organic farming systems.

3.1 Pest control efficacy

3.1.1 Spectrum of activity

Bt crops have been engineered to produce insecticidal proteins from *Bacillus thuringiensis* (Bt), which are effective against a wide range of pests, particularly lepidopteran and coleopteran species. These proteins, such as Cry1 and Cry2, target specific pests while having minimal impact on non-target organisms, including beneficial insects and humans (Tabashnik, 2015; Arends et al., 2021). The narrow and selective toxicity spectrum of Bt proteins helps in managing primary pests effectively without causing significant harm to the ecosystem (Catarino et al., 2016).

3.1.2 Field application successes

The implementation of Bt crops has led to significant successes in pest control in various regions. For instance, Bt cotton and maize have been widely adopted in the United States, India, and Australia, resulting in reduced pest populations and decreased reliance on chemical insecticides (Tabashnik, 2015; Xiao and Wu, 2019; Gassmann,

and Reisig, 2022). In the southwestern United States, a combination of Bt cotton and integrated pest management strategies, including the release of sterile moths, has successfully eradicated the pink bollworm, a major pest (Tabashnik and Carrière, 2019). These successes highlight the practical benefits of Bt crops in reducing pest-related crop damage and increasing agricultural productivity.

3.1.3 Resistance management

One key challenge in using Bt crops is the evolution of pest resistance. However, strategies such as planting refuges with non-Bt crops and using pyramided Bt crops that produce multiple Bt toxins have effectively delayed the development of resistance (Carrière et al., 2016). Cooperative management among government regulators, growers, and other stakeholders plays a critical role in effectively managing pest resistance (Figure 1). For example, in Australia, Bt cotton using Cry1Ac and Cry2Ab toxins helps manage resistance in the cotton bollworm (*Helicoverpa armigera*) (Tabashnik, 2015). Additionally, combining Bt crops with other pest management strategies, such as crop rotation and the use of non-Bt host plants, has been shown to enhance the sustainability of Bt crops by slowing the development of resistance (Gassmann, 2021).

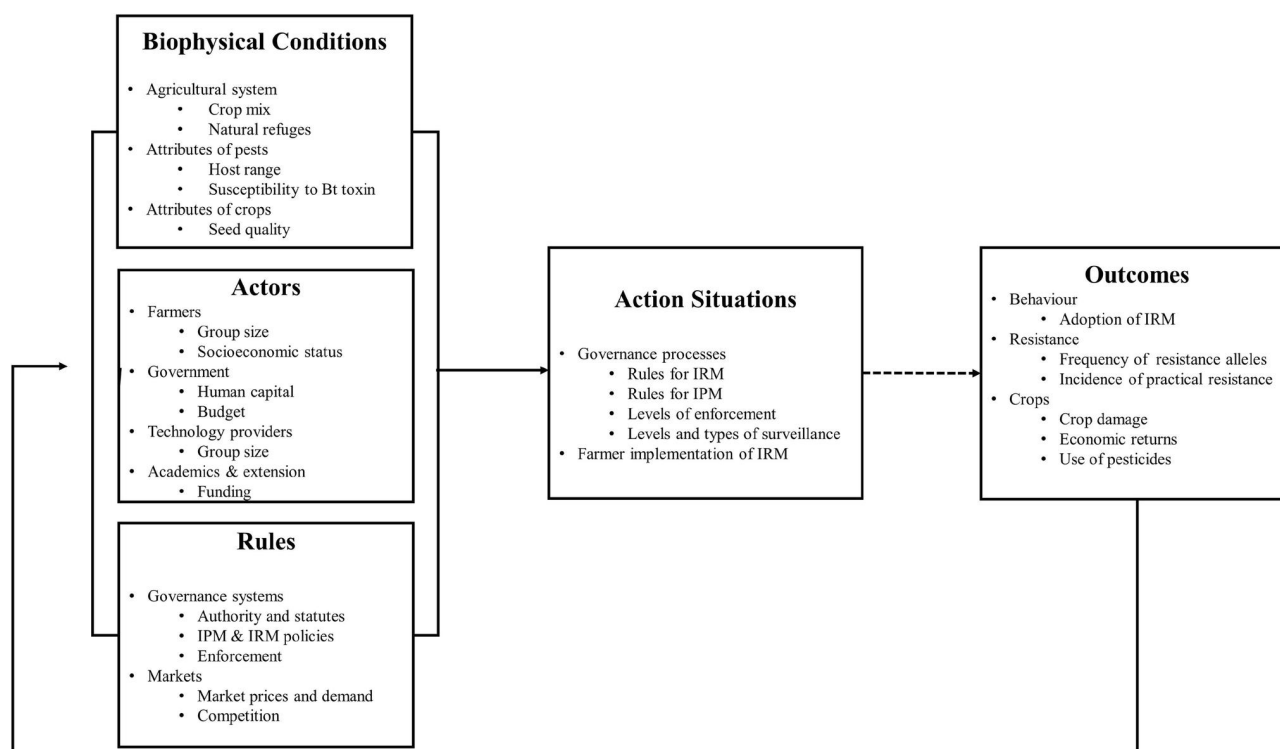


Figure 1 Overview of the Institutional Analysis and Development framework for governance of Bt crops (Adopted from Carrière et al., 2016)

The Institutional Analysis and Development (IAD) framework for the governance of Bt crops demonstrates how biophysical conditions, rules, and the actions of different stakeholders interact to influence resistance outcomes. Figure 1 emphasizes the importance of considering ecological and socio-economic factors when formulating and implementing resistance management policies. It underscores the integrated roles of government agencies, growers, and the industry in managing resistance to Bt crops to ensure sustainable pest control and long-term agricultural productivity.

3.2 Environmental benefits

Bt crops offer significant environmental benefits by reducing the need for chemical insecticides, which can have harmful effects on non-target organisms and the environment. The adoption of Bt crops has led to a decrease in insecticide use, thereby reducing the environmental footprint of agricultural practices (Xiao and Wu, 2019; Gassmann and Reisig, 2022). Moreover, Bt crops contribute to biodiversity conservation by preserving beneficial

insect populations and reducing the risk of pesticide runoff into water bodies (Arends et al., 2021). The environmental safety of Bt crops has been well-documented, with studies showing no adverse effects on non-target organisms, including mammals, birds, and beneficial insects (Koch et al., 2015).

3.3 Compatibility with organic farming principles

Bt crops align well with the principles of organic farming, which emphasize sustainable and environmentally friendly agricultural practices. The use of Bt crops can reduce the reliance on synthetic chemical insecticides, which are generally prohibited in organic farming (Xiao and Wu, 2019). Additionally, Bt crops can be integrated into organic farming systems as part of a broader integrated pest management strategy, which includes crop rotation, biological control, and the use of natural refuges (Carrière et al., 2016). This compatibility makes Bt crops a valuable tool for organic farmers seeking to manage pests effectively while adhering to organic farming standards.

4 Limitations of Bt in Organic Farming

4.1 Development of resistance

4.1.1 Mechanisms of resistance development

The development of resistance to Bt crops in organic farming is a significant limiting factor. Resistance mechanisms typically involve genetic mutations in target pests. For instance, mutations in genes encoding ATP-binding cassette (ABC) transporters have been associated with *Helicoverpa zea* resistance to the Cry2Ab toxin (Tabashnik, 2015). Additionally, resistance may also result from mutations in other genes that affect toxin binding sites or are involved in the mode of action of Bt toxins (Xiao and Wu, 2019; Jurat-Fuentes, 2021). The development of resistance is promoted by factors such as the inheritance of non-recessive resistance traits, low fitness costs associated with resistance, and continuous exposure to Bt crops (Gassmann, 2021). The introduction of Bt maize and Bt cotton has significantly suppressed pest populations in some regions, reducing the need for traditional insecticides and increasing farmers' profits. However, the evolution of pest resistance to Bt traits remains a critical challenge, leading to reduced effectiveness of Bt crops (Figure 2).

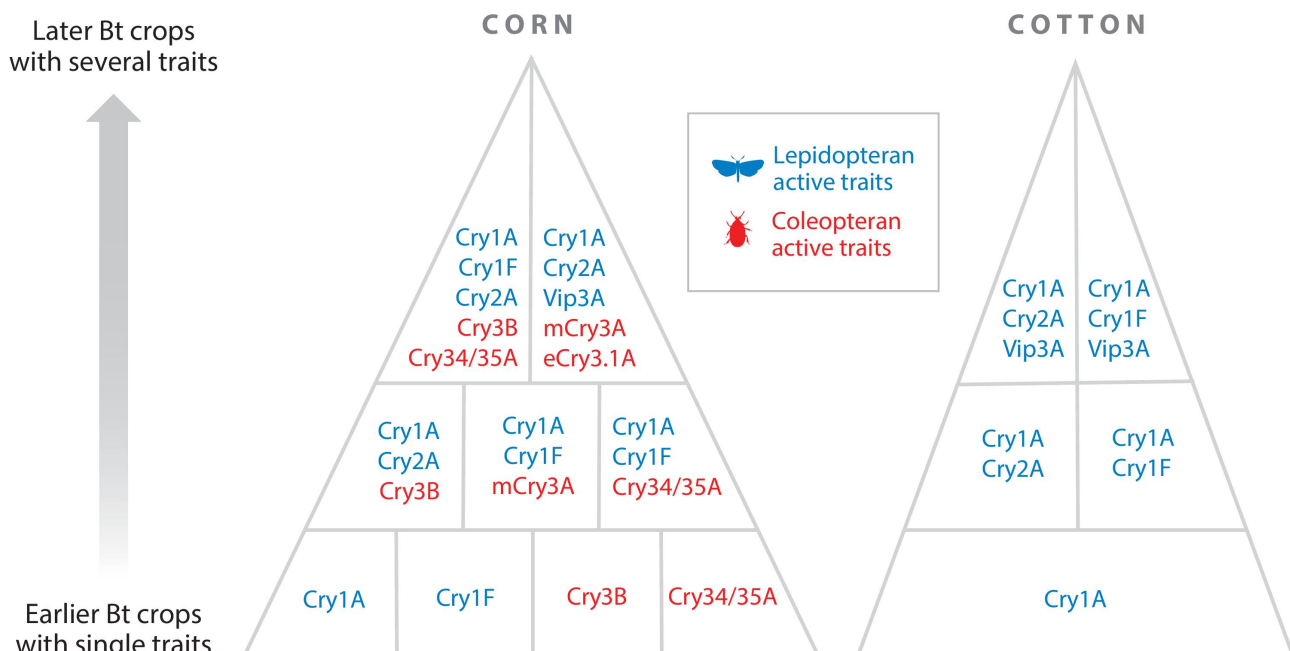


Figure 2 Pattern of pyramiding and stacking of *Bacillus thuringiensis* (Bt) traits over time for Bt corn and Bt cotton in the United States (Adopted from Gassmann, 2021)

Image caption: Subdivisions within larger triangles represent an array of Bt traits found within an individual crop plant. All past and current trait combinations are displayed for cotton, but only a subset of trait combinations are provided for corn. While lepidopteran traits target pests in multiple families, coleopteran traits are only used to manage species in the genus *Diabrotica* (Chrysomelidae) (Adopted from Gassmann, 2021)

The figure from Gassmann (2021) details the combination of different Bt toxins within a single crop, highlighting the cumulative effect of multiple Bt toxins on the same pest. This pyramiding strategy aims to delay the evolution of pest resistance by using multiple toxins, but the figure also reveals that cross-resistance and antagonism between the toxins can impact the effectiveness of resistance management. Therefore, optimizing the combination of Bt toxins is crucial for improving pest control efficacy.

4.1.2 Monitoring and detection

Effective monitoring and detection of resistance are crucial for managing Bt resistance. Techniques such as the F2 screen and genetic linkage analysis have been employed to detect resistance alleles in field populations. Regular surveillance and bioassays are essential to identify early signs of resistance and implement timely management strategies (Carrière et al., 2019). For example, monitoring data from Australia showed no significant increase in Cry2Ab resistance over eight years, highlighting the importance of robust monitoring programs (Tabashnik, 2015).

4.1.3 Strategies to mitigate resistance

Several strategies can mitigate the development of resistance to Bt crops. One approach is the use of multi-toxin Bt crops, which produce multiple Bt toxins to delay resistance (Tabashnik, 2015). Another strategy is the implementation of refuges, where non-Bt crops are planted to maintain a population of susceptible pests (Arends et al., 2021; Gassmann, 2021). Additionally, integrated pest management (IPM) practices, such as crop rotation and the use of non-Bt insecticides, can help manage resistance (Gassmann, 2021; Gassmann and Reisig, 2022). The success of these strategies depends on coordinated efforts among farmers, regulators, and other stakeholders (Carrière et al., 2019).

4.2 Environmental constraints

Environmental factors can also limit the effectiveness of Bt in organic farming. The interaction between Bt crops and the surrounding ecosystem can influence the development of resistance. For example, the local abundance of non-Bt crops and natural refuges can affect the effectiveness of resistance management strategies (Arends et al., 2021). Moreover, the nutritional status of insect herbivores can impact their susceptibility to Bt toxins, with optimal diets potentially reducing the efficacy of Bt crops (Deans et al., 2016). Understanding these environmental constraints is essential for developing sustainable pest management practices.

4.3 Regulatory and market challenges

Regulatory and market challenges pose additional limitations to the use of Bt in organic farming. Regulatory frameworks vary across countries, affecting the implementation of resistance management measures (Carrière et al., 2019). In some regions, the lack of mandatory refuges and insufficient monitoring can exacerbate resistance issues (Tabashnik and Carrière, 2019). Market acceptance of Bt crops also varies, with some consumers and organic certification bodies opposing genetically modified organisms (GMOs) (Xiao and Wu, 2019). Addressing these challenges requires harmonized regulations, effective communication among stakeholders, and public education on the benefits and risks of Bt crops.

5 Case Studies and Field Applications

5.1 Successful implementations

The adoption of *Bacillus thuringiensis* (Bt) crops has shown significant success in various agricultural settings. For instance, widespread Bt maize adoption in the Mid-Atlantic United States has led to marked decreases in the number of recommended insecticidal applications, insecticides applied, and damage to vegetable crops. This regional pest suppression has benefited not only Bt crop fields but also non-Bt crop fields, including those managed organically (Dively, 2018). Additionally, Bt crops have been associated with increased profits for farmers due to reduced conventional insecticide use and enhanced pest control.

5.2 Lessons learned

While Bt crops have demonstrated substantial benefits, there are critical lessons to be learned from their implementation. One significant challenge is the evolution of pest resistance to Bt traits. In some cases, pests have developed resistance, leading to increased crop damage and reduced effectiveness of Bt crops. To mitigate this, strategies such as increasing the prevalence of refuges and integrating pest management practices are essential. These measures can help delay resistance and sustain the efficacy of Bt crops over time (Gassmann and Reisig, 2022).

5.3 Comparative analysis with other organic methods

When comparing Bt crops with other organic farming methods, several factors come into play. Organic farming generally outperforms conventional systems economically due to lower production costs and higher market prices, despite lower yields (Durham and Mizik, 2021). However, the integration of Bt crops can enhance pest control and reduce the need for insecticides, which is beneficial for both conventional and organic systems (Dively, 2018; Gassmann and Reisig, 2022).

Moreover, organic farming practices, such as crop rotations and the use of organic fertilizers, contribute to soil fertility and environmental sustainability (Kolbe, 2022). However, the yield gap between organic and conventional farming remains a challenge, with organic systems producing on average 25% lower yields (Alvarez, 2021). The combination of Bt crops with organic practices could potentially bridge this gap by improving pest control and reducing crop damage, thereby enhancing overall productivity and sustainability.

The integration of Bt crops in organic farming presents a promising approach to address some of the limitations of organic systems, particularly in pest management. However, careful consideration of pest resistance and the adoption of integrated pest management strategies are crucial to ensure the long-term success and sustainability of Bt crops in organic farming systems.

6 Future Perspectives

6.1 Innovations in Bt formulations

Innovations in Bt formulations are crucial for enhancing the efficacy and sustainability of Bt crops in organic farming. Recent studies have highlighted the importance of developing transgenic crop pyramids that produce multiple Bt toxins to delay the evolution of pest resistance. For instance, research has shown that pyramided Bt crops, which produce two or more Bt toxins targeting the same pest, can be more effective in managing pest resistance compared to single-toxin Bt crops (Carrière et al., 2015). However, the effectiveness of these pyramids can be compromised by cross-resistance and antagonism between the toxins, which are often related to the similarity in their amino acid sequences (Carrière et al., 2015). Therefore, future innovations should focus on optimizing the combination of Bt toxins to minimize these issues and enhance pest control.

6.2. Integration with other organic practices

Integrating Bt crops with other organic farming practices can further improve pest management and sustainability. Organic agriculture, known for its environmental benefits and profitability, can be complemented by Bt crops to reduce pesticide use and enhance crop yields (Reganold and Wachter, 2016). For example, the use of refuges — areas planted with non-Bt crops — alongside Bt crops has been shown to delay pest resistance by allowing susceptible pests to survive and mate with resistant ones, thereby reducing the overall resistance in the pest population (Carrière et al., 2016). Additionally, combining Bt crops with other pest management tactics, such as crop rotation and biological control, can create a more robust and sustainable pest management system (Carrière et al., 2016; Gassmann and Reisig, 2022).

6.3 Research and development priorities

Future research and development should prioritize understanding the long-term impacts of Bt crops and improving resistance management strategies. Studies have shown that while Bt crops can initially reduce pesticide use and increase profits, the evolution of pest resistance can lead to increased pesticide use and reduced effectiveness of Bt

crops over time (Kranthi and Stone, 2020). Therefore, it is essential to focus on developing new Bt traits and improving existing ones to delay resistance. This includes increasing the prevalence of refuges and using integrated pest management strategies (Gassmann and Reisig, 2022). Additionally, research should explore the socio-economic impacts of Bt crops on farmers and communities to ensure that the benefits of Bt technology are equitably distributed (Reganold and Wachter, 2016; Kranthi and Stone, 2020).

7 Concluding Remarks

The application of *Bacillus thuringiensis* (Bt) crops has shown significant benefits in pest management and agricultural sustainability. The widespread adoption of Bt maize has led to regional pest suppression, reducing the need for insecticidal applications and decreasing crop damage in both Bt and non-Bt fields, including vegetable crops. Bt crops have also reduced insecticide usage and increased farmers' profits. However, the evolution of pest resistance to Bt traits remains a critical challenge, necessitating integrated pest management strategies to delay resistance. Additionally, while Bt crops have shown efficacy in pest control, their performance can be compromised under environmental stress conditions.

Bt crops play a crucial role in sustainable agriculture by reducing the reliance on chemical insecticides, thereby promoting environmental health and biocontrol services. They help lower pest management costs and increase crop yields, thus boosting farmers' profits. Bt crops have also been proven to provide indirect benefits to neighboring non-Bt crops by reducing pest pressure, which is particularly beneficial for organic farming systems. Despite the lower yields and higher variability in organic farming, integrating Bt crops can enhance the overall sustainability of agricultural practices by balancing productivity with environmental and economic benefits.

Future research should focus on several key areas to enhance the benefits of Bt crops in organic farming and reduce their limitations. Investigate and develop strategies to delay pest resistance to Bt crops, such as increasing the prevalence of refuges and employing integrated pest management practices. Conduct comprehensive studies to understand the impact of various environmental stressors on the efficacy of Bt crops and develop robust predictive models for their performance under extreme climatic conditions. Additionally, explore the interactions between Bt crops and non-target organisms, including beneficial insects and aquatic ecosystems, to ensure that Bt crop adoption does not inadvertently harm these populations. By addressing these research areas, we can optimize the use of Bt crops in organic farming systems, ensuring their benefits are maximized while minimizing potential drawbacks.

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Conflict of Interest Disclosure

The author affirms 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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Research Insight

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Comparative Genomics of Bt and Related *Bacillus* Species

Hui Xiang, Zhongqi Wu ✉

Institute of Life Science, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China

✉ Corresponding author: zhongqi.wu@jicaf.orgBt Research, 2024, Vol.15, No.4 doi: [10.5376/bt.2024.15.0018](https://doi.org/10.5376/bt.2024.15.0018)

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Abstract *Bacillus thuringiensis* (Bt) and related *Bacillus* species hold significant importance in the fields of biocontrol and biotechnology. This study employs comparative genomics to systematically analyze the genomic features of Bt and related *Bacillus* species, exploring gene expression, the structure and evolution of toxin gene clusters, and the roles of plasmids and mobile genetic elements. The research also includes a comparison with other *Bacillus* species, revealing their phylogenetic relationships, conserved and unique genomic regions, and mechanisms of horizontal gene transfer. Additionally, functional genomics studies investigate the expression profiles of specific genes, proteomics and metabolomics characteristics, and their roles in environmental adaptation and co-evolution with hosts. This study aims to provide scientific evidence for the agricultural, medical, and industrial applications of Bt, and addresses regulatory and safety considerations. Through this research, new directions for Bt genomics research are anticipated, and practical applications are suggested.

Keywords *Bacillus thuringiensis*; Comparative genomics; Toxin genes; Functional genomics; Biocontrol

1 Introduction

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacterium renowned for its insecticidal properties due to the production of crystal (Cry) proteins. These proteins are highly specific to various insect orders, including Lepidoptera, Diptera, and Coleoptera, making Bt a valuable tool in integrated pest management (IPM) and agricultural biotechnology (Hằng et al., 2021). The insecticidal activity of Bt results from the formation of pores in the midgut epithelial cells of susceptible insects, leading to cell lysis and death. Bt has been extensively utilized in the development of biopesticides and genetically modified crops, such as Bt cotton and Bt corn, which express Cry proteins to protect against insect pests (Hằng et al., 2021; Lazarte et al., 2021).

Comparative genomics, which involves the analysis and comparison of genetic material from different organisms, helps to understand their evolutionary relationships, functional genomics, and genetic diversity. In the study of Bt and related *Bacillus* species, comparative genomics is crucial for several reasons. It aids in identifying new insecticidal genes and understanding their mechanisms of action, thereby developing more effective biopesticides (Hằng et al., 2021). It provides insights into the genetic basis of insect resistance to Bt toxins, enabling the design of strategies to mitigate resistance development (Lazarte et al., 2021; Chen et al., 2021). Furthermore, comparative genomics can reveal the evolutionary adaptations and ecological niches of different *Bacillus* strains, contributing to a broader understanding of their roles in natural and agricultural ecosystems (Reyaz et al., 2019; Crickmore et al., 2020).

This study provides a comprehensive analysis of the comparative genomics of Bt and related *Bacillus* species, summarizing current knowledge on the genetic diversity and evolutionary relationships among Bt strains and related *Bacillus* species. It identifies and characterizes new insecticidal genes and their potential applications in pest management, discusses the mechanisms of insect resistance to Bt toxins and potential strategies to overcome resistance, and explores the ecological and functional roles of Bt and related *Bacillus* species in various environments. By synthesizing findings from multiple research studies, this study aims to advance our understanding of Bt genomics and its applications in sustainable agriculture and pest management.

2 Overview of *Bacillus* Genomics

2.1 General characteristics of *Bacillus* genomes

Bacillus species are known for their diverse metabolic capabilities and adaptability to various environments. The genomes of *Bacillus* species typically range in size from approximately 5 to 6.5 million base pairs (bp) and have a G+C content around 35%-36% (Liu et al., 2017). For instance, the genome of *Bacillus thuringiensis* strain BM-BT15426 is 5 246 329 bp long with a G+C content of 35.40% (Liu et al., 2017), while *Bacillus thuringiensis* HM-311 has a genome size of 6,019,481 bp and a G+C content of 35.85%. These genomes often contain numerous plasmids, which carry genes responsible for various functions, including virulence factors and antibiotic resistance (Reyaz et al., 2019; Zuo et al., 2020).

2.2 Sequencing technologies and methods

The sequencing of *Bacillus* genomes has evolved significantly with advancements in sequencing technologies. Early sequencing efforts involved labor-intensive methods, but modern techniques such as PacBio RS II and Illumina HiSeq 4000 platforms have streamlined the process, allowing for high-throughput and accurate sequencing (Liu et al., 2017; Zuo et al., 2020). For example, the genome of *Bacillus thuringiensis* strain BM-BT15426 was sequenced using the PacBio RS II sequencer, which provides long-read sequencing capabilities, and assembled de novo using the HGAP pipeline (Liu et al., 2017). Similarly, *Bacillus thuringiensis* HM-311 was sequenced using both PacBio RS II and Illumina HiSeq 4000 platforms, combining long-read and short-read sequencing to achieve a comprehensive genome assembly (Zuo et al., 2020).

2.3. Genome annotation and analysis

Genome annotation is a critical step in understanding the functional capabilities of *Bacillus* species. Automated annotation tools such as the RAST server and NCBI's annotation pipeline are commonly used to predict coding sequences, identify genes, and assign functions based on homology (Quan et al., 2016; Reyaz et al., 2019). For instance, the genome of *Bacillus thuringiensis* X022 was annotated using the RAST server, which identified genes coding for insecticidal proteins and metabolic pathways (Quan et al., 2016). Similarly, the genome of *Bacillus thuringiensis* isolate T414 was annotated to reveal the presence of parasporal crystal protein genes and various virulence factors (Reyaz et al., 2019).

Comparative genomics further enhances our understanding by identifying core and pan-genomes, which distinguish conserved genes from those unique to specific strains. This approach has revealed significant functional differences among *Bacillus* species, such as variations in carbohydrate utilization and signal transduction pathways (Alcaraz et al., 2010). Additionally, comparative analysis of genomic and proteomic data can provide insights into gene expression and regulation, as demonstrated in studies of *Bacillus thuringiensis* strains (Rang et al., 2015; Quan et al., 2016).

3 Genomic Features of Bt

3.1 Genome structure and organization

Bacillus thuringiensis (Bt) exhibits a complex genome structure characterized by a circular chromosome and multiple plasmids. For instance, the Bt GR007 strain has a circular chromosome and three megaplasms, with the two largest megaplasms containing multiple pesticidal protein genes (Pacheco et al., 2021). Similarly, the Bt isolate T414 contains a chromosome and 15 different plasmids, which harbor various insecticidal genes (Reyaz et al., 2019). The genome of Bt X022 consists of one circular chromosomal DNA and seven plasmids, which include genes coding for several Cry proteins and a vegetative insecticidal protein (Quan et al., 2016).

3.2 Toxin gene clusters

3.2.1 Types of toxins

Bt produces a wide variety of insecticidal proteins, including *Cry*, *Cyt*, and *Vip* toxins. The GR007 strain, for example, contains 10 *cry* genes, two *vip* genes, and two binary toxin genes (Pacheco et al., 2021). The T414 strain harbors parasporal crystal protein genes (*cryIAa*, *cryIAb*, *cryIAc*, *cryIIAa*, *cry2Aa*, *cry2Ab*, and *cytI*) and a vegetative insecticidal protein gene (*vip3Aa*) (Reyaz et al., 2019). Additionally, the X022 strain contains genes coding for *CryIAc*, *CryIIa*, *Cry2Ab*, and *Vip3A* proteins (Quan et al., 2016).

3.2.2 Gene expression and regulation

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

3.2.3 Evolution of toxin genes

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

3.3 Plasmids and mobile genetic elements

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

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

4 Comparative Genomics with Related *Bacillus* Species

4.1 Phylogenetic relationships

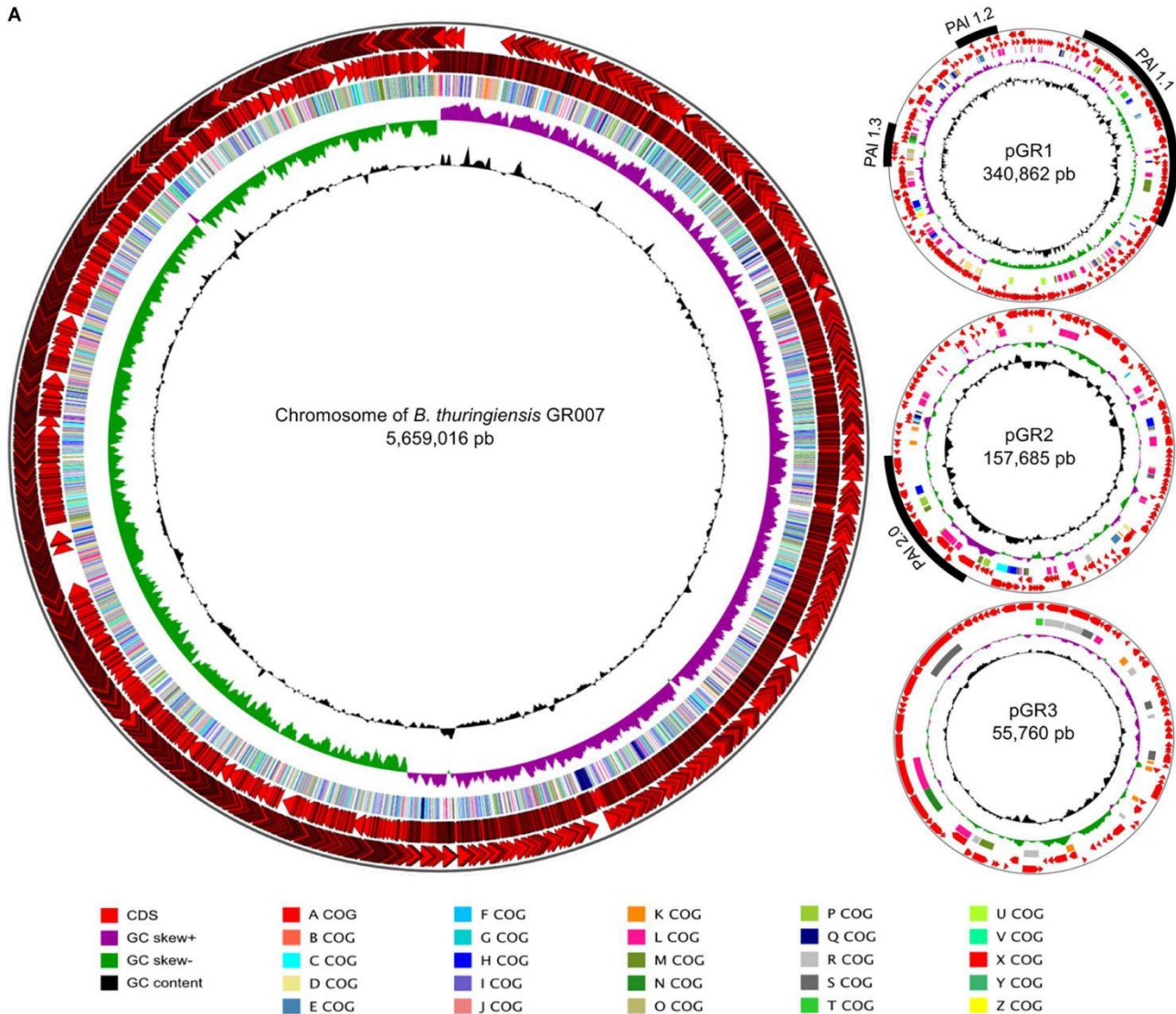
The phylogenetic relationships within the *Bacillus* genus, particularly between *Bacillus thuringiensis* (Bt) and other *Bacillus* species, have been extensively studied using whole-genome sequencing and phylogenomic analyses. A study involving nearly 900 whole genome sequences of *Bacillus cereus*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

4.2 Conserved and unique genomic regions

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

Furthermore, taxonomically restricted genes (TRGs) in *Bacillus* species, which are unique to specific lineages, have been identified and are thought to contribute to lineage-specific biological properties (Karłowski et al., 2023).

A



B

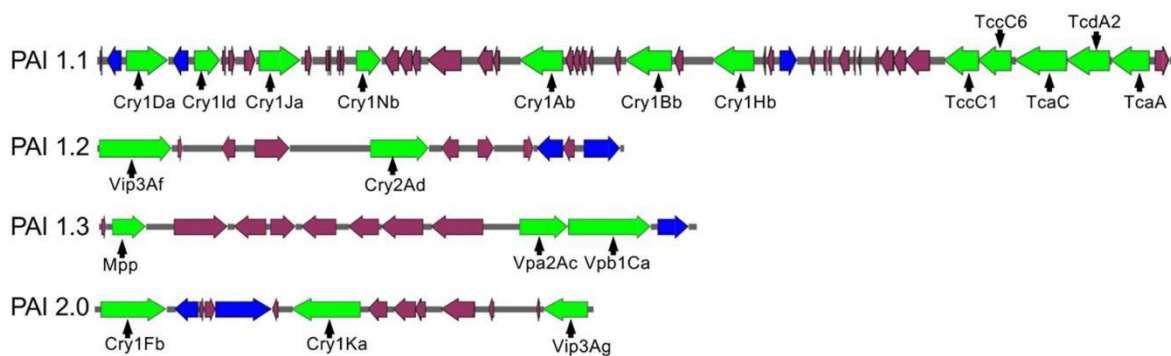


Figure 1 Genome of *B. thuringiensis* strain GR007. (A) Circular maps of chromosome and plasmids. The distribution of circles from outside to inside indicate CDS forward, CDS reverse, COG functional assignment, GC skew, GC content. The pathogenic islands (PAI) identified are indicated in the plasmids pGR340 and pGR157. (B) Representation of PAIs. Pesticidal proteins are represented in green arrows, transposases in blue arrows and additional ORF into the PAIs with purple arrows (Adopted from Pacheco et al., 2021)

4.3 Horizontal gene transfer

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

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

5 Functional Genomics

5.1 Gene expression profiling

Gene expression profiling in *Bacillus thuringiensis* (Bt) and related *Bacillus* species has been extensively studied to understand the regulation of insecticidal protein production and other metabolic processes. For instance, the comparative analysis of the Bt X022 strain revealed that the presence of Cu²⁺ significantly influences the metabolic regulation of carbon flux, leading to increased production of insecticidal crystal proteins (ICPs) during the spore-release period (Quan et al., 2016). Similarly, in Bt strain 4.0718, gene expression profiling indicated that certain genes, such as *cry2Ab* and *cryIIa*, were either silenced or expressed at very low levels due to the lack of functional promoters or the presence of transposons (Rang et al., 2015). These findings underscore the importance of gene expression profiling in identifying the regulatory mechanisms that control the production of key proteins in *Bacillus* species.

5.2 Proteomics and metabolomics

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

5.3 Functional studies of specific genes

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

Functional genomics approaches, including gene expression profiling, proteomics, metabolomics, and functional studies of specific genes, have significantly advanced our understanding of the complex regulatory networks and metabolic pathways in Bt and related *Bacillus* species. These insights are essential for harnessing the full potential of these bacteria in biotechnological and agricultural applications.

6. Ecological and Evolutionary Insights

6.1. Adaptation to environmental niches

Bacillus species exhibit remarkable adaptability to diverse environmental niches, driven by genomic variations and selective pressures. For instance, *Bacillus pumilus* group strains show distinct genomic features that facilitate their survival in marine and terrestrial environments. Marine strains are enriched with genes related to transcription, phage defense, DNA recombination, and repair, while terrestrial strains possess genes aiding survival in land-specific niches (Fu et al., 2021). Similarly, *Bacillus mycoides*, a member of the *Bacillus cereus* group, demonstrates significant genomic diversity, with adaptive genes enabling it to thrive in various ecological niches, particularly in cold climates (Fiedoruk et al., 2021). These adaptations are often facilitated by horizontal gene transfer and the presence of mobile genetic elements, which enhance the genomic plasticity of these bacteria (Du et al., 2023).

6.2 Co-evolution with hosts

The co-evolution of *Bacillus* species with their hosts is a significant driver of their evolutionary trajectory. *Bacillus thuringiensis*, for example, has evolved high virulence through the selective advantage of its *Cry* toxin genes during co-evolution with nematode hosts. This co-evolutionary process is distinct from unidirectional selection and involves the accumulation of multiple virulence factors, which enhance the pathogen's ability to infect and adapt to various hosts (Masri et al., 2015). Additionally, *Bacillus thuringiensis* has specialized to exploit multiple invertebrate hosts, with host switching occurring at both major clade and subclade levels. The transfer of plasmids carrying *cry* genes plays a crucial role in this adaptation, allowing the bacteria to target specific insect orders effectively (Zheng et al., 2017).

6.3 Implications for biocontrol and biotechnology

Insights into the ecology and evolution of *Bacillus* species are of great significance for biocontrol and biotechnology. It is known that *Bacillus amyloliquefaciens* strains with biocontrol properties possess a set of core genes involved in the production of secondary metabolites, volatile compounds, and biofilm formation, which are crucial for their beneficial interactions with plants. These genetic elements not only enhance plant growth but also induce host defense responses, making these strains valuable in agricultural applications (Magno-Pérez-Bryan et al., 2015). Moreover, the metabolic characterization of *Bacillus* species reveals their nutritional preferences and metabolic capabilities (Figure 2), information that can be used to develop targeted biocontrol strategies and enhance the efficacy of these bacteria under various environmental conditions (Chang et al., 2020). Additionally, the genomic analysis of Bt provides a theoretical basis for developing more effective biopesticides and highlights the potential of genomics in addressing insect resistance.

Chang et al. (2020) demonstrated the nutritional preferences in carbon utilization, highlighting the metabolic characteristics of *Bacillus* species. Through the analysis of utilization rates of nutrients with different chemical properties, the study revealed significant differences in the metabolic capacities of *Bacillus* species. The study showed that different strains have distinct utilization rates for carbohydrates, amino acids, and lipids. This information is crucial for developing targeted biocontrol strategies. Especially for Bt and related *Bacillus* species, analyzing their metabolic preferences and capacities under specific environmental conditions can enhance the efficacy of these bacteria in various environments, providing a scientific basis for biocontrol.

7 Applications and Implications

Bt's applications in agriculture, medicine, and industry highlight its versatility and potential for promoting sustainable practices. However, careful regulatory oversight is essential to mitigate any potential risks associated with its use.

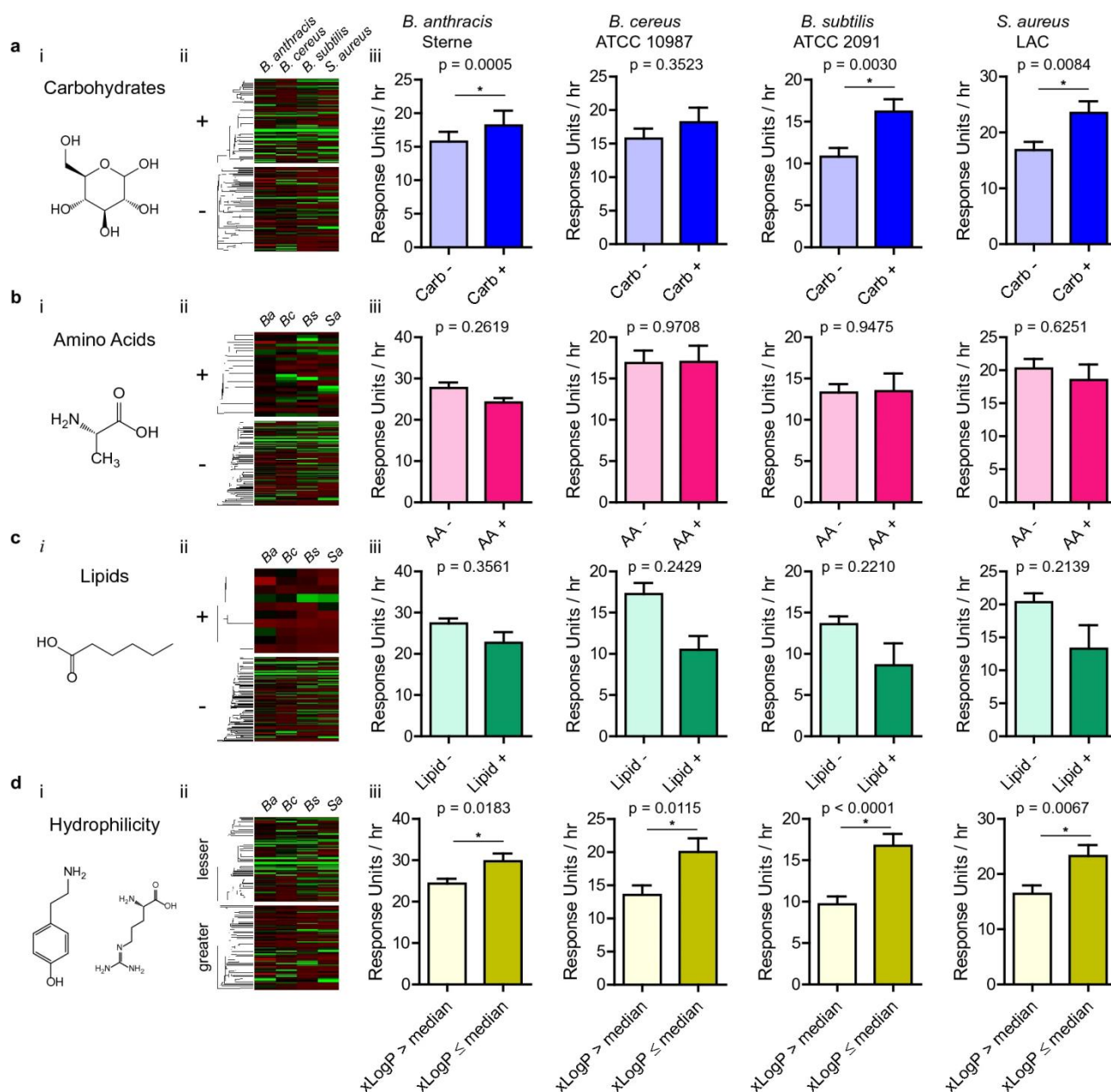


Figure 2 Metabolic rates correspond to certain chemical properties of nutrients (Adopted from Chang et al., 2020)

Image caption: (a–d) Four chemical properties of nutrients examined with the structure of an example from each category (i): (a) carbohydrates (shown: D-glucose), (b) amino acids (shown: L-alanine), (c) lipids (shown: caproic acid), and (d) hydrophilicity as represented by partition coefficient (shown: tyramine and L-arginine). (ii) Heat maps of maximum metabolic rates for nutrients with nutrients in the category for chemical property under question (+ or lesser) or did not (– or greater). Nutrients are hierarchically clustered by their chemical structural similarities using atom-pair distances. Ba: *B. anthracis*, Bc: *B. cereus*, Bs: *B. subtilis*, Sa: *S. aureus*. (iii) Average maximum metabolic rates for nutrients by chemical property (blue: carbohydrates, red: amino acids, green: lipids, yellow: hydrophilicity/partition coefficient). Bars represent averages of all nutrients categorized by chemical property. Error bars represent standard error of the mean. Maximum metabolic rate for each nutrient is an average from three independent experiments ($n = 3$, *: $p < 0.05$, unpaired Student's t-test). Figure created with R v3.5.3 (<https://www.R-project.org>) and GraphPad Prism 5 (<https://www.graphpad.com>) (Adopted from Chang et al., 2020)

7.1 Agricultural applications of Bt

Bacillus thuringiensis (Bt) has revolutionized agricultural practices through its use as a biopesticide and in genetically modified crops. Bt produces insecticidal proteins, particularly Cry proteins, which are highly effective against a variety of insect pests. These proteins have been incorporated into crops such as maize, cotton, and brinjal, providing them with inherent resistance to pests and reducing the need for chemical pesticides (Gutiérrez

et al., 2019). The use of Bt in agriculture not only enhances crop yields but also promotes environmentally friendly farming practices by reducing the reliance on synthetic chemicals (Jouzani et al., 2017; Dame et al., 2021). Additionally, Bt-based biopesticides are considered safe for humans and non-target organisms, making them a preferred choice for integrated pest management (Koch et al., 2015; Gutiérrez et al., 2019).

7.2 Medical and industrial uses

Beyond agriculture, Bt has shown potential in various medical and industrial applications. Recent studies have highlighted the use of Bt in bioremediation, where it helps in the detoxification of heavy metals and other pollutants (Jouzani et al., 2017). Bt has also been explored for its ability to produce polyhydroxyalkanoate biopolymers, which are valuable in the production of biodegradable plastics. In the medical field, Bt's parasporins have demonstrated anticancer properties, offering a novel approach to cancer treatment (Melo et al., 2016; Jouzani et al., 2017). Furthermore, Bt's chitinase enzyme, which degrades chitin, has industrial applications due to its abundance in nature and its role in enhancing the efficacy of Bt insecticides (Melo et al., 2016).

7.3 Regulatory and safety considerations

The widespread use of Bt in agriculture and other fields necessitates rigorous regulatory and safety evaluations. Bt proteins, particularly those expressed in transgenic crops, must undergo comprehensive biosafety assessments to ensure they do not pose ecological risks or harm non-target organisms (Li et al., 2022). These evaluations include studies on the environmental fate of Bt proteins, their impact on soil microbial diversity, and potential unintended effects on ecosystems. Additionally, the potential for gene flow from Bt crops to wild relatives is assessed to prevent ecological imbalances (Koch et al., 2015). Regulatory frameworks are in place to monitor and manage the use of Bt products, ensuring their safe and sustainable application in various industries (Koch et al., 2015; Li et al., 2022).

8 Concluding Remarks

Comparative genomics studies have revealed significant insights into the genetic diversity, insecticidal properties, and potential applications of *Bacillus thuringiensis* (Bt) and related *Bacillus* species. Bt strains exhibit a wide range of insecticidal proteins, such as Cry and Vip proteins, which are crucial for their effectiveness as biopesticides. For instance, Bt X022 contains genes for *Cry1Ac*, *Cry1Ia*, *Cry2Ab*, and *Vip3A*, while Bt GR007 harbors multiple *cry* and *vip* genes, demonstrating their genetic variability and potential for targeted pest control.

Proteomic analyses complement genomic data by identifying the expression of specific insecticidal proteins during different growth stages. For example, proteomic analysis of Bt X022 during the spore-release period detected Cry1Ca, Cry1Ac, and Cry1Da proteins, which could not be predicted solely by genomic data. Studies also identified various virulence factors and resistance genes in Bt strains, such as antibiotic and heavy metal resistance genes in Bt HM-311, which may contribute to their survival in contaminated environments.

Comparative genomic and proteomic techniques provide more accurate phylogenetic relationships among Bt strains than traditional serotyping methods, offering important insights into Bt classification and evolutionary history. Future Bt genomics research should further annotate Bt genomes functionally, particularly identifying and characterizing new insecticidal proteins and virulence factors, and exploring the regulatory mechanisms governing their expression under different environmental conditions.

In practical applications, utilizing genomic and proteomic data to develop Bt strains with specific insecticidal profiles tailored to particular pests can enhance biopesticide efficacy and reduce environmental impact. Advanced genomic tools should be used for routine monitoring of Bt biopesticide residues in food products to ensure food safety and regulatory compliance. Through these measures, future research and practical applications can maximize the benefits of Bt and related *Bacillus* species in sustainable agriculture and pest management.

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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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Research Insight

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Resistance Mechanisms to Bt Toxins in Insect Populations

Guanli Fu ✉

Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China

✉ Corresponding email: 158526138@qq.comBt Research, 2024, Vol.15, No.4 doi: [10.5376/bt.2024.15.0019](https://doi.org/10.5376/bt.2024.15.0019)

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Abstract Insect resistance to *Bacillus thuringiensis* (Bt) toxins has become a significant challenge in biological control and sustainable agriculture. With the widespread use of Bt toxins in genetically modified crops and biopesticides, insect populations have gradually evolved various resistance mechanisms. This study systematically analyzes the genetic, biochemical, and behavioral resistance mechanisms of insects to Bt toxins, revealing multiple pathways such as point mutations, gene amplification, epigenetic modifications, enhanced detoxification enzyme activity, and changes in behavioral patterns. Additionally, the study explores resistance management strategies, such as refuge strategies, Bt gene pyramiding, and integrated pest management (IPM), in delaying the development of resistance. Through case studies, this research summarizes successful resistance management experiences and the challenges faced, and offers directions for future research. This study aims to provide a scientific basis for the continuous improvement of resistance management strategies to ensure the long-term effectiveness of Bt toxins in agriculture.

Keywords Bt toxin; Resistance mechanism; Gene mutation; Resistance management; Insect behavior

1 Introduction

Bacillus thuringiensis (Bt) is a bacterium known for producing insecticidal proteins, commonly referred to as Bt toxins. These toxins have been widely used in sprayable insecticides and transgenic crops as an effective alternative to synthetic pesticides (Jurat-Fuentes et al., 2021). Bt toxins, particularly Cry and Vip proteins, exhibit highly specific insecticidal activity, effectively targeting a wide range of pests while having a relatively minimal impact on non-target organisms and the environment (Cao et al., 2020; Li et al., 2022). The incorporation of Bt genes into crops has revolutionized agricultural pest management, leading to the development of transgenic plants capable of self-protection and continuous pest resistance (Lazarte et al., 2021).

However, with the widespread use of Bt toxins, target insect populations have gradually evolved resistance, posing a significant threat to the long-term efficacy of Bt toxins (Wei et al., 2019; Jurat-Fuentes et al., 2021). Resistance to Bt toxins has been observed in several insect species, particularly those exposed to transgenic Bt crops (Naik et al., 2018; Jurat-Fuentes et al., 2021). Understanding the mechanisms by which resistance develops is crucial for developing effective management strategies and ensuring the sustainability of Bt technology. These resistance mechanisms may include genetic mutations in target receptors, changes in toxin binding sites, and alterations in midgut proteases (Pinos et al., 2021). By studying these mechanisms, we can gain insights into how resistance develops and spreads, enabling the design of more effective pest management strategies and the development of new Bt toxins with novel modes of action (Wang et al., 2019).

This study aims to compile and analyze current research on the mechanisms of resistance to Bt toxins in insect populations, discuss the implications of resistance for the sustainability of Bt technology, and propose potential management strategies to delay the development and spread of resistance. Through a comprehensive analysis of existing studies, this research hopes to provide a scientific basis for improving Bt technology and ensuring its continued application in agricultural pest management.

2 Overview of Bt Toxins

2.1 Types of Bt toxins

Bacillus thuringiensis (Bt) produces a variety of insecticidal proteins, commonly referred to as Bt toxins, which are utilized in both sprayable formulations and transgenic crops to control insect pests. The primary classes of Bt

toxins include Cry and Cyt proteins. Cry proteins, such as Cry1Ac and Cry1F, are the most widely used and are known for their specificity towards lepidopteran and coleopteran pests (Jurat-Fuentes et al., 2021; Kain et al., 2022). These proteins are produced as protoxins that require activation by insect midgut proteases to become toxic (Tabashnik et al., 2015; Guo et al., 2020). Additionally, there are other classes of Bt toxins, such as Vip (vegetative insecticidal proteins), which have different modes of action and target different insect receptors (Wei et al., 2019).

2.2 Mode of action

The mode of action of Bt toxins involves multiple steps. First, the toxin binds to receptors on the epithelial cells of the insect midgut, then forms pores that lead to cell lysis and insect death (Figure 1) (Heckel et al., 2021). The active toxin then binds to specific receptors on the midgut epithelial cells, such as cadherin and ATP-binding cassette (ABC) transporters (Zhu et al., 2019). This binding facilitates the formation of pores in the cell membrane, leading to cell lysis and ultimately causing insect death (Chen et al., 2015; Heckel et al., 2021). Recent studies suggest that protoxins and active toxins may have different modes of action, with protoxins potentially being more effective against resistant insect strains (Tabashnik et al., 2015; Guo et al., 2020).

Figure 1 illustrates the hypothetical model of the mode of action of Bt toxins, including the synergistic mechanisms of trans-action and cis-action. It details how toxin monomers bind to receptors to form prepores, which ultimately lead to pore insertion into the insect gut cell membrane, revealing the multi-step action pattern of Bt toxins. This provides an important reference for studying the mode of action of Bt toxins.

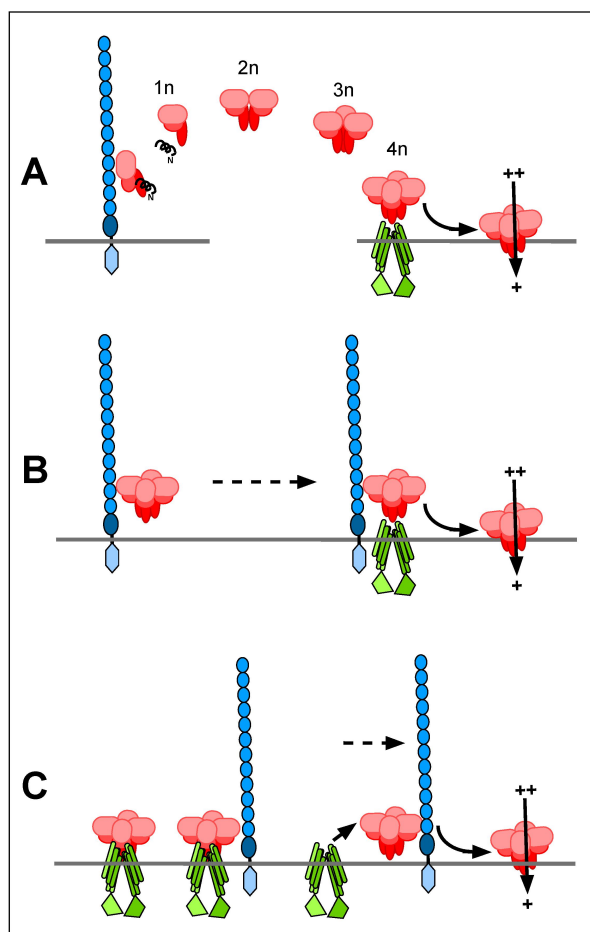


Figure 1 Hypothetical models of the mechanism of synergy between the 12-cadherin domain protein and an ABC transporter (Adopted from Heckel et al., 2021)

Image caption: (A) Trans-acting synergy, due to acceleration of oligomer formation following monomer binding to the cadherin. (B) Cis-acting synergy, due to the cadherin trapping the pre-pore and moving it to the ABC transporter. (C) Cis-acting synergy, due to the cadherin pulling the pre-pore away from the ABC transporter, freeing it to interact with another toxin pre-pore. Dotted lines represent movement of the cadherin within the membrane (Adopted from Heckel et al., 2021)

2.3 Target insect populations

Bt toxins are highly effective against a range of insect pests, particularly those in the orders Lepidoptera and Coleoptera. Lepidopteran pests, such as the cotton bollworm (*Helicoverpa armigera*), the diamondback moth (*Plutella xylostella*), and the cabbage looper (*Trichoplusia ni*), are among the primary targets of Cry1Ac and Cry1F toxins (Kain et al., 2022; Xiao et al., 2023). Coleopteran pests, such as the Colorado potato beetle (*Leptinotarsa decemlineata*), are targeted by other Cry proteins, such as Cry3A (Ren et al., 2021). The effectiveness of Bt toxins against these pests has led to their widespread use in transgenic crops, such as Bt cotton and Bt maize, which express these toxins to provide continuous protection against insect damage (Jurat-Fuentes et al., 2021; Heckel et al., 2021; Kain et al., 2022).

Bt toxins are a diverse group of insecticidal proteins with specific modes of action and target insect populations. Understanding the types, mechanisms, and target pests of Bt toxins is crucial for developing effective pest management strategies and mitigating the risk of resistance development.

3 Genetic Mechanisms of Resistance

The genetic resistance mechanisms of insect populations to Bt toxins include point mutations, deletions and insertions, gene silencing, gene amplification, and possible epigenetic modifications. These mechanisms alter the target sites or expression levels of proteins that interact with Bt toxins, thereby reducing the efficacy of these biopesticides and posing a significant challenge to their sustainable use in agriculture. In studies on the mode of action of Bt toxins, mutations in target genes are considered one of the key factors in the development of insect resistance (Figure 2). Research has shown that certain gene mutations in insects can alter the binding capacity of the toxin to its receptor, rendering the toxin inactive (Wang et al., 2019).

Figure 2 provides a detailed illustration of the receptor screening process for Bt toxins in insect cells. By overexpressing receptor proteins, the binding abilities of the toxin to different receptors were evaluated. These mutations weaken the binding capacity of Bt toxins to the receptors, leading to the development of resistance in insects. The varying binding effects of different toxins and receptors depicted in the figure offer critical experimental evidence for studying resistance mechanisms.

3.1 Mutations in target genes

3.1.1 Point mutations

Point mutations in target genes are a common mechanism by which insects develop resistance to Bt toxins. For instance, in the fall armyworm (*Spodoptera frugiperda*), point mutations in the *ABCC2* gene have been linked to resistance to the Cry1F protein (Guan et al., 2020). Similarly, a single point mutation in the cadherin gene of the cotton bollworm (*Helicoverpa armigera*) results in mislocalization of the cadherin protein, which underpins resistance to Cry1Ac toxin (Xiao et al., 2017). These mutations alter the structure and function of the proteins that Bt toxins target, thereby reducing the efficacy of the toxins.

3.1.2 Deletions and insertions

Deletions and insertions in target genes may also confer resistance to Bt toxins in insects. For example, in *Plutella xylostella* (diamondback moth), CRISPR/Cas9-mediated deletion mutations in the *PxABCC2* and *PxABCC3* genes resulted in high resistance to the Cry1Ac toxin (Guo et al., 2019). Studies have found multiple point mutations in target genes such as acetylcholinesterase 1 (*ace-1*), including A201S, G227A, and F290V, which are associated with resistance to organophosphates and pyrethroids (Figure 3). Similarly, in some populations of *Spodoptera frugiperda* in Brazil, a 12-base pair insertion mutation was found in the *ABCC2* gene, which is associated with resistance to Bt proteins (Guan et al., 2020). These genetic changes may lead to the production of truncated, non-functional proteins that are unable to effectively bind Bt toxins.

The study by Guo et al. (2019) illustrated four mutant alleles (r1-r4) of the *ABCC2* gene associated with resistance to Cry1F toxin, with r4 being a newly discovered mutation in this study. The figure details the positions of these mutations in both the genomic and protein structures, highlighting how these mutations may result in loss of function. This provides an important reference for understanding how deletions and insertions in target genes can confer resistance to Bt toxins in insects.

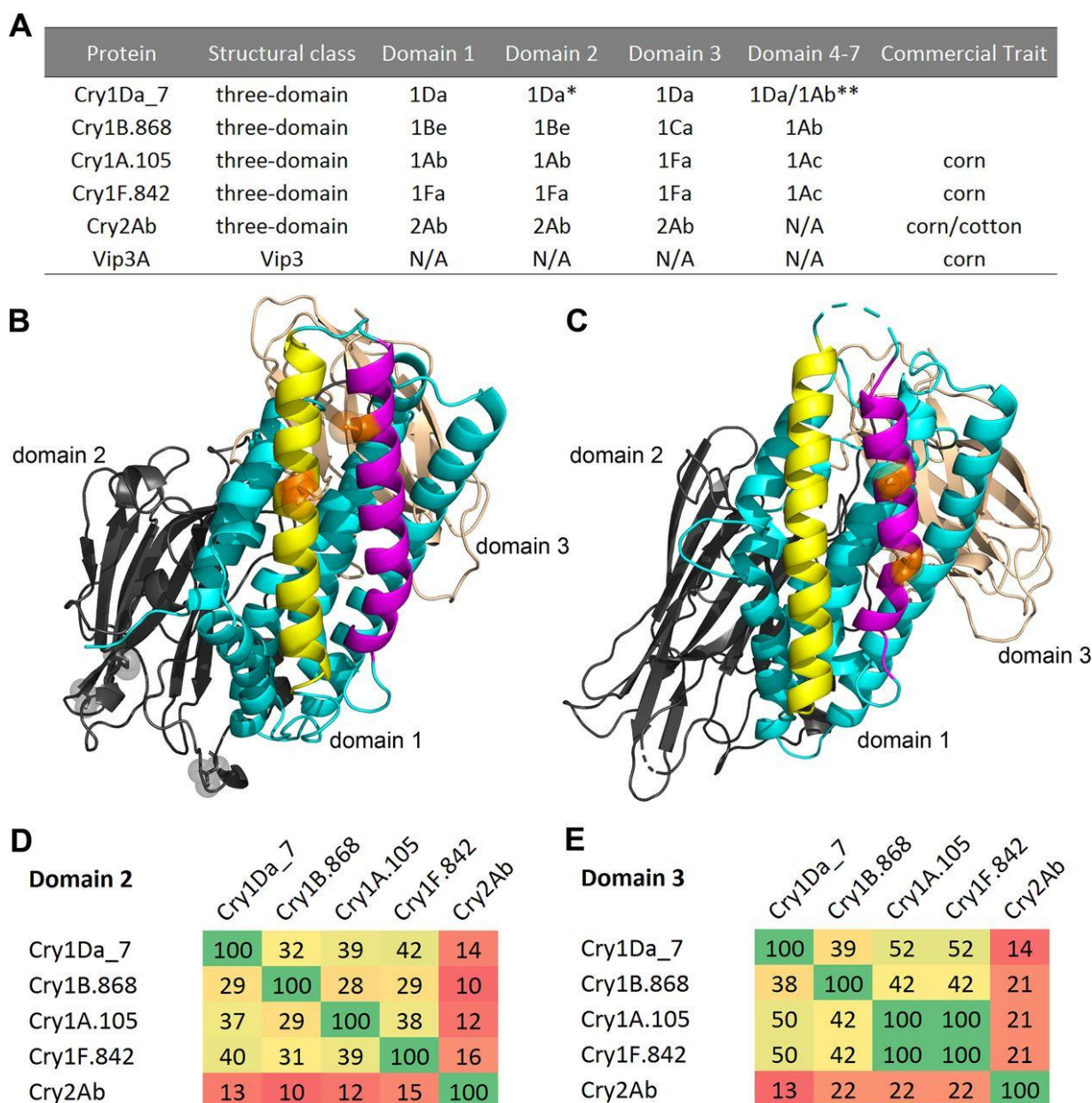


Figure 2 Sequence and structure relationship between Bt insecticidal proteins in current and next-generation above-ground traits (Adopted from Wang et al., 2019)

Image caption: (A) Protein sequence information on the different NIPs, indicated by Bt toxin holotype nomenclature. The asterisk indicates the Cry1Da domain, in which substitutions were made to enhance CEW activity. Domains 4 to 7 of the three-domain Cry1 proteins are protoxin domains that are digested in vivo and thus are not part of the active ingredient; the Cry1Da_7 active core was appended to both Cry1Da and Cry1Ab protoxin domains and tested separately (double asterisk). Cry2Ab does not have these protoxin domains. Vip3A is of a different structural class whose sequence is different and structurally distinct from those of three-domain Cry proteins. N/A, not applicable. (B) Crystal structure of Cry1Da_7-DIP showing the three-domain architecture of domain 1 (cyan), domain 2 (gray), and domain 3 (light pink) in cartoon representation as well as helix 3 (yellow) and helix 4 (magenta) in domain 1. The key domain 1-disabling cysteine substitutions V108C and E128C are highlighted with orange sticks and semitransparent spheres corresponding to their side chain. The gray sticks and semitransparent spheres in domain 2 indicate the side chains of substitutions (S282V, Y316S, and I368P) that confer increased CEW specific activity. (C) Model of the three-dimensional architecture of Cry1B.868-DIP protein in cartoon representation with the above-described color scheme. The key domain 1-disabling substitutions A160N and N167D are highlighted with orange sticks and semitransparent spheres corresponding to their side chain. (D) Percent sequence identity between domains 2 of FAW-active insecticidal proteins based on comparative sequence analysis by multiple-sequence alignment (74). (E) Percent sequence identity between these proteins in domain 3 (Adopted from Wang et al., 2019)

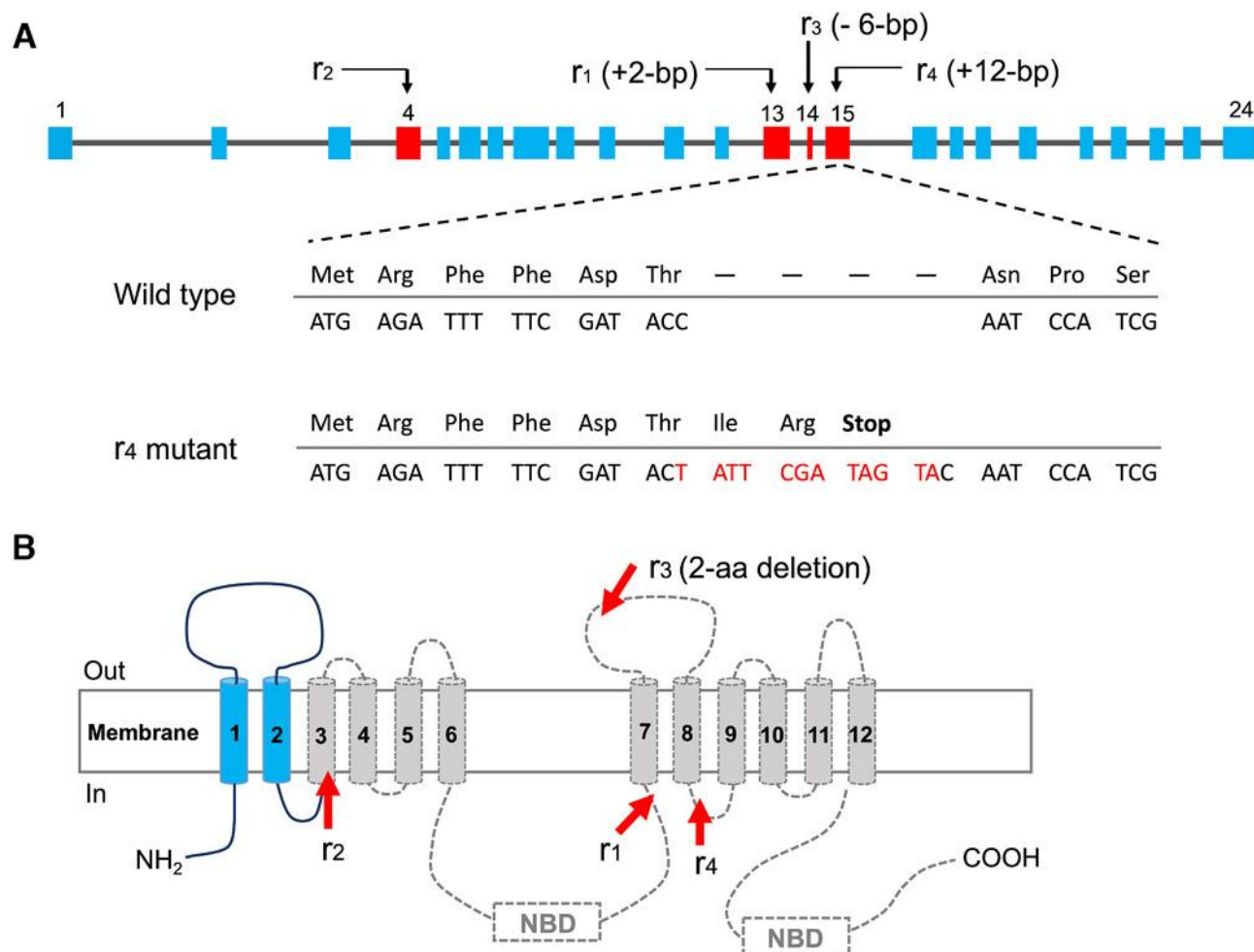


Figure 3 Four mutant alleles (r1–r4) of SfABCC2 associated with Cry1F resistance in *Spodoptera frugiperda*. (A) Genomic structure of SfABCC2. (B) Protein structure of SfABCC2. The r1, r2 and r3 alleles were reported respectively by Banerjee et al. (2017), Flangel et al. (2018) and Boaventura et al. (2019). The r4 allele was detected in the present study (Adopted from Guo et al., 2019)

3.1.3 Gene silencing

Gene silencing mechanisms, such as RNA interference (RNAi), can also play a role in resistance. Although specific examples of RNAi-mediated resistance to Bt toxins were not detailed in the provided papers, the down-regulation of key genes involved in Bt toxin binding and activation could theoretically contribute to resistance. For instance, the down-regulation of ABCC2 and ABCC3 genes has been linked to resistance in several lepidopteran species (Guo et al., 2019).

3.2 Gene amplification

Gene amplification, where multiple copies of a resistance gene are produced, can enhance resistance levels. While the provided papers did not specifically mention gene amplification as a resistance mechanism to Bt toxins, it is a well-documented phenomenon in other contexts of insecticide resistance. The increased expression of resistance genes can lead to higher levels of the corresponding proteins, which can sequester or degrade Bt toxins more effectively.

3.3 Epigenetic modifications

Epigenetic modifications, such as DNA methylation and histone modification, can alter gene expression without changing the DNA sequence. These modifications can potentially contribute to Bt resistance by regulating the expression of genes involved in toxin binding and activation. Although the provided papers did not explicitly discuss epigenetic modifications in the context of Bt resistance, this area remains an important avenue for future research to fully understand the complexity of resistance mechanisms.

4 Biochemical Mechanisms of Resistance

4.1 Altered toxin binding

Altered toxin binding is a common biochemical mechanism by which insects develop resistance to Bt toxins. This mechanism involves changes in the receptors on the insect midgut cells, where Bt toxins typically bind. Mutations or modifications in these receptors can reduce the binding affinity of the toxins, thereby diminishing their efficacy. This resistance mechanism has been observed in various insect species and poses a significant challenge in managing Bt resistance. Due to receptor alterations, the toxins cannot effectively bind to their target sites, reducing their toxic effect and allowing insects to survive exposure to Bt toxins. The widespread occurrence of this mechanism makes it a crucial area of study (Jurat-Fuentes et al., 2021).

4.2 Enhanced detoxification enzymes

Insects also resist Bt toxins through enhanced detoxification enzyme activity, which is another critical biochemical resistance mechanism. These enzymes can metabolize and neutralize Bt toxins before they reach their target sites, thereby reducing the toxins' effectiveness. Enhanced detoxification capacity is often associated with the overexpression or increased activity of these enzymes.

4.2.1 Cytochrome P450 monooxygenases

Cytochrome P450 monooxygenases (P450s) play a crucial role in detoxifying xenobiotics, including Bt toxins. These enzymes are involved in the metabolic detoxification of a wide range of substances, such as phytochemicals, insecticides, and environmental pollutants. The overexpression of P450s is closely linked to the enhanced detoxification capabilities observed in resistant insects. P450s can work through multiple pathways to detoxify xenobiotics, and their regulation involves complex networks of transcription factors and signaling pathways. Studies have shown that P450s can be induced through specific signaling pathways in resistant insects, significantly enhancing their detoxification capabilities (Lu et al., 2020; Nauen et al., 2021).

4.2.2 Glutathione S-transferases

Glutathione S-transferases (GSTs) are another group of critical detoxification enzymes involved in the resistance to Bt toxins. GSTs catalyze the conjugation of glutathione to toxic substances, making these substances more water-soluble and easier to excrete. Although the role of GSTs in Bt toxin resistance is less documented compared to P450s, studies indicate that they play a significant role in detoxifying other insecticides and xenobiotics. Therefore, GSTs' potential role in Bt resistance is an important area for further investigation (Jurat-Fuentes et al., 2021).

4.2.3 Esterases

Esterases are enzymes that hydrolyze ester bonds in various substrates, including insecticides. These enzymes can detoxify Bt toxins by breaking down their ester linkages, thereby reducing the toxins' potency. In resistant insect populations, the expression or activity of esterases is often significantly increased, further accelerating the detoxification process of Bt toxins. Esterases, like P450s and GSTs, contribute to the enhanced detoxification capabilities observed in resistant insects, and their role in Bt resistance is increasingly recognized (Nauen et al., 2021).

4.3 Changes in gut microbiota

Changes in the gut microbiota of insects can also play a critical role in Bt toxin resistance. The gut microbiota not only affects the overall health and metabolic capacity of the host insect but can also directly or indirectly participate in the detoxification of xenobiotics. Research has shown that changes in the composition and function of the gut microbiota can enhance insect resistance to Bt toxins in several ways. Some microbes can directly degrade Bt toxins, reducing their toxicity, while others may enhance the host's immune response and detoxification enzyme activity, further increasing resistance. Although this area of research is still developing, preliminary studies suggest that gut microbiota plays an essential role in Bt resistance, and future research will provide more scientific insights into this mechanism (Pinos et al., 2021).

By understanding these biochemical mechanisms in depth, researchers can develop more effective resistance management strategies to mitigate and delay the development of insect resistance to Bt toxins, ensuring the continued efficacy of Bt-based pest control methods.

5 Behavioral Mechanisms of Resistance

5.1 Avoidance behavior

Avoidance behavior is a critical mechanism by which insects can resist the effects of Bt toxins. Insects may develop the ability to detect and avoid Bt-treated plants or areas, thereby reducing their exposure to the toxins. This behavior can be particularly effective in heterogeneous environments where non-Bt plants are available as refuges. For instance, the presence of abundant refuges of non-Bt host plants has been shown to favor sustained susceptibility to Bt crops by providing alternative feeding sites for pests, thereby reducing the selection pressure for resistance (Tabashnik et al., 2023).

5.2 Changes in feeding patterns

Changes in feeding patterns represent another behavioral adaptation that insects can employ to mitigate the impact of Bt toxins. Insects may alter their feeding habits, such as feeding at different times of the day or targeting different parts of the plant that have lower concentrations of Bt toxins. For example, studies have shown that the western corn rootworm, a significant pest of maize, exhibits changes in its feeding behavior when exposed to Bt-expressing maize. Resistant insects were found to maintain a more stable microbiome compared to susceptible insects, suggesting that their altered feeding patterns might help them avoid the detrimental effects of Bt toxins (Paddock et al., 2021).

5.3 Altered life cycle

Insects may also develop resistance to Bt toxins through alterations in their life cycle. This can include changes in developmental timing, such as faster or slower growth rates, which can help them avoid peak periods of Bt toxin expression in plants. For example, the larvae of *Anoplophora glabripennis*, when fed on transgenic poplar lines expressing dual Bt toxins, showed significant changes in the expression of genes related to their growth and development. These changes suggest that the larvae are adapting their life cycle to mitigate the effects of Bt toxins (Ren et al., 2021). Additionally, the nutritional environment can influence the susceptibility of insects to Bt toxins. *Helicoverpa zea*, a polyphagous pest, showed a 100-fold increase in LC50 when reared on protein-biased diets compared to carbohydrate-biased diets, indicating that diet-mediated plasticity can alter the life cycle and resistance levels of insects (Deans et al., 2017).

6 Management Strategies to Combat Resistance

6.1 Refuge strategies

Refuge strategies involve planting non-Bt crops near Bt crops to maintain a population of pests that remain susceptible to Bt toxins. This approach helps to delay the evolution of resistance by ensuring that susceptible pests can mate with potentially resistant individuals, thereby diluting resistance genes in the pest population. The high-dose/refuge strategy has been particularly successful in North America, where it has helped maintain susceptibility in major pests like the European corn borer and the pink bollworm (Huang et al., 2011). However, the effectiveness of this strategy can be compromised by non-compliance with refuge requirements and the presence of pests with non-recessive resistance (Brewer and Bonsall, 2020). Natural refuges, such as non-Bt host plants, have also been shown to delay resistance, although they may not be as effective as structured non-Bt cotton refuges (Jin et al., 2014).

6.2 Pyramiding Bt genes

Pyramiding involves stacking multiple Bt genes that produce different toxins within the same crop. This strategy aims to provide multiple modes of action against pests, making it more difficult for them to develop resistance. Pyramided Bt crops, such as those expressing both Cry and Vip3Aa toxins, have shown promise in delaying resistance. The synergistic action of multiple toxins can effectively manage pest populations even when some pests have developed resistance to one of the toxins. However, the design of these pyramids must consider

potential cross-resistance and antagonism between toxins, which can reduce their effectiveness (Carrière et al., 2015). Products like SmartStax and PowerCore have demonstrated improved resistance management benefits compared to single-toxin products, allowing for smaller refuges and less dependence on high mortality rates among susceptible pests (Storer et al., 2012).

6.3 Integrated pest management (IPM)

Integrated Pest Management (IPM) combines multiple control tactics to manage pest populations sustainably. This approach includes the use of Bt crops, refuges, biological control agents, and cultural practices. IPM aims to reduce the reliance on any single control method, thereby delaying the evolution of resistance. For instance, combining Bt crops with the release of sterile insects or transgenic insects has been proposed as a method to manage pest populations and resistance simultaneously (Zafar et al., 2020; Brewer and Bonsall, 2020). Additionally, IPM strategies can be tailored to regional pest pressures, ensuring that Bt crops are used where they are most beneficial and reducing their use in areas with low pest pressure. By integrating various control methods, IPM can enhance the sustainability of Bt crops and delay the development of resistance in pest populations (Gassmann and Reisig, 2022).

7 Case Studies

7.1 Successful management of resistance

The successful management of resistance to Bt toxins in insect populations has been achieved through various strategies. One notable example is the implementation of proactive resistance management strategies such as the refuge strategy and the pyramid strategy. These strategies have been effective in sustaining the control of target pests for nearly two decades (Wu, 2014). Additionally, the use of multi-toxin Bt crops has been shown to delay resistance evolution by providing redundant killing mechanisms, which is supported by field outcomes and theoretical predictions (Tabashnik et al., 2013). In the United States, regional suppression of pest populations and even pest eradication have been achieved in some cases, leading to reduced reliance on conventional insecticides and increased profits for farmers (Gassmann and Reisig, 2022).

7.2 Challenges faced in different regions

Despite the successes, several challenges have been encountered in different regions. For instance, practical resistance to Bt crops has been documented in some populations of 11 pest species across seven countries, affecting nine widely used Bt toxins (Tabashnik et al., 2023). In the United States, pests have evolved resistance to multiple Bt traits, compromising the effectiveness of Bt crops and leading to increased crop damage (Gassmann and Reisig, 2022). The spatial heterogeneity of Bt crop deployment in small-holder farm systems has also been identified as a factor that can accelerate the regional evolution of resistance, highlighting the need for spatially explicit resistance management strategies (Huang et al., 2017). Furthermore, the genetic diversity of resistance mechanisms, including mutations in ABC transporters and enhanced immune responses in insects, poses additional challenges for understanding and managing resistance (Gahan et al., 2010; Heckel et al., 2012; Tay et al., 2015; Xiao et al., 2023).

7.3 Lessons learned

Several lessons have been learned from the management of Bt resistance in insect populations. The importance of maintaining abundant refuges of non-Bt host plants has been underscored as a key factor in delaying resistance evolution (Tabashnik et al., 2013; Tabashnik et al., 2023). Understanding the molecular genetic basis of resistance, such as the role of ABC transporters in toxin mode of action, is crucial for developing effective resistance detection methods and management strategies (Gahan et al., 2010; Heckel et al., 2012; Tay et al., 2015). The integration of multiple strategies, including the use of multi-toxin Bt crops and spatially explicit deployment patterns, can enhance the sustainability of Bt technology (Tabashnik et al., 2013; Huang et al., 2017). Finally, continuous monitoring and proactive adaptation of resistance management strategies are essential to address the evolving threat of resistance and ensure the long-term success of Bt crops (Wu, 2014; Gassmann and Reisig, 2022).

8 Concluding Remarks

Research on the resistance mechanisms of insect populations to *Bacillus thuringiensis* (Bt) toxins has revealed several key findings. The evolution of resistance to Bt toxins has become a major challenge to Bt-based pest control strategies, particularly in transgenic crops where lepidopteran and coleopteran insects have shown significant resistance. Key resistance mechanisms include mutations in ABC transporters, which play a crucial role in the mode of action of Bt toxins. Additionally, the dynamics of cross-resistance between Bt and other insecticides indicate that resistance development involves complex genetic and biochemical pathways. The continuous evolution of Bt toxins to overcome resistance underscores the need for ongoing innovation in Bt technology.

Continuous monitoring and research are essential for managing and mitigating the evolution of Bt resistance. The changes in global resistance patterns emphasize the importance of early detection and timely countermeasures. A deep understanding of the genetic and biochemical bases of resistance, particularly the role of ABC transporters, is crucial for developing effective management strategies. Moreover, research shows that spatial heterogeneity in the deployment of Bt crops can significantly influence the speed of resistance evolution, necessitating targeted management practices based on specific environmental conditions.

Future research needs to further explore how ABC transporters and other genetic factors contribute to Bt resistance, which will deepen the understanding of resistance mechanisms. Investigating the cross-resistance dynamics between Bt and other insecticides is also crucial, as it can lead to the development of pest management strategies that address multiple resistance pathways. Continuous development and testing of new Bt toxin variants will enhance their effectiveness against resistant insect populations. Expanding field studies and monitoring will provide more data to optimize resistance management strategies and ensure their effectiveness in different agricultural environments. Promoting Integrated Pest Management (IPM) practices that combine Bt crops with other control methods can effectively reduce the selection pressure for resistance.

By advancing these key research areas, future studies will not only enhance the sustainability of Bt technology but also provide innovative solutions for broader pest control. As the understanding of resistance mechanisms deepens and new Bt toxins are developed, scientists will be able to design more precise management strategies, delaying the onset of resistance and mitigating its impact on agricultural production. These efforts will ultimately ensure that Bt technology continues to serve as an effective and sustainable tool, contributing to global agricultural security and environmental protection.

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Conflict of Interest Disclosure

The author affirms 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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Feature Review

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Metabolic Engineering of Bt for Enhanced Production of Insecticidal Proteins

Wenfei Zhang ✉

College of Life Sciences, Hainan Normal University, Haikou, 570206, Hainan, China

✉ Corresponding email: wenfei2007@163.comBt Research, 2024, Vol.15, No.4 doi: [10.5376/bt.2024.15.0020](https://doi.org/10.5376/bt.2024.15.0020)

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Abstract *Bacillus thuringiensis* (Bt) is a key bacterial strain used for producing bioinsecticides, with its insecticidal proteins being widely applied in agricultural pest control. However, traditional Bt production faces challenges such as low protein yield and high production costs. To address these issues, this study explores strategies to enhance Bt metabolic pathways through metabolic engineering, aiming to increase protein production. The research covers Bt's primary and secondary metabolic pathways, their relationship with protein synthesis, and case studies of enhanced protein production achieved through genetic modification and metabolic flux optimization. This study aims to provide feasible improvements for the commercial production and application of Bt products in agriculture by offering theoretical insights into the metabolic engineering of Bt.

Keywords *Bacillus thuringiensis*; Insecticidal proteins; Metabolic engineering; Genetic modification; Agricultural application

1 Introduction

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacterium widely used in biological pest control due to its ability to produce insecticidal Cry proteins. These proteins are highly toxic to various insect orders, including Lepidoptera, Coleoptera, and Diptera, making Bt a crucial tool in agricultural pest management (Akhtar et al., 2021). Bt's insecticidal properties have been successfully applied in sprayable biopesticides and transgenic crops, providing an environmentally friendly alternative to chemical pesticides (Li et al., 2022; Yamamoto et al., 2022). Moreover, Bt's effectiveness against pests such as mosquitoes, which are vectors of disease, further underscores its importance in integrated pest management (Nair et al., 2020; Akhtar et al., 2021).

In modern agriculture, Bt insecticidal proteins play a vital role in crop protection. They are widely used in transgenic crops like cotton and corn, significantly reducing the use of chemical pesticides and boosting crop yields (Li et al., 2020; Yamamoto et al., 2022). The specificity of Bt proteins, which target only harmful insects while being safe for humans and other non-target organisms, makes them an ideal choice for sustainable agriculture (Chen et al., 2021; Singh et al., 2021). However, the emergence of insect resistance to certain Bt proteins necessitates ongoing research to design and develop new insecticidal proteins with enhanced efficacy and broader insecticidal spectra (Chen et al., 2021; Yamamoto et al., 2022).

This study focuses on the latest advancements in metabolic engineering to enhance the production of Bt insecticidal proteins. It analyzes and discusses various strategies to improve Bt protein yield and efficacy, including genetic modifications, co-expression systems, and the environmental impact of engineered Bt strains. Through these analyses, this study aims to provide a theoretical foundation for the future development of Bt insecticidal proteins and to promote the advancement of more effective and sustainable pest management solutions.

2 Overview of Bt and Insecticidal Proteins

2.1 Bt strains and their characteristics

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacterium widely recognized for its ability to produce insecticidal proteins during the sporulation phase of its growth cycle. These proteins, primarily Cry, Vip, and Cyt, are highly specific to certain insect orders, making Bt a valuable tool in pest management (Bravo et al.,

2015; 2017). Bt strains have been utilized in both sprayable pesticide formulations and transgenic crops to protect against insect damage. The diversity of Bt strains and their respective insecticidal proteins allows for targeted pest control, reducing the need for broad-spectrum chemical insecticides (Li et al., 2022; Yamamoto, 2022).

2.2 Types of insecticidal proteins produced by Bt

Bt produces several classes of insecticidal proteins, each with distinct target specificities and modes of action. The primary types include:

Cry Proteins: These are the most extensively studied and utilized Bt toxins. They are crystalline proteins that target specific insect midgut receptors, leading to cell lysis and insect death. Examples include Cry1Ac, Cry1Ab, and Cry2Ab, which are used in transgenic crops like cotton and corn to control pests such as the tobacco budworm and European corn borer (Bravo et al., 2017; Rathinam et al., 2019; Yamamoto, 2022).

Vip Proteins: Vegetative insecticidal proteins (Vip) are produced during the vegetative growth phase of Bt. They have a different mode of action compared to Cry proteins and can target a broader range of insect pests.

Cyt Proteins: Cytolytic proteins (Cyt) are less commonly used but are effective against certain insect orders and nematodes. They work by forming pores in the cell membranes of the target insects (Bravo et al., 2015; 2017).

2.3 Mechanisms of action of insecticidal proteins

The insecticidal proteins produced by Bt operate through several mechanisms to exert their toxic effects on target pests:

Cry Proteins: These proteins bind to specific receptors in the insect midgut, such as cadherin-like proteins, leading to pore formation in the gut epithelial cells. This results in cell lysis, gut paralysis, and ultimately, insect death. The specificity of Cry proteins to their receptors is a key factor in their effectiveness and safety (Bravo et al., 2017; Liu et al., 2018; Rathinam et al., 2019).

Vip Proteins: Vip proteins also target the insect midgut but have a different binding mechanism compared to Cry proteins. They can be effective against insects that have developed resistance to Cry proteins, providing an alternative mode of action (Bravo et al., 2015; 2017).

Cyt Proteins: These proteins form pores in the cell membranes of target insects, leading to cell lysis and death. Their mode of action is similar to that of Cry proteins but involves different target sites within the insect (Bravo et al., 2015; 2017).

The continuous evolution of insect resistance to Bt proteins necessitates ongoing research and development of new proteins and engineering of existing ones to maintain their efficacy. Strategies such as protein engineering, domain swapping, and bioconjugation have been employed to enhance the insecticidal activity and broaden the spectrum of Bt proteins (Pan et al., 2019; Rathinam et al., 2019; Yamamoto, 2022).

3 Metabolic Pathways in Bt

3.1 Primary metabolic pathways

Bacillus thuringiensis (Bt) primarily relies on standard bacterial metabolic pathways for its growth and survival. These include glycolysis, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation, which are essential for energy production and biosynthesis of cellular components. The primary metabolism in Bt is crucial for providing the necessary precursors and energy required for the synthesis of secondary metabolites, including insecticidal proteins.

3.2 Secondary metabolic pathways

3.2.1 Pathways leading to toxin production

The production of insecticidal proteins in Bt, such as Cry and Vip toxins, is a hallmark of its secondary metabolism. These proteins are synthesized during the sporulation phase of Bt and are encoded by specific genes

that are activated under certain environmental conditions. The Cry proteins, for instance, are produced as protoxins that require activation to exert their insecticidal effects (Tabashnik et al., 2015). The Vip proteins, on the other hand, are secreted during the vegetative growth phase and have distinct mechanisms of action compared to Cry proteins (Jin et al., 2022).

3.2.2 Regulatory networks

The regulation of toxin production in Bt involves complex networks of genetic and environmental factors. Key regulatory genes and proteins control the expression of toxin genes in response to specific signals. For example, the expression of Cry proteins is tightly regulated by sporulation-specific sigma factors and other regulatory proteins that respond to nutrient availability and other environmental cues (Figure 1) (Li et al., 2022). Additionally, the interaction between primary and secondary metabolic pathways plays a significant role in modulating toxin production (Tabashnik et al., 2015).

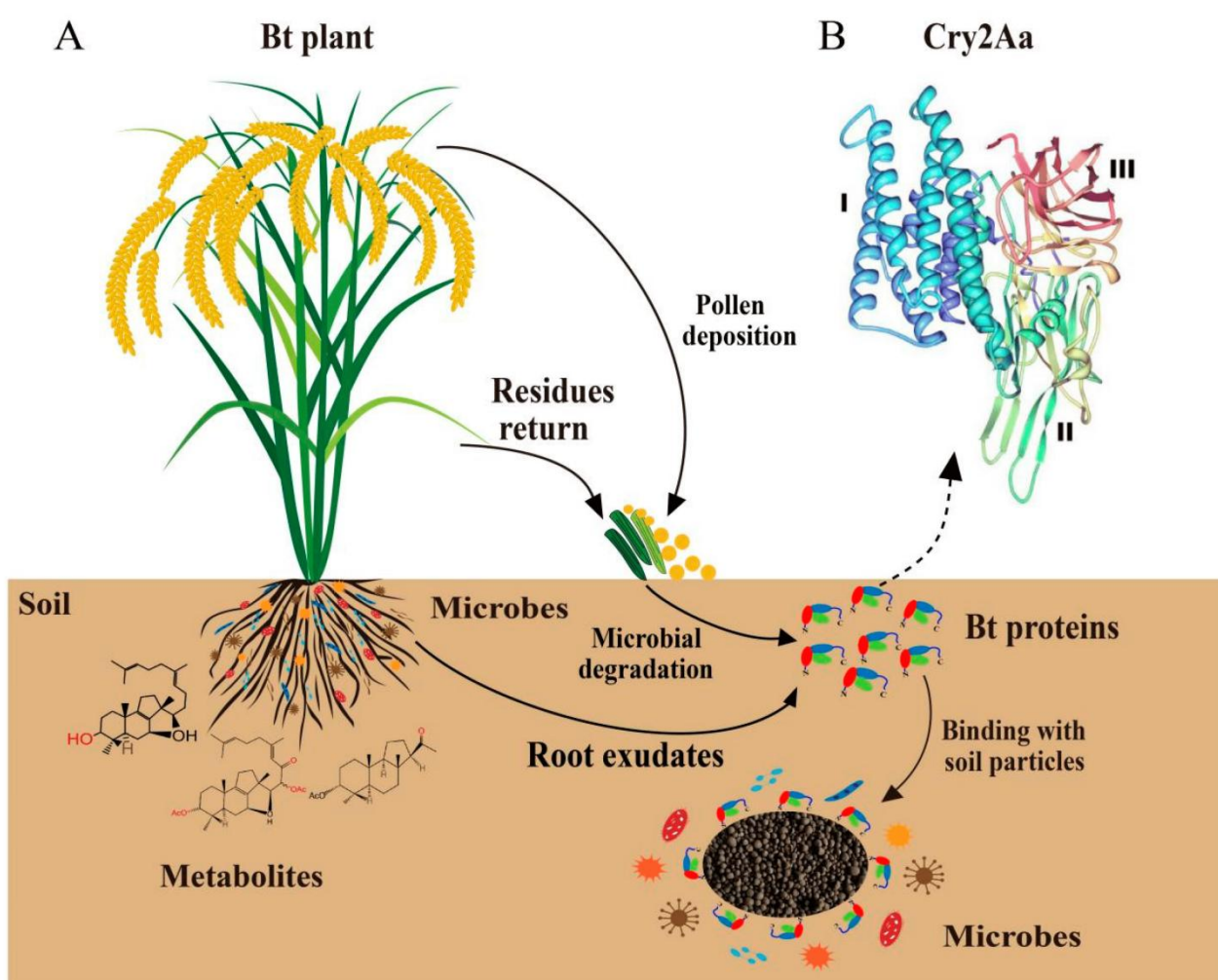


Figure 1 Environmental behaviors of Bt protein (A) and its three-dimensional structures (B). I, II, and III: domains I, II, and III (Adopted from Li et al., 2022)

The production of Bt toxin is influenced by both genetic regulation and environmental factors. At the genetic level, the expression of specific genes and regulatory networks play a key role in Bt toxin production, while environmental factors such as temperature, pH, and nutrients significantly affect the synthesis and release of the toxin. Figure 1 illustrates how high-throughput screening methods systematically assess these genetic and environmental variables to optimize Bt toxin production. This approach is crucial for improving the efficiency and stability of Bt toxin production and is an important tool in Bt metabolic engineering research.

3.2.3 Interactions with primary metabolism

The synthesis of insecticidal proteins in Bt is intricately linked to its primary metabolism. The energy and precursors required for the production of these proteins are derived from primary metabolic processes. For instance, the amino acids and nucleotides necessary for protein synthesis are generated through the central metabolic pathways. Moreover, the regulation of primary metabolic pathways can influence the efficiency and yield of toxin production, highlighting the interconnectedness of primary and secondary metabolism in Bt (Tabashnik et al., 2015; Li et al., 2022).

3.3 Role of metabolism in protein synthesis

Metabolism plays a crucial role in the synthesis of insecticidal proteins in Bt. The metabolic state of the bacterium determines the availability of resources required for protein synthesis. During the sporulation phase, when Cry proteins are produced, Bt undergoes significant metabolic shifts to allocate resources towards the synthesis of these proteins. Similarly, the production of Vip proteins during the vegetative phase is influenced by the metabolic activity of the bacterium. Understanding the metabolic pathways and their regulation in Bt is essential for optimizing the production of insecticidal proteins through metabolic engineering (Tabashnik et al., 2015; Li et al., 2022; Jin et al., 2022).

4 Metabolic Engineering Strategies for Bt

4.1 Genetic modifications for enhanced protein production

4.1.1 Overexpression of key genes

Overexpression of key genes in *Bacillus thuringiensis* (Bt) has been a pivotal strategy to enhance the production of insecticidal proteins. For instance, the introduction of a late embryogenesis abundant (LEA) peptide co-expression system has shown significant promise. By using the expression vector pHT01 with a strong σ^A -dependent promoter, the production of crystal proteins was enhanced threefold after 12 hours of induction with IPTG (Akhtar et al., 2021). This method was further optimized by using lactose as an inducer, which provided a more cost-effective and efficient alternative to IPTG, leading to enhanced Cry protein expression through intermittent induction (Akhtar et al., 2022).

4.1.2 Gene knockouts to remove bottlenecks

Gene knockouts have been employed to remove metabolic bottlenecks that hinder the efficient production of insecticidal proteins. By targeting and knocking out specific genes that compete for resources or produce inhibitory byproducts, the metabolic flux can be redirected towards the synthesis of desired proteins. This approach has been instrumental in increasing the yield and efficacy of Bt toxins, although specific examples in the context of Bt require further elucidation in the literature.

4.1.3 Use of synthetic biology tools

Synthetic biology tools have revolutionized the metabolic engineering of Bt. Techniques such as CRISPR-Cas9 and advanced gene editing have enabled precise modifications to the Bt genome, facilitating the creation of strains with enhanced insecticidal properties. For example, the engineering of Bt toxin Cry2Ab30 and its bioconjugation with 4''-O-succinyl avermectin (AVM) resulted in a bioconjugate with significantly higher insecticidal activity and binding affinity compared to the native Cry2Ab30 (Pan et al., 2019). This demonstrates the potential of synthetic biology in creating more potent and efficient Bt strains.

4.2 Optimization of metabolic flux

Optimizing the metabolic flux within Bt is crucial for maximizing the production of insecticidal proteins. This involves fine-tuning the metabolic pathways to ensure that precursors and energy are efficiently channeled towards the synthesis of target proteins. The use of stable isotope labeling, as demonstrated in the production of $^{13}\text{C}/^{15}\text{N}$ -labeled Cry1Ab/Ac proteins, provides insights into the metabolic fate of these proteins and helps in understanding and optimizing their production (Wang et al., 2020). Additionally, the co-expression of LEA peptides has been shown to enhance the metabolic flux towards crystal protein production, further highlighting the importance of metabolic optimization (Akhtar et al., 2021; Akhtar et al., 2022).

4.3 Enhancing precursor availability

Enhancing the availability of precursors is another critical strategy in the metabolic engineering of Bt. By ensuring a steady supply of essential building blocks, the production of insecticidal proteins can be significantly increased. For instance, the use of molecular docking and bioinformatic analysis to engineer chimeric proteins like Cry1AcF has shown broad-spectrum efficacy against pests, indicating that optimizing precursor availability can lead to the development of more effective Bt strains (Rathinam et al., 2019). Moreover, the bioconjugation of Cry2Ab with AVM not only improved binding affinity but also suggested potential mechanisms for enhancing precursor availability through structural modifications (Pan et al., 2019).

The metabolic engineering of Bt for enhanced production of insecticidal proteins involves a multifaceted approach, including genetic modifications, optimization of metabolic flux, and enhancing precursor availability. These strategies collectively contribute to the development of more potent and efficient Bt strains, thereby improving their efficacy in pest management.

5 Case Studies of Successful Engineering in Bt

5.1 Enhanced Cry protein production

By employing various genetic engineering strategies, the production of Cry proteins in *Bacillus thuringiensis* (Bt) has been significantly enhanced. For example, the developed chimeric protein Cry1AcF exhibits broad-spectrum insecticidal effects. This chimeric protein is engineered to effectively interact with aminopeptidase 1 receptors from different insect species, thereby enhancing its insecticidal activity (Rathinam et al., 2019). Studies have shown that genetically engineered Bt proteins have higher stability and effectiveness, showing significant resistance enhancement against multiple pests, effectively addressing the issue of resistance to traditional Bt proteins (Figure 2). Furthermore, the seed industry is also optimizing the activity of Bt insecticidal proteins by modifying the protein structure to improve their effectiveness against target pests (Yamamoto et al., 2022).

The study by Rathinam et al. (2019) demonstrates that through genetic engineering strategies, the production of Cry proteins in *Bacillus thuringiensis* (Bt) has been significantly improved. Specifically, these strategies include protein structure optimization and gene combination modifications, resulting in engineered Bt with higher insecticidal efficiency and broad-spectrum resistance. This enhanced production capability effectively addresses the challenge of pest resistance, providing a more powerful tool for agricultural pest management.

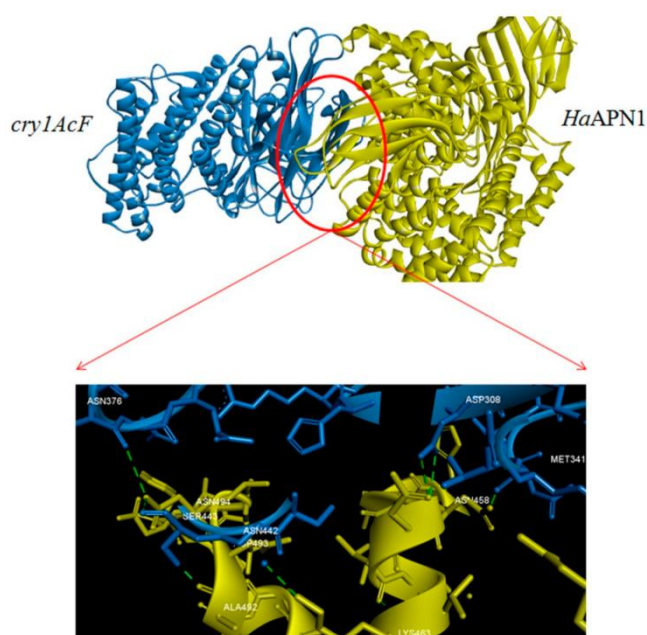


Figure 2 Molecular interaction between Cry1AcF and SIAPN1 (insect: specific region of interaction between the two proteins) (Adopted from Rathinam et al., 2019)

5.2 Improved stability and yield

Improving the stability and yield of Bt insecticidal proteins is crucial for their effectiveness in agricultural applications. One innovative approach involves the use of magnesium hydroxide nanoparticles (MHNPs) to control the loss of Cry1Ac protein. These nanoparticles enhance the adhesion of Cry1Ac to plant surfaces, thereby increasing its stability and insecticidal activity. The Cry1Ac-loaded MHNPs showed a significant increase in pest mortality and remained stable during the adsorption process, making them a promising adjuvant for biopesticides (Rao et al., 2018). Furthermore, studies have shown that the environmental behavior of Bt proteins, including their adsorption, retention, and degradation in soils, can impact their stability and efficacy. Understanding these behaviors helps in designing strategies to maintain the activity of Bt proteins in field conditions (Li et al., 2022).

5.3 Integration of multiple engineering strategies

The integration of multiple engineering strategies has been pivotal in enhancing the production and effectiveness of Bt insecticidal proteins. For example, the combination of different Cry proteins in transgenic crops has been employed to delay resistance and broaden the spectrum of pests controlled. Second-generation Bt crops producing multiple toxins, such as Cry1Ac and Cry2Ab, have been developed to combat resistance in pests like *Helicoverpa armigera*. This approach has shown success in maintaining the efficacy of Bt crops over time (Tabashnik et al., 2015). Additionally, the creation of chimeric proteins by combining domains of different Cry proteins has led to enhanced insecticidal properties and broader pest control (Koch et al., 2015). These integrated strategies not only improve the effectiveness of Bt proteins but also contribute to sustainable pest management practices.

6 Challenges and Future Directions

6.1 Technical challenges in metabolic engineering

Metabolic engineering of *Bacillus thuringiensis* (Bt) for enhanced production of insecticidal proteins faces several technical challenges. One significant issue is the optimization of Bt insecticidal proteins to overcome resistance in target pests. For instance, pests such as *Heliothis virescens* and *Ostrinia nubilalis* have developed resistance to Cry1Ac and Cry1Ab proteins, necessitating the engineering of new Bt proteins with higher activity levels (Yamamoto et al., 2022). Additionally, the structural complexity of polyketide insecticidal agents poses challenges for chemical modification, which is essential for enhancing their efficacy and production (Yi et al., 2023). The metabolic pathways involved in the production of these proteins are intricate, and any genetic modifications can have unintended effects on bacterial growth, sporulation, and protein formation, as seen with the *gabD* and *sucA* gene knockouts.

6.2 Ecological and safety concerns

The ecological and safety concerns associated with Bt proteins are paramount. Bt insecticidal proteins, when released into the environment through transgenic plants or biopesticides, can persist in the soil and potentially affect non-target organisms. The structural and functional differences between naturally occurring Bt proteins and those expressed in genetically modified organisms (GMOs) necessitate thorough biosafety evaluations before field deployment (Li et al., 2022). Moreover, the evolution of resistance in pests not only reduces the efficacy of Bt crops but also raises concerns about the long-term sustainability of Bt technology (Jurat-Fuentes et al., 2021; Tabashnik et al., 2023). While extensive studies have shown that current Bt crops do not adversely affect non-target species, continuous monitoring and assessment are essential to ensure environmental safety (Koch et al., 2015; Romeis et al., 2019).

6.3 Scaling up production and commercialization

Scaling up the production of Bt insecticidal proteins for commercial use involves overcoming several hurdles. The production of stable isotope-labeled Cry proteins, for instance, requires precise recombinant expression protocols and purification processes, which can be technically demanding and costly (Wang et al., 2020). Additionally, the optimization of Bt proteins for higher insecticidal activity, as required for commercial viability, involves complex protein engineering and metabolic engineering strategies (Rathinam et al., 2019; Yamamoto et al., 2022). The development of high-yield strains through metabolic engineering and combinatorial biosynthesis is crucial for the

economical production of these proteins (Yi et al., 2023). Furthermore, the commercialization of Bt crops and biopesticides must navigate regulatory frameworks that ensure their safety and efficacy, adding another layer of complexity to the scaling-up process (Koch et al., 2015).

While the metabolic engineering of Bt for enhanced production of insecticidal proteins holds great promise, it is fraught with technical, ecological, and commercial challenges. Addressing these challenges through innovative research and stringent safety evaluations will be key to the successful application and sustainability of Bt technology in pest management.

7 Advances in Biotechnology and Tools

7.1 CRISPR-Cas and genome editing

The advent of CRISPR-Cas technology has revolutionized the field of metabolic engineering, providing a precise and efficient method for genome editing. This technology allows for targeted DNA cleavage, enabling various genome engineering modes such as insertions, deletions, and chromosomal rearrangements (Nishida and Kondo, 2020). The versatility of CRISPR systems has led to the development of derivative technologies, such as deaminase-mediated base editing, which introduces point mutations with reduced cytotoxicity. Additionally, CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) enable temporary control of gene expression without altering the genomic sequence, further expanding the toolkit available for metabolic engineering (Nishida and Kondo, 2020).

CRISPR-Cas systems have been successfully applied to a wide range of organisms, including industrially relevant microbes like *Escherichia coli* and *Saccharomyces cerevisiae* (Mougiakos et al., 2016). These systems have facilitated the rapid development of microbial cell factories by enabling high-efficiency genome editing and transcriptional regulation (Jakočiūnas et al., 2016). For instance, CRISPR-Cas9 has been used to introduce multiple mutations simultaneously in *E. coli*, optimizing metabolic pathways for the overproduction of valuable compounds such as β -carotene (Li et al., 2015). The discovery of novel Cas9-like systems from diverse microbial environments continues to broaden the range of organisms that can be engineered using CRISPR technology (Mougiakos et al., 2018).

7.2 High-throughput screening methods

High-throughput screening methods are essential for the efficient evaluation of genetic modifications and the identification of optimal metabolic pathways. CRISPR-Cas technology has significantly enhanced the capabilities of high-throughput screening by enabling multiplexed genome editing and the construction of guide RNA (gRNA) libraries (Ding et al., 2020). These advancements allow for the comprehensive discovery and evaluation of metabolic pathways, accelerating the development of engineered strains with desired traits (Nishida and Kondo, 2020).

The integration of CRISPR-Cas systems with high-throughput screening has been particularly impactful in the field of microbial biotechnology. For example, CRISPR-based tools have been used to perform genome-wide perturbations and model-guided genome editing strategies, enabling the systematic exploration of metabolic networks (Jakočiūnas et al., 2017). This approach has been applied to both model organisms and non-model microbes, facilitating the development of novel production hosts for biotechnologically relevant products (Mougiakos et al., 2018).

7.3 Computational modeling and simulation

Computational modeling and simulation play a crucial role in the design and optimization of metabolic engineering strategies. These tools enable researchers to predict the effects of genetic modifications on metabolic pathways and to identify potential bottlenecks and targets for further engineering. The integration of CRISPR-Cas technology with computational modeling has further enhanced the precision and efficiency of metabolic engineering efforts (Jakočiūnas et al., 2017).

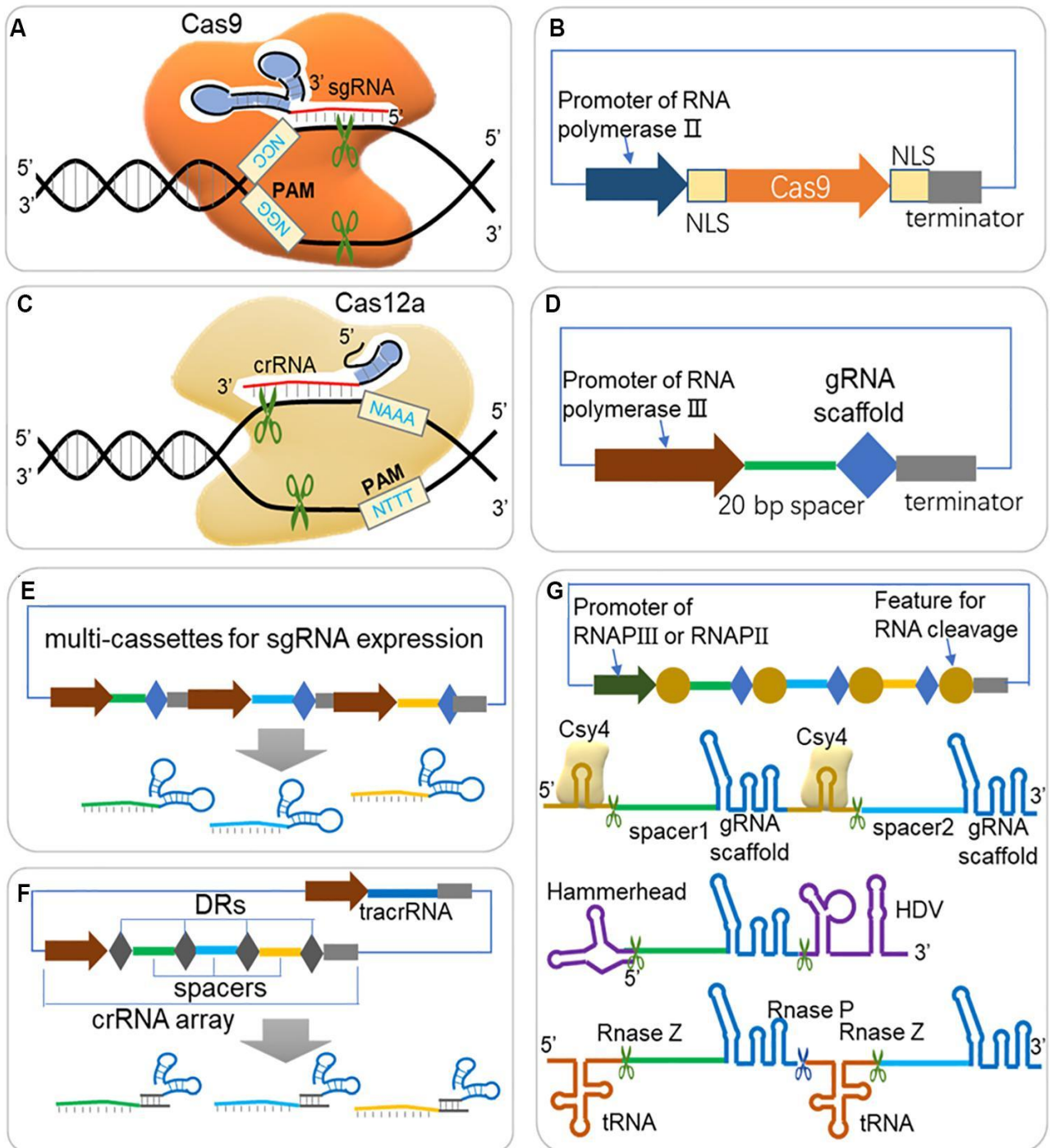


Figure 3 Guidelines for expression of Cas protein and sgRNA in CRISPR/Cas system (Adopted from Ding et al., 2020)

Image caption: (A) Scheme of CRISPR/Cas9 system. The Cas9-sgRNA (or Cas9-crRNA-tracrRNA) complex binds to DNA target arising from Watson-Crick base pairing of spacer sequence, and triggers double strand break (DSB) when next to a short protospacer adjacent motif (PAM, 'NGG' for Cas9 from *S. pyogenes*). (B) Expression cassette for Cas9. For efficient targeting to nucleus in eukaryotes, the Cas9 should be fused to NLS (nuclear localization sequence) at one end or both ends. (C) Scheme of CRISPR/Cas12a (Cpf1) system. Cas12a triggers DSB through a similar scheme of Cas9, but depends on different PAM ('NTTT') and less folded crRNA, and creates a sticky end at 18–23 bases away from the PAM. (D) Expression cassette for sgRNA. A promoter of RNA polymerase III (RNAP III) is usually required for directing sgRNA in nucleus and with less modification. (E) Multi-sgRNA expression through multi-cassettes. Repeated elements, such as promoters, gRNA scaffold and terminators are repeated for different spacer sequences. (F) Multi-sgRNA expression through crRNA array and tracrRNA (HI-CRISPR system). (G) gRNA multiplexing strategies. Both RNAP II and RNAP III promoter can be used for expression the sgRNA array, where sgRNAs are separated by features for RNA cleavage (Adopted from Ding et al., 2020)

Model-guided genome editing strategies, supported by CRISPR-Cas systems, allow for the rational design of metabolic pathways and the systematic testing of hypotheses (Jakočiūnas et al., 2017). Computational tools for the design of gRNAs and the prediction of off-target effects are also essential for the successful application of CRISPR technology in metabolic engineering (Jakočiūnas et al., 2016). These tools help to minimize unintended genetic modifications and to ensure the accuracy and efficiency of genome editing efforts.

The advances in CRISPR-Cas technology, high-throughput screening methods, and computational modeling have significantly enhanced the capabilities of metabolic engineering. These tools have enabled the precise and efficient modification of microbial genomes, facilitating the development of engineered strains with improved production of insecticidal proteins and other valuable compounds. The continued integration of these technologies promises to drive further innovations in the field of metabolic engineering.

8 Concluding Remarks

The systematic review of the metabolic engineering of *Bacillus thuringiensis* (Bt) for enhanced production of insecticidal proteins has highlighted several key findings. Bt proteins, particularly Cry and Vip toxins, have played a crucial role in pest management through their use in genetically engineered (GE) crops such as corn, cotton, and soybean. These proteins are highly specific to target pests while remaining safe for non-target organisms, including humans. However, the emergence of resistance in some pest populations presents a significant challenge, emphasizing the need for the development of new Bt proteins and strategies to manage resistance. Advances in protein engineering have led to the creation of chimeric and optimized Bt proteins with enhanced insecticidal activity and broader spectrum efficacy. Environmental and biosafety assessments of Bt crops generally support their safety, though ongoing monitoring and evaluation are essential.

The metabolic engineering of Bt for enhanced production of insecticidal proteins holds substantial potential for agriculture. Developing Bt proteins with higher potency and broader spectrum activity can improve pest control, reduce reliance on chemical insecticides, and promote sustainable agricultural practices. Enhanced Bt proteins can also help mitigate the issue of pest resistance, ensuring the long-term efficacy of Bt crops. Integrating Bt crops into integrated pest management (IPM) strategies can support the conservation of natural enemies and overall ecosystem health. The economic benefits for farmers, including increased crop yields and reduced pest management costs, further underscore the positive impact of metabolic engineering on agriculture.

Future research should focus on maximizing the benefits of metabolic engineering of Bt. Continued development and optimization of new Bt proteins are necessary to stay ahead of evolving pest resistance. Exploring novel protein engineering techniques and bioconjugation methods to enhance the efficacy and stability of Bt proteins is important. Comprehensive biosafety evaluations should be conducted to assess the environmental impact of new Bt proteins and ensure their safety for non-target organisms. Investigating the mechanisms of resistance in pests is essential for developing more effective resistance management strategies. Integrating Bt crops into broader IPM frameworks and promoting the use of refuges can help sustain the long-term effectiveness of Bt technology.

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Conflict of Interest Disclosure

The author affirms 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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