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Microbial control and biotechnology research on *Bacillus thuringiensis* in China

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Abstract

The current status of production and application of biopesticides for pest control in China is briefly reviewed, with a focus on research advances in microbial control with *Bacillus thuringiensis* (Bt). These have led to improvements in Bt production, exploitation of Bt gene resources, and development of engineered Bt insecticides and transgenic Bt crops that have expanded host ranges and increased efficacy against target pests. Both conventional and biotechnology approaches need to be employed to achieve further progress in discovery, production technology, formulation processing, development of quality standards and recommended use patterns.

Keywords: Microbial control; Biopesticide; *Bacillus thuringiensis*; Biotechnology; Bt crops

1. Introduction

China is a country with many agricultural pests. According to statistics from the Chinese Ministry of Agriculture (2007), the area of pest infestation during 1990–2000 was about 300 million hectares per year and the annual loss of food crops destroyed by pests was over 15 billion kilograms, even though various measures of control were applied. During this time, pest control was mainly dependent on chemical pesticides, and the yearly total amount applied was as high as 250,000 tons of active ingredient (Qiu, 2005). Owing to ever-increasing problems of environmental pollution and food safety caused by chemical pesticides, the Chinese government recently provided more support to the development of an environmental friendly strategy of biological control. As a result, China has made very significant progress in developing microbial control programs.

2. Current status of microbial control

2.1. Biopesticide production, microbial insecticides and pest control

Currently, China has established an integrated research and development system with multiple disciplines for biological control, including microbial control. Microbial control has played an important role in Chinese pest management. According to estimates by the Ministry of Agriculture in 2004, the output of all biopesticides (including microbial agents, agro-antibiotics, biochemical agents and botanical pesticides) and application area, respectively, accounted for 12% and 9.5% of total output (Table 1).

According to statistics produced by the Institute for the Control of Agrochemicals in the Ministry of Agriculture, more than 300 microbial insecticide products have been registered in China (Table 2).

There are also a number of microbial insecticides being used in large scale applications although the final registrations have not yet been granted. For example, *Beauveria bassiana*, for control of pine lappet caterpillar, *Dendrolimus*

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Table 1
Production and application of biopesticides China in 2004

	Biopesticide production[*,**]	Percentage due to biopesticides vs. total pesticides
Output	Over 12,000 ton	12
Output value	$250 million	10
Number of products	411	8
Number of active ingredients	140	15
Application area	30 million ha.	9.5

[*] Including microbial agents, agro-antibiotics, biochemical agents and plant-origin pesticide.
[**] *Source*. Chinese Ministry of Agriculture, http://www.agri.gov.cn.

Table 2
Microbial control products registered for use in China[*]

Number of products	Active ingredient	Target pest
276	*Bacillus thuringiensis*	Lepidopterans, Coleopterans, Dipterans (Mosquitoes)
21	*Helicoverpa armigera* SNPV[**]	Cotton bollworm
2	*Plutella xylostella* GV[***]	Diamondback moth
2	*Spodoptera littoralis* MNPV[****]	Cotton leaf worm
2	*Ectropis oblique* MNPV[****]	Tea geometrid
2	*Metarhizium anisopliae*	Locusts

[*] *Source*. Chinese Ministry of Agriculture, Institute for the Control of Agrochemicals.
[**] Singly embedded nuclear polyhedrosis virus.
[***] Granulosis virus.
[****] Multiply embedded nuclear polyhedrosis virus.

punctatus, and Asian corn borer, *Ostrinia furnacalis*, and the microsporidium, *Nosema locustae*, for the control of locusts and grasshoppers.

2.2. Microbial control with Bt products

More than 50 subspecies of Bt belonging to 41 H serotypes have been discovered in China since Bt products were introduced in 1955 (Yu et al., 2001). Bt serotypes found in China constitute 60% of the total recorded worldwide. Ten new subspecies of Bt were discovered in China (Lecadet et al., 1999). After 50 years of growth, a system of independent research, development, commercial production and application has been established. Because Bt products are the most widely used microbial pesticides in China in terms of both output and area applied, subspecies of this bacterium play a very large role in Chinese microbial control. Bt products are primarily used for the control of lepidopteran pests, such as cotton bollworm, *Helicoverpa armigera*, Asian corn borer, *O. furnacalis*, rice leaf folder, *Cnaphalocrocis medinalis*, rice skipper, *Parnara guttata*, cabbage worm, *Pieris rapae*, diamondback moth, *Plutella xylostella*, pine lappet caterpillar, *D. punctatus*, and the tea leaf-rollers, *Homona coffearia and Adoxophyes orana*. Over the past several years, fermentation technology and the quality of Bt products produced in China have improved remarkably. Various Bt formulations, such as powders, emulsions, concentrated suspensions, wettable powders, slow-release preparations, granules, water dispersible granules and ultra-low volume agents have been developed. Of these, wettable powders are becoming the leading formulation. At present, based on fermentation yields, the annual output of Bt products is about 40,000 metric tons (Qiu, 2005), a level that meets domestic and export requirements.

3. Research and development on Bt biotechnology

3.1. Exploitation of Bt gene resources

The discovery and application of novel Bt insecticidal crystal protein genes provides an important strategy for the improvement of biological control of insect pests. Since 1997, more than 50 Bt genes, comprising nearly one-fourth of the genes recorded worldwide, were isolated by Chinese scientists and named by the International Bt Insecticidal Protein Gene Nomenclature Committee (Crickmore, 2006; Song et al., 1998, 2003, 2005; Sun et al., 2000; Liu et al., 2004).

China's effort to identify Bt endotoxins that are active against soil inhabiting grubs is an example of a coordinated effort that yielded several new discoveries. It had been known that *cry8*-type genes were toxic to a number of coleopteran pests, especially to certain species of scarabs (Ohba, 1992; Sato et al., 1994; Michaels et al., 1994; Shin-ichiro et al., 2003), but these had a fairly narrow spectrum. To identify new *cry8*-type genes, a PCR-RFLP identification system was developed, and a scarab rearing method was established. Using large-scale isolation techniques, a novel Bt gene, designated *cry8Ca2*, was cloned from Bt strain HBF-1. This gene has only one amino acid difference with the known *cry8Ca1*, and it was highly toxic to larvae of the scarabaeid insects *Anomala exoleta* and *Anomala corpulenta*. Recently, novel *cry8Ea1*, *cry8Fa1* and *cry8Ga1* genes were successively identified (Yu et al., 2006). The sequence homologies of these were less than 78% compared with the known *cry8*-type genes. These novel genes were verified to be specifically toxic to two important grubs, the Asian cockchafer, *Holotrichia parallela* and the related species, *Holotrichia oblita* (Fig. 1). None of the other known Bt toxin genes has been reported to be effective against these two species.

Additional discoveries include a crystal protein gene, *cry51Aa1*, (GenBank DQ836184) that was isolated from Bt strain F14-1, which had very low identity to known *cry* genes (e.g., the maximum identity is 22% to *cry15Aa*). When cloned in an acrystalliferous Bt strain BMB171, crystals formed, and the protein showed toxicity to *Bombyx*