S. Wang[1]
A. Y. Guo[2]
W. J. Zheng[3]
Y. Zhang[1]
H. Qiao[1]
I. R. Kennedy[4]

[1]Tianjin Key Laboratory of Food Nutrition and Safety, Faculty of Food Engineering and Biotechnology, Tianjin University of Science and Technology, Tianjin, P.R. China.

[2]Hebei Normal University of Science and Technology, Hebei, P.R. China.

[3]Tianjin Entry-Exit Inspection and Quarantine Bureau of the People's Republic of China, Tianjin, P.R. China.

[4]Faculty of Agriculture, Food and Natural Resources, University of Sydney, Sidney, Australia.

Research Article

Development of ELISA for the Determination of Transgenic Bt-Cottons Using Antibodies against Cry1Ac Protein from Bacillus thuringiensis HD-73

The area cultivated with Bt-cottons expressing Cry1Ac gene increases year by year in China and other countries. To evaluate any potential adverse impacts on the environment from the release of Bt (*Bacillus thuringiensis*) technology, the development of a method for easily detecting the activity of the Cry1Ac toxins is of particular interest. The aim of this study was to develop sandwich-ELISA for the detection of Cry1Ac protein in Bt-cotton tissues. A specific antibody was obtained from rabbits inoculated with Cry1Ac protein derived from Bt strain HD-73 and a secondary antibody conjugated to HRP could combine the Bt Cry1Ac protein specifically. The limit of detection was 5 ng/mL and there were no cross-reactions between the positive control of Cry1Ab/1Ac, Cry1C, Cry2A, Cry3Bb1 and Cry9C. Extracts of proteins from cotton leaves were used to evaluate the suitability of the assay. Tris-borate buffer and sodium carbonate buffer were employed for the extraction of protein, the limit absorbance of detection was 0.134 and 0.449, respectively, and the latter produced a higher background. The results showed that cultivars GK-12, GK-22, insect-resistant cotton, bivalent transgenic cotton and shiyuan 321 assayed positively and NON was the negative sample. The PCR method was used for the validation of the developed assay. Although both methods allowed the same results to be obtained, ELISA needed simple equipment and took less time. The developed immunoassay method is considered reliable for the detection of Bt Cry1Ac protein.

Keywords: Biotechnology, PCR, Transgenic plants

Received: September 10, 2006; *revised:* January 28, 2007; *accepted:* February 5, 2007

DOI: 10.1002/elsc.200620179

1 Introduction

Bacillus thuringiensis (Bt) is a ubiquitous soil bacterium [1] which has the character of a Gram-positive, aerobic and rod-shaped microrganism [2, 3][1)] Its insecticidal properties were first discovered in Japan in 1901 by Ishawata and in 1911 in Germany by Berliner [4]. This bacterium produces crystalline parasporal inclusion bodies containing insecticidal proteins (δ-endotoxins) during its sporulation [2–4]. The insect larvae die of anorexia if the proteins are ingested [2, 5]. In 1938, Bt first became available as a commercial insecticide in France and in the 1950s entered commercial use in the United States [4]. Currently, transgenic plants such as tobacco, maize, potato, cotton, tomato, and rice expressing insecticidal Bt proteins have been shown to control certain chewing insects [6]. In China, transgenic cotton expressing Cry 1Ac insecticidal toxin protein has been commercially available to control cotton bollworms since 1998. The Bt cotton has demonstrated significant control of cotton bollworms in China [7], and the area now cultivated with genetically modified (GM) cotton has reached 2.8 million hectares in 2003 [8]. Bt cottons were commercialized in 1996/97 in Australia [9].

The problem of detecting genetically modified cultivars has been discussed previously. At present, the method used most

Correspondence: S. Wang (s.wang@tust.edu.cn), Tianjin Key Laboratory of Food Nutrition and Safety, Faculty of Food Engineering and Biotechnology, Tianjin University of Science and Technology, 1038 Dagunan Road, Tianjin, China, 300222.

1) List of abbreviations at the end of the paper.

frequently is the amplification of genetically modified organism (GMO) specific DNA by PCR followed by agarose gel electrophoresis, restriction fragment length analysis, southern blot hybridization or DNA sequencing [5]. This approach has been successfully applied on transgenic potato, tomato, herbicide-resistant maize, Bt-maize, soya bean and processed products [10]. However, this requires a well-equipped laboratory and suitable methods to optimize the results, and PCR-based methods are time consuming. Immunoassay provides an alternative means for the detection of GMO based on the determination of the protein product of the foreign gene. Polyclonal antibodies raised either in rabbits [11–13] or goats [14] have been used. Monoclonal antibodies were also used by Walschus et al. [5], but the production of monoclonal antibodies was complicated and required a specialized resource to achieve success. An immunoradiometric assay for the quantification of Cry1Ab in transgenic sugarcane plants was developed by Vazquez et al. [15], but the radioactivity is considered a potential risk to the investigators' health. The area cultivated with Bt-cottons, expressing the Cry1Ac gene, increases annually in China and in other countries [8, 9]. However, there is limited availability of specific methods to determine the gene of Cry1Ac and Cry1Ac protein in Bt-cotton.

To evaluate any potential adverse impact on the environment from the release of Bt technology, the development of a method for easily detecting the activity of the Cry1Ac toxins is of particular interest. Though many ELISAs described in the literature have been developed for the determination of Bt Cry toxins, the proteins of Bt Cry1A or Bt Cry1Ab toxins were obtained from Bt HD-1 [11–14]. However, there are not any reports on the usage of protein of Bt Cry1Ac obtained from Bt HD-73, which produced enriched Cry1Ac only to develop ELISA for the Bt Cry1Ac. The aim of this study was to develop sandwich-ELISA for the detection of Cry1Ac protein in Bt-cotton tissues, and the key Cry1Ac protein was obtained from Bt HD-73.

In this paper, the obtainment of Cry1Ac protein from *Bacillus thuringiensis* HD-73 and the acquisition of antibodies were described and discussed in detail. The developed ELISA method was applied to determine five different transgenic Bt-cottons and one traditional cotton. The reliability of the method was validated by the PCR method.

2 Materials and Methods

2.1 Fermentation and Extraction of Bt Cry1Ac Protein

The Bt HD-73 strain which produces enriched Cry1Ac protein [14] was inoculated in 50 mL of 50 % NB liquid medium [2.5 % [w/v] beef extract, 5 % [w/v] peptone and NaCl, pH 7.2] and cultured at 28 °C with shaking at 200 rpm. The cells were collected after a 3 day incubation. The culture solution was centrifuged at 7,500 g for 30 min and the pellet was firstly washed with 1 mol/L NaCl containing 0.1 % [w/v] Triton X-100 and rinsed with sterile water several times. Then it was dissolved in 50 mmol/L sodium carbonate buffer (pH 9.6) with 0.1 % 2-mercaptoethanol at room temperature for 2 h. After this, the lysate was treated by two methods: the high-speed centrifugation and isoelectric point deposition method. The first half lysate was directly centrifuged at 12,000 g and the Cry1Ac protein was obtained in the supernatant. HCl was added into the other half of the lysate until the pH reached ca. 5.0. Then it was centrifuged at 8000 g and the deposit protein was reconstituted with 20 mmol/L Tris-HCl buffer, respectively. The purity of the protein was estimated by a 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and the concentration was determined according to the Lowry method. Finally, the Cry1Ac protein was lyophilized in aliquots and stored at –20 °C for further analysis.

2.2 Preparation of Antibodies

Polyclonal antibodies were produced by the immunization of rabbits with Bt CrylAc protein. Rabbits were intradermally and subcutaneously immunized. Immunogen containing 0.5 mg of Cry1Ac protein with complete Freund's adjuvant (Sigma, USA) were used for the first immunization and 0.25 mg protein with incomplete Freund's adjuvant (Sigma, USA) was used for the second, third and fourth immunization. Sera that exhibited a strong immunogenic response were purified by affinity chromatography using a Protein A-Sepharose 4B column (Amersham Biosciences, Sweden).

2.3 Labeling of the Antibodies

The horseradish peroxidase (Sigma, USA) was dissolved in freshly prepared 0.025 mol/L sodium periodate, and the free sodium periodate was removed using gel filtration columns (PD-10, Amersham Biosciences, Sweden) with 1 mM sodium citrate buffer (pH 4.5), and the gold-colored fractions were collected. Approximately equal volumes of the purified antibodies (a ratio of 2 mg antibody : 1 mg HRP) were added into the above fractions. Then 1 mol/L sodium carbonate buffer (pH 9.6) was added to the reaction mixture until the pH reached 9.5. After stirring the solution gently for 3 h at room temperature, 10 % [v/v] 2 mol/L ethanolamine buffer (pH 9.6) was added and the mixture incubated overnight at 4 °C. The mixture was then freed of small molecules by gel filtration on a column of Sepharose CL-6B (Amersham Biosciences, Sweden) in phosphate-buffered saline (PBS, pH 7.2) and the fractions in the first peak where both $A280$ nm and $A403$ nm overlap were collected. Finally, BSA was added into the conjugated solution at 5 mg/mL, and the product stored in aliquots at –20 °C.

2.4 Extraction of Bt Protein from Cotton Leaves

Six different cotton leaf tissues (NON, GK-12, GK-22, insect-resistant cotton, bivalent transgenic cotton, shiyuan 321 (which were planted in the laboratory)) were ground in a mortar under liquid nitrogen with a pestle, and then were thoroughly mixed with the two different buffers [Tris-borate buffer (0.2 mg/mL) [5] and 50 mmol/L sodium carbonate buffer (pH 9.6) with 0.2 % L-ascorbic]. The mixtures were incubated at room temperature for 15 min while the samples were sha-

ken on a rotary shaker and the extracts were centrifuged at 8,000 g for 20 min to remove insoluble materials. The supernatant was used for the determination of Bt Cry1Ac protein by ELISA.

2.5 Sandwich-ELISA Assay

In this study, a sandwich-ELISA for Bt Cry1Ac protein analysis was developed. Microwell plates (Nunc, Denmark) were coated with 100 μL per well of the purified polyclonal antibody (5 μg/mL) diluted in coating buffer and incubated overnight at room temperature. After washing the plates three times with PBST (PBS with 0.05 % [v/v] Tween 20), the unbound sites were blocked with 200 μL of blocking solution at 37 °C for 1 h. Then the plates were washed three more times with PBST.

For the assay, 100 μL samples (either Bt protein, six different positive controls (Cry1Ab/1Ac, Cry1C, Cry2A, Cry3A, Cry3Bb1, Cry9C which were purchased from Agdia, USA) or extract of protein) were added into each well and incubated at room temperature for 1 h. After the plates were washed four times with washing solution, 100 μL of the secondary antibody conjugated HRP (1 : 100) diluted in PBST was pipetted into each well and incubated for 1 h. The plates were washed five times, and 150 μL of substrate solution were added into each well. The reactions were stopped after 30 min by adding 50 μL of 1.25 M sulfuric acid, and the absorbance was read in the microplate reader (Thermo, Finland) in a dual-wavelength mode (450–650 nm). Three samples for each concentration (or six different lyophilized Bt toxins or extract of protein) were analyzed. PBST or extract buffer of protein was used as a blank control.

2.6 Extraction of DNA

Plumules from six different cotton cultivars were crushed in a mortar under liquid nitrogen with a sterile pestle. The cetyltrimethyl ammonium bromide (CTAB) protocol was used for the genomic DNA extraction from cottons in this study. And finally DNA was taken up in 50 μL TE (10 mmol/L Tris-1 mmol/L EDTA) buffer and stored at –20 °C.

2.7 Polymerase Chain Reaction

The primer pairs that were used for amplification by PCR are listed in Tab. 1. A TE buffer was employed as a blank control. Each PCR reaction was carried out in 25 μL reaction mixtures [containing 2.5 μL with 10 times PCR buffer (Mg^{2+} plus), 2 μL of a deoxynucleoside triphosphate (dNTP) mixture (2.5 mM), 1 μL of each primer (20 μM), 0.2 μL of *TAKARA Taq* polymerase, 1 μL of each template and 17.3 μL of sterile bidistilled water] for 39 cycles at 95 °C for 30 s, 54 °C for 40 s and 72 °C for 30 s, with the specific primers. The mixture was heat-treated at 95 °C for 10 min at the beginning of the reaction and finally the reaction was further extended for 10 min at 72 °C. The products were analyzed with electrophoresis on agarose gel (1.5 %, w/v) and made visible by staining with EB at UV transillumination.

Table 1. The primer pairs used for the amplification by the polymerase chain reaction.

Genes	Primer pairs sequence	Target length [bp]
CaMV-35S	5cct aca aat gcc atc a-3′ 5agt ggg att gtg cgt ca-3′	195
Cry1Ab+Cry1Ac	5cgt tct cgg act agt tg-3′ 5gag ctg ggt tag tag ga-3′	215
Cry1Ac	5cca gct aca gct acc tcc-3′ 5cta aag ttt cta aca ccc ac-3′	119
18s rRNA	5gcc cta tca act ttc gat ggt a-3′ 5ttg cgc gcc tgc tgc ctt cct t-3′	137

3 Results and Discussion

3.1 Extraction of Cry1Ac Protein from Bt HD-73

Bacillus thuringiensis produces insecticidal proteins during its sporulation at the stationary phase. Poorer nutrition is of benefit to maximize the formation of spores and crystal protein. Furthermore, the bacterium requires sufficient oxygen to satisfy its needs for growth. In the course of fermentation, the shaking rate was increased to the maximum to obtain enough oxygen.

The results from SDS-PAGE indicate that the purity of protein obtained by high-speed centrifugation was better than that obtained by isoelectric point deposition. There was only one protein band visible (data not shown) indicating a purity of Cry1Ac greater than 90 % and this was considered sufficient to be used directly as an immunogen for producing antibodies.

3.2 Preparation of Antibodies

In this study, two methods for antibody preparation were adopted. The first used solubilization of protein sample in PBS buffer as an immunogen and the second used emulsification of the protein band obtained from polyacrylamide gels as an immunogen. The rabbits immunized by the second method showed a greater immune response in the course of full development of immunity. However, lower-titer antibodies were obtained compared to those obtained by the first method after the same time (data not shown). This result was presumably due to the polyacrylamide retaining the antigen in the animals, acting as an adjuvant [16]. Consequently, the immunization time should be prolonged to obtain higher-titer antibody when using proteins in gel. Because of their higher titer and sensitivity, antibodies from animals immunized by proteins in solutions (method 1) were chosen for ELISA.

3.3 Analysis of Sandwich-ELISA

In the preliminary work, the concentration of the coating and the secondary antibody was assessed for the polyclonal anti-

body-based assay. The results of this assessment are presented in Fig. 1, showing the limit of detection for Bt Cry1Ac protein to be 5 ng/mL, which was calculated as an average of the blank control values ± three standard deviations of the blank control values. The linear range was approximately 16–250 ng/mL of protein.

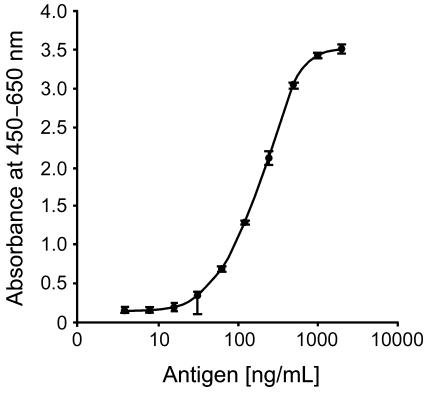

Figure 1. Determination of the Cry1Ac protein concentration using the ELISA developed. (Ten different concentrations of antigens (2000-3 ■? ng/mL) obtained by serial 1:1 diluted in PBST.)■concentration?■

3.4 Cross-Reaction with Other Bt Cry Proteins

Tests with controls of five different lyophilized Bt toxins (Cry1C, Cry2A, Cry3A, Cry3Bb1, Cry9C (purchased from Agdia)) were used to validate the specificity of the ELISA reaction with the positive control of Bt Cry1Ab/1Ac in this assay. The absorbance of the lyophilized positive control of Bt Cry1Ab/1Ac is shown in Fig. 2. The absorbance of other lyophilized positive controls is identical to that of Bt Cry1Ab/1Ac. Therefore, there were no cross-reactions between the analyte and the lyophilized Cry1C, Cry2A, Cry3Bb1 and Cry9C up to a concentration of 40 ng/mL (except for Cry3A for which the concentration was 12 ng/mL). Thus, these antibodies and antibodies conjugated to HRP could specifically determine the Bt Cry1Ab/1Ac proteins without significant cross-reaction to the other Bt toxins tested here.

3.5 Extraction of Proteins from Transgenic Cotton Tissue

Crude extracts of Bt proteins from cotton leaf tissues were used to evaluate the suitability of the assay. The result is shown in Fig. 3. The limit absorbance of two different buffers (Tris-borate buffer, sodium carbonate buffer) were 0.134 and 0.449, respectively, (calculated as an average of the zero values ± three standard deviations of the zero values). If the absorbance of the

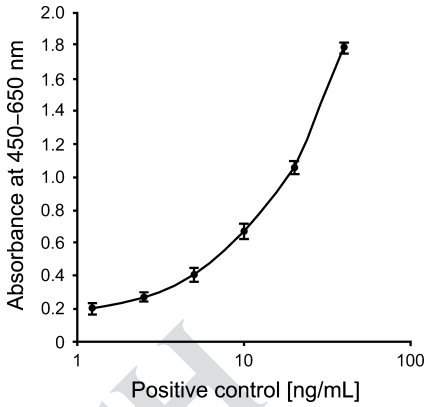

Figure 2. Determination of different concentrations of the commercial positive control of Cry1Ab/1Ac protein using the ELISA developed.

samples was higher than that of the blank, the samples were positive. Therefore, using assay conditions given in the methods, the leaf tests indicated that GK-12, GK-22, insect-resistant cotton, bivalent transgenic cotton and shiyuan 321 were positive samples and NON was the negative sample. At the same time, the extracts of samples made with sodium carbonate buffer produced a higher background value than tris-borate buffer using the current antibodies. These results corresponded with the published results [5, 14, 17]. Therefore, Tris-borate buffer was used for the extraction of Cry1Ac protein from the cotton leaves in this paper.

3.6 DNA Extraction and PCR Analysis

Generally, it was found that the cottons should be kept in a dark room for at least one day to reduce the amount of amylum when the experiment was prepared. In this assay, DNA extraction was repeated three times at different dates. The results showed that DNA was most feasibly obtained from GK-12, NON, insect-resistant cotton, bivalent transgenic cotton and shiyuan 321 in the preliminary growth period except for GK-22 from which DNA was easily acquired at any time (data

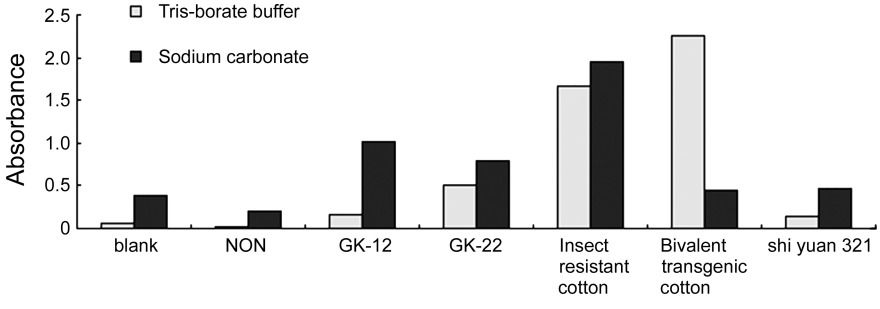

Figure 3. Determination of six different samples of different extract buffer using sandwich ELISA. (Three samples for each sample were analyzed and the extract buffer of protein was used as a blank control.

not shown). In this assay, the primer pairs CaMV-35S (Cauliflower Mosaic Virus), Cry1Ab + Cry1Ac, Cry1Ac as a detection gene and 18S rRNA as an inner gene were selected from the 5c-end and 3c-end of the coding region, respectively. The bands compared with the DNA markers confirmed the expected length of these products. The electrophoresis result also indicated that NON was a negative sample and the other five samples were positive. When 18S rRNA was used, the bands were shown in all samples. A comparison of the bands with the DNA markers confirmed the expected length of these products (data not shown).

3.7 Comparison between ELISA and PCR Results

In this study, the results for the detection of transgenic cottons obtained with ELISA were confirmed with PCR. The ELISA tests showed that GK-12, GK-22, insect-resistant cotton, bivalent transgenic cotton and shiyuan 321 were the positive samples and the NON-sample was the negative one. The same conclusions were obtained from tests using the PCR method, as demonstrated in Fig. 3. As shown in Fig. 3, the expected positive bands were all detected in the six cotton samples (NON was negative). These results indicated that the developed immunoassay method was reliable for the detection of Bt Cry1Ac protein.

4 Conclusions

In this study it was confirmed that a sandwich-ELISA method could be more readily developed for the detection of transgenic Bt-cottons using a selected strain of *B. thuringiensis*. A specific antibody was obtained from rabbits inoculated with Cry1Ac protein derived from Bt strain HD-73 and the secondary antibody conjugated to HRP could combine the Bt Cry1Ac protein specifically. The limit of detection was 5 ng/mL and there were no cross-reactions between the positive control of Cry1Ab/1Ac, Cry1C, Cry2A, Cry3Bb1 and Cry9C. Extracts of proteins from cotton leaves were used to evaluate the suitability of the assay. Tris-borate buffer and sodium carbonate buffer were employed for the extraction of six different cotton leaves. Sodium carbonate buffer produced a higher background than tris-borate buffer. The results showed that cultivars GK-12, GK-22, insect-resistant cotton, bivalent transgenic cotton and shiyuan 321 assayed positively and NON was the negative sample. For the validation of this method, a PCR method was used for the determination of samples, the primer pairs CaMV-35S, Cry1Ab + Cry1Ac, Cry1Ac were selected as a detection gene and 18S rRNA as an inner gene, and the same results were obtained as with the ELISA method. Although the two methods allowed the same results to be achieved, ELISA needed simpler equipment and less time. Therefore the developed immunoassay method is considered reliable for the detection of Bt Cry1Ac protein.

Acknowledgments

The authors are grateful for financial support from the Ministry of Science and Technology of the People's Republic of China (Project No. 2006BAD05A06), the New Century Talent Program of the Ministry of Education of the People's Republic of China (Project No. NECT-04-0243), and the Fok Ying Tung Education Foundation (Project No. 101073) .

Abbreviations

ELISA	enzyme-linked immunosorbent assay
Bt	*Bacillus thuringiensis*
HRP	horseradish peroxidase
PCR	polymerase chain reaction
GM	genetically modified
GMO	genetically modified organism
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PBST	PBS with 0.05 % [v/v] Tween 20
dNTP	deoxynucleoside triphosphate
CTAB	Cetyltrimethyl Ammonium Bromide
CaMV-35S	Cauliflower Mosaic Virus

References

[1] J. Raymond, C. Cannon, *Bt* transgenic crops: Risks and benefits, *Integrated Pest Manage. Rev.* **2000**, *5*, 151–173.

[2] M. Kirouac, S. Vachon, J. L. Rivest, R. Schwartz, R. Laprade, Analysis of the properties of *Bacillus thuringiensis* insecticidal toxins using a potential-sensitive fluorescent probe, *J. Membrane Biol.* **2003**, *196*, 51–59.

[3] G. Stotzky, Persistence and biological activity in soil of the insecticidal proteins from *Bacillus thuringiensis*, especially from transgenic plants, *Plant Soil* **2004**, *266*, 77–89.

[4] C. Camilla, B. A. Neppl, Managing resistance to *Bacillus thuringiensis* toxins, *Ph.D. Thesis*, University of Chicago **2000**.

[5] U. Walschus, S. Witt, C. Wittmann, Development of monoclonal antibodies against Cry1Ab protein from *Bacillus thuringiensis* and their application in an ELISA for detection of transgenic *Bt*-maize, *Food Agric. Immunol.* **2002**, *14*, 231–240.

[6] L. P. Fearing, D. Brown, D. Vlachos, M. Meghji, L. Privalle, Quantitative analysis of CryIA(b) expression in *Bt* maize plants, tissues, and silage and stability of expression over successive generations, *Mol. Breeding* **1997**, *3*, 169–176.

[7] M. Xingyuan, G. Feng, N. Erdal, Y. Megha, N. Parajulee, Behavioral response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to cotton with and without expression of the CrylAc δ-endotoxin protein of *Bacillus thuringiensis* Berliner, *J. Insect Behav.* **2005**, *18*, 33–50.

[8] Y. Litao et al., Validation of a cotton-specific gene, Sad1, used as an endogenous reference gene in qualitative and real-time quantitative PCR detection of transgenic cottons, *Plant Cell Rep.* **2005**, *24*, 237–245.

[9] G. P. Fitt, Implementation and impact of transgenic Bt cottons in Australia – Cotton production for the new millennium, in *Proc. of the 3rd World Cotton Research Conf.*, Cape Town, South Africa, March **2003**.

[10] C. Hupfer, H. Hotzel, K. Sachse, K. H. Engel, Detection of genetically modified insect-resistant *Bt*-maize by means of polymerase chain reaction, *Z. Lebensm.-Unters.-Forsch. A.* **1997**, *205*, 442–445.

[11] G. Tapp, G. Stotzky, Dot blot enzyme-linked immunosorbent assay for monitoring the fate of insecticidal toxins from *Bacillus thuringiensis* in soil, *Appl. Environ. Microbiol.* **1995**, *61*, 602–609.

[12] Y. Takahashi, H. Hori, H. Furuno, T. Kawano, M. Takahashi, Y. Wada, Enzyme-linked immunosorbent assays for rapid and quantitative detection of insecticidal crystal proteins of Bt pesticides, *J. Pesticide Sci.* **1998**, *23*, 386–391.

[13] H. Hori, Y. Takahashi, M. Takahashi, Y. Wada, Detection of the *Bacillus thuringiensis* serovar *japonensis* strain *Buibui protoxin* with enzyme-linked immunosorbent assay and its application to detection of the protoxin in soil, *Appl. Entomol. Zool.* **2000**, *35*, 401–411.

[14] S. R. Sims, S. A. Berberich, *Bacillus thuringiensis* CryIA protein levels in raw and processed seed of transgenic cotton: determination using insect bioassay and ELISA, *J. Econ. Entomol.* **1996**, *89*, 247–251.

[15] R. I. Vazquez, D. de la Prieto, G. A. Riva, G. Selman-Housein, Development of immunoradiometric assay for quantitative determination of Cry1A(b) in transgenic sugarcane plants, *J. Immunol. Methods* **1996**, *196*, 33–39.

[16] B. Bugler, M. Caizergucs-Fetter, G. Bouche, H. Bourbon, F. Alamric, Detection and localization of a class of proteins immunologically related to a 100 kDa nucleolar protein, *Eur. J. Biochem.* **1982**, *128*, 475–480.

[17] C. Palm, J. K. Donegan, D. Harris, R. J. Seidler, Quantification in soil of *Bacillus thuringiensis* var. *kurstaki* δ-endotoxin from transgenic plants, *Mol. Ecol.* **1994**, *3*, 145–151.

DOI: 10.1002/elsc.200620179

Research Article: Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects. In this paper, the obtainment of Cry1Ac protein from *Bacillus thuringiensis* HD-73 and the acquisition of antibodies are described. The developed ELISA method is applied to determine five different transgenic and one traditional Bt-cottons.

Development of ELISA for the Determination of Transgenic Bt-Cottons Using Antibodies against Cry1Ac Protein from *Bacillus thuringiensis* HD-73

S. Wang*, A. Y. Guo, W. J. Zheng, Y. Zhang, H. Qiao, and I. R. Kennedy

Eng. Life Sci. **2007**, *7 (2)*, XXX ... XXX