

Development of Monoclonal Antibodies Against Cry1Ab Protein from *Bacillus thuringiensis* and Their Application in an ELISA for Detection of Transgenic Bt-Maize

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Several transgenic insect resistant maize varieties, commonly referred to as Bt-maize, express the gene for the *Bacillus thuringiensis* toxin Cry1Ab. As a method for the determination of Bt-maize, a sandwich-type ELISA for Cry1Ab was established. Monoclonal antibodies were developed by immunization of mice with trypsin-activated Cry1Ab. The secondary antibody was labeled with horseradish peroxidase, and 3,3',5,5'-tetramethylbenzidine (TMB) was used as chromogene. The detection limit was 0.4 ng ml⁻¹ of Cry1Ab. No (Cry1Ac, Cry1C, Cry2A, Cry3A) or only little (Cry9C) cross-reactivity with other Cry proteins was observed. Furthermore, the assay was applied to protein extracts from maize powder containing different amounts of Bt-maize from three different lines (Bt-176, Bt-11 and MON810). Using 100 mg maize per sample and Tris-borate buffer for rapid protein extraction, the minimum detectable mass fraction was 0.10% for Bt-11 and 0.23% for MON810. In contrast, Bt-176 maize could not be detected due to the fact that the Cry1Ab gene is not expressed in seed tissue of Bt-176 maize.

Keywords: Cry1Ab, *Bacillus thuringiensis*, monoclonal antibodies, ELISA, transgenic Bt-maize

INTRODUCTION

The Gram-positive soil bacterium *Bacillus thuringiensis* produces inclusion bodies during sporulation which contain crystalline proteins (delta-endotoxins) with a potent and specific toxicity against different insect species (Höfte & Whiteley, 1989; Schnepf *et al.*, 1998). These proteins act as protoxins which are processed by the insects to toxins after ingestion. The toxic effects are based on the binding of the toxins to specific receptors in the insect midgut

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membrane, resulting in the formation of pores and subsequently an alteration of the osmotic balance, ultimately leading to death (Brousseau & Masson, 1988; English & Slatin, 1992; Schnepf *et al.*, 1998). Since the toxicity of the delta-endotoxins is restricted to insects, *B. thuringiensis* is an attractive agent for pest control in agriculture. Respective formulations of *B. thuringiensis* and its toxins have therefore been used worldwide since many years as safe biopesticides (Siegel, 2001).

In recent years, the genes for the crystalline proteins were successfully transferred by genetic engineering into the genome of different plants to develop insect resistant plant varieties for agriculture (Van Rie, 2000). For human and animal nutrition, the most widely adopted of these plants is maize. A number of transgenic insect resistant maize lines, commonly referred to as Bt-maize, was approved and commercialized for food and feed production in the USA, Japan, Argentina and some other countries (Betz *et al.*, 2000). In contrast, up to now the adoption of these plants in the countries of the European Union (EU) is negligible (Fulponi, 2000). The use of genetically modified organisms (GMO) for agriculture and food production in the EU is regulated by national law and European guidelines, of which the regulation No. 258/97 (EC), also known as the 'Novel Food Regulation', is most important (EU, 1997). Since July 2001 a revised regulation is pending the approval of the European Parliament and the European Council (EU 2001). Both the 'Novel Food Regulation' as well as the newly proposed regulation require the labeling of food products which contain GMO. As an approach for the practice, the new regulation proposal from July 2001 provides a threshold level of 1% for the GMO content of food. A GMO content above this threshold would require the labeling of the respective product, while a content below this threshold is considered an unpreventable contamination. A similar approach based on limit values is considered in other countries as well.

To enforce such regulations, reliable methods for the detection and quantification of GMO in plants and food products are needed. At present, the method used most frequently is the amplification of GMO-specific DNA by polymerase chain reaction (PCR) followed by an identification of its specific amplification products, for example by agarose gel electrophoresis, restriction fragment length analysis, southern blot hybridization or DNA sequencing (Meyer, 1999; Anklam *et al.*, 2002). The main advantage of DNA detection methods is their high sensitivity. On the other hand, these methods require a well-equipped laboratory and a careful adaptation of the method to the specific equipment by experienced investigators to optimize the results (Hübner *et al.*, 1999). Furthermore, PCR-based methods are time consuming and difficult to quantify.

Alternatively, immunoassays can be used for the detection and quantification of GMO based on the determination of the protein product of the newly introduced gene. In the case of Bt-maize, most of the lines which are currently approved and commercially used for food production contain the gene for the *B. thuringiensis* toxin Cry1Ab, thus expressing this protein in their tissue. A number of ELISAs for different *B. thuringiensis* toxins has been developed for the determination of these toxins in soil (Tapp & Stotzky, 1995; Hori *et al.*, 2000), commercial *B. thuringiensis* formulations (Takahashi *et al.*, 1998) and transgenic cotton (Sims & Berberich, 1996). All of these assays use polyclonal antibodies (PABs) raised either in rabbits (Tapp & Stotzky, 1995; Takahashi *et al.*, 1998; Hori *et al.*, 2000) or goats (Sims & Berberich, 1996). While PABs are easy to obtain in sufficient quantities, the amount of a specific polyclonal antiserum is nonetheless restricted by the limited availability of serum from a specific host animal, because antisera from different host animals may vary in their characteristics. Furthermore, PABs are a mixture of immunoglobulins with many different epitope specificities. In contrast, monoclonal antibodies (MAbs) are specific to a single epitope of their antigen and can be produced at virtually unlimited quantities. Additionally, all of the assays which are mentioned above deal with non-food relevant applications. An immunoradiometric assay for the quantification of Cry1Ab in transgenic sugarcane plants was developed (Vazquez *et al.*, 1996), but ELISA methods are usually favored by most investigators due to the potential health risks and waste disposal problems of radiometric

assays. Therefore, the aim of this study was to develop a set of monoclonal antibodies to Cry1Ab for use in a sandwich type ELISA, and the application of this assay to the detection and quantification of Bt-maize.

MATERIALS AND METHODS

Chemicals

Lyophilized purified trypsin-activated Cry1Ab protein for the immunization and the development of the antibodies was kindly provided by Luke Masson, Bt Project Leader, Biotechnology Research Institute of the National Research Council of Canada, Montreal (Masson *et al.*, 1989). Positive controls for six different Bt proteins (Cry1Ab, Cry1Ac, Cry1C, Cry2A, Cry3A and Cry9C), containing 80 ng lyophilized protein per control, were purchased from Agdia, Inc. (Elkhart, USA). Candidate reference materials (CRM) for the Bt-maize varieties Bt-176 (IRMM-411), Bt-11 (IRMM-412) and MON810 (IRMM-413), consisting of maize powder with 0%, 1% and 2% (w/w) genetically modified maize of the respective line (Kramer *et al.*, 2001), were obtained from the Institute for Reference Materials and Measurements (Geel, Belgium). Azaserine, bovine serum albumin (BSA), glutamine, hypoxanthine, incomplete Freund's adjuvant, 2-mercaptoethanol, penicillin, polyethylene glycol/dimethylsulfoxide (PEG/DMSO) solution, RPMI 1640, sodium pyruvate, streptomycin, thymidine, TiterMax and tris[hydroxymethyl]aminomethane (Trizma base) were purchased from Sigma (Taufkirchen, Germany). 3,3',5,5'-tetramethylbenzidine (TMB) and Tween-20 were supplied by Merck (Darmstadt, Germany). Fetal calf serum (FCS) was obtained from Seromed (Berlin, Germany), and gentamycin was purchased from Biochrom (Berlin, Germany). Blocking buffer, a horseradish peroxidase-labeled antibody to mouse immunoglobulin for antibody screening and an immunoglobulin subclass typing kit were obtained from Roche Diagnostics (Berlin, Germany). All other reagents were of reagent grade or better and were supplied either by Sigma or Merck.

Equipment

A Columbus microplate washer and a SLT Spectra II microplate reader, both from SLT Labinstruments (Crailsheim, Germany), were used to perform the Cry1Ab sandwich ELISA. Further laboratory equipment includes a Biofuge pico centrifuge from Heraeus Instruments (Osterode, Germany) and a Vortex Genie 2 mixer from Scientific Industries, Inc. (Bohemia, USA).

Other Materials

Maxisorp F96 respectively F8 microtiter plates from Nalge Nunc International (Roskilde, Denmark) were used for the ELISA. PD-10 desalting columns for buffer exchange were obtained from Amersham Pharmacia Biotech (Freiburg, Germany). For the purification of antibodies from ascites fluid, a HiTrap Protein G HP column with a bed volume of 1 ml (Amersham Pharmacia Biotech) was used.

Buffers and Solutions

The following buffers were used: (a) sodium carbonate buffer (SCB), 50 mM, pH 9.6, for coating the microtiter plates; (b) phosphate-buffered saline (PBS), 50 mM, pH 7.4, for purified antibody preparations and to dilute toxin controls and antibody conjugates; (c) PBS, 8 mM, containing 0.5% (v/v) Tween-20 (PBS-T) as washing buffer for microtiter plates; (d) Tris-borate buffer (TBB), pH 7.5, containing 100 mM Tris-HCl, 10 mM boric acid, 0.05% (v/v) Tween-20, 5 mM magnesium chloride and 0.2% (w/v) L-ascorbic acid for protein extraction from maize powder (Sims & Berberich, 1996). TMB solution was used as enzyme substrate for the horseradish peroxidase (HRP) and prepared as follows: 400 µl of a stock

solution (30 mg TMB in 5 ml DMSO) were diluted in 25 ml sodium acetate citric acid buffer (pH 5.5), and 3.3 μ l hydrogen peroxide (30%) was added.

Immunization

Three eight-weeks-old female Balb/e mice were intraperitoneally, intradermally and subcutaneously immunized three times at fortnightly intervals with 50 μ g Cry1Ab protein and 100 μ l TiterMax for first immunization or incomplete Freund's adjuvant for second and third immunization. On day 37 as well as one day prior fusion (day 40) the mouse with the highest antibody titer in serum received a booster of 50 μ g Cry1Ab in 100 μ l PBS intravenously.

Hybridoma Cells

Medium. RPMI 1640 containing 10% (w/v) FCS was supplemented with 2 mmol l⁻¹ glutamine, 1 mmol l⁻¹ sodium pyruvate, 100 000 U l⁻¹ penicillin, 100 mg l⁻¹ streptomycin, 100 mg l⁻¹ gentamycin and 5 mmol l⁻¹ 2-mercaptoethanol. The standard medium was used for growing of myeloma and hybridoma cells.

Myeloma cell line. The HAT sensitive Balb/c mouse myeloma cell line SP2/0-Ag14 (Ozato & Sachs, 1981) obtained from the cell bank of the Federal Research Centre for Virus Diseases of Animals (Isle of Riems, Germany) was used in fusion experiments.

Hybridoma generation. One day after the last immunization, the mouse with the highest antibody titer was killed and its spleen was obtained aseptically. For the fusion procedure 5×10^7 splenic lymphocytes were incubated with 2×10^7 mouse myeloma cells and 1 ml PEG/DMSO for 30 s according to the method of Lane (1985) as detailed previously (Witt *et al.*, 1991). The fused cells were aliquoted into five 96-well microculture plates and incubated with 1×10^4 mouse peritoneal cells per well as feeder layer for 24 h. The cells were grown in standard medium supplemented with 10% (w/v) FCS, 10^{-4} mol l⁻¹ hypoxanthine and 5×10^{-6} mol l⁻¹ azaserine as selection reagents. After two weeks the cells were cultivated in standard medium supplemented with 10% (w/v) FCS, 10^{-4} mol l⁻¹ hypoxanthine and 1.6×10^{-5} mol l⁻¹ thymidine. One week later the hybridoma cells were grown in standard medium.

As soon as the hybrid cells grew semiconfluently (according after two weeks) the hybridoma culture supernatant was assayed for antibodies reacting with Cry1Ab by ELISA. Cells from positive wells were twice cloned by limiting dilution and were grown by mass culture.

Antibody screening. ELISA plates were coated with 3 μ g ml⁻¹ Cry1Ab in SCB overnight at 4°C. After washing twice with PBS-T the plates were blocked with blocking buffer, washed twice with PBS-T and incubated with cell culture supernatant at room temperature by shaking for one hour. After washing twice, the plates were incubated with HRP-labeled antibody to mouse immunoglobulin, washed three times and incubated with TMB solution. After 15 min, the reaction was stopped with 2 N-sulfuric acid. The color intensity of the product was measured with a microplate reader from Berthold Technologies (Bad Wildbad, Germany) at 450 nm.

Preparation and Labeling of the Antibodies

Three clones (3A11C3, 2C4G11, 3E7E9) with a high growth rate and high antibody titers in the culture supernatant were selected for antibody production by ascites. The antibodies were

purified from ascites fluid by affinity chromatography using a protein G column. Determination of the immunoglobulin isotype was performed using a subclass typing kit. All three clones produced antibodies of the IgG1 isotype. The antibodies were conjugated to horseradish peroxidase using EZ-Link™ Plus activated peroxidase (an amine-reactive HRP derivative) from Pierce (Rockford, USA). All chemicals which were used for the coupling reaction were supplied ready-made by Pierce. Briefly, the purified MAb was buffer exchanged into 0.2 M carbonate buffer and diluted to a concentration of 1 mg ml⁻¹. 1 mg of lyophilized activated peroxidase was reconstituted with 100 µl water and added to 1 ml of the MAb preparation. The reaction mixture was incubated for 1 h at room temperature. Subsequently 10 µl of a 5 M sodium cyanoborohydride solution were added to stabilize the coupling between the HRP and the IgG, and the mixture was incubated for another 15 min. This step was followed by the addition of 20 µl 3 M ethanolamine and an additional 15 min of incubation. The resulting conjugate was buffer exchanged into PBS. BSA was added to the conjugate solution at a concentration of 10 mg ml⁻¹, and the solution was subsequently filtered through a 0.45 µm filter. Finally, the conjugate was frozen in aliquots at -20°C.

By crosswise testing of the three antibodies in a sandwich-type ELISA, clone 3E7E9 was selected as primary antibody and 2C4G11 as secondary antibody for further studies (data not shown).

Protein Extraction from Maize Powder

100 mg of maize powder containing different amounts of genetically modified maize (CRMs IRMM-411, IRMM-412 and IRMM-413) were thoroughly mixed with 500 µl TBB using a Vortex mixer and incubated for 10 min at room temperature while shaking the samples by hand. After the incubation, the samples were centrifuged at 13 000 rpm for 10 min to remove insoluble material from the supernatant. The liquid protein extract was used in the assay.

Microplate ELISA

In all procedures, washing of the microtiter plates was performed by applying 300 µl PBS-T per well three times using an automated plate washer. The microplates were coated with 100 µl of a 1:5000 dilution of the primary antibody in SCB and incubated at 4°C overnight. After washing the plates, blocking of residual protein binding sites was performed at 37°C for 1 h with 200 µl of BSA diluted in SCB at a concentration of 1% (w/v). After the blocking step, the plates were washed again.

For the assay, samples of 100 µl (either toxin controls or protein extracts from maize powder) were pipetted into each well and incubated for 2 h. After washing the plates, 100 µl of the secondary antibody conjugated to HRP was pipetted into each well at a dilution of 1:1000 and incubated for 1 h. The plates were washed again, and 100 µl TMB solution was added to each well. After 30 min, the reaction was stopped with 4 N-sulfuric acid. The color intensity of the reaction product was measured with a microplate reader at a wavelength of 450 nm.

RESULTS

Sandwich-ELISA for Cry1Ab Protein

To determine the limit of detection for Cry1Ab protein, an assay was performed with samples containing decreasing concentrations of Cry1Ab (160; 80; 40; 20; 10; 5; 2.5; 1.25; 0.63; 0.31 ng ml⁻¹; obtained by serial 1:1 dilution) diluted in PBS from a lyophilized Cry1Ab control. PBS was used as a blank control. Three samples for each concentration were analyzed. The results are shown in Figure 1. From these results, the limit of detection was determined as 0.4 ng ml⁻¹ with a linear range from approximately 1 to 40 ng ml⁻¹ protein (the limit of detection was calculated as average of the zero values ± three standard deviations of the zero values). The Cry1Ab concentration at 50% of the HRP activity was determined as 7.7 ng ml⁻¹.

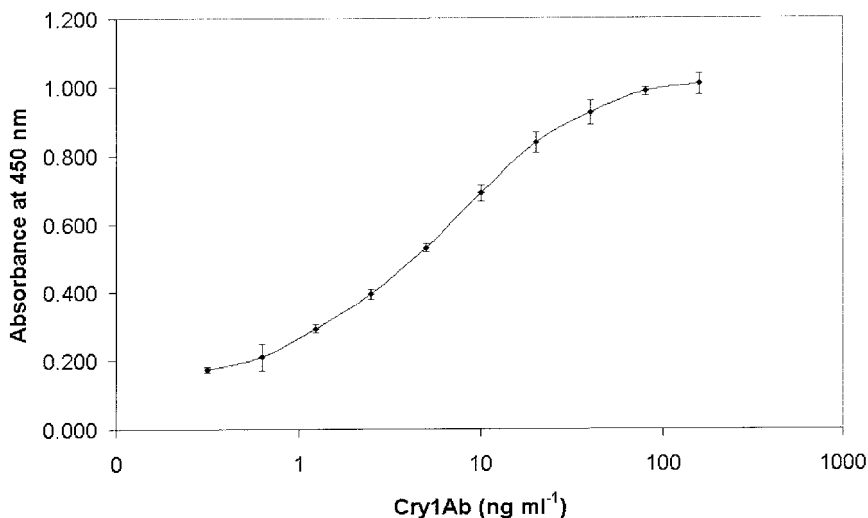


FIG. 1. Determination of the Cry1Ab concentration by ELISA; the 2-fold standard deviations ($\pm 2s$, representing 95% confidence intervals) are indicated as error bars.

Cross-reactivity with Other Cry Proteins

Lyophilized protein controls of five different Bt toxins (Cry1Ac, Cry1C, Cry2A, Cry3A and Cry9C) were used to evaluate the cross-reactivity of the assay. The controls were reconstituted in PBS and diluted to concentrations of 80, 40, 20, 10, 5, 2.5, 1.25 ng ml⁻¹, and an assay according to the conditions outlined in the methods section was performed using these dilutions. In addition, PBS was used as blank control. The assay showed no correlation between the analyte concentration and the color intensity for Cry1Ac, Cry1C, Cry2A, and Cry3A up to a concentration of 80 ng ml⁻¹. The analyte concentration at 50% of the HRP activity was below the limit of detection for these toxins. A correlation was observed for Cry9C, and the Cry9C concentration at 50% of the HRP activity was 41.6 ng ml⁻¹. Based on this concentration, the cross-reactivity for Cry9C was determined as 18.5% with a limit of detection of 26.2 ng ml⁻¹. The results are shown in Figure 2.

Detection and Quantification of Transgenic Maize

Protein extracts from maize powder made from the transgenic maize varieties Bt-176, Bt-11 and MON810 were used to evaluate the suitability of the assay for the detection of transgenic maize. From each maize variety, samples containing 0%, 1% and 2% GM maize were analyzed, with three samples per GMO content level. In addition, samples of extraction buffer served as a blank control. The results as mean of three determinations are illustrated in Figure 3. Furthermore the limit of detection by means of GMO content was calculated from these results. The minimum detectable mass fraction of transgenic maize was 0.10% for Bt-11 maize and 0.23% for MON810 maize (calculated as average of the zero values $\pm$ three standard deviations of the zero values). In contrast, Bt-176 maize was not detectable with this assay.

DISCUSSION

The aim of this study was to develop MAbs against the insecticidal protein Cry1Ab from *B. thuringiensis* and to establish an ELISA based on these antibodies suitable for the detection and quantification of Bt-maize. Such an assay appears to be suitable for application to other

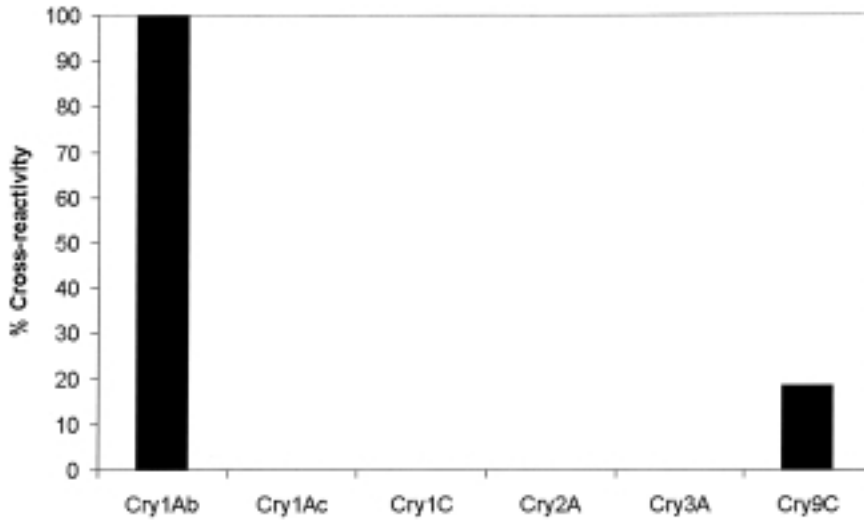


FIG. 2. Cross-reactivity of the assay with different Bt-proteins

fields of interest as well, for example the quantification of Cry1Ab levels in soil or in industrial biopesticides. But since a number of immunoassay-based methods was already published which deal with these specific areas (Tapp & Stotzky, 1995; Takahashi *et al.*, 1998; Hori *et al.*, 2000), only the application to food-related samples was studied.

The linear range of the assay is approximately 1 to 40 ng ml⁻¹ protein concentration and the detection limit for purified Cry1Ab protein was determined as 0.4 ng ml⁻¹. Since the limits of detection of other published immunoassays for Bt toxins are given in other units, for example ng toxin per microgram soluble protein (Vazquez *et al.*, 1996) or ng of toxin per g dry weight

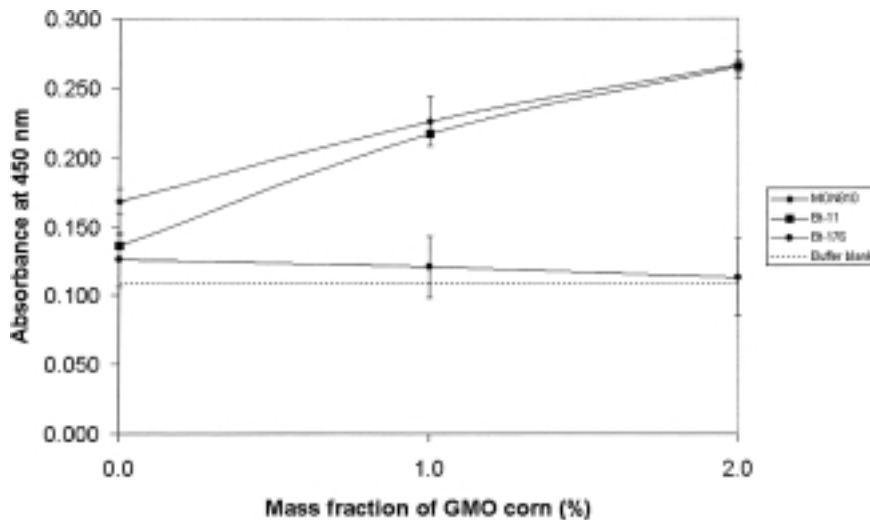


FIG. 3. Determination of the GMO content of maize samples from 3 different transgenic maize varieties; the 2-fold standard deviations ($\pm 2s$, representing 95% confidence intervals) are indicated as error bars.

soil (Palm *et al.*, 1994), they are difficult to compare. However, the detection limit of the assay is in the range which can be expected for an sandwich-type enzyme-linked immunoassay. Based on the determination of five different Cry proteins, the assay showed no cross-reactivity to Cry1Ac, Cry1C, Cry2A and Cry3A and 18.5% cross-reactivity to Cry9C. Although many more Bt toxins have been isolated and described (Crickmore *et al.*, 1998), the proteins which were selected for this study are of special interest in agriculture as biopesticides, particularly due to their use in transgenic plants (EPA, 2001). For example, they have been incorporated into maize (Cry1Ac, Cry9C), cotton (Cry1Ac, Cry2A) or potatoes (Cry3A). Since no or only little cross-reactivity was observed to any of the five tested proteins, the assay therefore provides a method for specifically detecting Cry1Ab.

Furthermore, the suitability of the assay for the detection of Bt-maize based on the determination of Cry1Ab in maize seed tissue was tested. When the assay was applied to protein extracts from maize seed powder, matrix effects could be observed as demonstrated by comparison between the values for the buffer blanks and the values for the maize powder extracts at 0% GMO content, and between the values for the maize powder extracts for the three different maize varieties at 0% GMO content. Comparable results for the blank sample and the 0% sample were observed by Stave (1999) in an ELISA for the detection of RoundUp Ready® soybean crops using IRMM reference materials for this soybean variety. For a quantitative determination of GMO in food and agricultural products it is therefore necessary to use matrix specific reference materials with defined amounts of GMO content. Similar conclusions were drawn by the authors of an interlaboratory study using an immunoassay for the detection and quantification of genetically modified soybean (Lipp *et al.*, 2000). The influence of the sample matrix on the results of immunochemical methods for analysis of food-related samples was also discussed by Anklaam *et al.* (2002). Possible reasons for such matrix effects include for example non-specific interactions of the antibodies with proteins, surfactants or phenolic compounds from the sample, the denaturation of the antibodies by fatty acids or the presence of endogenous phosphatases or enzyme inhibitors (Anklaam *et al.*, 2002). In the present study, the samples for the Bt-11 and the Bt-176 maize gave comparable background values at 0% GMO content, while the background value for the 0% samples of MON810 maize was considerably higher. This may be due to a modification in the procedure for the preparation of these reference materials (Kramer *et al.*, 2001). According to the documentation from the Institute for Reference Materials and Measurements, which manufactured these materials, finer powders and a different mixing procedure was used for the preparation of the MON810 samples. When the samples were compared visually after the centrifugation step in the extraction method, the supernatant in the Bt-11 and Bt-176 maize samples appeared clearer than in the MON810 maize samples. Thus it is possible that the higher background values in the 0% sample of MON810 maize are due to a higher content of very small powder particles remaining in the samples after the centrifugation step, as well as differences in the sample composition as the result of the different preparation procedures.

For the extraction of the Cry1Ab protein from maize seed powder, Tris-borate buffer with a pH of 7.5, containing Tween-20 as a detergent and L-ascorbic acid was used as published by Sims and Berberich (1996). Tween-20 and ascorbic acid at comparable concentrations were also used by Palm *et al.* for the extraction of *B. thuringiensis* var. *kurstaki* toxin from soil (Palm *et al.*, 1994). According to their results, the addition of ascorbic acid specifically reduced the background reading in the ELISA when extracts from samples containing plant material were analyzed. Initially, sodium carbonate buffer (50 mM, pH 9.6) with 0.1% Tween-20 was used for protein extraction in this study. Compared to the extraction with Tris-borate buffer, this resulted in higher background values in the buffer blanks as well as in the samples with 0% GMO content and a higher limit of detection (data not shown). Therefore, Tris-borate buffer as outlined in the Methods section was used for the extraction of Cry1Ab protein from the maize samples. It should be noted that the rapid protein extraction which was used in this study is only applicable for a quantification by means of GMO content using

matrix-matched reference materials. If a quantification of the Cry1Ab protein concentration in plant tissue is necessary, more sophisticated extraction methods are probably needed to achieve optimal protein recovery from the samples. On the other hand the level of Cry1Ab protein in plant tissue and processed samples depends on a number of plant-specific and environmental factors like differences in the gene expression in different tissue types and maize lines as well as the specific storage conditions and processing steps. Since samples with the same mass fraction of Bt-maize may differ significantly in their content of Cry1Ab protein depending on these factors, a determination of the GMO content of agricultural and food samples requires the use of matrix-matched reference standards as discussed before. Therefore, the rapid protein extraction method which was used is sufficient and suitable for quantification by means of GMO content if such reference materials with defined GMO content are used as controls.

Using the assay, no transgenic maize was detectable in the Bt-176 maize samples. This is consistent with the fact that the Cry1Ab gene is not expressed in the seed tissue of this variety. Thus the application of the assay could give false-negative results if Bt-176 maize is actually present in given samples. Since the US registration for Bt-176 maize expired in 2001 and will not be extended (EPA, 2001), it can be expected that Bt-176 maize will be out of commercial use in the USA in the near future. Furthermore, the US registration for Cry1Ac maize was voluntarily cancelled by the manufacturer in 1998. In addition the US registration for Cry9C maize, which was only approved for industrial and feed use, was cancelled in 2001 (EPA, 2001). In the future, it can be expected that the registration of transgenic plants in the European Union and other countries will closely resemble the registration of these plants in the USA. This leaves the lines MON810 and Bt-11 as the only Bt maize varieties which are currently approved for food production. Both lines can be detected by the assay in maize seed powder as demonstrated by the results. The limits of detection for both varieties, 0.10% for Bt-11 and 0.23% for MON810, are sufficient for detecting these maize lines at a threshold level of 1% as outlined in the 2001 proposal for a revised EC regulation. The difference in the detection limit between Bt-11 and MON810 maize is probably due to differences in the preparation of the samples as mentioned above as well as differences in the gene expression in these plants.

In conclusion, an ELISA for the detection and quantification of the *B. thuringiensis* toxin Cry1Ab was developed. Furthermore, it was demonstrated that this assay can be used for the determination of the GMO content of maize powder made from transgenic Bt-maize. For further characterization of the assay, the evaluation of a greater range of samples from different food products varying in their preparation methods, as well as a validation using established reference methods are needed.

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