Research Note

Analysis of Flour and Food Samples for cry9C from Bioengineered Corn

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ABSTRACT

StarLink corn is a variety of yellow corn that has been genetically modified by the insertion of an altered cry9C gene into the plant genome, resulting in expression of the insecticidal Cry9C protein. The U.S. Environmental Protection Agency has approved StarLink corn for use in animal feed but not in food intended for human consumption. Therefore, under the U.S. Food, Drug, and Cosmetic Act, any food intended for human consumption in which the presence of StarLink corn is indicated by the presence of either the Cry9C protein or the cry9C gene would be considered adulterated. Extraction and PCR-based methods were used to detect the presence of the cry9C DNA initially in corn flour and corn meal, and then these methods were extended to the analysis of processed corn products, including taco shells, cereals, baby foods, party snacks, and chips, for the presence of this modified genetic material. In a survey of 63 products, the cry9C transgene was detected in 4 taco shells.

Cry9C is one of over 60 members of the Cry protein family. The Cry proteins are natural toxins produced by strains of Bacillus thuringiensis and are notable for their potent insecticidal activity. Other members of the Cry protein family have been approved by the U.S. Environmental Protection Agency for use on food crops both by spraying the toxin onto crops and by expression of chimeric genetic constructs within the plant. Crops containing cry transgenes, including corn and soybeans, are widely planted in the United States. Aventis CropScience isolated Cry9C from B. thuringiensis and found that its toxicity was mediated through a receptor other than the receptor that was found to mediate the toxicity of previously studied Cry proteins (8). In addition, Cry9C was shown to target a wider variety of pests. The insecticidal activity of Cry9C was further improved during cloning through the removal of a proteolytic cleavage site associated with inactivation in the insect gut (8). The resulting protein was also more resistant than were previously approved Cry protein insecticides to breakdown by heat and digestion under conditions similar to those in the human intestinal tract (1). Aventis CropScience used this genetic construct to develop StarLink corn, a variety of yellow corn that expresses the pesticide Cry9C throughout the plant.

In 1998, the U.S. Environmental Protection Agency approved StarLink corn for animal feed (2) but not for human consumption because of concern over the potential allergenicity of the genetically modified and artificially expressed Cry9C protein (1). Therefore, under the U.S. Federal, Drug, and Cosmetic Act, the presence of either the Cry9C protein or the cry9C gene in any food intended for human consumption renders that food adulterated. On 17 September 2000, a private testing company publicly claimed that the genetic material encoding Cry9C was present in taco shells distributed in retail markets nationwide (3). After a second, independent, laboratory confirmed the presence of cry9C in these taco shells, the manufacturer issued a voluntary recall to remove the product from grocery shelves (6). The finding of cry9C in taco shells immediately raised the possibility that Cry9C and cry9C might be present in other foods as well.

Two strategies, one to detect proteins and the other to detect DNA, are available for the analysis of genetically modified foods such as StarLink corn (5, 7). Antibody-based methods (enzyme-linked immunosorbent assay and a lateral flow strip test) are used to detect protein molecules, while the polymerase chain reaction (PCR) targets the gene that encodes the protein. Immunological methods tend to be faster and less expensive than PCR methods. However, because it can amplify a specific gene construct, the PCR may be more sensitive and specific.

Detection of DNA or proteins associated with bioengineered plants is impeded by degradation of the target during food processing and by interference from other components of the food matrix. The use of PCR primers designed to amplify small, unique regions of the transgene can reduce problems associated with DNA degradation. However, the presence of food-derived ingredients (e.g., oils) in processed foods that may inhibit PCR amplification remains a major concern. The relative sensitivity of the PCR and antibody-based tests for the detection of StarLink...
corn in processed food products has not been firmly established.

Soon after the announcement of the recall of taco shell products by Kraft, the Center for Food Safety and Applied Nutrition at the U.S. Food and Drug Administration initiated a program to analyze yellow corn flour, corn meal, and taco shells. While Aventis CropScience provided reference materials and PCR primer sequences, no validated method for StarLink corn detection was available from Aventis or from any other source. PCR analysis confirmed that the cry9C genetic material was present in Kraft taco shells. Since then, over 60 different food products made from yellow corn have been analyzed for presence of the cry9C gene.

MATERIALS AND METHODS

DNA extraction of samples. Each sample was placed in a resealable zip-lock plastic bag. This bag was then placed in a second bag to allow the sample to be crushed without creating cross-contaminating aerosols. A rolling pin was used to crush and powder the material. Powdered samples (100 mg) were transferred to 1.5-ml microcentrifuge tubes. Each sample was paired with a duplicate sample to which 10 mg of StarLink flour was added (cry9C spike). The spiked sample served as a means to evaluate the inhibition of PCR due to material extracted from the food sample. An open tube was present during sample crushing, weighing, and transfer to monitor for cross-contamination. DNA was extracted by the addition of 860 μl of TNE buffer (10 mM Tris [pH 8.0], 150 mM NaCl, 2 mM EDTA [pH 8.0], 1% SDS), 100 μl of guanidine-HCl (3 M; Promega Corp., Madison, Wis.), and 40 μl of proteinase K (20 mg/ml; Promega). The contents were mixed in a vortex mixer, and the tubes were then incubated at 56°C for 3 h. The contents were mixed by inversion every 30 min. Samples were clarified by centrifugation at 14,000 rpm for 15 min at room temperature, and 500 μl of extract was applied to a DNA purification column (Wizard PCR Prep DNA Purification System, Promega). The purified DNA template was then eluted with 100 μl of nuclease free H₂O (molecular biology grade; Quality Biological, Inc., Gaithersburg, Md.) at 70°C according to the manufacturer’s instructions. Extraction of 5-g samples was performed with a DNA purification system employing magnetic beads (Wizard Magnetic DNA Purification System for Food, Prom-
<table>
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<th>Food item</th>
<th>Brand name</th>
<th>PCR detection results</th>
<th>cry9C-spiked control</th>
<th>Corn aldolase control</th>
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* Samples were collected from retail markets in and around Washington, D.C., over a 3-month period. Food items for which multiple analyses were performed were derived from different production dates or lot numbers.

The amount and integrity of the extracted DNA was analyzed by gel electrophoresis. PCR. PCR conditions and primer sequences used in this study were similar to those used by Aventis CropScience (9). Extracts were analyzed by PCR with reagents from Promega Corp. Each reaction contained 5 μl 10× thermophilic DNA polymerase buffer, 2.5 μl 25 mM MgCl2, 1 μl 10 mM PCR nucleotide mix, 0.4 μM each primer (see below), 1 U of Taq DNA polymerase in storage buffer A, 5 μl of DNA template, and 5 μl of molecular biology grade water to a final volume of 50 μl. Each series of reactions contained a PCR reagent blank in which 5 μl of molecular biology grade water was substituted for the DNA template to serve as a negative control. Reactions were executed in 0.65-μl thin-walled tubes with a PTC200 thermal cycler (MJ Research, Waltham, Mass.) with a heated lid. An initial denaturation step of 4 min at 95°C was followed by five cycles of 1 min at 95°C, 1 min at 57°C, and 2 min at 72°C, which were followed by an additional 30 cycles of 30 s at 92°C, 30 s at 57°C, and 1 min at 72°C. Reactions were completed after an additional 5 min at 72°C.

Two primer pairs were used in parallel reactions. One pair was specific to the transgenic construct of StarLink, and one pair targeted the multicopy corn aldolase gene. This latter set of reactions was used as a positive control for the presence of corn DNA.

Agarose gel electrophoresis. Electrophoresis was performed with the Run-One gel system (Embi-Tec, San Diego, Calif.) in 0.5× Tris-acetate EDTA (TAE) buffer (pH 8.3; Promega) using 55-mm-long agarose gels with 3-mm wells. Isolated genomic DNAs (8 μl) and PCR products (10 μl) were analyzed with 0.8% and 1.5% agarose gels, respectively, containing 0.2 μg/ml ethidium bromide. A 100- or 25-bp DNA ladder was used as the molecular size standard (Promega), and Orange-G (Mallinckrodt, Phillipsburg, N.J.) was used as a loading dye. The expected PCR amplicon sizes were 174 bp for the cry9C transgene and 172 bp for the amplified aldolase gene. PCR products were visualized and recorded with a FOTO/Analyst documentation system (Fotodyne, Harland, Wis.).

Confirmation by restriction digestion. The specificity of the amplification product in PCR reactions targeting the cry9C transgene was confirmed by restriction digestion of the PCR product. The 174-bp product (15 μl of PCR reaction mixture) was combined with 10 U of NcoI (New England Biolabs, Beverly, Mass.) together with 2 μl of 10× buffer D in a total volume of 20 μl at 37°C for 2 h. These products were analyzed on a 5% NuSieve agarose (3:1 agarose; FMC BioProducts, Rockland, Maine) gel in 1× TAE buffer with the Run-One electrophoresis system with a 25-bp DNA ladder (GIBCO-BRL, Rockville, Md.) as the size standard. The expected restriction pattern from digesion of the cry9C transgene amplicon was two bands of 128 and 46 bp, respectively. While the smaller band was rarely visible, the larger band ran at a location distinctly different from that of the uncut 174-bp starting product.

FIGURE 3. Confirmation of cry9C PCR amplification by NcoI restriction endonuclease digestion and nested PCR. (A) Restriction endonuclease digestion of PCR amplicons with NcoI. The molecular weight marker is a 25-bp ladder. (B) Nested PCR amplification of the primary cry9C amplicon.
DNA sequence analysis. Amplicons were sequenced with both amplification primers by a contract laboratory. Primers and nucleotide phosphates were removed from the PCR reactions with exonuclease I and shrimp alkaline phosphatase.

Nested PCR. A third method of confirmation was nested PCR. The product of the initial PCR amplification of the cry9C transgene (3 μl) was either used neat or diluted 1:100 and amplified without further purification. Reaction conditions were as described above except that MgCl₂ was increased to 4 mM and the reaction times and temperatures were 2 min at 95°C followed by 30 cycles of 30 s at 92°C, 30 s at 58°C, and 30 s at 72°C, followed by an additional 5 min at 72°C. Reaction products were analyzed by agarose gel electrophoresis for a 111-bp product.

RESULTS AND DISCUSSION

Flour, meal, and processed foods made from yellow corn or its derivatives were analyzed for adulteration by StarLink corn. Samples were crushed and extracted to isolate DNA, which was then purified and tested for the presence of modified cry9C DNA by PCR. For most samples, the extraction protocol provided DNA that was suitable for PCR. Comparable quantities of DNA were routinely and reproducibly isolated between replicate extractions of the same commodity. However, the amount and quality of the DNA obtained varied greatly with the type of product being analyzed. Food processing conditions known to influence DNA extraction include the milling process (wet or dry milling), the steeping techniques, and the processing conditions used to manufacture each individual type of food (4). Conditions such as high temperature or extreme pH can degrade DNA as a template for PCR, as indicated by a low-molecular-weight product seen in an agarose gel electrophoretic analysis of a sample. In our study, examination of purified DNA from food extracts by agarose gel electrophoresis (Figs. 1 and 2A) indicated varying degrees of DNA degradation. In most instances, however, the extraction and isolation procedure delivered sufficient DNA of >400 bp that was suitable for use as a template in PCR analysis, as indicated by the successful PCR amplification of the corn aldolase gene.

Whereas 0.1 g of most of the foods sampled was sufficient to yield DNA for PCR analysis, extracts from corn flakes and cornstarch did not produce detectable levels of DNA. Moreover, increasing the amount of product extracted to 5 g and using an alternative DNA purification system employing magnetic beads did not substantially improve the DNA yield. Thus, the presence or absence of StarLink could not be determined for these products.

PCR analysis relied on the specificity of the primers to detect the cry9C transgene associated with StarLink. A representative PCR analysis series is shown in Figure 2. One primer used was in the region 5’ of the coding sequence for the gene, annealing to regulatory DNA sequences derived from sources other than bacteria or maize. The other primer targeted the coding region for the modified cry9C gene. Thus, successful amplification relies on the proximity of these sequences, from disparate sources, to one another in the extract being analyzed. However, because of the presence of inhibitors and the potential for low target concentration, the PCR contained several cycles with relatively “relaxed” PCR conditions, increasing the possibility of amplification events, including amplification of an inappropriate target. To exclude nonspecific amplification events, all potentially positive samples were subjected to a confirmatory test as described below.

PCR amplification of test samples, spiked samples, and control samples revealed the presence or absence of the cry9C transgene (Fig. 2). Of the 63 products analyzed, only four samples contained the target transgene, as seen by the resulting 174-bp ampiclon obtained with cry9C specific primers (Table 1). All of the samples that were found to contain the cry9C transgene were from Taco Bell taco shells, provided by Kraft. This report does not include the results of larger surveys conducted at other Food and Drug Administration facilities. No amplification of the aldolase gene occurred during analysis of corn flake and cornstarch samples, and therefore no genomic DNA analyses were conducted for these products. One baby food product failed to produce a PCR product when spiked with StarLink flour, suggesting the presence of inhibitors that would prevent detection of the modified cry9C transgene.

To provide an estimate of the limit of detection of the cry9C transgene using this technique, we analyzed mixtures of non-StarLink corn flour spiked with StarLink flour. Samples containing 0.1% (100 μg/100 mg sample) StarLink flour always yielded a distinct 174-bp band on an agarose gel. Samples containing 0.01 to 0.075% StarLink flour were detected in some experiments and not in others (data not shown). It is unclear how to extrapolate this result to estimate the limits of detection of cry9C in the processed food products analyzed.

Sequencing of putative PCR-positive ampiclons provides the most definitive confirmation of specific amplification. However, this approach is time-consuming and costly for large-scale surveys. During the early phase of our investigations, restriction endonuclease digestion was used as the confirmatory test (Fig. 3A). NcoI digestion of PCR products of the cry9C transgene yielded two fragments, one of 128 bp and one of 46 bp. It is unlikely that the 6-nucleotide recognition sequence would be present in such a short piece of DNA randomly amplified during PCR. However, some PCR products were not suitable for restriction fragment length polymorphism analysis. For example, some samples contained faint bands that might have been ampiclons but were too faint to be expected to generate a visible product on agarose gels after dilution and restriction analysis.

To provide a more robust confirmatory test, nested PCR primers based on the sequence of the PCR product were developed. These primers, like the original ones, hybridized to both coding and regulatory DNA sequences and were specific to the chimeric nature of the cry9C transgene. Agarose gel electrophoresis analysis of positive control samples subjected to nested PCR revealed multiple bands. The amplified products were generated as a result of the presence of not only the nested PCR primers but also the original primers carried over from the initial PCR amplification. The extraneous bands were eliminated by dilution.
of the template before PCR, thus reducing the concentration of the primers used in the initial PCR reaction. Dilution of a positive control sample 10,000-fold still produced a distinct 111-bp band with nested PCR (see Fig. 3B), indicating the sensitivity of the test.

Use of the nested primers allowed the definitive rejection of the hypothesis of potential StarLink adulteration in several samples with faint bands seen by agarose gel electrophoresis analysis of the initial PCR products. While the use of nested PCR may increase the overall sensitivity of the assay, no samples analyzed were found to be adulterated solely on the basis of the results of the nested PCR reactions.

ACKNOWLEDGMENTS

The authors thank Marc De Beuckeleer for his assistance and technical expertise and Larry Summerville of Aventis CropScience for his cooperation in providing valuable reagents.

REFERENCES

9. Van der Meer, K., and M. De Beuckeleer. “Detection of transgenic DNA sequences in dry milled fractions, wet milled fractions and masa processed fractions and processed foods made from 100% StarLink grain.” Detection of Cry9C protein in dry milled, wet milled and masa processed fractions and processed foods made from 100% StarLink™ grain. [Internet, WWW]. ADDRESS: http://www.epa.gov/oppbppd1/bioppesticides/otherdocs/starlink/B003244%20CM00B014-Final.pdf.