Potential Botanical Evidence for Crime Scene Investigation

By

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Abstract

A forensic scientist's most important work is to find a potential evidence for crime scene investigation and solving a crime. The individualization of a plant species from stomach contents that came from autopsy or vomit by using DNA typing methods is a novel area of research. Stomach content analysis will also provide qualitative information concerning the decedent's last meal and to link the suspect or a location through the identification of source of the food. Ingested items such as seeds could become key evidence for providing leads in criminal investigations, estimate the time of death or verify an alibi.

Currently, the identification of botanical evidences still relies on microscopical examination. We found that it is hard to distinguish between the seeds of cultivars of tomato and between tomato and pepper. In this research, tomato and pepper seeds were selected as a model system for the species identification and cultivars identification since they are commonly found in a variety of cuisines. Our results revealed that(1) high quality DNA from a half tomato seed could be isolated; (2) Find one optimal primer's DNA-AFLP pattern could be used to discriminate between tomato and pepper species or different tomato cultivars; (3) DNA could not be isolated from tomato seeds of 12 commercial products, home cooked and baked.

Our research established a new botanical method to distinguish different species and cultivars. The results of such examination often provide valuable information for forensic purposes. Identification of seeds from stomach contents can verify the deceased last meal, provide linkages between victim and suspect or utilize traceable seed evidence in death investigations. The AFLP is a viable procedure for the identification of seed, thus adding an additional tool to identify botanical evidence for criminal investigations.

Key words: forensic science, forensic botany, crime scene, tomato seed, amplified fragment length polymorphism

Introduction

Plant and animal products not only provide important information for criminal investigations by linking a victim, suspect, witness or weapon to a crime, but also may verify an alibi, or provide investigative leads during criminal investigations. Tomato (Lycopersicum esculentum) seeds were selected as a model system since tomato is commonly found in a variety of cuisines around the world. The examination of ingested food from stomach contents, small and large intestine are important steps in medicolegal investigation. The results of such examination often provide valuable information for forensic purposes. Identification of seeds from stomach contents can verify the deceased last meal, provide linkages between victim and suspect or utilize traceable seed evidence in death investigations. In addition, stomach contents collected at autopsy may contain identifiable plants and seeds that can be used for estimating the time of death or verifying an alibi.

Our study has shown that high quality DNA could be recovered from ingested tomato seeds and DNA extraction from seed embryo will avoid the maternal component or other surface contaminants. Amplified fragment length polymorphism (AFLP) technique was utilized to identify DNA form tomato seeds. The AFLP technique is based on the detection of genomic restriction fragments after PCR amplification. Band patterns are produced without prior sequence knowledge, using a limited set of amplification primers. This technique has been used to identify fresh tomato samples but not for ingested samples. AFLP method permits the inspection of polymorphisms at a large number of loci within a short period of time and requires only a small amount of DNA (about 10 ng). AFLP analysis is the method of choice since it has general applicability to any single source plant sample regardless of the species. It is envisioned that AFLP can be performed to link a seed back to a parent plant or original fruit to aid in any criminal investigations.

As a model system, tomato seeds were examined microscopically after different cooking treatments and assessed for the potential to DNA type seeds for cultivar identification. A sufficient quantity and quality of DNA was recovered from uncooked tomato seeds for AFLP analysis; however, any form of cooking destroyed the seed DNA. A simple microscopic analysis was able to distinguish between a cooked tomato seed versus an uncooked seed. This study is intended to provide an overview of case examples and current techniques for the forensic examination of seeds as plant-derived evidence.

At crime scenes, an investigator may find various types of tomato evidence such as spaghetti sauce, canned tomatoes, Bloody Mary mix, fresh tomatoes, tomato juice or tomato seeds on weapons or on clothing due to vomiting, defecation or in autopsied stomach contents. This study was designed to determine whether the DNA

of the commercial and home cooked tomato seed could be recovered; and if there are morphological differences between fresh, commercial and home cooked tomato seeds.

Materials and Methods

Simple Method for the Recovery of DNA from a Single Tomato Seed

Tomato seeds were collected from different fresh tomatoes obtained from the local grocery. DNA extractions were performed as recommended by the manufacturer's protocol unless otherwise stated. Seed preparation method: mechanical grinding of samples in the presence of liquid nitrogen was performed using the Mixer Mill MM300 following the manufacturer's protocol. For the final step, 50 µL of preheated (65°C) buffer AE was added onto the DNeasy membrane to elute the extracted plant DNA. DNA yields were estimated by comparison with genomic DNA standards after electrophoresis on 1% agarose gels containing ethidium bromide for visualization. A portion of each DNA sample (10 µL) was loaded on the agarose gel for estimating yield. Some minor modifications to the manufacturer's protocol were made for isolation of DNA from single seeds.

The tomato seeds were processed as dissected embryos (cut in half) and intact tomato seed (embryo plus seed coat, cut in half). The average yield from three DNA extractions of a single tomato seed was 100 ng and 62.5ng for exterior seed coat and interior embryo respectively. The above result showed that high quality DNA (40 ng total) could be extracted from only half a seed embryo. It means that the small amount of tomato sample is sufficient for further DNA testing. We found that the plant DNeasy kit from QIAGEN was used to efficiently process intact tomato seeds and dissected tomato seed embryos to obtain PCR-quality plant DNA.

AFLP Method to Identify the Tomato with Other Plant Species

Ingested tomato and pepper seeds were used as a model system to test the feasibility of performing DNA testing for plant species identification. Seeds were collected from different types of tomato and pepper. Fresh tomato and pepper seeds were selected as known control samples for this study. Additional seeds were selected for use in a blind test. This approach can be applied to other plant species in addition to tomato and pepper seeds.

From the tomato and pepper DNA extraction results, it is clear that high quality DNA could be extracted from either a single fresh seed. The DNA quality and quantity

were sufficient for the PCR methods using the DNA-AFLP technique. To select optimal primer sets, sixty four possible primers in the AFLP Plant kit were screened. We selected the AFLP pattern with some peaks at the same position and common to all tomato types but different with all pepper types; those peaks were valuable for recognizing the species and may be the tomato specific markers. We could use those peaks to differentiate those species (pepper). The sequence information of the selected PCR primers are EcoRI + 3-AAC and MseI + 3-CAA as referenced in our published data. Using the primer combinations, it is easy to obtain the AFLP patterns with many peaks that differed between tomato and pepper samples. Those peaks may be of the species-specific markers.

From the above results, we showed that AFLP analysis can be performed to distinguish two plant seed samples at the species level, thus adding an additional tool for criminal investigations. Using the AFLP method, it is possible to use a DNA-based system to discriminate different species and even closely related plant sources.

AFLP Method to Identify the Cultivar Tomatos

Tomato seeds were used as a model system to test the feasibility of performing DNA testing on cultivar identification within species. Five phenotypically different fresh tomatoes were selected from the local grocery supermarket. No genetic variety names were available from the grocery or their produce supplier. DNA extractions were performed as recommended by the above protocol. The identification of candidate markers for distinguishing phenotypically distinct tomatoes indicates it is possible to use a biological method (DNA-based system) with sufficient markers to discriminate even closely related plant sources (e.g. two groups of seed from the same tomato plant as well as potentially for cultivar identification). The AFLP patterns showed us that many of the peaks differed between different cultivar of tomato; these were useful for discriminating different cultivar of plant and may prove to be variety-specific markers. Those peaks were valuable for tracing the cultivar of the tomato. From the binary Excel file data, we can easily distinguish the different patterns for each tomato type. Here, we also show that AFLP analysis was performed to generate an individualizing band or peak pattern from tomato seeds. It is envisioned that AFLP can be performed to link a seed back to a parent plant or original fruit to aid in any criminal investigations.

The arrows showed different peaks in the AFLP pattern and it illustrates some representative DNA profiles generated from those tomato seeds. DNA typing with the AFLP method can be useful for further individualization of the tomato samples.

Interestingly, these same markers were observed in whole seeds as well as from the three extracted embryos per tomato, which suggests that it may not be necessary to dissect the seeds for cultivar identification applications.

Commercial* & *Home Cooked Tomato Products

Twelve different brands of commercial tomato products were used in this study. There were five kinds of canned tomatoes including “Hunt’s tomatoes petites diced”, “Weis quality diced tomatoes”, “Big Top tomatoes”, “Del Monte Quality diced tomatoes” and “Contadina Roma Style tomatoes”. Seven kinds of tomato spaghetti sauces were used including “Prego pasta sauce”, “Newman’s Own Marinara pasta sauce”, “Health Choice Traditional Pasta sauce”, “Francesco Rinaldi tomato & basil”, “Barilla Basilico tomato & basil”, “Classico traditional favorites tomato& basil” and “DelGrosso tomato basil spaghetti sauce”. Nine of the twelve above listed commercial tomato products contained seeds. DNA extractions were carried out for both separated tomato materials (flesh, tissue) and the seed.

Home-made tomato products were prepared by cooking fresh tomato with three methods for different time periods:

A) Fresh tomatoes were boiled at 100°C for 1, 5, 10, 15, 20, 30 seconds; 1, 3, 5, 10, 20, 30 minutes; or 1, 2, 3 hours.

B) Baked tomatoes were prepared in oven at 218°C for 10, 20 or 30 minutes.

C) Pan fried tomatoes were prepared at above 100°C for approximately 3 minutes.

Tomato seeds were collected after each of the cooking treatments and at the specified time intervals.

Comparison of Tomato Seed Morphology

Fresh tomato was used as the positive control in this study. Seeds were manually separated from the tomato tissue and DNA was extracted accordingly. The morphology of the fresh tomato seed and the seed recovered from home made products and commercial products were examined and compared using an Olympus microscope (model BH-2) at 40 X magnification.

Result

Seeds from stomach contents can verify a last meal or provide traceable evidence in criminal investigations. Many seeds with tough, durable seed coats that remain intact after passing through the human digestive system. These seeds can be

retrieved from a crime scene where a suspect may have vomited. In addition, stomach contents collected at autopsy may contain identifiable plants and seeds that can be used for estimating the time of death or verify an alibi. These information may become key evidence and very useful in forensic investigations. Our study show DNA could be recovered from tomato seeds in fecal material, after passing through the stomach, small intestine and large intestine. Therefore, seeds collected from stomach contents, an earlier step in the digestive pathway, will yield equivalent results

The recovered tomato seed was useful for DNA typing by the amplified fragment length polymorphism (AFLP) method. From our discussion, a sufficient quality and quantity of DNA can be obtained to generate an individualizing DNA profile. The species/cultivar specific markers could be easily distinguished by AFLP method. Only using the selected PCR primers EcoRI + 3-AAC and MseI + 3-CAA combinations, we could easily obtain the AFLP patterns with many peaks that differed between species/cultivar samples. Those peaks were valuable and may be used as species-specific markers for the further forensic application. Once additional cultivar-specific markers have been identified and catalogued into a database, this method should be useful and provides a model system for tracing edible plant matter for criminal investigation.

Seeds were recovered from all five brands of canned tomatoes and four of the seven brands of tomato sauce. However, none of our DNA extraction protocols produced detectable amount of DNA from these commercial tomato samples. Additional seeds were extracted and the results were consistent with those from the single seed study. DNA was extracted from 1 mL of tomato material from all 12 commercial tomato products. No detectable amount of DNA was recovered from any of those samples. Our results indicate that DNA could not be recovered from tomato seeds that had been cooked in boiling water for more than 20 seconds, and no detectable amount of DNA was obtained from oven baked or pan fried tomato samples. The surface characteristics of tomato seeds is under microscopic examination. In fresh tomato seeds, a smooth surface was observed. However, after the seed was cooked for sauces or an omelet, the epidermal hairs on the seed surface became more obvious. A comparative study was conducted by examining tomato seeds boiled for different lengths of time. Epidermal hairs were observed after the seed was boiled for 1 minute. After 3 minutes of boiling, the surface hairs became quite evident. These types of hairs were also observed on the seeds recovered from commercial products. The result shows the seed surface morphology of many different commercial tomato products. The color of tomato seed darkens with oven baked samples and the seed lost its epidermal hairs after baking.

Discussion

In this study, we also show that AFLP analysis was performed to generate an individualizing band or peak pattern from tomato seeds. It is envisioned that AFLP can be performed to link a seed back to a parent plant or original fruit to aid in any criminal investigations. Our data show that it is possible to identify different tomato types. Once additional cultivar-specific markers have been identified and catalogued into a database, this method should be useful and provides a model system for tracing edible plant matter for forensic identification.

Our results also show that a sufficient quantity and good quality DNA could be extracted from a fresh seed embryo or seed coat; however DNA could not be extracted from a entire seed of a canned tomato. To resolve this concern, we further found that after high temperature treatment, the DNA of most tomato seeds will be destroyed. A microscopic examination could be useful to quickly screen tomato seeds to identify characteristic features of a tomato seed's surface to further distinguish between fresh and cooked tomato samples. The fresh seed had a smooth appearance and the cooked seed had obvious epidermal hairs. This discovery could be used to screen valuable seed quickly for DNA value in forensic casework.

The plant DNeasy kit from QIAGEN was used to efficiently process intact seeds and dissected embryos to obtain PCR-quality DNA. The use of forensic botanical evidence from crime scenes for DNA typing is still in the developmental stages for difficult samples such as plant matter from stomach contents and excrement. In our study, we extracted and analyzed tomato seed's DNA from commercial products such as spaghetti sauce, tomato sauce and canned tomato. We found that none of our DNA extraction protocols produced satisfactory yields for quantity and quality of DNA when using commercial tomato seeds as starting materials. Our results suggest that the DNA yield is directly related to the temperature and/or pressure of how the tomato products were processed.

In addition, a novel procedure was developed to combine the microscopic analysis of seed morphology and the yield determination of DNA from the seed. This procedure could be used to distinguish whether a tomato seed originated from fresh tomato, home made tomato sauce or a commercial product and if it is likely to yield a DNA profile. While the microscopic identification of a plant seed to the species level may be sufficient for analyzing some forensic cases, many other types of seeds will require molecular biological (DNA) identification either because they are not morphologically distinguishable as intact seeds or because fragmentation has limited any identifiable features for further characterization. This study was

designed to further define the criteria for choosing to process seed evidence for a DNA profile for identification and individualization.

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Table. Binary Coding for12 Variable AFLP Markers for Tomatoes A-E

Sample	01	02	03	04	05	06	07	08	09	10	11	12
A1	0	0	0	0	0	0	1	0	0	1	0	0
A2	0	1	0	0	0	0	1	0	0	1	0	0
A3	1	0	0	0	0	0	1	0	0	1	0	0
A4	0	1	0	0	0	0	1	0	1	1	0	0
B1	0	0	0	0	1	1	0	0	0	0	1	1
B2	1	0	0	0	1	1	0	0	0	0	1	0
B3	0	0	0	0	1	1	0	0	0	0	1	0
B4	0	0	0	1	1	1	0	1	0	0	1	1
C1	0	1	0	0	1	1	0	0	0	0	1	0
C2	0	0	0	0	1	1	0	1	0	1	1	1
C3	0	0	0	0	1	1	0	1	0	0	1	1
C4	0	0	0	0	1	1	0	1	0	0	1	1
D1	0	0	1	0	1	0	0	1	0	0	1	1
D2	0	0	0	0	1	1	0	1	0	0	1	0
D3	0	0	1	0	1	0	0	1	0	0	1	0
D4	0	0	1	0	1	1	0	1	0	0	1	1
E1	1	0	1	1	1	0	0	0	0	0	0	0
E2	1	0	0	1	1	1	0	0	0	0	0	0
E3	1	0	0	1	1	1	0	0	0	0	0	0
E4	1	0	0	1	1	1	0	0	1	1	0	0