Research Notes

Random Amplified Polymorphic DNA Fingerprints for Identification of Species in Poultry Pâté[1]

J. H. Calvo,[2] P. Zaragoza, and R. Osta

Laboratorio de Genética Bioquímica, Facultad de Veterinaria, c/Miguel Servet 177, 50013 Zaragoza, Spain

ABSTRACT Because some fraudulent or unintentional mislabeling occurs that can be undetected, resulting in lower quality pâté, and because some population groups, for philosophical or religious reasons, do not wish to eat meat from certain species, a new procedure was developed and evaluated to detect pâté species composition by randomly amplified polymorphic DNA (RAPD).

The RAPD method was used to generate fingerprint patterns for pork, chicken, duck, turkey, and goose meats. Ten DNA samples from pork, chicken, turkey, and duck meats were tested to confirm the effectiveness and specificity. Specific results for each species were obtained by the RAPD method. Sensitivity of the method was studied by DNA dilution in each species, detecting as little as 250 pg of DNA. Isolations of DNA from 30 pâtés (tinned and untinned) were carried out, and an optimal DNA was obtained for using as template DNA in polymerase chain reaction (PCR). The RAPD-PCR pattern was useful to identify species composition of pork, duck, duck-pork, goose, and poultry pâtés. This study demonstrates the usefulness of RAPD fingerprinting to distinguish between species in pâtés.

(*Key words*: DNA fingerprint, poultry, pig, pâté, authentification)

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INTRODUCTION

The problem of substitution or adulteration of good quality, high-priced pâtés, whether by accident or intention, is not new. Determining pâté species is an integral part of food regulatory control with respect to economic fraudulence. Apart from possible economic loss, correct species identification is important for the consumer for other reasons, such as medical requirements of individuals who may have specific food allergies or religious restrictions. This situation has prompted research to find methods for the detection of the origin of pâtés.

Numerous analytical methods, which rely on protein analysis, have been used for pork and poultry identification, such as electrophoresis techniques (Kim and Shelef, 1986; Skarpeid et al., 1998), liquid chromatography (Ashoor et al., 1998), and immunoassays (Jones et al., 1985; Hsieh et al., 1998). However, proteins lose their biological activity after an animal dies, and their presence and characteristics depend on the cell types. Furthermore, most of them are heat labile. Thus, for species identification, DNA analysis would be preferable to protein analysis.

The dot-blot technique was the first genetic approach for determination of species identity (Wintero et al., 1990; Ebbehoj and Thomsen, 1991). At present, however, polymerase chair reaction (PCR) is the technique of choice for species identification. Some PCR approaches are random-amplified polymorphic DNA (RAPD)-PCR (Lee and Chang, 1994), DNA mithochondrial D-loop analysis (Murray et al., 1995), and restriction fragment length polymorphism (RFLP) analysis of different PCR fragments (Borgo et al., 1996). Identification of poultry has been carried out in meat samples by different authors (Meyer et al., 1995; Chikuni et al., 1990). Up to now, identification of species in pâté has not been attempted. Because our purpose was to identify species used in pâté, a method for poultry pâté identification is presented on the basis of RAPD-PCR, which can be applied to the detection of fraudulent or unintentional mislabeling of this species.

MATERIALS AND METHODS

Sample Selection and DNA Extraction

In order to test the specificity of the technique, 10 unrelated blood and muscle samples of chicken, duck, turkey, and pork were analyzed (10 of each). Genomic DNA from blood was extracted according to a previously described procedure (Lahiri et al., 1992). Genomic DNA from muscle was extracted according to a previously described

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[2]To whom correspondence should be addressed: jhcalvo@posta.unizar.es.

Abbreviation Key: PCR = polymerase chain reaction; RAPD = random amplified polymorphic DNA fingerprints; RFLP = restriction fragments length polymorphic.

procedure for muscle tissue. Protocol I for isolation of DNA from tissue samples from Sambrook et al. (1989) was used.

Furthermore, 30 different pâtés (10 pork, 10 duck, 6 duck-pork, 3 goose, and 1 poultry) were analyzed. These pâtés were commercial brands purchased in a store. Ten of these were tinned pâtés, labeled as sterilized products (four duck, one pork, three duck-pork, and two goose). Genomic DNA was extracted according to Protocol I for isolation of DNA from tissue samples (Sambrook et al., 1989).

DNA Fingerprinting and Gel Electrophoresis

The set of primers used for RAPD amplification were designed as follows: AGGACGCCTTCCGATACATG and CAGAAGGTCTTGGAGATGGC. Double-stranded amplifications were carried out in a final volume of 50 μL, containing 10 m*M* Tris-HCl, pH 8.8; 2.5 m*M* $MgCl_2$; 50 m*M* KCl; 0.1% triton X-100; 0.2 m*M* each of dATP, dTTP, dGTP, and dCTP; 15 pmol of each primer; 50 ng of template DNA and 2 U of Taq polymerase.[3] The DNA was amplified in a Biometra Thermal cycler.[4] Thirty-five cycles were run with the following step-cycle profile: strand denaturation at 94 C for 1 min, primer annealing at 47 C for 1 min, and primer extension at 72 C for 1 min. The final extension step was 5 min longer. An initial denaturation at 94 C for 4 min was performed to improve the final result. Electrophoresis of a 10-μL portion of the amplification was carried out for 45 min at 100 V in a 3% agarose gel, containing ethidium bromide (1 μg/mL) in TBE buffer (0.045 *M* Tris-borate, 0.001 *M* EDTA, pH 8.0). DNA fragments were visualized by UV transillumination and were photographed with Type 55 Polaroid film.[5]

Sensitivity

Samples containing 2,500, 250, 100, 50, 25, 10, and 5 pg/μL chicken, duck, turkey, and porcine DNA by dilution were taken. For this purpose, PCR was carried out in a final volume of 50 μL, with the PCR conditions, primers, and electrophoresis conditions described in the previous section.

RESULTS AND DISCUSSION

The RAPD profiles were analyzed visually. The obtained fingerprint patterns were reproducible within the particular species under the same controlled conditions. Reproducibility was checked by subjecting 10 unrelated animals from each species to the same PCR and electrophoresis conditions. Primers used showed good and specific fingerprint patterns for each species studied (Figure 1). With these patterns we could discrimate between the five species. No amplifications were obtained from goose samples.

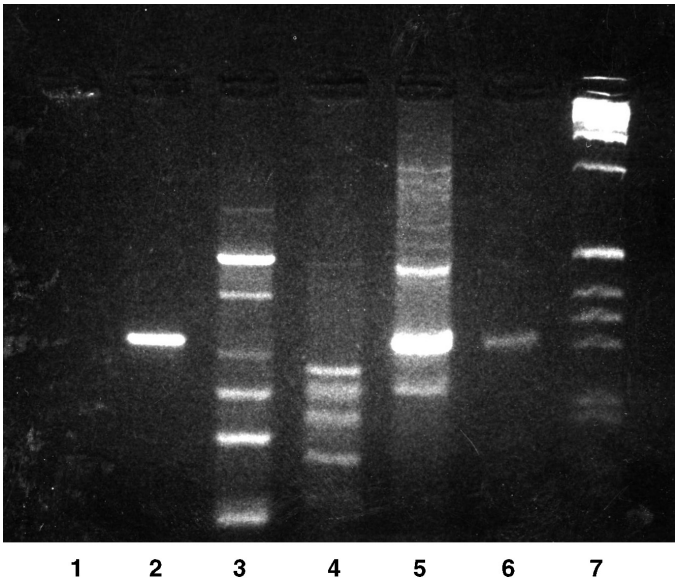

FIGURE 1. Results of random amplified polymorphic DNA fingerprint-polymerase chain reaction (RAPD-PCR) from blood and muscle samples. Lane 1: goose; Lane 2: duck; Lane 3: turkey; Lane 4: chicken; Lane 5: pork; Lane 6: duck; Lane 7: 1-kb marker (Gibco).

In order to verify the sensitivity of the method, several DNA dilutions were made. It was possible to detect as little as 250 pg of DNA for the species studied. This quantity of DNA corresponded to 1/1,000 dilution of DNA.

For identification of pâté species composition, PCR amplifications were carried out with several pâtés (tinned and untinned). Extraction of DNA from pâtés and other food matrices for subsequent use in PCR is often considered to be a problem (Rossen et al., 1992). Our work has shown that DNA extracted from pâté, by the method described, can be successfully used as template DNA in PCR. Fingerprints from duck, pork, goose, and chicken

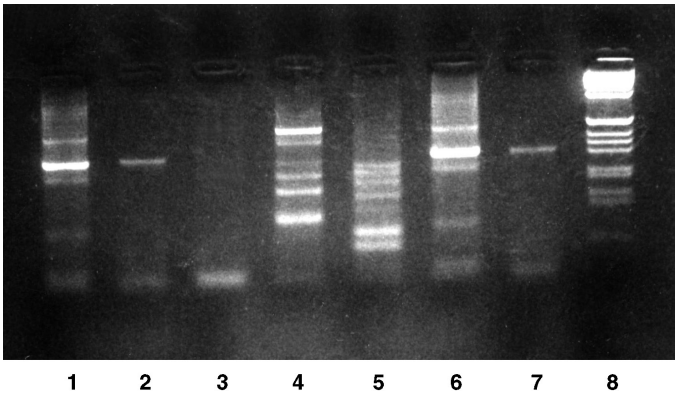

FIGURE 2. Results of random amplified polymorphic DNA fingerprint-polymerase chain reaction (RAPD-PCR) from pâtés. Lane 1: pig-duck; Lane 2: duck; Lane 3: goose; Lane 4: turkey; Lane 5: chicken; Lane 6: pig; Lane 7: duck; Lane 8: 1-kb marker (Gibco).

[3]Promega Corporation, Madison, WI 53711-5399.
[4]Biometra Ltd., Whatman House, St. Leonard's Road, 20/20 Maidstone, Kent, ME 16 OLS, UK.
[5]Polaroid Corporation, St Albaus, Hertfordshire, UK.

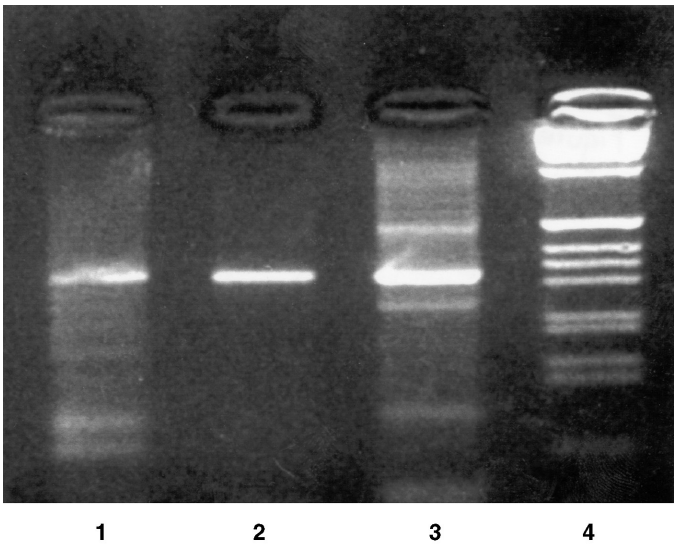

FIGURE 3. Results of random amplified polymorphic DNA fingerprint-polymerase chain reaction (RAPD-PCR) from fraudulent pâtés. Lane 1: duck (chicken and duck profiles were detected in this pâté); Lane 2: duck; Lane 3: duck (pork and duck profiles were detected in this pâté), Laine 4: 1-kb marker (Gibco).

showed the same DNA fingerprint patterns as those described for blood and muscle samples (Figure 2). Any band in goose pâtés would be considered fraudulent, because no amplification was observed in 100% goose pâtés. Chicken and duck profiles were found in the poultry pâté. Pork pâtés showed a specific profile, in which one band was the same size as the specific band in duck pâtés (Figure 2).

Duck pâtés showed one band, but duck-pork pâtés showed the specific profile amplified from pork. Thus, pork adulteration in pâtés labeled as 100% duck can be detected by amplificaton of the specific pork profile. In our work, some fraudulent pâtés were detected. Good quality, high-priced duck pâtés did not present pork as component. However, low quality duck pâtés, included pork or chicken that had not been specified on the label (Figure 3). In Figure 3, the specific pork profile can be observed in a duck pâté, showing pork adulteration. The chicken profile can also be observed in other duck pâtés, showing chicken adulteration. These findings support the identification of fraudulent pâtés by using RAPD-PCR. To make the species composition identification easy, known samples profiles of pork, duck, and poultry can run together with the pâté samples from an unknown species.

Thus, RAPD-PCR is a good method for rapid and qualitative pâté speciation. However, further research is needed to develop a quantitative method because this simple PCR analysis is only qualitative. In conclusion, results of this study clearly demonstrate the capability of RAPD fingerprint to distinguish pâté mixtures with simple PCR. This method is less expensive than others based on RFLP-PCR (which requires further digestions) and ELISA or other techniques that require expensive equipment.

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