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Cytochrome *b* for identification of animal species in processed food

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Abstract. The objective of this work has been to develop a suitable methodology based on mitochondrial cytochrome *b* gene (cytb) to allow the identification of animals species used as raw material in the manufacture of processed food. In some cases, more detailed information on the kind of food sample found in the stomach of a victim may be of particular importance. A number of specimens of processed food with bovine, pork and chicken origin were analyzed. DNA was extracted by two methods: (1) Cell lysis using proteinase K and SDS, and phenol-chloroform DNA purification; (2) NucleoSpin Food (Macherey-Nagel™). PCR amplification of 358 pb of the cytb was done. The species of the samples were identified by aligning to the cytb gene sequence entries using BLAST of the National Center for Biotechnology Information. Both the DNA extraction procedures yielded enough DNA to be analyzed by PCR. Sequences obtained were searched against nucleotide sequence database (GenBank). With this procedure, it is possible to classify each sample correctly according to its biological species. © 2003 Published by Elsevier B.V.

Keywords: Cytochrome *b*; Processed food; Bovine; Pork; Chicken

1. Introduction

The cytochrome *b* gene (cyt b) from the mitochondrial genome contains species-specific information and has been used in phylogeny as well as in forensic investigations in previous studies. Thus, it has been confirmed the usefulness of cytb analysis in identifying the biological origin of casework specimens [1]. In some cases, more detailed information on the kind of food sample found in the stomach of a victim may be of particular importance. This information can be used to estimate the postmortem interval and may provide information about the place of decease itself.

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In addition, identification of processed food is necessary as the customer has the right to be informed about products being bought and consumed [2]. Law requires that products should be labeled with official names, thus creating a foundation for discouraging fraud. Also, enforcement of protected species requires techniques to verify authenticity.

Improved techniques for the identification of food components are urgently required, and DNA-based technologies are providing answers to many of the questions being asked [3].

The objective of this work has been to develop a suitable methodology based on mitochondrial cytochrome *b* gene to allow the identification of animals species used as raw material in the manufacture of processed food.

2. Methodology

A number of specimens of processed food with bovine, pork, chicken and fish origin were analyzed. DNA was extracted by two methods: (1) Cell lysis was performed using proteinase K and SDS. The DNA was purified using the phenol-chloroform method. (2) NucleoSpin Food (Macherey-Nagel™) was used following the manufacturer's protocol. PCR amplification of 358 pb of the cytb was done under conditions described by Parson et al. (2000). All the samples were sequenced using the dRhodamine Terminator kit (Applied Biosystems) method, in an automatic ABI Prism 310 DNA sequencer. The obtained sequences were used to identify the species of the samples by aligning to the cytb gene sequence entries using the online BLAST search engine of the National Center for Biotechnology Information (NCBI).

3. Results and discussion

DNA was extracted from processed food using the methods above described. In both the cases, DNA extraction procedures yielded enough DNA to be analyzed by PCR. PCR products were sequenced. The sequencer identified most of the bases unambiguously (Fig. 1).

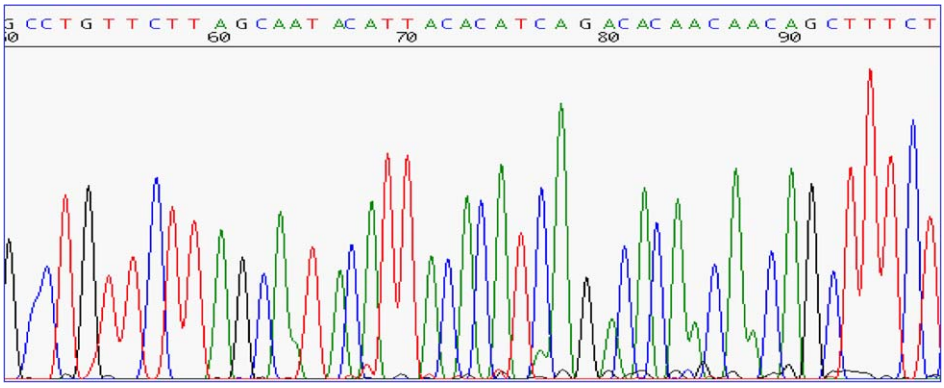

Fig. 1. Partial sequence of cytb obtained of DNA from sausage extracted by Proteinase K and SDS and purified with phenol-chloroform.

Table 1
Results from BLAST for identification of species from processed food

Processed food	Label of manufacturer	Species GenBank	Score (bits)	*E* value	Coincidence between label and DNA cytb analysis
Sausage	Pork	*Sus scrofa*	563	e – 158	+
Hard pork sausage	Pork	*Sus scrofa*	563	e – 158	+
Meatball	Bovine	*Box taurus*	525	e – 147	+
Stew chicken	Chicken	*Gallus gallus*	517	e – 144	+

Sequences obtained from processed food were searched against nucleotide sequence database (GenBank). The results of a sequence search contains a list of database entries, the score and e-value displayed high similarity between the laboratory results and the corresponding gender or species specified by the manufacturer (Table 1).

These results indicate that animal samples extracted from processed food can be successfully identified to specific level, even from degraded material using the cytb analysis. With this procedure, it is possible to classify each sample correctly according to its biological species.

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