APPLICABILITY OF THE mtDNA CYTOCHROME C OXIDASE SUBUNIT 1 GENE (*COI*) IN SPECIES IDENTIFICATION OF PORK, BEEF AND DUCK MEAT

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Abstract – Species identification concerns health-related, economic and occasionally religious issues significant from the point of view of consumers. Researchers investigating species identification increasingly focus on the cytochrome c oxidase subunit 1 gene (*COI*). In this study species specific PCR primers were applied, designed on the basis of a fragment of the *COI* sequence, in the identification of meat coming from three animal species, i.e. swine, cattle and the duck, evaluated individually and in combinations. It was found that the *COI* gene may be applied in the qualitative identification of the above mentioned meat species both when analysed individually and in mixtures of two, four and six components comprising raw ground meat.

Key Words – PCR (*Polymerase Chain Reaction*), Species specific primers, Meat authenticity

I. INTRODUCTION

Analytical methods based on DNA analysis are considered to be of greatest value in studies on species meat authentication. This results from the specific structure of the deoxyribonucleic acid and the contained data [1]. They are most frequently based on sequences of mitochondrial DNA (mtDNA).

Researchers investigating species identification increasingly focus on the cytochrome c oxidase subunit 1 gene (*COI*). In 2003 this gene was proposed as a candidate gene for the assessment of global biodiversity of eukaryotic organisms in the so-called *DNA barcoding* [2]. Using this gene [3] adulteration was shown in meat of a fish Red Snapper (*Lutjanus campechanus*) with different other fish species and in meat of albacore tuna with tilapia. This gene was also used when distinguishing meat of cattle (*Bos taurus*) and southern reedbuck (*Redunca arundinum*) [4]. Using universal primers a fragment of the *COI* gene of cattle and chicken was amplified, also indicating the suitability of the sequencing method in distinguishing these animal species [5]. The sequence of the *COI* gene along with seven restriction endonucleases was used in the identification of raw meat from cattle, the chicken, turkey, sheep, pig, camel and donkey [6]. The method using the *COI* gene made it possible to distinguish even such closely related species as cattle and the buffalo or the chicken and turkey [6].

The aim of this study was to determine the suitability of the cytochrome c oxidase subunit 1 gene (*COI*) in the identification of meat coming from swine, cattle and the duck using species specific PCR primers. The analyses concerned both individual meat species and mixtures of ground meat.

II. MATERIALS AND METHODS

Sample preparation

Analyses were conducted on meat from three animal species, i.e. swine (*Sus scrofa f. domestica*), cattle (*Bos taurus*) and the duck (*Anas platyrhynchos)*. Collected material was placed in sterile test tubes and stored at -80°C until DNA isolation.

Additionally, meat mixtures were prepared, which apart from the three above mentioned meat species contained also meat of the chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) and the goose (*Anser anser*).

These meat mixtures comprised 9 two components mixtures and additionally two,

from which one mixture contained meat from 6 species and one from 4 species. Meat species were denoted using the first letter of the name of the species which meat was analysed, i.e. S – swine, B – beef, D – duck, C – chicken, T – turkey and G - goose. Proportions of meat in a 2-component mixture were 9:1 and 1:1. In the former sample type the abbreviated denotation consisted in the presentation of the species name accounting for 90% mixture followed by that added at 10%, with letters denoting the species separated with ":". In the latter sample type the letters ascribed to individual meat species are separated with "/". Denotation, the qualitative and quantitative composition of 6- and 4-component mixtures were as follows:

- a 6-component mixture M6 contained 20% beef (B), 20% pork (S), 20% chicken (C), 20% turkey (T), 10% duck (D) and 10% goose meat (G),
- a 4-component mixture M4 contained 25% B, 25% S, 25% C and 25% T.

Isolation and analysis of DNA concentration and purity

DNA from individual meat species as well as mixtures was isolated using a *PureLinkTMGenomic DNA kit* (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. DNA concentration and purity were determined using spectrophotometric measurements of light absorbance at a wavelength of 260 nm and 280 nm (Nanodrop, ThermoScientific, Wilmington, DE, USA). During analyses DNA was stored at +4°C.

Designing of species specific PCR primers

Species specific primers for the detection of pork, beef and duck meat were designed based on the sequence of the *COI* gene collected from the GenBank of the NCBI database (*National Center for Biotechnology Information*, Bethesda, MD, USA) available at http://www.ncbi.nlm.nih.gov/. The sequences with the following accession numbers were selected: NC_012095.1 for swine, AF492351 for cattle and L22480 for the duck. Primers were designed using the Primer3 software (http://biotools.umassmed.edu/bioapps/primer3_www.cgi). Primers were synthesised by TIB MOLBIOL (Syntheselabor GmbH, Berlin, Germany).

The designed primer pairs had the following sequence: for cattle DNA the forward primer 5' GAACTCTGCTCGGAGACGAC 3', the reverse primer: 5' GGTACACGGTTCAGCC TGTT 3', for the DNA of swine, the forward primer GGAGCAGTGTTCGCCATTAT, the reverse primer TTCTCGTTTTGATGCGAATG, for DNA of the duck the forward primer TAATTGGCAC AGCACTCAGC, the reverse primer TTATCAGG GGGACCAATCAG. PCR primers were designed to amplify a product of 294 bp for DNA of swine, 255 bp for cattle and 192 bp for the duck, respectively.

Optimization of conditions for polymerase chain reaction

The total volume of the PCR mixture was 20 µl. Each time the reaction mixture contained 40 ng DNA, 2 µl 10x concentrated PCR buffer (Sigma, Saint Louis, MO, USA), 1 µM each of primer pair (TIB MOLBIOL), 0.25 mM dNTP (Sigma) and 0.03 units of Taq polymerase DNA (Sigma). Reagents by Invitrogen were used in the case of primers for the detection of duck meat. The PCR sample contained 2 µl 10x concentrated PCR buffer without $MgCl_2$ (Invitrogen), 5 mM $MgCl_2$ (Invitrogen), 1 µM each of the primer pair (TIB MOLBIOL), 0.25 mM dNTP (Sigma) and 0.03 units of Taq polymerase DNA (Invitrogen).

The PCR on DNA isolated from meat from the three animal species was run in two replications for each species with the negative control (NK) and human DNA (HS).

DNA from the mixtures was analysed by PCR including the positive (PK) and negative (NK) controls. PCR was run in a peqSTAR thermocycler (PEQLAB, Erlangen, Germany).

PCR conditions were as follows: initial denaturation at 95°C for 9 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 59°C for swine primers, at 60°C for cattle primers, at 71°C for duck primers for 1 min and extension at 72°C for 3 min. The final extension was done at 72°C for 10 min.

Electrophoresis of PCR products

Obtained PCR products were separated in 1% agarose gel (Sigma) supplemented with 0.003% ethidium bromide (Sigma).The separation was run for 45 min at a voltage of 100 V (Power PAC 300, Bio-RAD, Hercules, CA, USA) in 1x TBE buffer (90 mM boric acid, 90 mM Tris, 3 mM EDTA). In order to identify the sizes of the DNA amplification products the *PCR 100 bp LowLadder* (Sigma) size marker was used. Separation products were observed using a G:Box transilluminator (Syngene, Frederik, MD, USA).

Sequencing of PCR products

Sequencing was performed by the cyclic method using capillary electrophoresis. Produced DNA sequences were analysed using the *ChromasPro* software (TechnelysiumPtyLtd) and next their homology with reference sequences was compared using the BLAST programme (NCBI).

III. RESULTS AND DISCUSSION

The aim of these analyses was to provide information whether on the basis of the nucleotide sequences of the *COI* gene found in the database it is possible to design PCR primers, which would selectively identify meat of the three animal species, i.e. swine, cattle and the duck. As a result of PCR followed by electrophoresis of the formed reaction product it was found that all the three pairs of primers designed on the basis of the *COI* gene showed specificity towards DNA obtained from the three animal species, i.e. swine, cattle and the duck, and provided products with the size assumed at their designing (Fig. 1).

As a result of the analyses of PCR product sequences it was found that all the three PCR primer pairs, i.e. those designed for the amplification of DNA from swine, cattle and the duck, hybridised with the fragment of *COI*. Analysis of samples containing DNA coming from

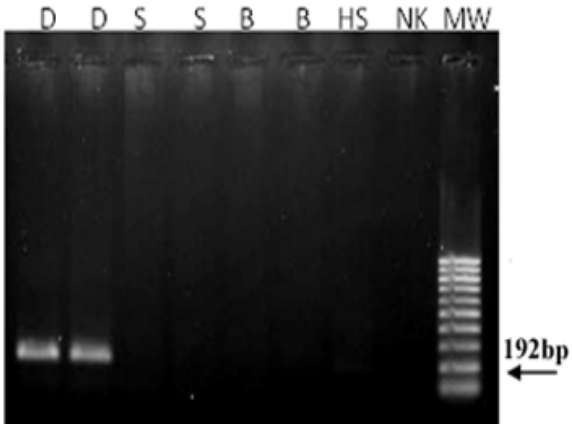

Figure 1. An example of PCR products electrophoretic separation in 1% agarose gel. PCR was performed using for duck specific primers on DNA isolated from individual meat species.

Denotation of letter abbreviations used in Figures: D – duck DNA, S - swine DNA, B - cattle DNA, G – goose DNA, C – chicken DNA, T – turkey DNA, HS - human DNA, NK - negative control, PK – positive control, MW – molecular weight marker

mixtures of meat from different animal species is much more demanding than that connected with single DNA samples. The presence of DNA from different meat species within one sample may disturb the formation of PCR products. DNA of a meat species contained in the mixture as quantitatively predominant over the other components may be excessively amplified, while those found in smaller amounts may be underestimated [5, 7, 8].

Results of the conducted analysis showed that all three PCR primer pairs designed for the amplification of DNA sequences of swine, cattle and the duck correctly identified these meat species in meat mixtures (Fig. 2).

Each of the primer pairs amplified DNA from meat found in the mixture at 10%. This reaction occurred also in the 6-component mixture in the case of the duck primers.

Recorded results indicate the potential for species identification in meat mixtures composed of pork, beef and duck meat using primers designed based on the *COI* gene.

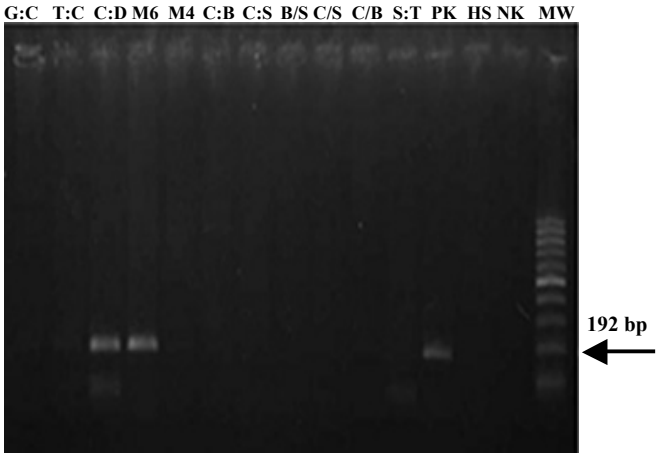

Figure 2. An example of electrophoretic separation of PCR products in 1% agarose gel. PCR products of predicted size 192 bp were observed only for samples: C:D, M6, PK. Specificity of each PCR products was confirmed by DNA sequencing

IV. CONCLUSION

Analysis of nucleotide sequences of the *COI* gene showed its high applicability in the identification of meat coming from three species of slaughter animals, i.e. swine, cattle and the duck. The cytochrome c oxidase subunit 1 gene proved suitable also in the analysis of DNA collected from two-, four- and six-component mixtures prepared from raw meat. Each of the designed PCR primer sets made it possible to identify a given meat species found in the mixture at 10%.

ACKNOWLEDGEMENTS

This study was financially supported by Grant N N312 205636 from the Polish Ministry of Science and Higher Education.

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