IDENTIFICATION OF AMERICAN BISON TISSUE BASED ON THE MITOCHONDRIAL *CYTOCHROME B* GENE

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Introduction

Bison is reared in North America (USA and Canada) at a significant extent and exported into the European Union. Bison meat is classified as a "gourmet product" because of its uncommon and exceptional taste. However, with respect to its meat texture it is very similar to beef and for this reason it should be unequivocally labeled as bison. The exact taxonomy of the American bison (bison bison) is as follows: kingdom – animal; phylum – chordata; subphylum – vertebrates (having a spine); class – mammalians (having mammary glands); subclass – eutheria (having placenta); super order (ungulates); order - artiodactyls (having an even number of fingers); suborder – ruminants (having the rumen); family – bovidae, subfamily – bovinae; genus – bison; species – bison bison (Wilson and Reeder, 1993). Although buffalo (bubalus bubalis), cattle (bos taurus) and bison belong to the same subfamily (bovinae) they differ from each other by genus. Bison has to be legally treated in accordance with the regulation of the European Parliament (EC) 1760/2003 dealing with the labeling of beef and beef products. On this account the Federal Ministry for Nutrition, Agriculture, and Consumer Protection as well as the German Association for Meat Business argued for obligatory labeling of bison and bison products. As a consequence, there exists a need for molecular biological methods to differentiate between bison, buffalo and cattle. Based on mitochondrial DNA a polymerase chain reaction (PCR) system was developed to provide a possibility to distinguish between tissues of the mentioned animals.

Materials and Methods

DNA used for PCR was isolated from 50 mg meat in each case applying a modified CTAB protocol (Binke *et al.*, 2003). The DNA content was determined according to Warburg and Christian (1942). PAGE was performed as described by Altmann *et al.*, (2004) using the following molecular weight standards: λ-DNA/Eco RI + Hind III and pBR322/HaeIII. DNA amplification was carried out in a *Perkin Elmer Gene Amp System 9600*.
Primer design focusing on mitochondrial DNA was implemented by means of *HUSAR Data Base* (German Cancer Research Center, Heidelberg). The following primer pair was used for the amplification of a bison (bison bison) specific DNA fragment of a length of 138 bp:
CY-Bis/forward: 5′ - AAATCCACTCAATACACCTCCC - 3′ (22 bp);
CY-Bis/reverse: 5′ - CTAATCCTTGCCCTCATTCC - 3` (20 bp).
In addition a buffalo specific primer system was applied resulting in a buffalo (bubalus bubalis) specific DNA fragment consisting of 242 bp:
CY-Buf/forward: 5′ - TAGGCATCTGCCTAATTCTG - 3′ (20 bp);
CY- Buf/reverse: 5′ - ACTCCGATGTTTCATGTTTCT - 3′ (21 bp).

Results and Discussion

In order to identify the animal species and to investigate the specificity of the PCR system based on the *cytochrome b* gene for bison, various animal species were tested by PCR for cross similarity. No PCR product was amplified for goat, pig, horse, chicken, buffalo and cattle (Fleckvieh and Schwarzbunt) (Figure 1A: lane 2- 9). However, a distinct band with a length of 138 bp can be observed in the case of bison (Figure 1A: lane 10).
Further studies were performed to exclude a cross similarity between buffalo and bison, as well as the other above mentioned animals using a buffalo specific primer system, which is also based on the mitochondrial *cytochrome b* gene (Rajapaksha *et al.*, 2003). Figure 1B clearly shows, that there is only a distinct band in lane 7 reflecting an amplification product of 242 bp, that is specific for buffalo, but not for bison and all the other animal tissues tested. Only in the case of pig, a weak band was observed indicating a cross similarity between pig and buffalo. In Figure 2 the primer sequences in the mitochondrial *cytochrome b* gene region between bison, buffalo and cattle is depicted visualising the number of existing nucleobase mismatches.

Conclusions

The results demonstrate clearly, that the primer system designed for bison based on the mitochondrial *cytochrom b* gene provides the possibility for unequivocal differentiation between bison and the other animal species tested. As there was no animal material available originating from yak (bos grunniens) and the East European bison (bison bonasus), the so called wisent, further investigations have to be performed to prove that no cross similarity exists in the case of the chosen primer system between bison on the one hand and yak and wisent on the other hand.

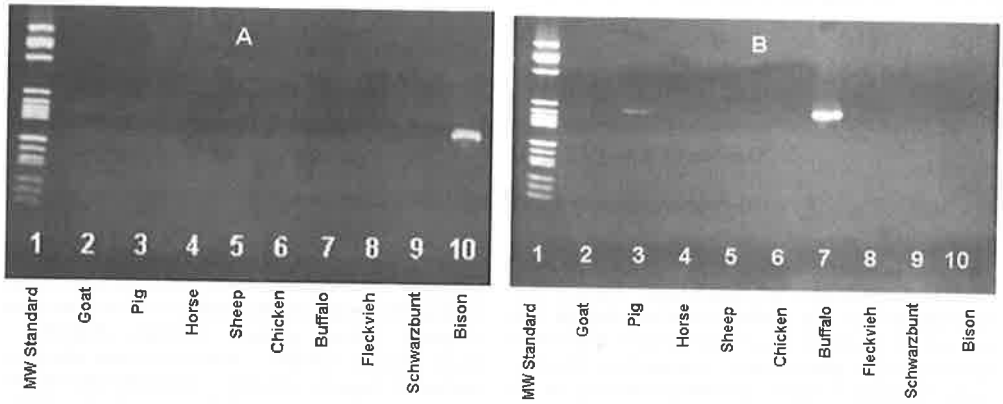

Figure 1: PAGE of various amplification products visualized by Ethidiumbromide intercalation: Gel A on the left shows only one distinct DNA band (lane 10) with 138 bp reflecting the specificity of the CY-Bis primer system for bison (bison bison) solely. There are no amplicons for tissue in the case of the other animals. On Gel B depicted on the right there is a clear DNA band (lane 7) with 242 bp for buffalo. This demonstrates the specificity of the CY-Buf primer system for buffalo (bubalus bubalis). Merely a weak band has been observed in lane 3 where DNA of pig tissue was isolated and amplified.

Forward primer:

Bison bison	AAATCCACTC AATACACCTC CC	
Bubalus bubalis	AAACCCACTC AACACACCTC CC	2 Mismatches
Bos taurus	CAATCCACTC AACACACCCC CT	4 Mismatches

Reverse primer:

Bison bison	CTAATCCTTG CCCTCATTCC	
Bubalus bubalis	CTAATCCTCA TTCTCATGCC	5 Mismatches
Bos taurus	CTAATTCTTG CTCTAATCCC	4 Mismatches

Figure 2: Forward and reverse primer sequences in comparison of Bison (bison bison), Buffalo (bubalus bubalis) and cattle (bos Taurus). Concerning taxonomic aspects the three animals belong to the subfamily bovinae. However, they are different with respect to genus. The specificity of the primer system for bison is reasonable because of the number of existing mismatches (underlined) in the case of buffalo and cattle.

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