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Evidences reveal that cattle and buffalo evolutionary derived from the same ancestor based on cytogenetic and molecular markers**

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Muscle-DNA from cattle and buffalo was extracted to amplify the mitochondrial DNA segment (cytochrome *b* gene) and the gene encoding species-specific repeat (SSR) region. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and SSR techniques were used to identify of species origin. Restriction analysis of PCR-RFLP of the mitochondrial cytochrome *b* segment and SSR analysis showed no differences between cattle and buffalo. Where, the fragment length (bp) generated by *Alu*I PCR-RFLP were 190, 169 and PCR amplification size of the gene encoding SSR region was 603 bp in both cattle and buffalo. Consequently, finding from this study could be revealed that cattle and buffalo are evolutionary derived from the same ancestor.

Keywords: cattle; buffalo; PCR; PCR-RFLP; mt-DNA; cytochrome *b;* species-specific; conservation

Introduction and literature review

Comparative cytogenetics, accompanied by comparative gene mapping, show that chromosome band homology is a good indicator of genetic homology, and that chromosome conservation indicates syntenic conservation (Lalley et al. 1978 and O'Brien & Nash 1982). Cattle karyotype comprises 60 chromosomes, whereas in the river buffalo there are 50 chromosomes. Although the diploid chromosome numbers of cattle and buffalo are different, the fundamental number of both is 62. Where, in cattle

58 acrocentric autosomes and two bi-armed sex chromosomes, while in buffalo 38 acrocentric autosomes, five pairs of bi-armed autosomes and two bi-armed sex chromosomes. The buffalo chromosomes include five bi-armed chromosome pairs which correspond to ten one-armed pairs of the cattle chromosomes. These five buffalo bi-armed chromosome pairs (1-5) originated from centric fusion translocations between cattle chromosomes: 1-25, 2-23, 8-19, 5-28 and 16-29, respectively (*Di Berardino et al. 1981; Di Berardino & Iannuzzi 1984; Iannuzzi et al. 1987 & 1990*).

In this research we report the use of the polymerase chain reaction (PCR) to amplify 359 bases of mitochondrial cytochrome *b* gene and species-specific repeat (SSR) gene in both cattle and buffalo.

Materials and methods

Genomic DNA included mitochondrial DNA (mt-DNA) was extracted from muscle tissue according to *Bardakci and Skibinski* (1994), where 0.5 g of the tissue was homogenized and suspended in 1 ml STE buffer (0.1 M Na Cl, 0.05 M Tris and 0.01 M EDTA, pH 8). The mixture was treated with 30 µl SDS (10 %) and 30 µl Proteinase K (10 mg/ ml), followed by phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1), successively. DNA was precipitated with cold 95% ethanol, then dissolved in an appropriate volume of double distillated water (ddH_2O).

The segment of mt-DNA and SSR gene were amplified with the use of Primers sequences (Lenstra et al. 2001): 5'- CCA TCC AAC ATC TCA GCA TGA TGA AA-3' (forward)/5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3' (reverse) and 5'-AAG CTT GTG ACA GAT AGA ACG AT-3' (forward)/5'- CAA GCT GTC TAG AAT TCA GGG A-3' (reverse), respectively. PCR was performed in a reaction volume of 25 µl contained 50 ng of genomic DNA, 25 pmol dNTPs, 25 pmol of the primer, 1.0 U Taq DNA polymerase and reaction buffer (Finnzymes). After 35 cycles (94°C, 30 sec; 56°C for cytochrome *b* gene and 60°C for SSR gene, 30 sec; 72°C, 30 sec), the presence of the 359 bp and species-specific gene products were checked on 3% agarose gel. For restriction analysis, digestion of 12 µl of each PCR product (359 bases of mitochondrial cytochrome *b* gene) was accomplished with 10 units *Alu*I restriction enzyme for 4 hours at 37°C.

Digested DNA was separated on 3% agarose gels in 1x TBE buffer, stained with ethidium bromide, visualized under UV light and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Results and discussion

In the present study, genomic DNA included mitochondrial DNA (mt-DNA) from muscle tissues of cattle and buffalo was extracted to amplify both the gene encoding cytochrome *b* and the gene encoding species-specific repeat (SSR). The amplification product of the gene encoding cytochrome *b* in both cattle and buffalo was 359-bp in length (Fig. 1A). Two different patterns were detected after the *Alu*I digestion and the sizes were 190- and 169-bp in both cattle and buffalo. Because of the little difference in length (21 bp) between these two fragments, they appear tightly close to each other and look likes one thick band (Fig. 1B). In relation to SSR, PCR amplification of the gene encoding species-specific repeat yielded 603-bp in length in both cattle and buffalo. It should be noted that the size and the position of the generated fragment (603 bp) are the same with the fragment of the molecular weight marker (ΦX174 DNA ladder) as shown in Fig. 2.

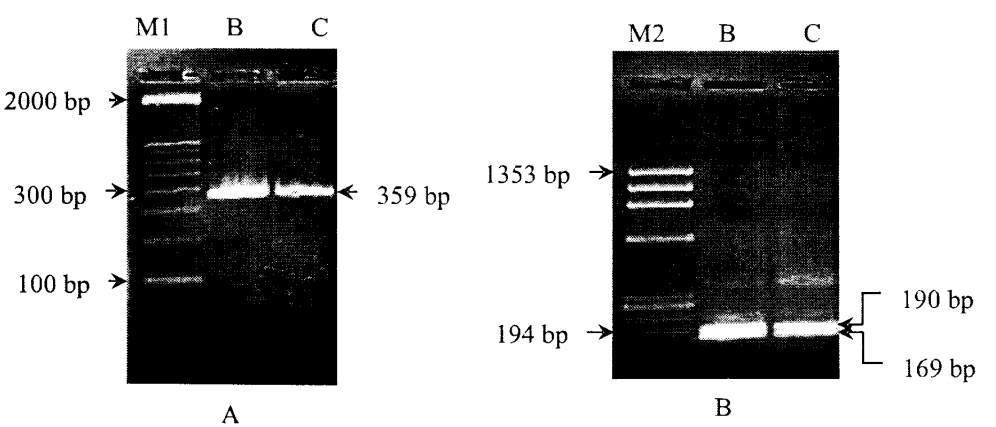

Figure 1. Agarose gel electrophoresis of amplified *cytochrome b* gene (A) following digestion with *Alu*I generated two fragments with a size of 190 and 169 bp (B). Lane B is buffalo, lane C is cattle and lanes M1 and M2 are molecular weight markers (100-bp ladder and ΦX174 DNA – *Hae* III Digest, respectively).

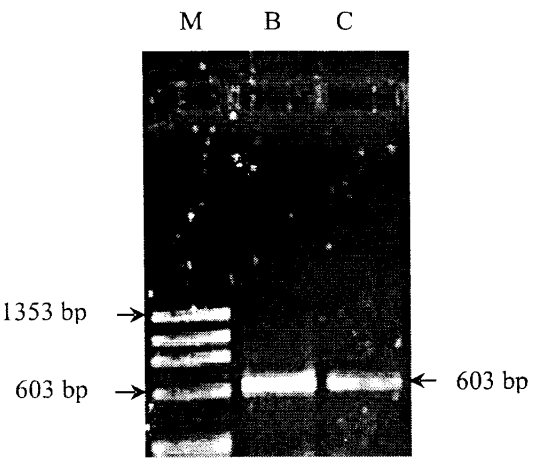

Figure 2. PCR products (603-bp) generated by primers species-specific oligonucleotide. Lane B is buffalo, lane C is cattle and lane M is a molecular weight marker (ΦX174 DNA – *Hae* III Digest).

Discussion

This research was mainly performed for species-specific identification of cattle and buffalo using PCR-RFLP mitochondrial cytochrome *b* gene and species-specific repeat (SSR) gene techniques. The results of these two techniques showed good evidence for molecular markers linked to genetic similarity between cattle and buffalo. Where, PCR amplification of the gene encoding cytochrome *b* gene generated the same fragment (359-bp) in both cattle and buffalo. *Alu*I digestion of the PCR fragment (359-bp) resulted the same restriction pattern fragments 190- and 169-bp in both cattle and buffalo. Also, PCR amplification of the species-specific repeat (SSR) gene, using the same primers, yielded the same PCR fragment (603-bp in length) in both cattle and buffalo.

Obviously, cattle and buffalo belong to family *Bovidae*, which is notable for its conservative mechanism of karyotype evolution, that seems to have proceeded almost exclusively by centric fusion of acrocentric chromosomes. This is evidenced by the small variation in the number of chromosome arms (58-62) and the strikingly similar banding patterns in the chromosomes of various species (*Buckland and Evans 1978*). The cattle autosomes show a banding pattern which is similar to that of buffalo, confirming the extensive chromosome homology between them as summarized in Table 1 (*Iannuzzi et al. 1990 and Report of the committee for the standardization of banded karyotypes of the river buffalo 1994*).

Table 1. Homologous river buffalo and cattle G- and R-banded autosomes

River buffalo	1q	1p	2q	2p	3q	3p	4q	4p	5q	5p	6	7	8	9	10
Cattle	1	25	2	23	8	19	5	28	16	29	3	4	6	7	9
River buffalo	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
Cattle	10	11	12	13	14	15	17	18	20	21	22	24	26	27	

Several genes assigned to cattle chromosomes were assigned to buffalo chromosomes with similar banding pattern. However, *El Nahas et al.* (1993) assigned three genes encoding isoenzymes GAPD, TPI and LDHB to the long arm of buffalo chromosome 4. These three genes were assigned to cattle chromosome 5 (*Fries et al. 1991*), which is homologous to buffalo chromosome 4q as shown in Table 1. The immunoglobulin gamma heavy chain (IGHG), previously mapped to cattle chromosome 21q24 by *Gu et al. (1992*), was assigned to the homologous buffalo chromosome 20q23-q25 by *Hassanane et al.* (1993). The omega (IFNW) and trophoplast (IFNT) interferon genes were assigned to cattle chromosome 8q and to the homologous buffalo chromosome 3q at the band 15 in both cattle and buffalo (*Iannuzzi et al. 1993a*). The bovine major histocompatibility complex (MHC), previously assigned to cattle chromosome 23q13-q23 by Fries et al. (1986) and Hediger et al. (1991), was mapped to the homologous river buffalo chromosome 2p17 *(Iannuzzi et al. 1993b*).

Ryan et al. (1994) mapped the uridine monophosphate synthase (UMPS) gene to cattle chromosome 1q31, which was assigned by *Iannuzzi et al.* (1994a) to the homologous buffalo chromosome 1q. The interferon gamma (IFNG) gene was mapped to cattle chromosome 5q25-q27 (*Chowdhary et al. 1993*), which was later assigned to the homologous buffalo chromosome 4q23-q26, using the same cDNA probe (*Hassanane et al. 1994*). In a study of *Iannuzzi et al.* (2003) by fluorescence in situ hybridization and R-banding, all mapped loci from 26 bovine syntenic groups were assigned to homologous chromosomes and chromosome regions of river buffalo chromosomes.

Conclusion

As a consequence, our results, in combination with the previous findings, indicate that there is considerable evidence that cattle and buffalo are evolutionary derived from the same ancestor. This is speculation, however.

Dokazi na bazi citogenetskih i molekularnih markera o poreklu goveda i bivola od istog predaka

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Rezime

U ovom istraživanju, korišćene su tehnike PCR-RFLP i SSR za identifikovanje porekla vrste. PCR – lančana reakcija polimeraza je korišćena za pojačavanje 359 baza mitohondrijalnog citohroma *b* gena, a gen SSR – ponavljanje specifično za vrstu i kod goveda i kod bivola. Proizvod amplifikacije citohroma *b* gena je bio 359-bp po dužini i dva različita obrasca su otkrivena nakon *Alu*I digestije i veličine su bile 190- i 169-bp i kod goveda i kod bivola. Nasuprot tome, PCR amplifikacija gen kodirajućeg ponavljanja specifičnog za vrstu SSR dala je 603-bp po dužini I kod goveda I kod bivola.

Rezultati dobijeni primenom ove dve tehnike su pokazali dokaze za molekularne markere vezane za genetsku sličnost i idnetifikaciju specifičnu za vrstu između goveda i bivola. Goveda i bivoli pripadaju familiji *Bovidae*, koja je posebna zbog svog konzervativnog mehanizma evolucije kariotipa. Autozomi goveda pokazuju obrazac sličan onom kod bivola, čime se potvršuje ekstenzivna homologija hromozoma među njima. Međutim, nekoliko gena i sinteničkih grupa dodeljenih hromozomima goveda su dodeljeni hromozomima bivola sa sličnim obrascem bandova. Nakon razmatranja dobijenih rezultata I prethodnih pronalazaka zaključili smo da goveda I bivoli evoluciono potiču od istog predaka.

Ključne reči: goveda; bivo; PCR; PCR-RFLP; mt-DNA; citohrom *b;* specifično za vrstu; konzervacija

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