Fast and sensitive identification of species-specific milk using PCR and PCR-RFLP techniques

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Introduction

- Many centuries ago, perhaps as early as 6000-8000 BC, ancient man learned to domesticate species of animals (cows, buffaloes, sheep, goats and camels) for the production of milk for human consumption.
- Cows: Nine out of every ten glasses of milk consumed by people come from cows.
- Buffaloes: Water buffalo produce half of the milk consumed in India.
- Goats: Some people find goat's milk easier to digest than cow's milk and fat globules in goat's milk are smaller than in cow's milk.
- Sheep: Milk from sheep has twice the fat content of cow's milk.
- Camel: In the hot desert, camel milk lasts longer than other types of milk and it can last for three months when properly refrigerated.

So, milk species identification has a remarkable importance for the following reasons:

- **Human adverse reactions toward some species milk proteins and government regulations.**
- **The common fraudulent practice found in the dairy production line is the use of a less costly type of milk in substitution of more expensive ones.**
- **Avoiding unfair competition and to assure consumers of accurate labeling.**

Consequently, PCR and PCR-RFLP techniques were developed for rapid and sensitive identification of species-specific milk.

Materials and MethodS

- **Milk Samples.** Milk samples (100 µl) were collected from buffalo, camel, cattle, goat and sheep.

- **DNA Extraction.** Genomic DNA included mitochondrial DNA (mt-DNA) was extracted according to Sharma et al. (2000) with some modifications by Abdel-Rhaman and Ahmed (2007).

- **PCR Amplification.** Buffalo, cattle, sheep, goat and camel species-specific DNA sequences and a segment of mt-DNA (359 bp) in both buffalo and cattle were amplified with the use of primer sequences as can be seen in Table 1 (Lenstra et al., 2001, Abdel-Rahman, 2006 and Abdel-Rahman et al., 2015).

- **PCR-RFLP Technique.** Restriction fragment length polymorphisms (RFLP's) for the amplified cytochrome-*b* gene (359 bp) were generated by *Taq*I restriction enzyme to differentiate between some species such as buffalo's and cattle's milk.

Table 1: Primer sequences and annealing temperatures of buffalo, cattle, sheep, goat and camel SSR, in addition to *cytochrome b* gene for both buffalo and cattle.

Species	*Primer sequence 5' - 3'*	*Annealing temperature (°C)*
Buffalo/Cattle	AAGCTTGTGACAGATAGAACGAT CAAGCTGTCTAGAATTCAGGGA	60
Sheep	GTTAGGTGTAATTAGCCTCGCGAGAA AAGCATGACATTGCTGCTAAGTTC	62
Camel	ACTGGAATCTATCTGCTGCTC GCTGCTGATGCCAAAGAGG	58
Goat	CGACAAGGCAAAACGGACAC TCCTGGCAGAGGAAGACTCCA	51
Cytochrome-*b*	CCATCCAACATCTCAGCATGATGAAA GCCCCTCAGAATGATATTTGTCCTCA	57

Species-specific regions (SSR) of follicle stimulating hormone receptor (FSHR) gene in both camel and goat.

Results and Discussion

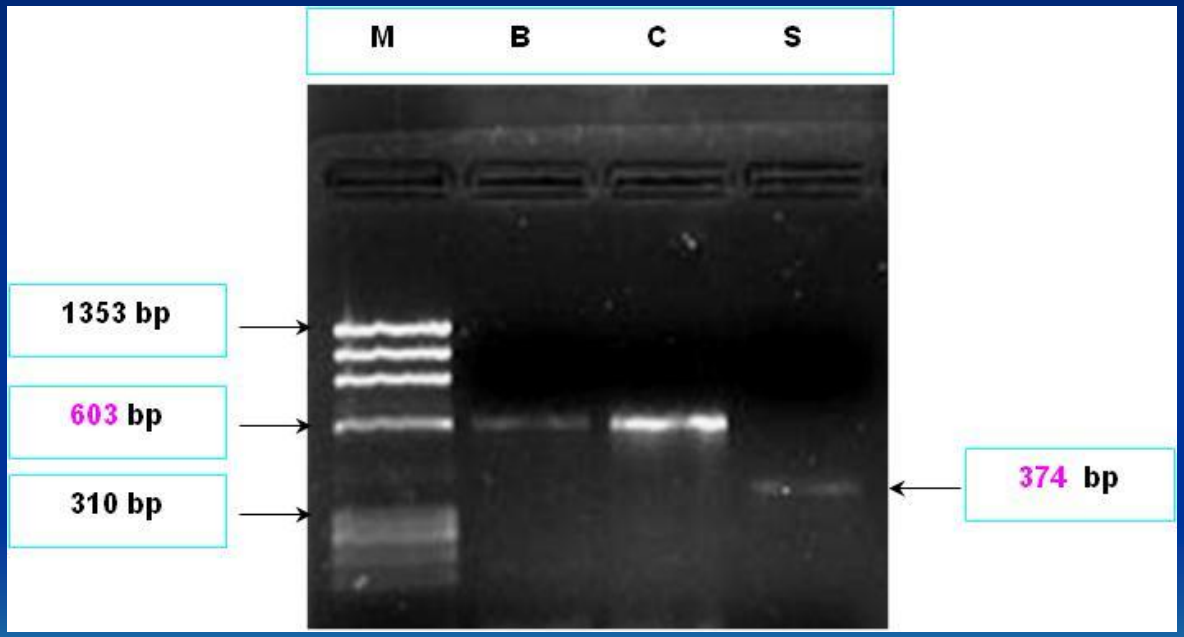

Figure 1: PCR products (603 and 374 bp) generated by primers species specific oligonucleotide. Lane B is buffalo, lane C is cattle, lane S is sheep and lane M is a marker.

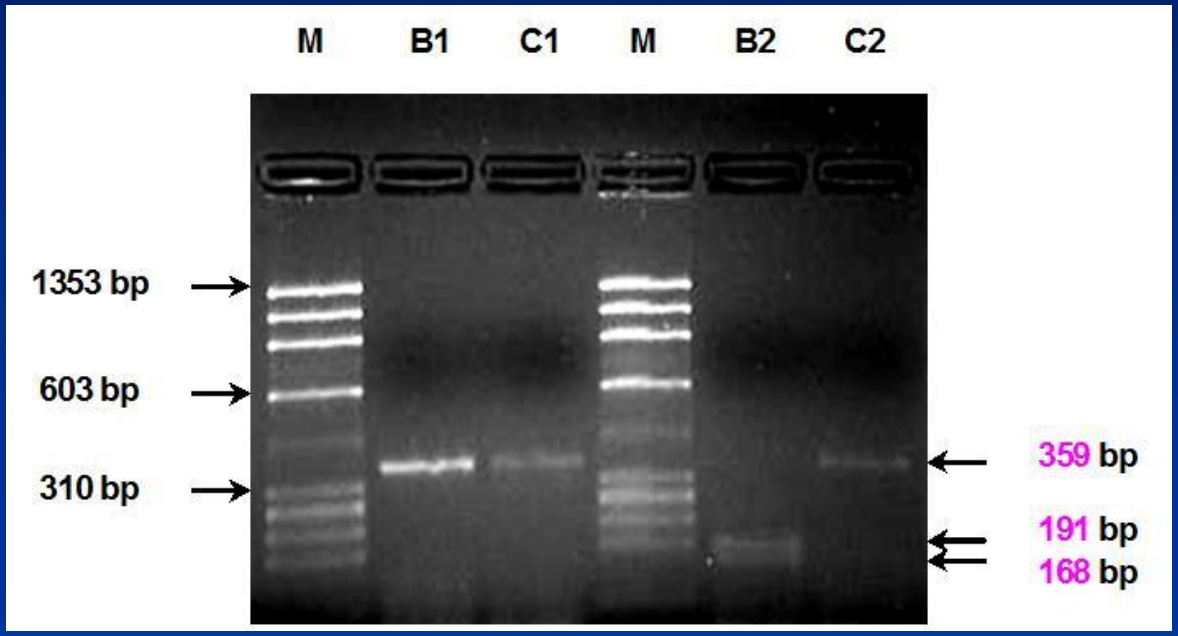

Figure 2: PCR amplification (359 bp) of cytochrome *b* gene in both buffalo (lane B1) and cattle (lane C1) following digestion with *Taq*I generated two fragments with sizes of 191 and 168 bp in buffalo (lane B2), while in cattle no digestion (359 bp) was obtained (lane C2). Lane M is a marker.

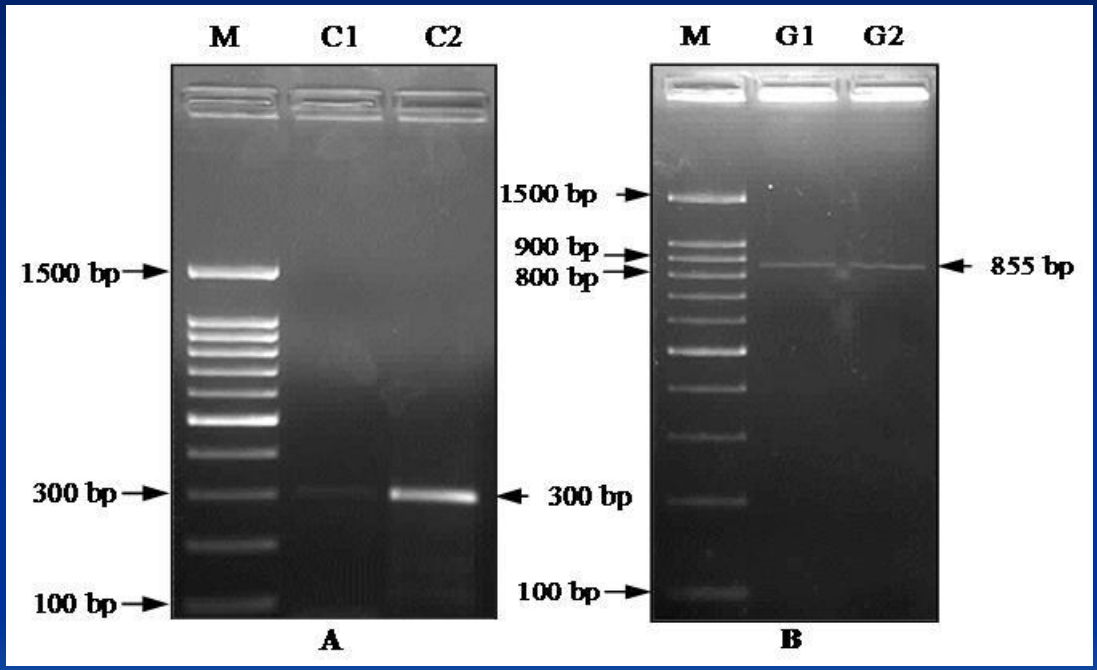

Figure 3: PCR products generated by species-specific designed primers in both camel (A) and goat (B). Lane C2 is camel's milk fragment size (300 bp). Lane G2 is goat's milk fragment size (855 bp). Lane M is a marker.

Conclusion

Table 2: PCR products of the species-specific milk descending ordered according to the fragment size.

Specie	*Band position (bp)*	*Cyt-b product size (bp)*	*Restriction enzyme*	*Fragment size (bp)*
Goat	855			
Buffalo	603	359	*Taq*I	359
Cattle	603	359	*Taq*I	191 and 168
Sheep	374			
Camel	300			





THANKS