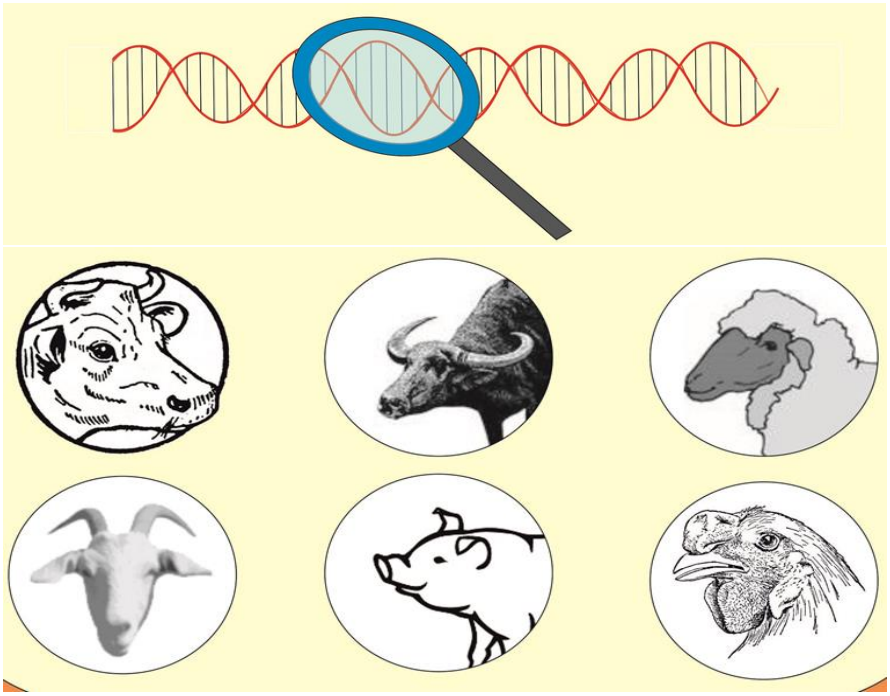
Molecular tools for Identification of Species and Sex of Meat



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1.0 Preamble

Identification of species and sex of meat is a very important requirement in Indian context as consumer is concerned about authenticity of meat. Reasons for it are selective preference for meat originated from particular species and sex of meat animals and religious concerns towards consumption of meat derived from a particular animal species. Some of the reasons which necessitate technologies for species and sex identification are as follows: (a) prevention of economic fraudulence of misrepresenting costlier meat with cheaper meats; (b) implementation of statutory slaughter restrictions on cattle especially cow; (c) verification of export consignments; (d) preventing poaching and trading of wild animals, (e) detection of animal derived material in vegetarian diet etc. Several techniques have been developed for authentication of meat but DNA based molecular techniques are the most reliable among them and hence widely used for identification of species. Under different research projects undertaken at ICAR – National Research Centre on Meat, Hyderabad array of molecular techniques have been developed for species and sex identification of meat. These techniques are being used for forensic evaluation of meat samples thereby technological support is given to different implementation agencies.

2.0 Brief description of technologies

2.1 Species identification of meat by sequence analysis of mitochondrial Cytochrome B gene:

Techniques is based on Forensically Important Nucleotide Sequencing method which involves extraction of DNA from meat, PCR amplification of mitochondrial Cytochrome B gene, sequencing of amplicon followed by alignment of sequence in Basic Local Alignment Search Tool (BLAST) of National Centre for Biotechnology Information (NCBI) database www.ncbi.nlm.nih.gov. For species identification of unknown meat samples, DNA need to be extracted from meat samples, PCR amplification to be undertaken using novel universal primers, followed by sequencing of amplicons. Aligning of the nucleotide sequence of Cytochrome B gene of reference sample with that of sequences of database will show highest score for corresponding species hence it can clearly and unambiguously identify species of meat.

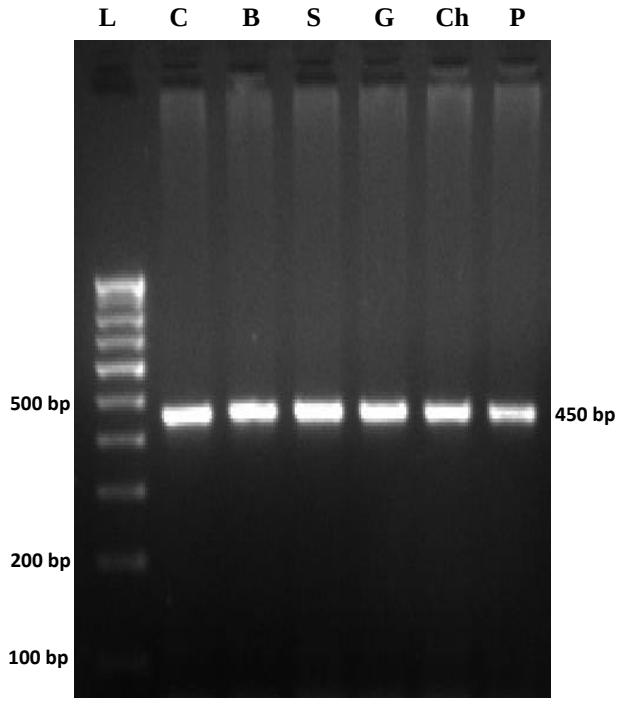

Figure 1: Polymerase chain reaction amplification of mitochondrial Cytochrome B gene of meat samples run on 1.5 % agarose gel
L: 100 bp ladder; C: Cattle; B: Buffalo; S: Sheep; G: Goat; Ch: Chicken; P: Pig

2.2 Species identification of beef by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism of mitochondrial Cytochrome B gene

Technology involves extraction of DNA from meat using standard protocol, PCR amplification of mitochondrial Cytochrome B gene using the novel primers which yields amplicon of size 450 bp followed by restriction digestion of amplicons using *Msp* I. *Msp I* restriction enzyme was selected as it has restriction site only in cattle at 252 bp, hence RFLP yields DNA fragments of size 252 bp and 198 bp upon restriction digestion in cattle. No cross reaction was found in closely related species like buffalo, sheep, goat and chicken. Beef was detectable up to 10% level in buffalo meat adulteration samples. Different cooking methods also did not affect RFLP pattern of beef samples and results were similar in beef samples thermal processed at 72^{o} C, 90^{o} C and 121^{o} C, 15 lb for 30 min.

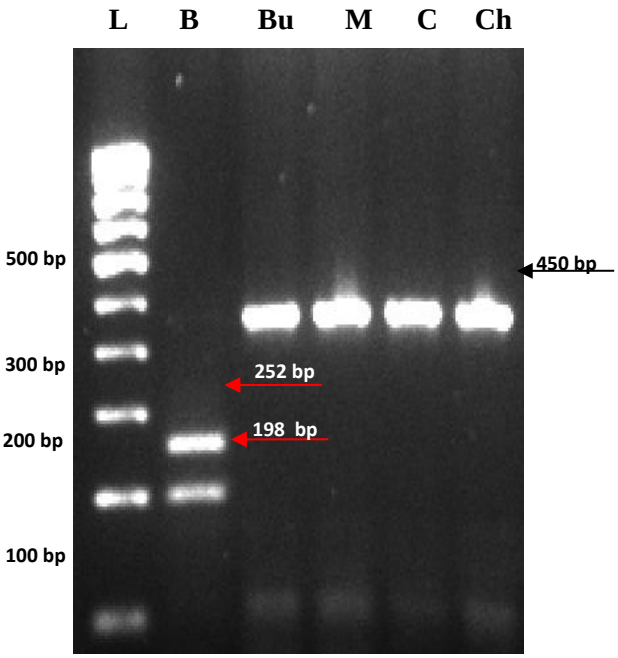

Figure 2: Polymerase Chain Reaction- Restriction Fragment Length Polymorphism of mitochondrial Cytochrome B gene of meat animals using *Msp I* enzyme.
L: 100 bp ladder; B: Beef; Bu: Buffalo meat; M: Mutton; C: Chevon; Ch: Chicken

2.3 Meat species identification by species specific polymerase chain reaction

Species specific PCR involves amplification of specific region of the gene by utilizing set of specific primers targeting a species. It takes lesser assay time as it does not involve any post reaction processing except electrophoresis. Primers yield amplicon only in the species for which they have been designed and no amplification is expected in other species. Species specific primers have been designed and tests have been standardized for identification of buffalo meat, chevon and mutton. This techniques obviates the requirement of sequencing as in FINS and restriction enzyme digestion as in PCR RFLP based techniques thereby saving cost and time.

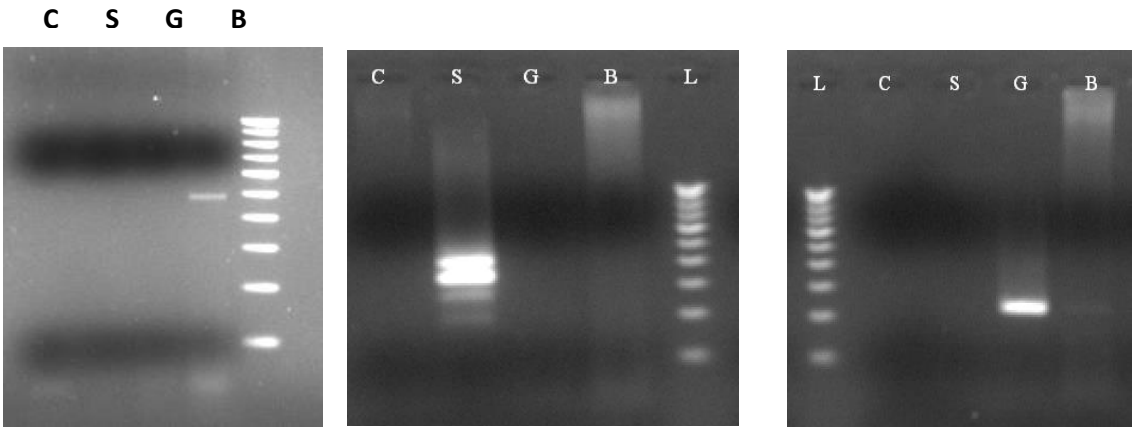

Figure 3: (a) Buffalo specific PCR (b) Sheep specific PCR (c) Goat specific PCR

2.4 Identification of sex of beef by duplex PCR amplification of SRY and mitochondrial 12S rRNA gene

SRY (Sex-determining Region Y) is a sex determining gene located on Y chromosome. The SRY gene encodes the testes determining factor, which is also referred to as the SRY protein. PCR amplification targeting sex specific SRY gene can reliably authenticate sex of meat. For the purpose of PCR, primers were designed for SRY gene which would give an amplicon of about 195 bp in male and no amplification in female. To obviate the chances of false negative results due to failure in PCR, mitochondrial 12S rRNA gene primers are used in the reaction mix to act as internal control which will give amplicon of about 456 bp in both male and female samples.

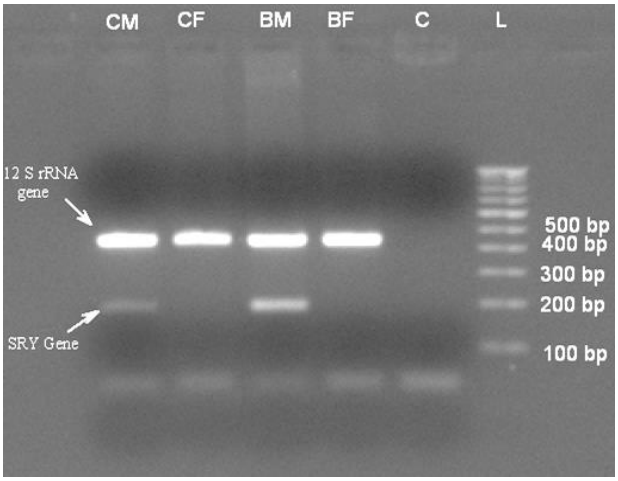

Figure 4: Duplex PCR targeting SRY and mt 12S rRNA gene for identification of sex of meat
CM: Cattle Male; CF: Cattle Female; BM: Buffalo Male; BF: Buffalo Female
C: Negative control; L: 100 bp Ladder

2.5 Proteomic based technique for meat species identification

Proteomic-based technology using in-gel (two-dimensional gel electrophoresis, 2DE) and OFFGEL-electrophoresis for authentication of meat species from three closely related ruminant species viz, water buffalo, sheep and goat in both raw and cooked conditions was developed. The MALDI-TOF/TOF MS analysis of proteins separated using 2DE or OFFGEL electrophoresis delineated species-specific peptide biomarkers derived from myosin light chain 1 and 2 (MLC1 and MLC2) of buffalo, sheep and goat meat mix in different proportions that were found stable to resist thermal processing. The 2DE and tandem mass spectrometry based in-gel method can detect up to 1.0 per cent substitution of sheep and goat meat in buffalo meat, whereas OFFGEL electrophoresis and tandem MS approach can detect even up to 0.1 per cent substitution of sheep and goat meat in buffalo meat. The effectiveness of OFFGEL electrophoresis over in-gel based method is its efficiency to concentrate and enrich the low abundant proteins mainly originating from myosin light chain 1 and 2. We demonstrated the accuracy and robustness of OFFGEL-based method over 2DE based in-gel approach. The aforesaid technology envisages the robustness of high throughput proteomic approach coupled with OFFGEL electrophoresis as an alternative to other existing methods for meat speciation and pave the way for future requirements of food safety and authenticity.

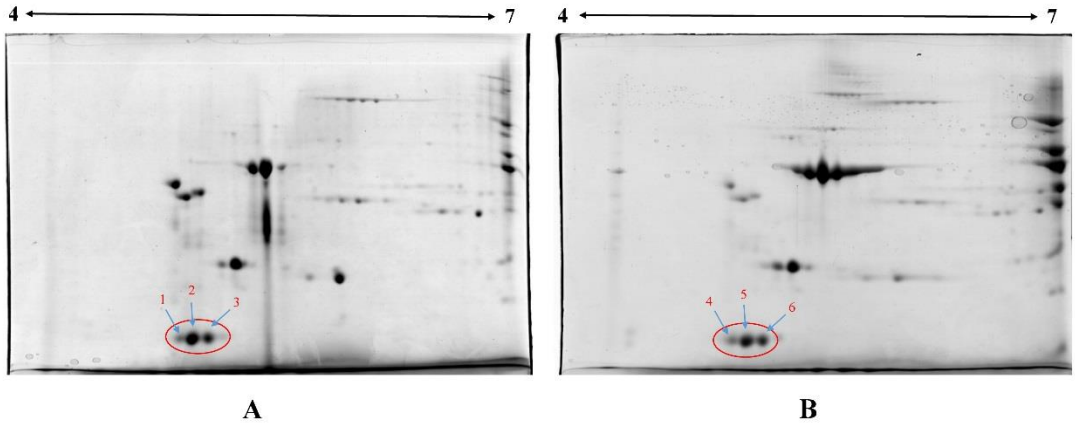

Figure 5: Representative 2DE gel of total muscle proteins extracted from (A) raw and (B) cooked meat mix containing buffalo: sheep: goat (98:1.0:1.0).

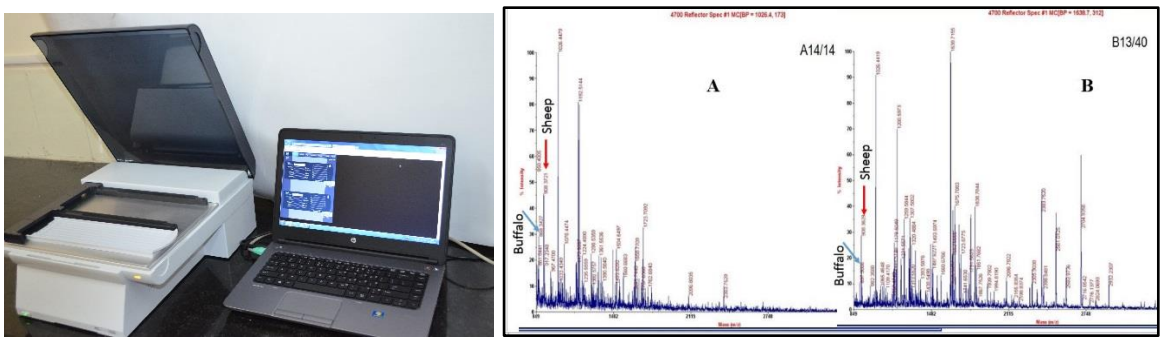

Figure 6: OFFGEL Electrophoresis and MS-based technique for meat species identification

3.0 Benefit to stakeholders

State-of-the-art laboratory for identification of species and sex of meat has been established at the Institute. Funding was taken from Ministry of Food Processing Industries, New Delhi for upgradation of the laboratory. Species identification service facility is provided to stakeholders at a cost of Rs 10,000 per sample. So far about 100 samples have been analyzed by the Institute which helped different organizations involved with implementation of statutory rules on maintaining authenticity of meat. Revenue of about Rs 10,00,000 was generate by providing service activities.

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