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Research Article

DETECTION OF DIFFERENT MEAT SPECIES BY MULTIPLEX PCR ASSAY

MAKWANA M.D.[1*], RATHOD P.T.[2], ROY S. K.[3] AND PATEL B.K.[4]

[1,3]Department of Livestock Products Technology, College of Veterinary Science and Animal Husbandry, SDAU, SK Nagar, 385506, Gujarat
[2]Department of Veterinary Public Health), Slaughter house, Jamalpur, Ahmedabad
[4]Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, SDAU, SK Nagar, 385506, Gujarat
*Corresponding Author: Email-mahendra2803@gmail.com

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Abstract- In presence study four meat Species (Buffalo, Sheep, Goat and Chicken) were selected for identification of raw meat. Mitochondrial cytochrome b gene was use as a marker gene for species identification in multiplex PCR. Common forward primers and species-specific reverse primers were used for the development of multiplex PCR by mixing of primers in the different ratio *viz.* 2.5:1.5:1.5:1.5:3 for forward: Goat: Sheep: Chicken: buffalo, species-specific reverse primers respectively. PCR primers were designed such a way to give different lengths of amplicon for different species, there is 157 bp for goat, 227 bp for chicken, 274 bp for buffalo and 331 bp for sheep in agarose gel electrophoresis, respectively. The present study was undertaken for detection of animal species in meat samples by multiplex PCR assay using cytochrome b gene variability.

Keywords- Multiplex PCR assay, Mixing of primer, Cytochrome b gene, Agarose gel electrophoresis.

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Introduction

Meat is considered as one of the important nutritious diet. The production and consumption of meat of various species of food animals are increasing in national and international markets. The preference of the consumer always remained towards the certain specific meat. In the greater part of the world, national or religious regulation restricts the use of meat from certain species as a human food. Food authenticity is currently an issue of foremost concern for food authorities, since improper marking of animal foods may have remarkable negative consequences. In the previous few decades, debasement of food products has become a problem in many countries as well as India [1]. The detection of adulteration of meat has always been a source of concern for various reasons such as public health, religious problems and harmful competition in meat marketplace and also, consumers have become more conscious about the basis of food and expect the source of food to be reliable [2].

Consumer should be protected from these malicious practices of meat adulterations by different DNA-based techniques used for animal species identification include DNA hybridization [3,4], polymerase chain reaction (PCR) and its variants [5] polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) [6] random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) [7-9], PCR-SSCP [10] and PCR-sequencing [11]. However, multiplex PCR assay was found to be rapid and cost effective for identification of meat species and successfully used for identification of various species of meat [12-14]. These PCR assays targets genomic as well as mitochondrial DNA for the purpose of meat species identification.

In the present study, Multiplex PCR assay simultaneously identifies multiple meat species in single PCR reaction using different primers sets for each species or common forward and species-specific reverse primer by targeting mitochondria DNA. Keeping in view the above facts and considering future implications in the meat trade, the present study is planned to develop simple, quick, sensitive, precise, cost effective and applicable method for detection of buffalo, sheep, goat and chicken meat processed under different processing conditions by PCR Assay.

Materials and Methods

Experimental materials for the present study comprised of meat samples of different species of animals like buffalo, sheep, goat and chicken randomly collected from the municipal/local slaughter houses and meat markets (15 samples from each species.) at Palanapur, Gujarat.High quality mitochondrial DNA was extracted using DNeasy Blood and Tissue Kit (Qiagen) as per the manufacturer's guidelines. The superiority and purity of DNA were checked and quantification done by Pico drop spectrophotometry and agarose gel electrophoresis. Showed 1.7 to 2.0 values for OD ratio (260 nm/280 nm) were considered of good quality of DNA samples and was used in the present study. A set of primers specific to cyt b gene family [5,15] was custom synthesized at sigma Aldrich. In the present study cattle, primer was used as a buffalo primer [15].

PCR reactions were carried out in a final volume of 25 µl encompassing of 3 µl of DNA Template, 2µl of primer mix, 12.50µl PCR master mixes and 7.50 µl DNase-RNase free water. Multiplex PCR was done by mixing of primers in the dissimilar ratio viz. 2.5:1.5:1.5:1.5:3 for forward: Goat: Sheep: Chicken and buffalo specific reverse primers. PCR cycling protocol included initial denaturation at 94°C for 5 minutes then monitored by 34 cycles of 94°C for 30 seconds, 62°C for 30 seconds 72 °C for 30 seconds and final extension at 72° for 10 minutes.

PCR amplicons were resolved by 4% agarose gel electrophoresis. The 5 µl PCR products mixed with 1µl of 6X gel loading dye were loaded @ of 6 µl per well on 4 % agarose gel and electrophoresed at 80 V for 30 min using 0.5X TAE buffer.

Results and Discussion

Meat identification by species - specific primers

Cytochrome b gene amplicon were amplified from DNA of meat samples using species specific primers by conventional and multiplex PCR to identify meat species.

Table-1 *The primer sequences are given below [15].*

Sr. no	Name	Primer	Sequences (5' – 3')	No. of Bases
1.	SIM	Forward	GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA	38
2.	Goat	Reverse	CTCGACAAATGTGAGTTACAGAGGGA	26
3.	Chicken	Reverse	AAGATACAGATGAAGAAGAATGAGGCG	27
4.	Sheep	Reverse	CTATGAATGCTGTGGCTATTGTCGCA	26
5.	Buffalo	Reverse	CTAGAAAAGTGTAAGACCCGTAATATAAG	29

Conventional PCR

Initially species specificity of primers was tested by conventional PCR using a pair of the common forward primer and the species specific reverse primers with DNA of meat of different known species. Thus, PCR for each species was developed. In each conventional PCR, 100 ng/µl of DNA and 10pmoles of each primer pair were used. All the PCR amplified the target cyt. b gene of meat of different known species. There did not give any non-specific amplification in any of the reaction i.e. goat specific PCR gave amplified product only in goat DNA and not in any of the other DNA. Amplified PCR products from goat, chicken, buffalo and sheep produced amplicon of expected size 157, 227, 274 and 331 bp, respectively [Fig-1] were same as a previous study [5, 15].

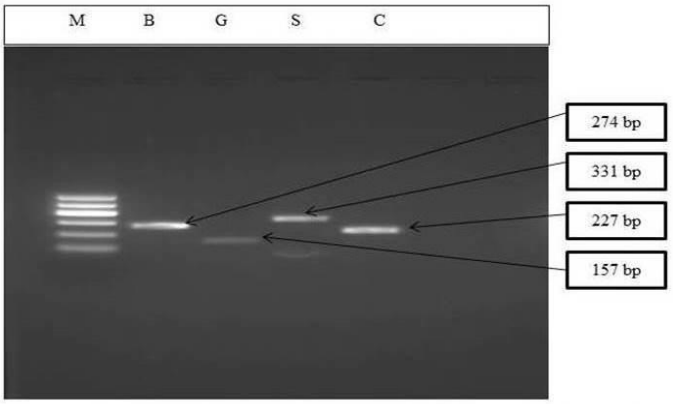

Fig-1: 4 % agarose gel electrophoresis of conventional PCR products from the *cyt b* gene of different meat species. M: Marker, 100 bp ladder, B: Buffalo (274 bp), G: Goat (157 bp), S: Sheep (331 bp), C: Chicken (227 bp)

Multiplex PCR

The primers were planned to amplify target arrangements of the four species at similar efficiency. However, multiplex PCR using equal amount of primers did not result into a species specific single band same as a previous study [5]. In general, quantitative PCR is difficult because of unequal efficiency of amplification. Amplification productivity is affected by the dissimilarity of primer sequences. The common forward primer was designed to be shared by four species; therefore amplification efficiency of PCR was affected by only reverse primers. A little difference of Tm among the reverse primers would distress the productivity. In order to control efficiency, the ratio of the primers was change according to the results. Hence, the primers ratio 2.5:1.5:1.5:1.5:3 for SIM: G: S: C: B was used in subsequent PCR testing which successfully amplified only the target species cyt b gene. In the present study multiplex PCR amplified amplicons specific to each species produce characteristic single band pattern on agarose gel electrophoresis for single species and a multiple band pattern for DNA mix showed in [Fig-2]. Amplified PCR products from goat, chicken, buffalo and sheep produced amplicon of 157, 227, 274 and 331 bp respectively. The four meats could thus be identified based on the size of PCR amplicons. Results obtained in the present study was similar to the previous study [5,15-17].

Conclusion

The present study was undertaken for detection of animal species in meat samples by multiplex PCR assay exhausting cytochrome b gene changeability. Meat samples from buffalo, sheep, goat and chicken were utilized for molecular investigation of every species as per standard technique. Specificity of each primer pair was confirmed by conventional PCR. After confirmed species specificity of each primer a multiplex PCR was designed by mixing all primers in a single reaction multiplex PCR assay was successfully amplified for DNA of meat samples of goat, chicken, buffalo and sheep. Agarose gel electrophoresis of multiplex PCR yields from goat, chicken, buffalo and sheep produced amplicon of 157, 227, 274 and 331 bp, respectively.

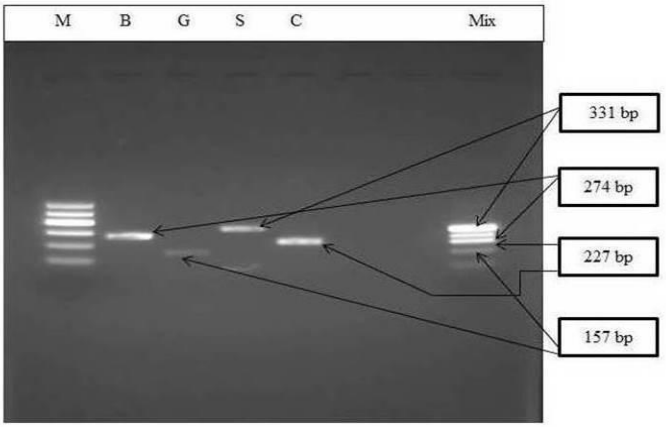

Fig- 2: 4 % agarose gel electrophoresis of multiplex PCR products from the *cyt b* gene of different meat species. M: Marker 100 bp ladder, B: Buffalo (274 bp), G: Goat (157 bp), S: Sheep (331 bp), C: Chicken (227 bp), Mix: PCR products of mixed meat species DNA

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Conflict of Interest: None declared

References

[1] Malmheden Y. I. and Emanuelsson R. (1998) New technology for faster disclosure of meat adulteration VarFada. 3, 6-7 (in Swedish).

[2] Kumar D., Singh S. P., Umapathi V., Nagappa K. and Gaurav A. (2011) *Buffalo Bulletin,* 30 (3), 170-176.

[3] Baur C., Teifel-Greiding J. and Leibhardt, E. (1987) *Archiv Fur Lebensmittel hygiene,* 38,172-184.

[4] Janssen F. W., Hagele G. H., Buntjer J. B. and Lenstra J. A. (1998) *Journal of Indian Microbiology and Biotechnology,* 21 (3),115-120.

[5] Matsunaga T., Chikuni K., Tanabe R., Muroya S., Shibata K., Yamada J. and Shinmura Y. (1999) *Meat Sciences,* 51,143-148.

[6] Girish P. S., Anjaneyulu A. S. R., Viswas K. N., Shivakumar B. M., Anand M., Patel M. and Sharma B. (2005) *Meat Sciences,* 70,107-112.

[7] Sebastio P., Zanelli P. and Neri T. M.(2001) *Journal of Agricultural Food Chemistry,* 49, 1194-1199.

[8] Mane B. G., Tanwar V. K., Girish P. S. and Dixit V. P. (2006) *Journal of Veterinary Public Health,* 4, 87-90.
[9] Mane B. G., Tanwar V. K., Girish P. S., Sharma D. and Sonawane A. A. (2008) *International Journal of Food Safety, Nutrition and Public Health,* 1(1), 51-57.
[10] Jurgen K. P., Weder R.and Kaiser K. (2001) *European Food Research and Technology,* 213, 139-144.
[11] Bartlett S. E. and Davidson W. S. (1992) *Journal of Biotechnology,* 12, 408-411.
[12] Ilhak O. I. and Arslan A. (2007) *Turkey Journal of Veterinary and Animal Science,* 31,159-163.
[13] Martin I., Garcia T., Fajardo V., Lopez-Calleja I., Rojas M., Hernandez Y. E., Gonzalez I. and Martin R. (2007) *Meat Science,* 76(4), 721-729.
[14] Frezza D., Giambra V., Chegdani F., Fontana C., Maccabiani G.and Losio N. (2008) *Innovative Food Science and Emerging Technology,* 9, 18-23.
[15] Jain S., Brahmbhatt M. N., Rank D. N., Joshi C. G. and Solanki J. V. (2007) *Indian Journal of Animal Sciences,* 77(9), 880-881.
[16] Obrovska I., Steinhauserova I. and Nebola M. (2002) *Folia Veterinaria,* 46(3), 113- 118.
[17] Dharanesh M. G. (2007) Identification of meats of different animal species using PCR based molecular techniques. M.V.Sc thesis (Unpublished). G.B. Pant University of Agricultural and Technology Pantnagar, India.