INTERNATIONAL JOURNAL OF RESEARCHES IN BIOSCIENCES, AGRICULTURE AND TECHNOLOGY
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www.vmsindia.org

SPECIES SPECIFIC IDENTIFICATION OF TEXTILES AMINAL FIBRE PASHMINA GOAT WOOL AND SHEEP WOOL BY MEANS OF MITOCHONDRIAL 12S rRNA-PCR MOLECULAR TOOLS

R. P. Sontakke and D. H. Tambekar
Post Graduate Department of Microbiology, SGB Amravati University, Amravati 444602, India
sontakke.raj@gmail.com

Abstract:
Due to the development of molecular biology, classification of various species by molecular phylogenetic analysis is performed, and it is most definitive to use the genome itself for identification, appraisal and phylogenetic analysis of organisms. At present, as a method of discriminating animal hair which has been put to practical use, it is carried out by an expert observation using an optical microscope. Therefore, in this research, PCR method which is one of genetic techniques is used, and method to differentiate wool and cashmere by analyzing specific parts of each DNA. By amplifying DNA specific to each part, it is possible to differentiate between wool and cashmere. In addition, the development of such technology will enable accurate determination of the composition of unknown animal fibre blends. This will help to give full-proof concerning about the dissimilarity in the DNA sequence of Individual animal and helping in differentiating them when present as blends to control adulteration and false declaration of the fibres.
Key words: Pashmina goat; wool; sheep; PCR; Textile fibre.

Introduction:

Textile Testing and Quality Control is responsible to ensure the quality of the products. Animal fibers are high commercial value and Wool is the most commonly used animal fiber. The fiber is obtained from the soft, hairy covering of sheep and sometimes goats. India produces the finest Pashmina (Cashmere) wool in the world, coming from the Changthang plateau of Ladakh region of J&K State. Pashmina wool is produced by Changra (Pashmina) goat in Ladakh and Chegu breed of goat in the eastern parts of Himalayas [6].

Under the microscope, the wool fiber looks like a long cylinder with scales on it. Animal hair fibres are based on cross-linked proteins known as keratins. The major fibre in this group is sheep's wool but other fibres of considerable commercial importance are those obtained from animals such as goats (cashmere and mohair), the bactrian camel and the South American camelids [3].

Currently, there is no precise method locally available to identify and differentiate the fibres. Animal fibres are highly valuable industrial products often adulterated during marketing. Identification of both raw and processed speciality animal fibres is important to help combat adulteration or false declaration and ensure adherence to international trading agreements. Cashmere, in particular has frequently been found to be adulterated with much cheaper fibres such as sheep's wool or yak hair [9].

In the textile quality labeling regulations, it has become possible to display the type of animal hair in the past, which could only display the hair, differentiation of the animal hair and measurement of mixing ratio is increasing. In addition, due to the development of wool drawing and descaling processing and diversification of animal hair, even more advanced discrimination techniques are being demanded. Differentiation of animal hair currently being put into practice is carried out by an expert on appearance observation using an optical microscope.

Advances in molecular biology in recent years are remarkable, DNA analysis is used not only for research in the fields of medicine and agriculture but also in the field of foods, differentiation of meat types used in processed foods. There are also some reports on the differentiation of animal hair. Therefore, we studied a method to discriminate between wool and cashmere by analyzing the difference of each DNA by PCR which is one of gene amplification techniques. The progress of molecular biology introduced a new approach, which is based on nucleotide sequence diversities among species in particular regions of DNA [7, 8].

Experimental method:

Sample: Pasmina Hair (*Capra hircus laniger)* sample was collected from Pasmina research Center ladakh India in form of raw wool fibre of Pasmina goat and Sheep wool *(Ovis aries)* sample collected from authentic sources from different regional office laboratories of the Textile Committee. Randomly 7 unknown woollen samples mark as Wol-1, Wol-2, Wol-3,Wol-4,Wol-5,Wol-6 and Wol-7 were scrutinized for Molecular study. A sheep of Merino type having an average fiber diameter of about 28 µm (Hereinafter referred to as wool) and about 18 µm raw wool (hereinafter referred to as " Lower cashmere)

Mitochondrial DNA extraction and preparation: Mitochondrial DNA was extracted using the method as described by Geng *et al.,* 2012 [1]. Briefly, all goat cashmere and sheep wool samples were cleaned

prior to mitochondrial DNA extraction by successively shaked 10 s in 95% ethanol to wipe off sundries. The air dried samples (about 15 mg) were then cut into 1 mm fragments and put into 2 ml microcentrifuge tube. The mitochondrial DNA was extracted using Tissue and Hair Extraction Kit (DNA IQ™, Promega, Madison, WI, USA) according to the manufacturer's protocol. DNA concentration was assessed by spectrophotometry (NanoDrop 1000 spectrophotometer, Thermo Scientific, Wilmington, USA). DNA extracts of dissolved in TE buffer and diluted so as to get 50 ng/ml DNA concentration.

PCR amplification of 12S rRNA factor:

The partial mitochondrial 12S rRNA factor was amplified by employing a try of universal primers (forward: 50-AAACTGGGATTAGATACCCCACTAT- 30; reverse: 50-GAGGGTGACGGGCGGTGTGT-30) found in Kocher *et al.,* (1989)[4], PCR amplification was performed in a final volume of 25 ml containing 2.5 ml of 10 _ PCR buffer (100 mM Tris-HCl, pH 9.0, 15 mM MgCl2, 500 mM KCl and 0.1% gelatin), 1 ml of 10 mM dNTPs mix, 1 ml each of primer (10 mM), 1 U of Taq DNA polymerase and 100 ng of DNA template. Amplification was performed in a Thermal Cycler C1000 (Bio-Rad, USA). PCR cycling parameters were as follows:

35 cycles each consisted of 94^{O}C for 30s, 57^{O}C for 30 s, and 72^{O}C for 30 s, with an initial hot start at 94^{O}C for 5 min and a final extension at 72^{O}C for 10 min. The PCR products were electrophoresed in 1% agarose gel and stained with ethidium bromide.

Detection of DNA amplified fragments:

5 µl or 10 µl of the PCR reaction solution was transferred to a 2% agarose gel, Electrophoresis was carried out, and the gel was dissolved in ethidium bromide. Then use an image analyzer on the UV irradiator, a gel photograph was taken and the amplified DNA fragment. Detection was done.

Results and Discussion

The PCR method described enabled the detection of the amplified fragments of goat DNA, size 336 bp, and sheep DNA, size 158 bp, respectively. This is clearly shown in Figure 1. Using DNA extracted from both wool and cashmere as a template, specific primers were designed for each experiment. As a result, it was found that it is possible to distinguish by presence or absence of amplification. Furthermore, by designing primers so that fragments of PCR products that amplify using DNA extracted from wool and cashmere as a template are different lengths, it is possible to judge both at the same time with one reaction solution. Kocher *et al.,*(1989) demonstrated dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Differential marker with meat assay kit mtDNA sequence is very preserved in several animal species facultative style universal primers for amplification of 12S rRNA factor [4] and Girish *et al.,* (2005) and Mahajan *et al.,*(2011) reported the meat identification of animal species[2,5].

It's been incontestable that mtDNA is pure from hairshafts, whereas nuclear deoxyribonucleic acid can't be systematically obtained[11]. Though hair shafts contain no vital nuclear deoxyribonucleic acid, they're a fashionable supply of mtDNA. The isolated mtDNA from hair shaft is used for the identification of animal fiber [10].

From the Figure 2, it is conformed that out of 7 unknown sample 4 belongs to Goat (Pashmina) i.e. Wol-2,Wol-3,Wol-6 & Wol-7 a fragment of about 336 bp, and remaining 3 were mating with the Sheep Prime i.e. Wol-1,Wol-4 and Wol-5. In this study, mitochondrial 12S rRNA sequence has been used as a molecular marker for goat cashmere and sheep wool for molecular identification. The used PCR-RFLP analysis technique was found to be effective in correct identification.

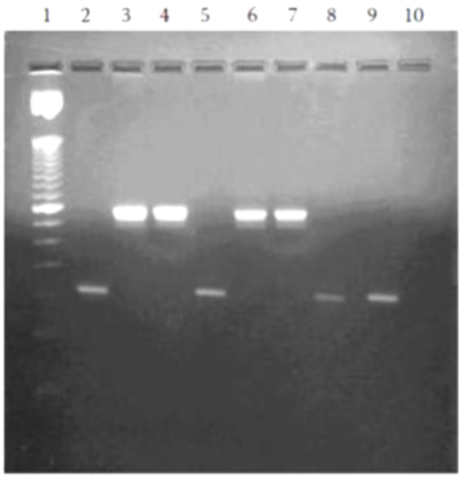

Figure 1: Detection of goat and sheep DNA (3% agarose gel)

Lane 1 – 50 bp DNA Ladder

Lane 2 – sheep Primer

Lane 3 – goat Primer

Lane 4 – PCR product of goat DNA (Pashmina)

Lane 5 – PCR product of sheep DNA

Lane 6 – PCR product of goat DNA (Pashmina)

Lane 7 – PCR product of goat DNA (Pashmina)

Lane 8 – PCR product of sheep DNA

Lane 9 – PCR product of sheep DNA

Lane 10 – Negative control

Figure 2: Differential marker with species specific primer of Sheep (Wool), Goat (Pashmina fiber) and 7 Unknown wool samples

Conclusion:

The method of amplifying using the common primer of the meat assay kit, judging with the fragment, the method of judging by the presence or absence of amplification using primers specific to wool and cashmere, respectively as a method of discrimination was found to be possible. Therefore, it is considered that the method using primers specific to species is most suitable for discrimination between wool and cashmere using PCR method.

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