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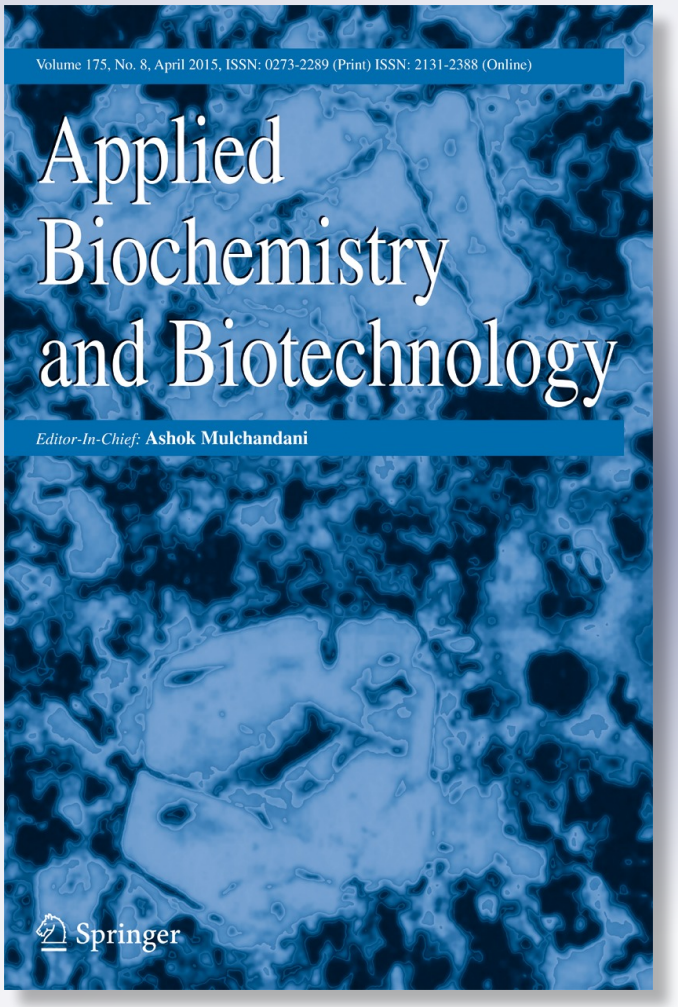

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Development of PCR-Based Technique for Detection of Purity of Pashmina Fiber from Textile Materials

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Abstract Pashmina fiber is one of major specialty animal fiber in India. The quality of Pashmina obtained from Changthangi and Chegu goats in India is very good. Due to restricted availability and high prices, adulteration of natural prized fibers is becoming a common practice by the manufacturers. Sheep wool is a cheap substitute, which is usually used for adulteration and false declaration of Pashmina-based products. Presently, there is lack of cost-effective and readily available methodology to identify the adulteration of Pashmina products from other similar looking substitutes like sheep wool. Polymerase chain reaction (PCR)-based detection method can be used to identify origin of animal fiber. Extraction of quality DNA from dyed and processed animal fiber and textile materials is a limiting factor in the development of such detection methods. In the present study, quality DNA was extracted from textile materials, and PCR-based technique using mitochondrial gene (12S rRNA) specific primers was developed for detection of the Pashmina in textile blends. This technique has been used for detection of the adulteration of the Pashmina products with sheep wool. The technique can detect adulteration level up to 10 % of sheep/goat fibers in textile blends.

Keywords Pashmina · Cottage-based industry · Fiber identification · PCR · Textile materials

Introduction

Specialty hair enjoys a unique place in shawl manufacturing cottage-based industry in India. These specialty animal fibers include Pashmina obtained from Changthangi and Chegu goats,

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angora fiber from Angora rabbits, and some others like down hair fibers from Yak. India produces about 40–50 tonnes of Pashmina fiber annually [1]. Due to restricted availability and high prices, adulteration of natural prized fibers is becoming a common practice by manufacturers. Sheep wool is one of the cheaper substitutes for adulteration with specialty animal fiber primarily because of its low cost and adequate availability and secondarily as scaly surface appearance and chemical composition of wool matches with specialty animal fibers. Moreover, Australia has developed ultrafine Merino wool of 14–16 μm diameter which is quite similar to Pashmina (cashmere) and easily admixture with cashmere for production of high-quality value added products [1]. Furthermore, OPTIM fiber processing technology has been developed by Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia which provides an opportunity to extend the wool up to 30 % of its original length which reduces the fiber diameter to 15–16 nm, hence appears quite similar to cashmere. Angora rabbit fibers could be readily identified visually on microscope from Pashmina due to ladder type medulatory structure of rabbit hair. Scanning electron microscopy (SEM) is a gold standard method available for exact identification of fibers of animal and plant origin but its high cost, requirement of technical expertise, and problem of uniform random sampling make it practically unsuitable for fiber identification [2]. Numerous efforts have been made in the previous years by using different imaging techniques for accurate analysis of sheep and goat origin fibers from blends [3–6]. Most of these techniques are based on the differences in surface structure of the natural fibers like scale heights, scale patterns, wavelet texture, etc. Emergence of modern technologies can modify the surface texture of the blends, and identification of fiber origin could be more difficult by conventional methods. Thus, difficulty associated with SEM imaging to identify sheep wool and Pashmina fibers warrant other readily available, sensitive techniques for identification of animal fibers.

Recent advances in isolation of quality DNA and availability of huge amount of nucleotide database of different organisms made PCR-based diagnostics easy, rapid, and species-specific. Since the woolen sector in India plays an important role by linking the rural economy with the manufacturing industry, hence, reliable methods are required to determine the composition of distinct animal fiber in yarns, fabrics, and woolen garments. By using PCR-based methods, it is now possible to identify species-specific animal fibers from raw and processed fiber samples. DNA amplification strategy was used to identify cashmere/cashgora, fine wool, yak and camel hair in treated and untreated fiber samples along with fiber blends [2]. Recently, real-time PCR has shown the applicability of fiber identification and quantitative estimation of cashmere and sheep wool in blends [7]. For developing a molecular test, quality of DNA extracted from treated textile is the limiting factor. Furthermore, wool and hair is a highly keratinized tissue, and the process of keratinization initiate vicious cycle of apoptotic changes in hair cells and nuclear DNA fragmentation occur [8]. Additionally, DNA deterioration occurs during textile manufacturing process which involves acid/alkali washes and dying process. In the present study, DNA from chemically finished woven clothes was extracted, and PCR-based detection of sheep and goat fibers was successfully done.

Materials and Methods

Natural animal fibers and silk were collected from authentic sources from different parts of the country. Briefly, sheep wool and Pashmina fiber were collected from crossbred sheep namely Bharat Merino developed at Central Sheep and Wool Research Institute (CSWRI) and Changthangi goat from Leh region (India), respectively. Angora rabbit hair, Camel hair, Yak down hair, Silk, and processed textile materials of known origin were procured from Division

of Textile Manufacturing and Textile Chemistry (TMTC), CSWRI, Avikanagar. DNA from raw fibers was isolated using previously published protocols [9] with modification. Briefly, wool and fiber materials (0.5 g) were washed with 2 % sodium dodecyl sulphate (SDS) and water. Then 5.0 ml of lysis solution containing 10 mM Tris HCl, 1 % Triton X-100, 100 mM NaCl, 40 mM $CaCl_2$, 10 mM EDTA, and 40 mM DTT was added and incubated overnight at 37 °C in shaker incubator. Furthermore, 100 μl (20 mg/ml) of proteinase K was added and further incubated at 56 °C for 4–6 h. Tris-saturated phenol (5.0 ml) was added to each tube and it was centrifuged at 5000*g* for 30 min. Supernatant was collected in a fresh tube and equal volume of chloroform: isoamyl alcohol was added and mixed thoroughly. It was centrifuged at 5000*g* for 30 min. Supernatant was collected and three volume of molecular biology grade absolute ethanol was added and tubes were kept in −20 °C for overnight. Precipitated DNA was collected by centrifugation at 10,000*g* for 10 min and washed with 70 % ethanol. Samples were air-dried and dissolved in 100 μl of TE buffer. Chemically finished textile materials were processed for DNA extraction using modified method. Briefly, textile materials were given prewashes with benzene and nitrobenzene to remove excess of dye materials. DNA precipitation was done following standard phenol-chloroform method. Precipitated DNA was further purified through commercially available silica gel column and washed thrice with washing buffer before being eluted. The quality and quantity of DNA was ascertained through 1.5 % agarose gel electrophoresis and spectrophotometric analysis, respectively. PCR amplification was performed in a 20 μl reaction volume containing 1X PCR buffer, 200 μM dNTP mix, 20 picomol each forward and reverse primer, 2.5 units of *Taq* DNA polymerase, and 5 μl (100–300 ng) of template DNA. Gene (12S rRNA)-specific primers for sheep: forward 5′-atatcaaccacacgagaggagac-3′; reverse 5′-taaactggagagtgggagat-3′ (reverse primer is specific for 16S rRNA) and goat: forward 5′-cgccctccaaatcaataag-3′; reverse 5′-agtgtatcagctgcagtagggtt-3′ were used [10]. PCR products were resolved and analyzed in agarose gel electrophoresis using ethidium bromide. Specificity of amplicons was ascertained by nucleotide sequencing. Species specificity of developed PCR technique was further confirmed with DNA isolated from Angora rabbit hair, Camel hair, Yak fibers, and Silk fibers. Additionally, PCR amplification from sheep and goat origin textile materials was also performed. PCR additives viz. BSA, DMSO, and Betaine in various concentrations were used in PCR reactions having template DNA from dyed textile materials. Where required, water was replaced with required amount of PCR additives. To check the sensitivity of PCR amplification, tenfold dilutions of sheep and goat DNA were used. Furthermore, PCR reactions were run with different combinations of DNA from sheep and goat fiber, i.e., 1 (up to 9) part of DNA from sheep wool admixed with 9 (down to 1) parts of DNA from goat Pashmina fiber.

Results

DNA Quality and Quantity DNA isolated from raw and chemically finished textile materials was resolved on 1.5 % agarose gel. Both intact and smeared bands are observed as evident from Fig. 1a, b. Spectrophotometric analysis of extracted DNA was carried out to measure the yield and purity. The total yield and its quality (Table 1) indicated that the DNA was pure and free from protein contamination. Total amount of DNA (per 0.5 g sample) was sufficient for 10–15 PCR reactions.

Sensitivity and Specificity of Developed PCR Method PCR amplification was done with raw and processed textile materials using sheep- and goat-specific primers. Single amplicon was obtained by both sheep- and goat-specific primers (Fig. 2). Nucleotide sequence of Pashmina-specific PCR product was submitted to the NCBI GenBank with accession number JF514286.

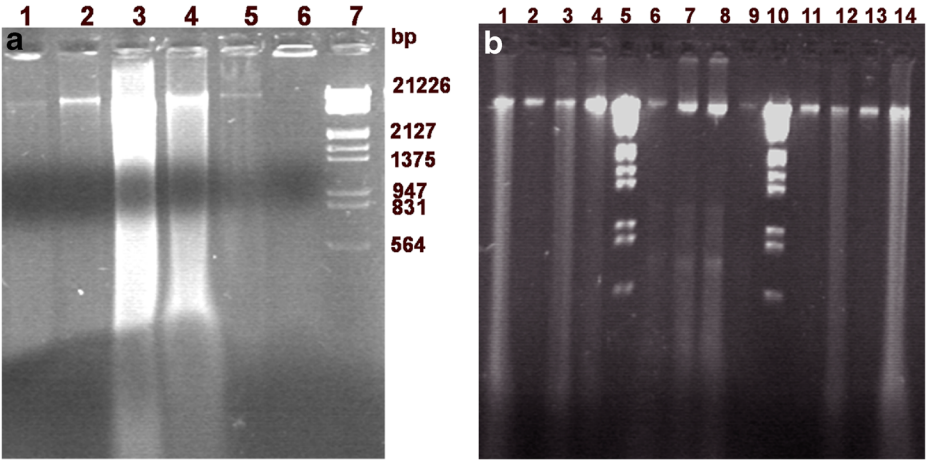

Fig. 1 **a** DNA from raw animal fibers resolved on 1.5 % agarose gel electrophoresis: *lane 1* Pashmina fiber, *lane 2* sheep wool, *lane 3* Angora rabbit hair, *lane 4* Yak down fiber, *lane 5* camel hair, *lane 6* silk fiber, and *lane 7* double digest DNA marker (Fermentas; *Cat*#SM0192). **b**:DNA from chemically finished textile materials resolved on 1.5 % agarose gel electrophoresis: *lane 1* Pashmina dyed with natural dye, *lane 2* Pashmina dyed with synthetic dye, *lane 3* Pashmina dyed with natural dye and iron sulphate ($FeSO_4$), *lane 4* acid-treated Pashmina, *lane 6* Pashmina treated with polyvinyl alcohol (*PVA*), *lane 7* sheep wool dyed with natural dye, *lane 8* sheep wool dyed with synthetic dye, *lane 9* sheep wool bleached, *lane 11* sheep wool dyed with natural dye and iron sulphate ($FeSO_4$), *lane 12* solvent-treated textile, *lane 13* formic acid-treated textile, and *lane 14* textile of unknown origin collected from local market; *lanes 5* and *10* double digest DNA marker (Fermentas; *Cat*#SM0192)

Additionally, different ratio of sheep- and goat-specific fiber DNA was used to analyze its detection limit. With raw fiber DNA, the detection limit was up to 5 %; however, with processed textile fiber DNA material, it was up to 10 % level, i.e., it can detect an adulteration level up to 90 % (Fig. 3). Additionally, PCR amplifications were further checked with purified DNA samples extracted from Angora rabbit hair, Camel hair, Yak Down hair, and Silk. The absence of PCR bands in these samples clearly indicated that there is no cross reactivity with these species.

Table 1 Spectrophotometric analysis of extracted DNA from raw animal fibers and chemically finished textile materials for its quantity and purity estimation

Animal fiber	DNA (μg/ml)[a]	Ratio $A_{260/280}$[a]
Raw sheep wool	523.50	1.863
Raw Pashmina	429.50	1.864
Pashmina treated with polyvinyl alcohol (PVA)	413.00	1.826
Pashmina dyed with natural dye	407.50	1.882
Pashmina dyed with synthetic dye	430.50	1.876
Pashmina dyed with natural dye and iron sulphate ($FeSO_4$)	419.80	1.886
Acid (HCl)-treated Pashmina	483.80	1.793
Sheep wool dyed with natural dye	463.00	1.828
Sheep wool dyed with synthetic dye	411.00	1.845
Sheep wool dyed with natural dye and iron sulphate ($FeSO_4$)	451.00	1.812

[a] Values are from one independent experiment only

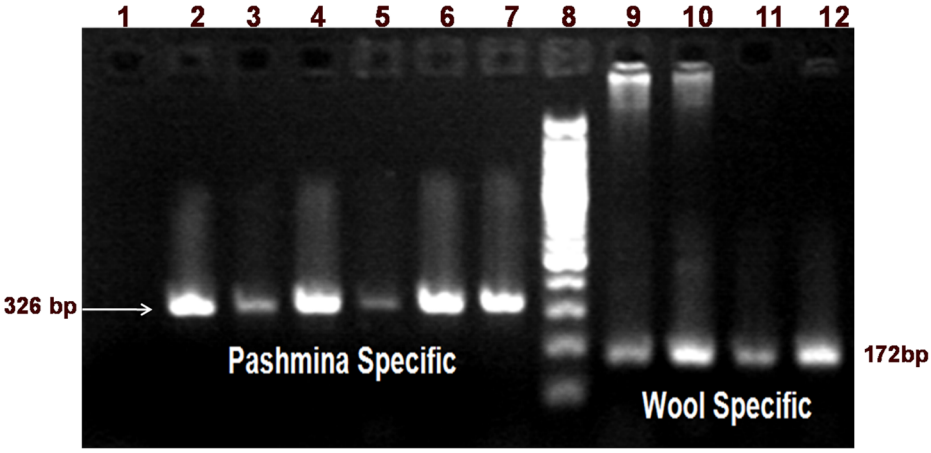

Fig. 2 PCR products resolved on 2 % agarose gel electrophoresis: *lane 1* non template PCR control, *lane 2–7* Pashmina-specific PCR band (326 bp), i.e., template DNA used from raw Pashmina, Pashmina dyed with natural dye, Pashmina dyed with synthetic dye, Pashmina dyed with natural dye and FeSO4, acid-treated Pashmina, Pashmina treated with PVA, and fiber of unknown origin, respectively; *lane 9–12* wool-specific PCR band (172 bp), i. e., template DNA used from raw sheep wool, sheep wool dyed with synthetic dye, sheep wool dyed with natural dye, and solvent treated textile, respectively; *lane 8* 100 bp plus DNA ladder (Fermentas; *cat*#SM0323)

Discussion

Extraction of quality DNA from textile materials is one of the limiting steps in the development of DNA-based techniques for detection of origin of natural fibers from textile materials. However, with certain modification in standard phenol-chloroform method, DNA extracted

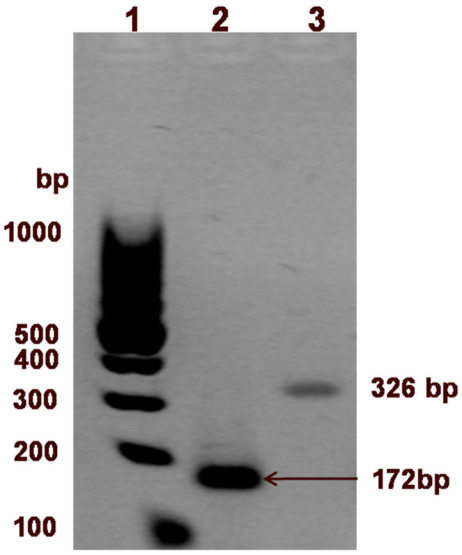

Fig. 3 PCR products resolved on 2 % agarose gel electrophoresis: identification of textile fabrics blended from sheep and goat origin fibers (9:1 ratio): *lane 1* 100 bp DNA ladder (Fermentas; *cat*#SM0243), *lane 2* PCR with sheep specific primers, and *lane 3* PCR with goat-specific primers

from chemically finished textiles are able to react with PCR. Wool and hair is highly keratinized in tissue where cell death initiated just after formation of anagen [11]. Apoptosis (cell death) leads to disintegration of nuclear genetic materials; however, the mitochondria of animal cells carry certain amount of genetic material. Mitochondrial DNA can be used for species-specific detection as shown by several workers [10, 12]. Additionally, mitochondrial DNA is easier to extract from hair and wool samples because of its presence in high copy numbers [13]. In the present study, 12S rRNA gene-specific primers were used for detection of sheep wool and Pashmina from textile blends. The exact identification of wool fibers is an important measure in the textile trade and manufacturing. Diagnostic PCR may prove highly successful and rapid method for detection of species specificity of natural fibers. However, its usefulness is limited partially due to presence of inhibitory substances like dye components, melanin pigment, phenols, etc. in template DNA. In the present study, PCR from iron sulphate-dyed textiles encountered inconsistent amplifications. Thus, we used certain PCR facilitators like BSA (0.2 μg/μl) (cat#AM2616, Ambion), DMSO (1 %) (Cat#D9170-1VL, Sigma), and Betaine (1–2 M) (cat#B0300-1VL, Sigma) for reproducible PCR amplification from mordant and synthetic dye-finished fabrics. The real hurdle in such diagnostic technique would be species-specific amplification of gene fragments from treated fibers which have highly disintegrated nucleic acids. The study would be highly applicable in development of a reliable method to determine the origin of distinct animal fiber used in textile industry. PCR-based technique would be a method of choice to discriminate Pashmina from sheep wool. The absence of cross reactivity with other common natural fibers further establishes its ability as diagnostic test for checking purity of Pashmina products. In the long run, a real-time PCR-based enumeration method could be developed for exact quantification of animal fibers in woolen garments as shown in a similar study [7].

Pashmina is one of the finest natural fibers obtained from domesticated goats and also is a mainstay of livelihood of certain pastoral community in arid rangeland of northern India. Thus, PCR-based method for identification of adulteration in Pashmina products with sheep wool would be an important tool to check false declaration in textile industries. Additionally, by using real-time chemistries, sensitivity limits can be further enhanced.

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References

1. Shakyawar, D. B., Raja, A. S. M., Kumar, A., Pareek, P. K., & Wani, S. A. (2013). Pashmina fiber-production, characteristics and utilization. *Indian Journal of Fibre & Textitle Research, 38*, 207–214.
2. Kerkhoff, K., Cescutti, G., Kruse, L., & Mussig, J. (2009). Development of a DNA-analytical method for the identification of animal hair fibers in textiles. *Textile Research Journal, 79*, 69–75.
3. Langley, K. D., & Kennedy, T. A., Jr. (1981). The identification of specialty fibers. *Textile Research Journal, 51*, 703–709.
4. Robson, D. (1997). Animal fiber analysis using imaging techniques-part I: scale pattern data. *Textile Research Journal, 67*, 747–752.
5. Robson, D. (2000). Animal fiber analysis using imaging techniques-part II: addition of scale height data. *Textile Research Journal, 70*, 116–120.
6. Zhang, J., Palmer, S., & Wang, X. (2010). *Identification of animal fibers with wavelet texture analysis*. London, UK: Proceedings of the World Congress on Engineering (WCE).

7. Ji, W., Bai, L., Ji, M., & Yang, X. (2011). A method for quantifying mixed goat cashmere and sheep wool. *Forensic Science International, 208*, 139–142.
8. Linch, C. A., Whiting, D. A., & Holland, M. M. (2001). Human hair histogenesis for the mitochondrial DNA forensic scientist. *Journal of Forensic Sciences, 46*, 844–853.
9. McNevin, D., Wilson-Wilde, L., Robertson, J., Kyd, J., & Lennard, C. (2005). Short tandem repeat (STR) genotyping of keratinised hair Part 1, review of current status and knowledge gaps. *Forensic Science International, 153*, 237–246.
10. Bottero, M. T., Civera, T., Nucera, D., Rosati, S., Sacchi, P., & Turi, R. M. (2003). A multiplex polymerase chain reaction for the authentication of cows', goats and sheep's milk in dairy products. *International Dairy Journal, 13*, 277–282.
11. Soma, T., Ogo, M., Suzuki, J., Takahashi, T., & Hibino, T. (1998). Analysis of apoptotic cell death in human hair follicles in vivo and in vitro. *The Journal of Investigative Dermatology, 111*, 948–54.
12. Rodriguez, M. A., Garcia, T., Gonzalez, I., Hernandez, P. E., & Martin, R. (2005). TaqMan real-time PCR for the detection and quantitation of pork in meat mixtures. *Meat Science, 70*, 113–120.
13. Stenn, K. S., & Paus, R. (2001). Controls of hair follicle cycling. *Physiological Reviews, 81*, 449–494.