ToxEHS

# Forensic Analysis using Microbial Community between Skin Bacteria and Fabrics

**So-Yeon Lee[1], Seung-Kyun Woo[1], So-Min Lee[2] & Yong-Bin Eom[1,2]**

[1]Department of Medical Science, College of Medical Sciences, Soonchunhyang University, Asan, Chungnam 31538, Republic of Korea
[2]Department of Biomedical Laboratory Science, College of Medical Sciences, Soonchunhyang University, Asan, Chungnam 31538, Republic of Korea
Correspondence and requests for materials should be addressed to Y.-B. Eom (omnibin@sch.ac.kr)

Received 24 June 2016 / Received in revised form 18 August 2016
Accepted 25 August 2016
DOI 10.1007/s13530-016-0284-y

pISSN : 2005-9752 / eISSN : 2233-7784

**Electronic supplementary material**
The online version of this article (doi:10.1007/s13530-016-0284-y) contains supplementary material, which is available to authorized users.

## Abstract

**Microorganisms flourish on and in the human body and skin surfaces are the largest. The skin surface microbiota can be transferred to an object upon touch. This has forensic implications. This study explored the hypothesis that residual skin bacteria left on fabrics could be valuable for forensic analysis. A non-culture based approach was used, since it can reveal a more diverse microbiome than culture-based methods. Fabrics examined were 100% cotton, 55% cotton - 45% polyester fabric, and 100% polyester. Three volunteers firmly grasped each fabric, and the DNA of all the samples was extracted and analyzed for the 16S rRNA gene. The 454-Next generation sequencing was used to observe the microbiome community relation between the individual and the fabrics in dendrogram and PCoA graph analyses. The analyses confirmed that the touched fabrics retained microorganisms from the individual. The study reveals the potential value of the approach in forensic examinations.**

**Keywords:** Microorganism, Fabrics, 16S rRNA, Next generation sequencing, Forensic

## Introduction

The human body is composed of an estimated hundred trillion bacteria residing externally and endogenously[1]. These bacteria constitute the human microbiota. The microbiota provides us with traits; for example, we rely on them to help us digest our food, resist pathogens, and educate our immune system[2,3].

The skin is a body habitat with complex regional variation. The skin houses one of the largest human-associated microbiota[4]. Although bacteria are common on all skin surfaces, we focused on bacteria found on the fingertips and palm, because of the tendency for these surfaces to come in contact with other surfaces. Also, these dynamic skin microbial habitats provide nearly constant and varied exposures to environmental surfaces. Because the microbiota survives environmental stresses like dryness, ultraviolet radiation, and temperature, these skin bacteria may persist on touched objects for a lengthy period of time[5,6].

Scientific fields like ecology and fermentation science have long recognized the importance of microbiology. In contrast, forensic science has largely ignored the microbiome[7]. Because it is estimated that less than 1% of bacterial species can be cultivated in artificial environment, microorganisms have been limited to culture-dependent assay[8,9]. However, this is likely to change as rapid advances in molecular sequencing and computational techniques change the way we approach the study of these organisms[10,11]. For example, it is no longer necessary to culture organisms to identify them. Metagenomics enables characterization of hundreds or thousands of microorganisms that constitute the microbial community, or microbiome, of an ecosystem. Analyses of bacterial communities target the 16S ribosomal RNA (rRNA) gene, which encodes the small subunit of the bacterial ribosome. The 16S rRNA gene is common among prokaryotes, but is not present in eukaryotes. The gene contains species-specific variable regions that are useful for identifying phylogenetic relationships[12]. The omnipresence and diversity of microbiota have a more diverse application scope than human DNA, therefore making it a potential source of forensic evidence. This has been reflected in the increasing recognition and use of microbial forensics in forensic investigations[13].

In many crime scenes, the suspect leaves behind biological evidence[14-16]. Human DNA in critical evidence, such as bloodstain or semen, can be valuable. DNA typing using short tandem repeat (STR) can be easily performed to identify the suspect. However, the evidence at crime scenes may feature inadequate quality and low copy number of DNA, which cannot be detected with the stringency needed for prosecution of crimes, including those involving violence. Also, an intelligent offender can take steps to eliminate residual evidences of the crime scene with blood, semen, and fingerprints, which can complicate offender detection. Also, in most cases, human DNA found on a latent sample is difficult to type and cannot be used effectively in a criminal investigation[15,17]. Therefore, a major challenge in forensic analysis is to develop the effective utilization of samples that complement the existing techniques. Even if human DNA cannot be detected from the casual contact of the suspect, bacterial DNA residing in and on the human body can be ubiquitously detected[2,18].

The present study examined the hypothesis that analysis of the microbiome present at a crime scene will enable to find the relationship between the legitimate individual (offender and victim). In this study, we examined the microflora deposited on fabrics following hand contact (fingertips and palm). We selected fabric, reflecting the great likelihood of contact between a suspect's hand and victim's clothes at a crime scene. The objectives of the current study were (1) to ascertain diversity of sample's microbiome, (2) to understand the connection between an individual hand with fabrics and (3) to see the difference of microbiome on fabric, before and after contact.

## Results

### Comparison of Non-culture Method versus Culture-based Method

We wanted to compare the efficacy of the non-culture method with culture-based method. The aim was addressed in two different ways. After collection of the sample, the fingertips and palm of the same individuals were swabbed using a cotton swab premoistended in normal saline and Tween 20. Half the samples were incubated in BHI broth for a day. The remainder was directly used to extract DNA for comparison of the results. The microbiome composition on fingertips and palm of the same individual were markedly different,

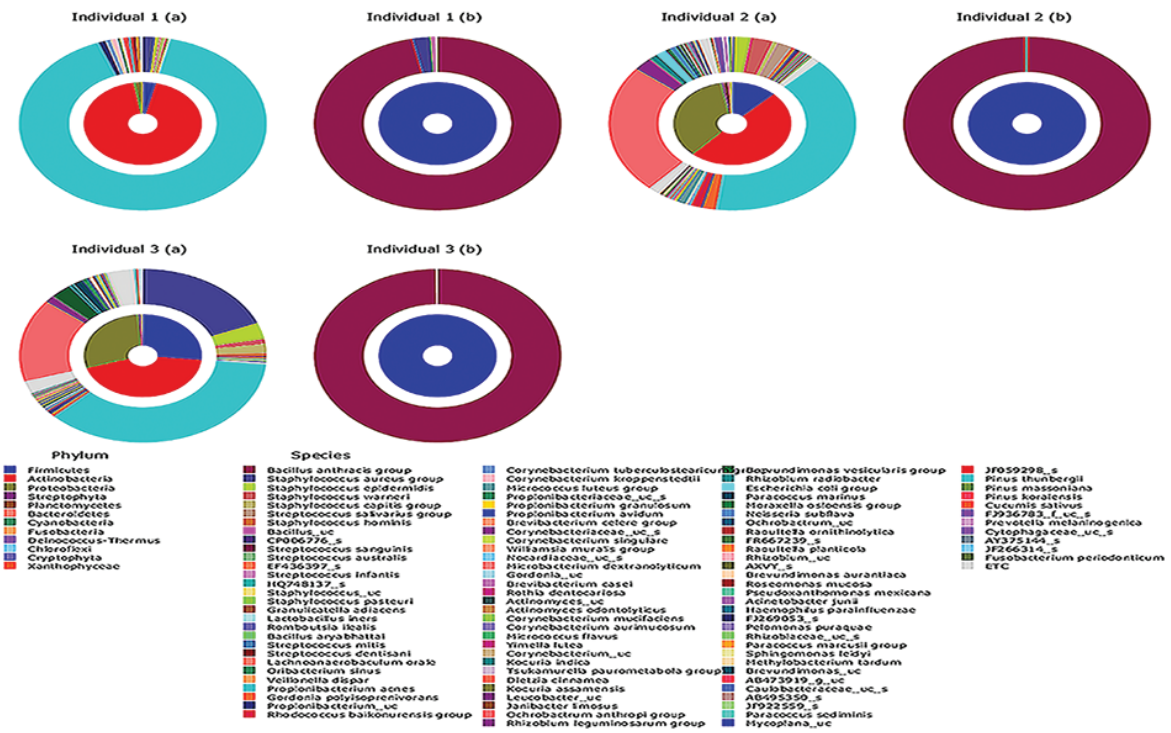


**Figure 1.** Comparison of non-culture and culture-based results. The (a) samples represent the non-cultured method and (b) are the results from cultivated analysis. The inner pie indicates the phylum composition and the outer pie chart indicates the species composition. The names for each color appear below the figure. The nomenclature for each phylotype is based on the Extaxon-e database. Species group of (a) indicates more diversity than species group of (b).

based on whether or not the sample was cultivated (Figure 1). Taxonomic composition of the non-cultured samples revealed marked species diversity in the outer circle. On the other hand, only one species (*Bacillus anthracis* group) was predominant in cultured samples. Also, phylum Firmicutes were dominant in the inner circle of sample (b). But sample (a) showed more diverse phyla Actinobacteria and Proteobacteria than sample (b). The findings in species and phylum levels indicated that non-cultured analysis can produce varied information about the microbiota than the cultivation method. We thus confirm our objective (1), which ascertains the diversity of the non-cultured sample's microbiome.

## Relation between Number of Bacterial Gene Sequence Reads and Species Richness

Bacterial DNA was extracted from the samples swabbed directly from the fingertips and palm. The bacterial community composition was determined using the barcoded fusion primer pyrosequencing procedure. DNA purity and concentration of PCR products were quantified by the BioDropμLITE; also the bacterial genes were analyzed by NGS (Table 1). In the table, samples represent the data from the fingertips and palm, and fabrics surfaces. The concentration of sample (a) was much higher than the other samples. The higher number of bacteria allowed greater bacterial gene sequences to be obtained (9,493 bacterial gene sequences from sample (a)). Figure 2 is a plot of a rarefaction curve (the number of species per sample) which is a technique used to standardize and compare species richness (or the number of species) that is computed from differently sized samples[19]. The green line (c) in Figure 2 denoted a slightly higher sequence read than the other samples, but sample (a) had a markedly lower number of OTUs compared to the other samples. Based on the data, this result indicated a lack of connection between the number of gene sequence reads and bacterial diversity.

## Bacterial Community Composition

To confirm objective (2), we assessed differences in overall bacterial community composition using the UniFrac phylogeny-based metric[20]. We used the UniFrac distance metric, a widely-used measure for comparison of two or more microbial communities, and analyzed the dendrogram to hierarchically visualize the microbiome among these samples. An Unweighted Pair Group Method with Arithmetic Mean (UPGMA) dendrogram was generated from the Fast UniFrac matrix which is a modification of the original UniFrac algorithm[21]. We identified clustering between each individual hand and fabric they touched (Figure 3 (A)). Cluster between Individual 1 (hand, cotton 100% fabric) and Individual 2 (hand, blended fabric) showed the different branch. After UPGMA dendrogram classification was carried out among these samples and analyzed, the Principal Coordinates (PCoA) were demonstrated. PCoA is a method to represent on a 2 or 3 dimensional chart, objects described by a square matrix containing resemblance indices between these objects. Samples of individual 1 and touched cotton 100% fabric grouped based on the Fast UniFrac, also individual 2 and individual 3 samples were grouped with touched fabric (Figure 3 (B)). Bacterial communities on the fabrics of a given individual were far more likely to be similar compared to communities on the fingertips and palm or fabrics of other individuals (Figure 3). Also, the bacterial communities on the fin-

**Table 1.** Number of bacterial gene sequence read, PCR product concentration and purity from each sample.

| Sample | Concentration (ng/μL) | Purity (A260/A280) | Bacterial gene sequences |
|---|---|---|---|
| a | 317.87 | 1.810 | 9,493 |
| b | 262.41 | 1.813 | 8,285 |
| c | 292.05 | 1.822 | 5,350 |
| d | 186.62 | 1.820 | 3,357 |
| e | 281.37 | 1.840 | 2,166 |
| f | 174.25 | 1.791 | 1,844 |
| g | 289.46 | 1.811 | 1,704 |
| h | 270.43 | 1.805 | 7,327 |
| i | 262.38 | 1.815 | 7,266 |

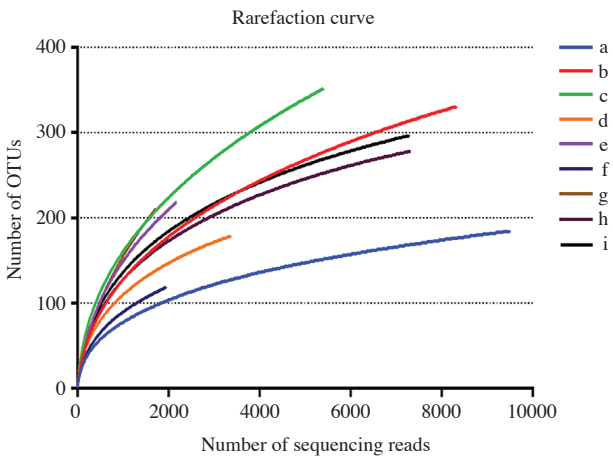


**Figure 2.** Rarefaction curves obtained following a normalized read size in each sample. The X axis indicates the number of sequencing reads and the Y axis indicates the number of OTUs. OTU refers to a group of sequences that are mathematically defined with a sequence similarity of 97% (the cut-off boundary). Sample (a) is the highest number in the sequence reads, while number of OTUs shows the lowest curve.

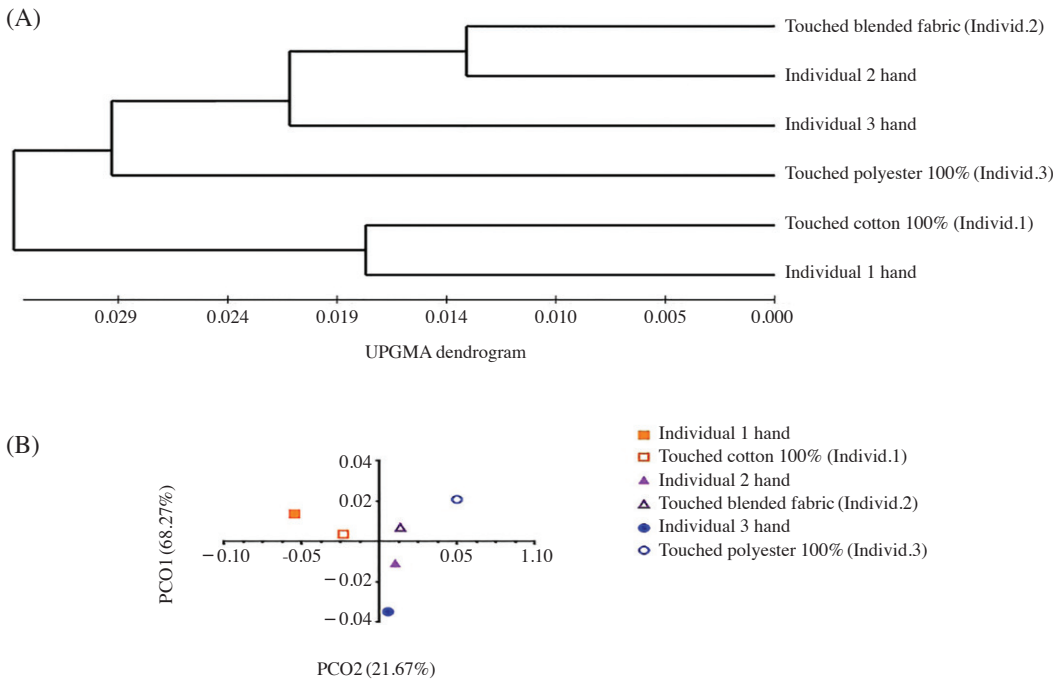


**Figure 3.** Unweighted Pair Group Method with Arithmetic Mean dendrogram (A) and Principal coordinate analysis plot (B). (A) UPGMA data shows visualize how communities are grouped. (B) The plot shows the degree of cluster pattern between each individual and touched fabric based on a PCoA. Together these two results indicate a relation between the fabric and the fingertips used to grip the fabric.

gertips and palm of individuals closely matched the fabrics that the individual had gripped. The results suggest that differences in fabric-associated bacterial communities are likely caused by direct transfer of the bacteria which reside on the fingertips. Together, these two results demonstrate the similarity in bacterial community structure between an individual's fingertips and the fabric that the individual gripped.

### Microbiome Analysis of Fabric before and after Being Touched

We wanted to confirm the changes in the microbial community composition of the fabric following hand contact (the objective (3)). We sampled the fabric before and after hand contact. Taxonomic composition analysis was conducted by the genus level (Figure 4). A total of twenty genera were identified. Five genera among the total of twenty genera were dominant in all the samples tested: *Propionibacterium*, *Corynebacterium*, *Ochrobactrum*, *Staphylococcus* and *Rhizobium*. The microbiome composition of the fingertips and palm matched the composition recovered from the touched fabrics. Several genera were detected between touched fabrics and after contact. Table S1 shows all the genera detected in the samples. The predominant genus of the several genera shows in the Figure 4. In the results of individual 1, the genus *Raoultella* was not detected on cotton fabric before contact (c) however, this genus was detected in the hand of individual 1 (a) and the cotton fabric after contact (b). The proportion of the genus *Raoultella* was 1.190% (a) and 1.178% (b), respectively (Table S1). The genus *Micrococcus* was detected in the hand of individual 2 (d) and the blended fabric after contact (e), while the genus was not recovered from the blended fabric before contact (f). *Micrococcus* was detected in (d) (1.057%) and (e) (3.007%) (Table S1). Also, the samples of individual 3 showed the movement of genus that associated before and after hand contact. The genus *Clostridium* was detected in the hand of individual 3 (g) and the polyester 100% after contact (h), but the genus was not detected on polyester before contact. The proportion of the genus *Clostridium* was 0.349% (g) and 0.052% (h), respectively (Table S1). We thus assume that the genus *Raoultella* existed in the hand of individual 1, *Micrococcus* existed in the hand of individual 2 and *Clostridium* existed in the hand of individual 3, and these were transferred to the fabrics after contact.

## Discussion

In this study, we investigated whether there is a bac-

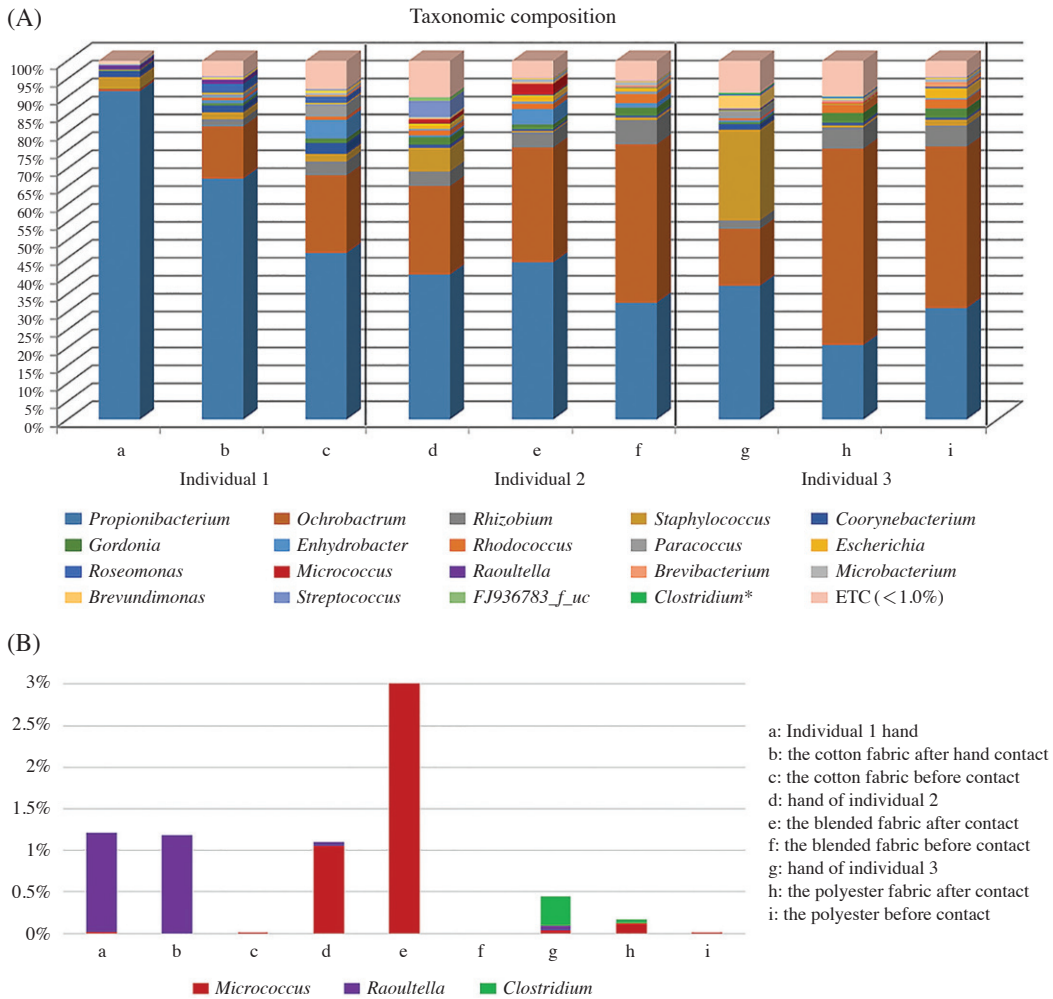


**Figure 4.** Taxonomic composition analysis at the genus level. (A) The total genus, and (B) a focus on *Raoultella*, *Micrococcus* and *Clostridium*. The genus *Raoultella* was present on the (a) and sample (b) that has been touched. The Raoultella was not present on the sample (c). The genus *Micrococcus* was present on the (d) and sample (e) that has been touched. But the genus was not detected in (f). Also, the genus *Clostridium* was present on the (g) and (h), but sample (i) not presented. ETC is a collection of minor components whose portion is below the cutoff value (<1.0%). *The genus *Clostridium* accounts for less than 0.05%.

terial connection between an individual's hand and fabrics contacted by the fingertips and palm. Barcoded fusion primer pyrosequencing obtained an average of 4,599 bacterial 16S rRNA gene sequences. This is a markedly more diverse microbiome analysis than our earlier research[22] that was conducted using culture-based method in all samples. The culture-based studies found that the genus *Bacillus* dominated, being present in 90% of samples. Typically, <1% of bacteria can be cultivated in media[4]. Figure 1 shows that culture-based analysis has a limited cultivated microbiome.

Individuals vary in their microbiota pattern in terms of genus dominance, and the pattern in the human stabilized even if the time elapsed[23,24]. We presently confirmed that predominanted microorganisms differed in individuals (Figure 1). For example, *Propionibacterium* dominated in individual 1 (91.51%) but not in individual 2 (*Propionibacterium* 40.35%; *Ochrobactrum* 24.72%) and individual 3 (*Propionibacterium* 37.27%; *Staphylococcus* 25.29%; *Ochrobactrum* 15.87%).

The hand of individual 3 and touched polyester 100% in UPGMA dendrogram seems like the different cluster based on the Fast UniFrac distance (Figure 3(A)). To accurately identify similarity and dissmilarity between the samples (individual hand and touched fabric), we used the PCoA analysis. PCoA results were slightly different than the dendrogram results; for instance, Individual 3 and touched fabric grouped one circle in the PCoA plot (Figure 3(B)). In our study, the results of dendrogram and PCoA allow the more exact

analysis for microbime similarity between the samples.

We confirmed a connection between the fabrics and after hand contact (Figure 4). Proportion of the genus *Raoultella*, *Micrococcus* and *Clostridium* account for 1-3% of taxonomic composition genus level. The proportion of about 1-3% among the total 100% of the Taxonomic composition can be considered to be a low proportion. However, a relatively small percentage of the microbial community on the touched fibers is significant. This is because the presence of the genus *Raoultella*, *Micrococcus* and *Clostridium* accounted for 0% of the total 100% on the non-touched fabrics.

A similar finding raised the possibility of personal identification using the bacterial flora of human skin following use of a computer keyboard and mouse[25]. Another recent study quantified DNA recovered following touching of glass, fabric, and wood; however, in these instances, there was a low recovery of DNA[17], Since this study focused on recovery of human DNA. Our study was more successful at recovery of bacterial DNA, and demonstrated the close dependence of the microbiome of fingertips and the touched object (Figure 3). It may be easier to recover bacterial DNA rather than human DNA from touched object surfaces. The present results must be considered provisional, given the low numbers of sampled fabrics and volunteers. Nonetheless, we confirmed a relation with between the bacteria resident on hand and the touched objects.

To the best of our knowledge, this study documents the microbial analysis approach by associating the bacterial community in fabric touched by a person. Our results indicate that the non-culture method shows various microbiomes (Figure 1), there is a less correlation between the number of bacterial gene sequence read and bacterial diversity (Figure 2; Table 1). And there is correlation the bacterial community between touched fabric and individual hand (Figure 3; Figure 4). We think that the current studies between hand and touched fabrics would be a beginning for future research to further explore the application of microbial communities in the forensic sciences. Further research is required to assess how the accurancy of this technique might compare with the more standard, and widely accepted, forensic tools.

## Materials and Methods

### Sample Collection and DNA Extraction

Samples were collected from three Korean adult volunteers. All three individuals were healthy at the time of sampling. Because the microbiome composition was significantly influenced by antibiotics, diet, and smoking[26], the volunteers were not being treated with antibiotics. To compare the bacterial communities on the fabrics with the microbial content of the volunteers, each volunteer was asked to hold a $5 \times 5$ cm piece fabric tightly in their fist for 5 min. There were three fabrics: 100% cotton (fabric 1), 55% cotton - 45% polyester blended (fabric 2), and 100% polyester (fabric 3). The fabrics were acquired at a drapery store. Each of the fabrics, as well as the individual fingertips and palms of each volunteer, were swabbed as described below to explore the correspondence between the bacterial communities of each person and the bacterial fingerprints recovered from the three fabrics. For a precise comparison, we also swabbed the fabric immediately prior to the contact. Skin surface and fabric samples were collected using autoclaved cotton-tipped swabs moistened with 0.15 M NaCl and 0.1% Tween 20[27]. Swabbing is as effective as other skin sampling methods for surveying bacterial diversity[28]. To analyze the different microbiome with fabrics, genomic DNA was extracted from the half of swabs using the PowerSoil DNA isolation kit (MoBio, Solana Beach, CA, USA). The cotton tip of each swab was broken off directly into a bead tube. The power bead tubes were incubated at 65°C for 10 min, and then shaken horizontally at maximum speed for 10 min. The remaining steps were performed as directed by the manufacturer. The other half of cotton-tipped swab was inoculated in 10 mL Brain Heart Infusion (BHI) broth (Difco, Detroit, MI, USA), and then incubated for 24 h at 37°C in a $CO_2$ incubator. Genomic DNA was extracted using the aforementioned DNA isolation kit.

### PCR Amplification and Next Generation Sequencing

PCR amplification was performed using primers targeting the V1 to V3 regions of the 16S rRNA gene of extracted DNA. For bacterial amplification, the barcoded primers were 27F (5′-CCTATCCCCTGTGTGCCTTGGCAGTC-TCAG-AC-GAGTTTGATCMTGGCTCAG-3′; the underlined sequence indicates the target region primer; 'TCAG' indicates the key sequence; 'AC' is the linker sequence) and 518R (5′-CCA TCTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-WTTACCGCGGCTGCTGG-3′) (http:/oklbb.ezbiocloud.net/content/1001). The amplifications were carried out using an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, primer annealing at 55°C for 30 sec, extension at 72°C for 30 sec, with a final elongation at 72°C for 5 min. PCR products were confirmed using 1% agarose gel electrophoresis with 0.5X TAE buffer, and were visualized using a Gel Doc system (BioRad, Hercules, CA,

USA). The amplified products were purified with a MinElute PCR purification kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The concentration and purity of the resulting DNA were measured with a BioDropμLITE (BioDrop, Cambridge, UK). Equal concentrations of purified products were pooled together and short fragments (non-target products) were removed using an Ampure bead kit (Agencourt Bioscience, Beverly, MA, USA). Quality and product size were assessed on a Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA) using a DNA 7500 chip. Mixed amplicons were used for emulsion PCR and deposited on Picotiter plates. Sequencing was carried out at Chunlab, Inc. (Seoul, Korea) using a 454 GS FLX titanium next generation sequencing (NGS) system (Roche, Branford, CT, USA) according to the manufacturer's instructions.

### Pyrosequencing Data Analysis

The basic analysis was conducted as previously described[29-31]. Obtained reads from the different samples were sorted by unique barcodes of each PCR product. The sequences of the barcode, linker, and primers were removed from the original sequencing reads. Any reads containing two or more ambiguous nucleotides, low quality score (average score $<25$), or reads shorter than 300 bp were discarded. Potential chimera sequences were detected by the bellerophone method, which compares the BLASTN search results between the forward-half and reverse-half sequences[32]. After removing chimera sequences, the taxonomic classification of each read was assigned against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net)[33], which contains the 16S rRNA gene sequence of type strains that have valid published names and representative species level phylotypes of either cultured or uncultured entries in the GenBank database with complete hierarchical taxonomic classification from the phylum to the species. The richness and diversity of samples were determined by Chao1 estimation and Shannon diversity index at the 3% distance. Random sub sampling was conducted to equalize read size of samples for comparing different read sizes among samples. The overall phylogenetic distance between communities was estimated using the Fast UniFrac[21]. To compare Operational Taxonomic Units (OTUs) between samples, shared OTUs were obtained with the XOR analysis of the CLcommunity program (Chunlab Inc.).

## Acknowledgements

This study was supported by the Soonchunhyang University Research Fund, by Laboratory Safety Management Program, through the National Research Foundation of Korea, funded by the Ministry of Science, ICT and Future Planning (NRF-2015M3B6A5022471) and by Basic Science Research Program, through the National Research Foundation of Korea, funded by the Ministry of Education (NRF-2015R1D1A1A02061773).

## References

1. Shrivastava, P., Jain, T. & Gupta, M. K. Microbial Forensics in Legal Medicine. *SAS J. Med.* **1**, 33-40 (2015).
2. Dethlefsen, L., McFall-Ngai, M. & Relman, D. A. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* **449**, 811-818 (2007).
3. Wilson, M. in *Bacteriology of humans: an ecological perspective* (John Wiley & Sons, 2009).
4. Fredricks, D. N. Microbial ecology of human skin in health and disease. *J. Investig. Dermatol. Symp. Proc.* **6**, 167-169 (2001).
5. Smith, S. M., Eng, R. H. & Padberg, F. T., Jr. Survival of nosocomial pathogenic bacteria at ambient temperature. *J. Med.* **27**, 293-302 (1996).
6. Brooke, J. S., Annand, J. W., Hammer, A., Dembkowski, K. & Shulman, S. T. Investigation of bacterial pathogens on 70 frequently used environmental surfaces in a large urban U.S. university. *J. Environ. Health* **71**, 17-22 (2009).
7. Alan, G. & Sarah, J. P. Microbes as forensic indicators. *Trop. Biomed.* **29**, 311-330 (2012).
8. Torsvik, V., Goksøyr, J. & Daae, F. L. High diversity in DNA of soil bacteria. *Appl. Environ. Microbiol.* **56**, 782-787 (1990).
9. Torsvik, V. & Øvreås, L. Microbial diversity and function in soil: from genes to ecosystems. *Curr. Opin. Microbiol.* **5**, 240-245 (2002).
10. Li, K., Bihan, M., Yooseph, S. & Methe, B. A. Analyses of the microbial diversity across the human microbiome. *PLoS One* **7**, e32118 (2012).
11. Segata, N. *et al*. Metagenomic microbial community profiling using unique clade-specific marker genes. *Nature Methods* **9**, 811-814 (2012).
12. Amann, R. I., Ludwig, W. & Schleifer, K. H. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* **59**, 143-169 (1995).
13. Budowle, B., Murch, R. & Chakraborty, R. Microbial forensics: the next forensic challenge. *Int. J. Legal. Med.* **119**, 317-330 (2005).
14. van Oorschot, R. A. & Jones, M. K. DNA fingerprints from fingerprints. *Nature* **387**, 767 (1997).
15. Raymond, J. J., van Oorschot, R. A., Gunn, P. R., Walsh, S. J. & Roux, C. Trace evidence characteristics of DNA: A preliminary investigation of the persistence of DNA at crime scenes. *Forensic Sci. Int. Genet.* **4**, 26-33 (2009).

16. Rudbeck, L. & Dissing, J. Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. *BioTechniques* **25**, 588-590, 592 (1998).
17. Daly, D. J., Murphy, C. & McDermott, S. D. The transfer of touch DNA from hands to glass, fabric and wood. *Forensic Sci. Int. Genet.* **6**, 41-46 (2012).
18. Turnbaugh, P. J. *et al*. The human microbiome project: exploring the microbial part of ourselves in a changing world. *Nature* **449**, 804-810 (2007).
19. Heck Jr, K. L., van Belle, G. & Simberloff, D. Explicit calculation of the rarefaction diversity measurement and the determination of sufficient sample size. *Ecology* **56**, 1459-1461 (1975).
20. Lozupone, C. & Knight, R. UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228-8235 (2005).
21. Hamady, M., Lozupone, C. & Knight, R. Fast UniFrac: facilitating high-throughput phylogenetic analyses of microbial communities including analysis of pyrosequencing and PhyloChip data. *ISME J.* **4**, 17-27 (2010).
22. Lee, S., Woo, S., Choi, G., Hong, H. & Eom, Y. Microbial Forensic Analysis of Bacterial Fingerprint by Sequence Comparison of 16S rRNA Gene. *J. Forensic. Res.* **6**, 2 (2015).
23. Costello, E. K. *et al*. Bacterial community variation in human body habitats across space and time. *Science* **326**, 1694-1697 (2009).
24. Pennisi, E. Microbiology. Bacteria are picky about their homes on human skin. *Science* **320**, 1001 (2008).
25. Fierer, N. *et al*. Forensic identification using skin bacterial communities. *Proc. Natl. Acad. Sci. U S A* **107**, 6477-6481 (2010).
26. Leake, S. L. Is human DNA enough? - potential for bacterial DNA. *Front. Genet.* **4**, 282 (2013).
27. Paulino, L. C., Tseng, C. H., Strober, B. E. & Blaser, M. J. Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. *J. Clin. Microbiol.* **44**, 2933-2941 (2006).
28. Grice, E. A. *et al*. A diversity profile of the human skin microbiota. *Genome. Res.* **18**, 1043-1050 (2008).
29. Chun, J., Kim, K. Y., Lee, J. H. & Choi, Y. The analysis of oral microbial communities of wild-type and toll-like receptor 2-deficient mice using a 454 GS FLX Titanium pyrosequencer. *BMC Microbiol.* **10**, 101 (2010).
30. Hur, M. *et al*. Effect of genetically modified poplars on soil microbial communities during the phytoremediation of waste mine tailings. *Appl. Environ. Microbiol.* **77**, 7611-7619 (2011).
31. Kim, B. S., Kim, J. N., Yoon, S. H., Chun, J. & Cerniglia, C. E. Impact of enrofloxacin on the human intestinal microbiota revealed by comparative molecular analysis. *Anaerobe* **18**, 310-320 (2012).
32. Huber, T., Faulkner, G. & Hugenholtz, P. Bellerophon: a program to detect chimeric sequences in multiple sequence alignments. *Bioinformatics* **20**, 2317-2319 (2004).
33. Kim, O. S. *et al*. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716-721 (2012).