Research Article

Estimation of Personal Environment Via Fingertip Microbiome and Mobile Phone Surfaces

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Background: Fingerprints can serve to identify individuals, but fingerprint quality may be deteriorated, even to the point of eliminating fingerprints, due to the external environment.

Objective: Poor fingerprint quality cannot be effectively used to identify individuals; hence, the need for other methods.

Materials and Methods: We investigated the utility of bacterial communities and the only microorganisms present in the sample to identify internal and external factors in individuals. Samples included eight participants' fingerprints and their mobile phone surfaces. Bacterial DNA in the samples was sequenced using next-generation sequencing to target the V3–V4 region in the 16S ribosomal RNA gene. The QIIME program was used to perform a taxonomic assignment and alpha diversity and beta diversity analyses based on the sequence data.

Results: Until now, personal identification has only relied on microbial communities. However, this study identified microbial differences according to Korean mobile phones, fingertips, or gender, and confirmed the possibility of characterization of samples when it was difficult to identify individuals by the microbial community. The biodiversity and composition of individual bacterial communities were affected by internal and external environments. Bacteria from individuals and mobile phones were shared due to contact between mobile phone surfaces and fingertips. Of the eight Koreans, six of the fingertips and mobile phone samples matched each other for personal identification.

Conclusions: This study confirmed that the bacteria from an individual could be matched with the contact object and could be used as forensic evidence. Such bacterial profiling of individuals may confer forensic evidence and serve as a basis for improving the accuracy of forensic verification.

Keywords: 16S ribosomal RNA; Bacterial communities; Fingertips; Forensic verification; Internal and external factors; Mobile phone

1. Background

A variety of biological and physical evidence is used to identify individuals at crime scenes. Primary methods of personal identification are classified as a phenotypic method using fingerprints or facial features (1, 2) and a genotypic method using DNA or RNA (3, 4). However, the phenotypic method has disadvantages such as subjective factors, resulting in decreased accuracy. For example, the analysis of fingerprint evidence depends on the subjective expertise of inspectors.

B.T. Ulery *et al*. identified that 3% of inspectors obtained a false positive result in fingerprint analysis (1). The vast majority of deaths can be identified by family members/loved ones and individuals' physical appearances. However, additional verification may be necessary if the event persons are unrecognizable due to severe deformation or decomposition (2). Studying personal identification methods has demonstrated that the commonly used technology of comparative dental identification (phenotypic method) has several

disadvantages and that personal identification by DNA fingerprinting (genotypic method) is more accurate (5). Individual identification via gene analysis is the most accurate of currently available methods, but it is often difficult to obtain sufficient quantities of DNA due to external environmental factors, resulting in limited use as evidence owing to the inadequate quality and low copy numbers (6, 7). Evidence destruction in the form of DNA contamination often occurs through the actions of the investigating officers, wherein the deposition of hair, fibers, and trace material from clothing destroy the existing DNA at the scene of an investigation. Environmental factors like weather and climate phenomena also play important roles in evidence destruction (8). Microbial communities can also be affected by the external environment; the human microflora has a profound effect on the composition of microbial communities in crime scenes. A large number of skin bacterial communities can be transferred to surfaces by contact alone that can aid in forensic identification. Furthermore, characterization of the microbial communities can help in making associations between the bacterial communities dislodged on the surfaces and that on human skin. These bacterial profiles, which can vary between individuals, can act as markers to help in the identification of suspects (9, 10). Besides, identification of identical twins is difficult due to similar genotypes (3). Thus, there is a critical need for an effective identity verification method.

2. Objectives

In this study, bacteria were used to investigate a novel identity verification method; the skin's surface has a significant number of bacteria that can be easily transferred onto objects' surfaces by touch (11). Such interactions may serve and provide forensic evidence, similar to human fingerprints and traces. Certain bacteria species may remain on surfaces for extended periods due to conferred resistance to external factors such as moisture, temperature, and UV radiation (12-14). Previous works have shown that the composition of individuals' skin bacterial community/microbiome transferred to surfaces is stable (7) and can be recovered within a few hours (12, 15). Therefore, forensic identification studies were herein conducted using skin bacterial communities resistant to external environmental factors. The possibility of personal identification using individual skin bacterial communities demonstrated that (i) bacterial composition is influenced by individual internal and external factors and is different among individuals and (ii) certain bacterial components leave traces

through contact and allow one to track an individual's surrounding environment through bacteria profiling.

Herein, we employed bacterial 16S ribosomal RNA sequencing via the Illumina MiSeq platform, a next-generation sequencing platform (7, 16). In addition, in the past, personal identification was performed solely by the microbial community, but the results of this study additionally used statistical methods to identify important taxonomic biomarkers that can be expressed by gender and mobile phone.

3. Materials and Methods

3.1. Participant Selection and DNA Extraction

Four men and four women were participants in our study, wherein individuals' fingertips and mobile phones were sampled. Equal representation between male and female participants was part of our study design as the bacterial composition may vary by sex. The participants were in their early 20s and go to the same university (15, 17). Those who were in the same school year spent most of their time in the same environment. Sample collection was performed after the participants completed all daily activities on campus; they visited the laboratory at 5–6 pm. Samples were collected three times at 3–4-day intervals. The participants consented to the study, which was approved by the Eulji University Institutional Bioethics Committee (EUIRB2017-18).

Samples were collected using cotton swabs soaked in sterile phosphate buffer solution. The fingertips of both hands and the surface of the mobile phones were sampled. After sampling, the swab was cut at the head and stored in a sterile Eppendorf Tube at −70 °C until DNA extraction. Metagenomics approaches were employed as outlined in (18). To extract bacterial DNA from samples, the DNeasy® PowerSoil kit was used (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). The kit's C1 solution was added to the samples and incubated at 60 °C for 20 min. The samples were then agitated using a vortex and sonicated using a water bath to further break the bacterial cell wall and extract a sufficient amount of DNA. Bacterial DNA was extracted per the manufacturer's protocol. DNA was eluted into 80 of pure tertiary distilled water. Sample extractions were performed in triplicate. For each sample, extracted DNA was mixed, pooled, and stored until further processing.

3.2. Amplification and Sequencing

DNA concentration was quantified using PicoGreen (Invitrogen, Grand Island, NY, USA). Primary PCR was only carried out on samples with a DNA concentration

of 1.0 ng.µL⁻¹ or more. Universal primers for the V3–V4 regions of the 16S ribosomal RNA gene (16, 19, 20) amplified the target DNA. The universal primers included the Illumina® flow cell adapter sequences: 341F (5′ – TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC A – 3′) and 805R (5′ – GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C – 3′). PCR mix consisted of 2.5 µL DNA sample (5 ng.µL⁻¹), 5 µL forward primer, 5 µL reverse primer, and 12 µL 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA) in a total volume of 25 µL. The thermal cycling program was as follows: initial denaturation at 95 °C for 3 min followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, with extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. Amplicons were sequenced after library preparation via secondary PCR. For the secondary PCR, a Nextera Index PCR primer (Illumina®, USA) pair (forward: 5′ – AAT GAT ACG GCG ACC ACC GAG ATC TAC AC - [i5] – TCG TCG GCA GCG TC –3′ and reverse: 5′ – CAA GCA GAA GAC GGC ATA CGA GAT - [i7] - GTC TCG TGG GCT CGG – 3′) was used. PCR mix consisted of 5 µL sample DNA, 5 µL Nextra XT Index primer 1 (N7XX), 5 µL Nextra XT Index primer 2 (S5XX) (Illumina®, USA), 25 µL 2X KAPA HiFi HotStart ReadyMix (KAPA Biosystems, Wilmington, MA, USA), and 10 µL PCR Grade Water. Thermal cycling amplification was performed by initial denaturation at 95 °C for 3 min. Next, eight PCR cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s were carried out followed by a final extension step at 72 °C for 5 min. Amplicons were quantified using TapeStation DNA screentape D1000 (Agilent, USA) and Picogreen assay. Generated libraries were sequenced using the Illumina Miseq® platform (300 cycles * 2). The above process and sequencing were performed on the Illumina MiSeq platform according to the manufacturer's instructions (21).

3.3. Sequence Analysis

Preprocessing and clustering steps were performed; low-quality, ambiguous, and chimeric sequences were removed using CD-HIT-Operational taxonomic unit Miseq/FLX. After clustering among sequences with more than 97% sequence similarity, species-level operational taxonomic units were obtained (22). We then performed clustering using the average neighbor method. This method was performed using rDNA Tools PacBio. As a result, clustering was cut off at 97%. As a result, a total of 935 operational taxonomic units

were obtained (23). We used representative sequences from each operational taxonomic unit for taxonomic assignments. One representative sequence was obtained by performing against the 2018 reference database (NCBI 16S database), with a raw-sequence data-matching rate of at least 85% (24). Phylogenetic trees were then constructed from the aligned and filtered representative sequences of the operational taxonomic units. Data visualization was performed using the QIIME program (UCLUST; version 1.8) (25).

3.4. Data and Statistical Analyses

We conducted a number of analyses to identify participants' bacteria-related information. The statistical significance was analyzed using a statistical package for social science software (v. 21.0). A p-value of 0.05 was considered statistically significant. DNA concentration of each sample was visualized as a box plot graph using the R program (v 3.2) (6, 26). The one-way analysis of variance (ANOVA) test was performed to compare gDNA mean concentrations among male and female fingertips. The Mann–Whitney U test was performed to compare the average gDNA concentration among male and female mobile phone samples. The biodiversity within a group of samples (alpha diversity) was analyzed and visualized using rarefaction curves (27). In the rarefaction curves, the x-axis indicated the number of reads, the y-axis indicated the biodiversity rate in one sample, and the graph was a direct proportional curve. As an index for confirmation of diversity, we used the Chao1 index (17, 23). The Chao1 index values among the samples were compared based on the final value (Final Chao1 index). The final values were used to compare mean biodiversity between males and females and among the sample types. Principal coordinates analysis was used to assess the similarity between samples (beta diversity) (28). Principal coordinates analysis graphs were generated using weighted and unweighted based UniFrac. The UniFrac is the fraction of the branch length linked to offspring in one environment of the phylogenetic tree. This method can determine the dissimilarity of phylogenies and the cause of such differences. The composition of the existing bacterial community in individual skin (fingertips) was visualized using a bar graph based on taxonomic assignments (17). Subsequently, the bacterial profiling yielded identification at the species level based on taxonomic assignments. The percentage of bacteria presented is a ratio of total taxa present. The questionnaire was applied to determine if internal and external environmental factors affecting the bacterial composition of participants. The categories

of personal environment included: (1) dog-related strains, (2) oral disease-related strains, (3) female-associated strains, (4) ocular disease strains, and (5) respiratory disease strains. Only bacteria with a ratio of 0.1% or more in the categories consisting of unique taxa were considered present in an individual. Notably, bacteria with a ratio of 1% or more in the oral and respiratory disease categories were considered present in an individual because a number of these bacteria are resident flora. Taxonomic biomarker discovery was performed using EZbiocloud, a commercially available ChunLab bioinformatic cloud platform for microbiome research (https://www.ezbiocloud.net/). We used the Kruskal-Wallis H test to evaluate the differences in the number of OTUs and used the Shannon, phylogenetic, and Pielou indexes to compare microbiome diversity between the mobile phone and fingertips of 8 people. Significant microorganisms resulted in only p-values less than 0.5.

4. Results

4.1. Measured Genomic DNA Concentration
Extracted genomic DNA concentrations are shown in **Table 1**. Samples were collected in triplicate, and extracted DNA was pooled. The box plot was generated based on **Table 1** using the R program (**Fig. 1**) (6, 26). Graphs were generated based on sex and sample type. The DNA concentrations from males' and females' fingertips were similar (One-way ANOVA, $p > 0.05$), but there was a clear difference in the DNA concentration between sex from mobile phone surfaces (Mann–Whitney U test, $p < 0.05$).

4.2. Biodiversity According to Sex and Sample Types
Our alpha diversity analysis revealed the level of biodiversity for the bacteria present in samples. Rarefaction curves were drawn based on the Chao1 matrix. We first investigated the degree of diversity rate based on sex **(Fig. 2a)**. The average biodiversity was determined for fingertips and mobile phones (a), only fingertips (b), or only mobile phones (c). The Final Chao1 index value of average biodiversity between males and females per category is as follows: (a) male, 111.902; female, 138.106; (b) male, 140.801; female, 176.471; and (c) male, 95.369; female, 115.792. As a result, female biodiversity was higher than male biodiversity. Next, the degree of diversity among sample types was compared **(Fig. 2d)**. The Final Chao1 index value of average biodiversity between fingertips and mobile phones is as follows: (d) fingertips, 191.751 and mobile phone, 184.129. The degree of biodiversity

Table 1. Total gDNA concentration (ng/μL)

	M1	M2	M3	M4	F1	F2	F3	F4	Mean ± S.D.
F	5.992	9.570	6.973	6.788	5.907	6.031	10.119	5.791	7.15 ± 1.6
MP	1.498	1.500	1.419	1.333	13.635	6.595	6.711	5.946	4.83 ± 4.1

Note: M1–M4 represent male samples, and F1–F4 represent female samples. F represents fingertip samples, and MP represents mobile phone samples.
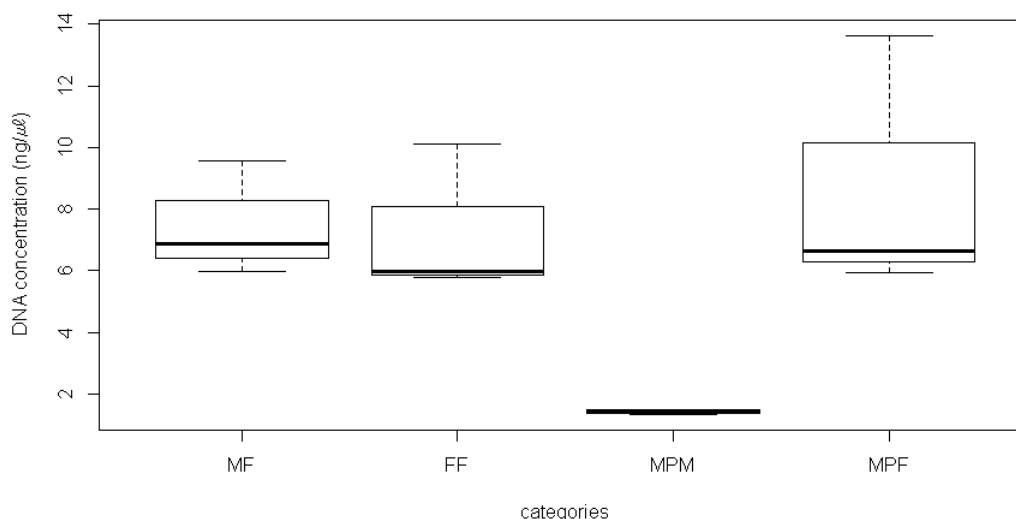

Figure 1. DNA concentration according to sex and sample types. The x-axis represents DNA concentration, and the y-axis represents sample types. MF : Fingertips of males, FF: Fingertips of females, MPM : Mobile phones of males, MPF : Mobile phones of females.

of fingertip samples was slightly higher than that of mobile phones. The range of biodiversity (standard deviation) of the mobile phones was much larger than that of fingertips, yielding the same result as our DNA concentration data (**Table 1**).

4.3. Clustering Analyses Between Samples

Clustering analysis was performed to confirm that specific strains were shared by contact and could be a trace. The principal coordinates analysis graphs were used to identify clustering of participants' fingertips and mobile phones (**Fig. 3**). Both unweighted and weighted UniFrac distances were used to improve accuracy. As a result, clustering among fingertips and mobile phones of M1, M3, and F3 was accurate based on the unweighted UniFrac matrix (Left). The clustering among fingertips and mobile phones of M1, F1, F2, and F4 was accurate based on the weighted UniFrac matrix. M2 and M4 did not cluster at both matrices (Right).

4.4. Bacterial Fingerprint Patterns

Taxonomic assignment was carried out by clustering analysis based on phylogenetic distances to identify the participant's bacterial fingerprint pattern, and it was confirmed that the bacterial patterns in the fingertips of M1, M3, F1, F2, F3, and F4 were similar to those on their mobile phones (**Fig. 3**). We observed that bacterial fingerprint patterns varied among individuals, and the compositions of the bacterial communities were distinct. In our taxonomical analyses, F4 showed the most varied bacterial composition among participants (**Fig. 4**, Supplementary **Fig. 1**).

4.5. Bacteria Profiling

Taxonomic assignments were also performed to bacteria profile each participant. Categories included (1) dog-related strains, (2) oral disease-related strains, (3) female-associated strains, (4) ocular disease strains, and (5) respiratory disease strains. Bacteria profiling was
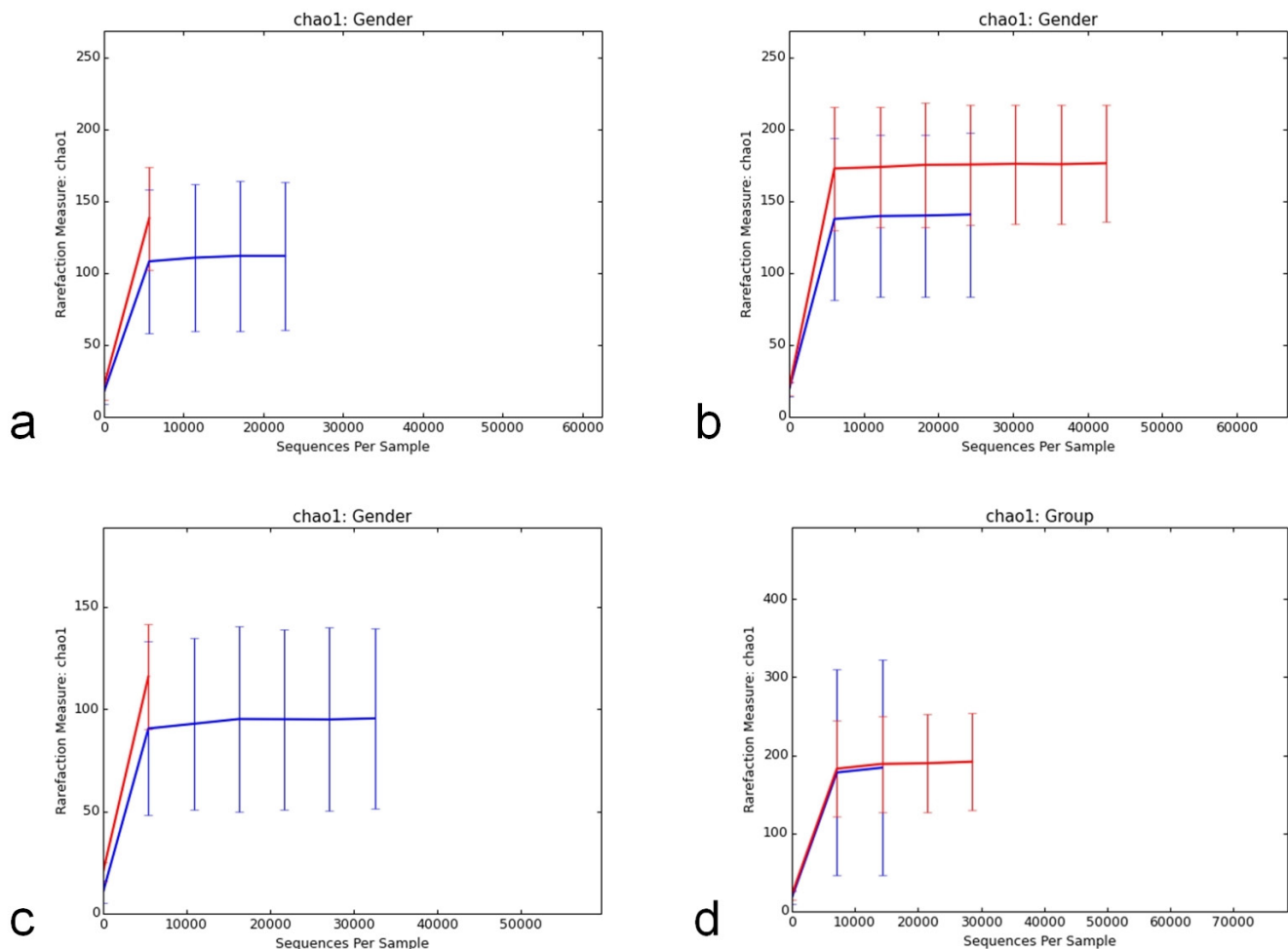

Figure 2. Biodiversity of samples. The rarefaction curves indicated biodiversity at **a, b, c** (red : women, blue : men). **a** is the average of the fingertips and mobile phones. **b** indicate the fingertips. **c** indicate mobile phones. **d** represents biodiversity according to sample types (red : fingertips, blue : mobile phones).
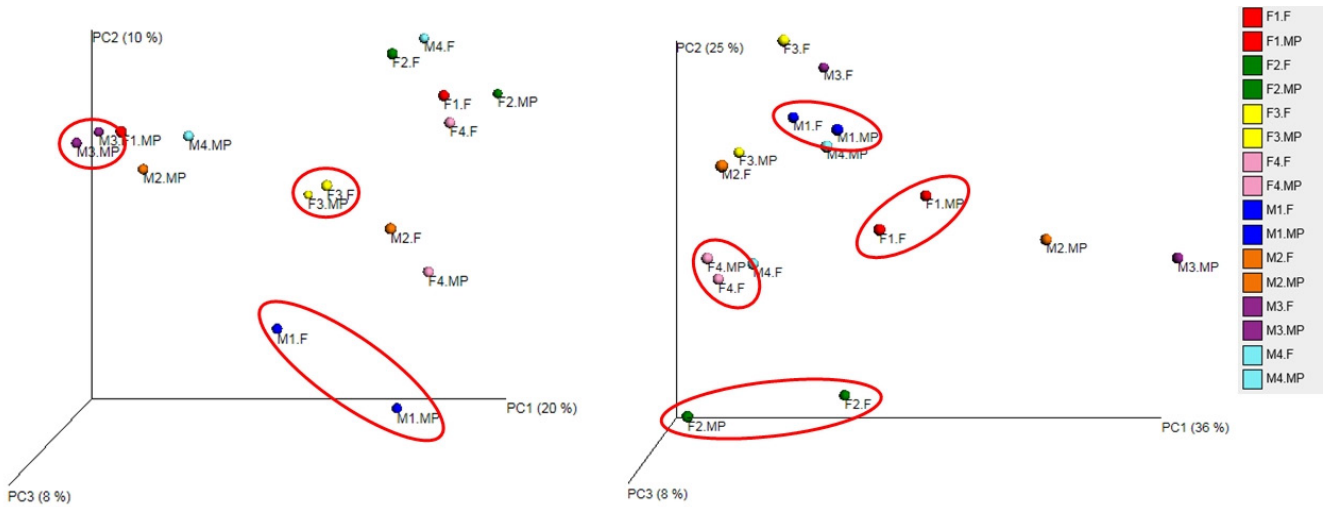
Figure 3. Similarity analysis through principal coordinates analysis graphs. The cluster is represented by a red circle in all samples. (blue : M1, orange : M2, purple : M3, sky blue : M4, red : F1, green : F2, yellow : F3, pink :F4). F, fingertips. MP, mobile phones.
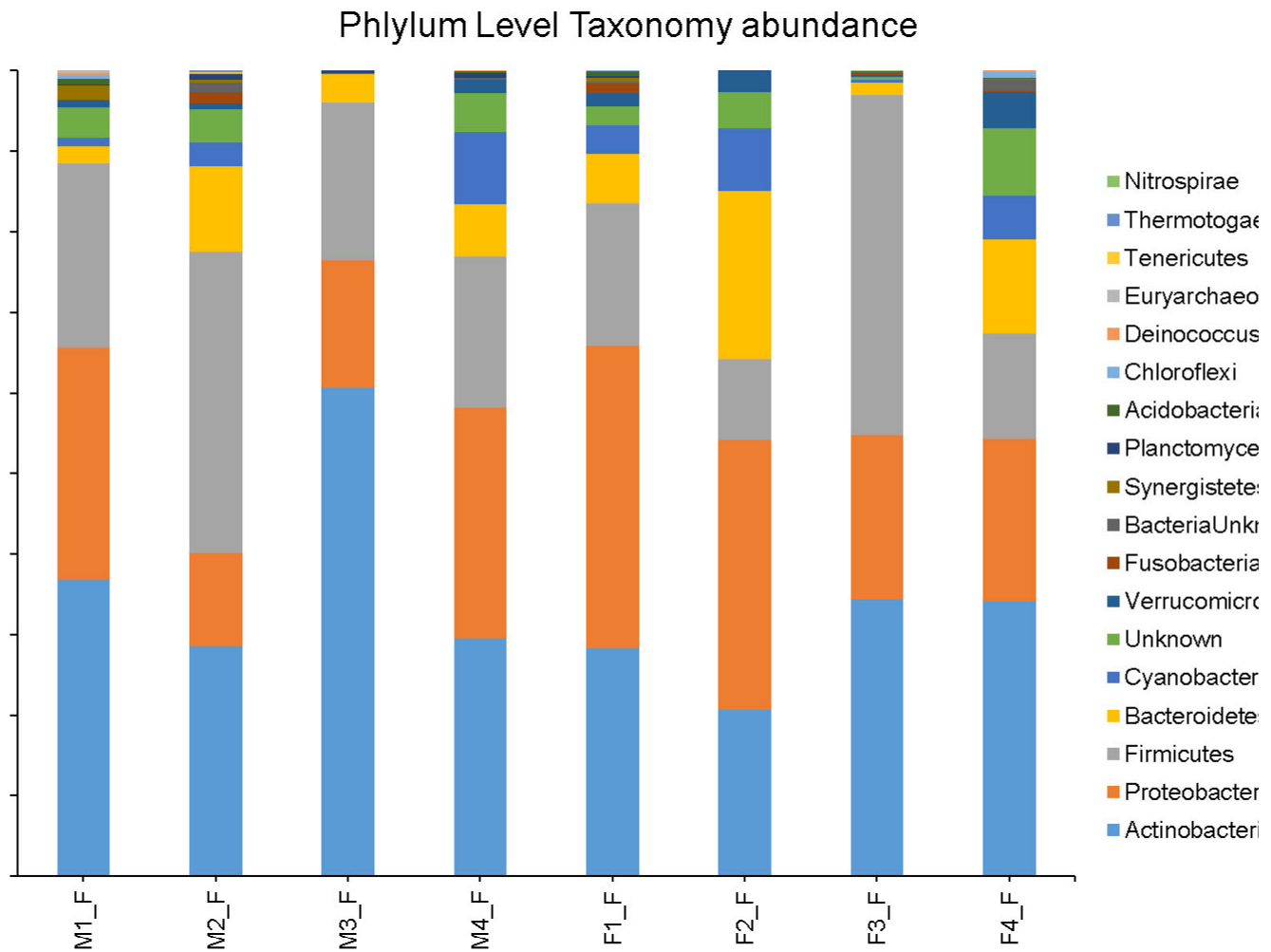

Phlylum Level Taxonomy abundance

Figure 4. Bacterial composition of individuals' fingertips. The taxanomic level of the bar graph is the Phylume level. Bar graphs of the family and species taxanomic levels are shown in Supplementary figures 1a and 1b.. Each bar represents individual participants (M1–M4 and F1–F4).

Table 2. Participant questionnaire

Participants	Sex	University year	Activities and uniqueness
M1	Male	Freshman	- More than once a week football - Living in a school dormitory - Nutrient intake (vitamins, lutein) - Hyperhidrosis, dental caries, purulent keratitis
M2	Male	Sophomore	- Had a cold for a week during the experiment - Intake of antibiotics for 1 week - More than once a week football - Had a lot of outside activities
M3	Male	Freshman	- Nutrient intake - More than once a week football - Uses public bus twice a week - Hyperhidrosis
M4	Male	Freshman	- Volunteer activities once a week - More than once a week football - Drink alcohol 3–4 times a week - Has a girlfriend
F1	Female	Sophomore	- Had dental caries
F2	Female	Sophomore	- Working at a hospital - Uses public buses three times a week
F3	Female	Sophomore	- Arrhythmia - Working at a hospital - Uses public buses three times a week - Oily skin - Frequent drinking with other people - Had dental caries
F4	Female	Sophomore	- Working at a school - Uses public bus more than 5 days a week - Hormonal control disorder - Co-residence with family - Often visited dog house

Note: In order to guarantee the anonymity of the subjects, names have been encrypted. The information in the table contains experimental variables.

also conducted to determine whether individuals were affected by internal and external environmental factors. **Table 2** shows the results of the participant questionnaire. Among the participants, F4 had constant contact with a dog in our study. There was more than 0.1% of dog-related bacteria in F4's fingertips and mobile phones (**Table 3**). Among M1, F1, and F3 participants who had an oral disease, M1 and F3 showed more than 1% of oral disease bacteria in their fingertips and mobile phones. F1 had more than 1% of oral disease bacteria only in the fingertips, but there was also a relatively high proportion of bacteria on F1's mobile phone. Female-associated strains were found in most female participants (> 0.1%), but not in F4, who had a hormonal disorder. However, M4, who had a girlfriend at the time of the study, was the only male participant with more than 0.1% female-associated bacteria in his fingertips and mobile phone. M1, who has purulent keratitis, was the only participant with ocular disease bacteria. More than 1% of respiratory bacterial strains were found on both the mobile phone and fingertips of M2, who had a cold for a week during the time of the study.

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Table 3. Unique distribution of bacteria in participants' fingertips and mobile phones

	Dog (0.1%)	Oral disease (1%)	Female (0.1%)	Ocular disease (0.1%)	Respiratory disease (1%)
M1.F	0.01	**2.05**	0.00	**4.44**	0.38
M1.MP	**0.75**	**2.45**	**1.05**	**0.12**	0.66
M2.F	**0.20**	**2.23**	0.00	0.00	**1.70**
M2.MP	0.00	0.00	0.00	0.01	**1.01**
M3.F	**1.21**	0.00	0.01	0.00	0.75
M3.MP	0.00	0.00	0.00	0.00	0.19
M4.F	0.00	0.23	**0.17**	0.08	0.18
M4.MP	0.00	0.28	**0.66**	**0.13**	**2.12**
F1.F	0.00	**6.01**	**0.14**	0.01	**2.11**
F1.MP	**0.76**	0.76	**0.75**	0.00	0.45
F2.F	0.00	0.00	**0.26**	0.05	0.12
F2.MP	0.00	0.15	**0.24**	0.04	0.01
F3.F	0.00	**4.20**	**0.26**	**0.14**	**6.66**
F3.MP	0.01	**1.48**	**0.21**	0.00	0.48
F4.F	**1.35**	0.15	0.00	0.07	0.00
F4.MP	**1.36**	**2.41**	0.00	**0.36**	0.06

Note: The percentage of bacteria presented is a ratio of the total taxa present. Dog, female, and ocular disease-related strains were regarded as strains of which only bacteria with a ratio of 0.1% or more were constantly present in individuals. Oral and respiratory disease strains were identified based on a ratio of more than 1% due to the presence of a large amount of resident flora. Ratio values of bacteria that meet the criteria are set in bold.

4.6. Bacteria Biomarker Discovery

Samples of eight human fingertips and eight mobile phones were compared by fingertip and mobile phone groups. Two genus levels (*Brevibacillus*, *Planococcus*) and two species levels (*Nesterenkonia lacusekhoensis* group and *Planococcus kocurii* group) were only identified in fingertip samples. The only microorganisms found in the mobile phone samples were 2 phylum levels, 4 class levels, 4 order levels, 4 family levels, 8 genus levels, and 5 species levels (**Supplementary Table 1**). The samples of eight human fingertips and eight mobile phones were then grouped and compared by gender. Microorganisms found only in the male microbial community include 1 order level (*Solirubrobacterales*), 1 family level (*Pseudoxanthobacter*_f), 1 genus level (*Pseudoxanthobacter*), and 4 species (*FJ542954*_s, *Dorealongicatena*, *Prevotella pallens*, and *Tannerella forsythia*). Microorganisms found only in the female microbial community were identified as 1 phylum level, 1 class level, 3 order level, 8 family level, 19 genus level, and 35 species-level (**Supplementary Table 2**).

5. Discussion

The study aimed to verify whether external and internal environmental factors of individuals could be estimated through bacterial communities. The concentration of genomic DNA present in samples was determined (**Fig. 1, Table 1**), and the gDNA concentration at the fingertips of participants was similar according to sex.

However, the gDNA concentration of mobile phones was found to be different according to sex. Male participants had outdoor activities once or more a week. In contrast, female participants had little outdoor activity. Thus, the male participants in our study may have had relatively low contact with their mobile phones compared to female participants. It confirmed that DNA concentration may reflect the frequency of contact between an individual and an external object (9, 29, 30).

Rarefaction curves visualized the degree of biodiversity by sex and sample type (31). As a rarefaction curve indicator, we used the Chao1 index, which accurately assesses species richness to confirm the results at the species level (17, 23). The biodiversity of women in all samples was higher than that of men (**Fig. 2**). Previous studies also compared biodiversity according to sex at various body sites. When examining overall phylogenetic structure or the average number of phylotypes per palms, female biodiversity was higher than male diversity (15). In another study, when bacteria of older men and women were compared, the alpha diversity of men was lower than women (32). When alpha diversity of rural-dwelling males and females were compared, diversity in male participants was significantly lower than women participants. Our results corroborated these findings regarding biodiversity and sex. In our work, fingertip biodiversity was higher than that of mobile phones. This was probably because fingertips are more exposed to various external

environments than mobile phones (29). Individuals in contact with various external surfaces may have a more diverse bacterial composition. Thus, our work revealed that the sex of individuals and external surfaces that an individual may come in contact with are reflected in biodiversity.

Clustering analysis (beta diversity) was performed to determine whether specific strains from individuals could leave traces through contact (**Fig. 3**) and to measure the distance between communities, using UniFrac. UniFrac employs standard multivariate statistics using principal coordinates analysis (28). The unweighted UniFrac distance method only considers the presence or absence of a species and can effectively confirm the richness of rare lineages. The weighted UniFrac distance is a sensitive method because it considers species abundance and is measured using absolute abundance (33). To improve accuracy, both weighted and unweighted UniFrac distances were used. Our results revealed clustered microbial communities in participants, except M2 and M4. A possible explanation could be that during the time of the study, M2 had a cold and took antibiotics for 1 week, and M4 had sexual contact which might have altered their bacterial profiles (**Table 2**). In fact, among the male participants, a large number of female-associated strains were detected in only M4 (**Table 3**). R.R. Marples *et al*. assessed for 3 weeks whether oral antibiotics could affect the skin microbial community and confirmed that the skin bacterial composition changed (34). Oral antibiotics administered to M2 may have altered the skin bacteria composition of his fingertips. M2's mobile phone had a unique bacterial community perhaps due to his catching of a cold; it would not have been consistent with the altered bacterial composition in his fingertips due to taking antibiotics. This study did not apply any restrictions to check whether it was possible to check personal identity information even in an unrestricted environment. As a result, except for the participants with specific characteristics, the bacterial populations of the remaining participants were found to have left traces. Weighted and unweighted UniFrac methods were used to analyze the similarities of microbial communities. The Unifrac results revealed a lower matching rate than expected. The unweighted and weighted Unifrac methods are the most widely used similarity measurement methods, and both methods are suitable for analyzing samples of rare or abundant bacterial communities. However, as in our experiment, they are not suitable for the analysis of a moderately abundant bacterial community, or in cases where the experimental and environmental microbial communities are mixed

(32). Therefore, we conducted a parallel analysis using bacterial profiling and statistical methods. Bacterial profiling can help identify the effect of the surrounding environment on the microbial communities in a sample; additionally, when multiple suspects are present in forensic cases, the scope of the suspect can be narrowed by the characterization of the suspect's microbiome.

We detailed herein whether bacteria could reflect individual internal and external factors using taxonomic assignment (**Fig. 4, Table 2, Table 3, and Supplementary Fig. 1**). Fingers are parts of the body that are in direct contact with various external environments. The taxonomic distribution of the bacterial composition of fingertips showed that the bacterial community of each individual was distinct (17). F4 was the most diverse and the only participant who lived with family and who is most likely to use public transportation. Thus, the bacterial composition diversity was the highest because F4 was exposed to many external environments (29). In each sample, taxonomic biomarkers were identified in fingertips, mobile phones, and gender. Each taxonomic biomarker microorganism was identified in statistical analysis, and since there is no previous study showing that it is directly related to Korean fingertips, mobile phone, and gender, it is not yet a complete biomarker to confirm the characteristics of the sample. Therefore, further analysis of 200 Koreans is underway to identify complete biomarker microorganisms. In six categories of bacterial profiling, six fingertips and mobile phones matched each other. This allows the bacteria to stay in contact, reflecting the internal and external factors of the individual and can be used as a forensic personal identification tool. After confirming the similarity between samples using both the Unweighted and Weighted UniFrac methods, characteristic strains were also identified by bacterial profiling and statistical methods, and the association of environmental covariates with the experimental group was confirmed. However, since the number of experimental groups was very small, only characteristic strains of the categories, namely pet dogs, women, oral diseases, respiratory diseases, and eye diseases have been identified. Therefore, it is necessary to identify microbial communities as biomarkers representing more characteristics such as gender, age, occupation, residence, and disease with large-scale follow-up studies.

6. Conclusions
Bacterial communities left traces of contact, presenting distinct and unique bacteria for each individual in our study. Accordingly, the composition of bacterial

communities of each individual was affected by internal and external environments. The bacteria on the individual and the objects the individual had contact with have revealed information about the identity of the individual. In conclusion, the verification of individual identity and environmental information can be traced through bacteria profiling. For more accurate bacterial profiling, it is necessary to establish a bacteria list (biomarkers) for identification information. Bacterial composition based on several factors may serve as an important clue to obtain information about suspects. Bacterial communities can be used as evidence in crime scenes if information about individuals' identities can be accurately identified via bacterial profiling.

Compliance with ethical standards
Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals
This article does not contain any studies involving animals performed by any of the authors.

Declaration of conflicting interests
Declarations of interest: none

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