Journal of Forensic Research

Lee et al., J Forensic Res 2015, 6:5
DOI: 10.4172/2157-7145.1000297

Research Article Open Access

Microbial Forensic Analysis of Bacterial Fingerprint by Sequence Comparison of 16S rRNA Gene

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Rec date: April 21, 2015 **Acc date:** June 25, 2015 **Pub date:** June 30, 2015

Abstract

Human have the microorganisms in the skin, gut and mouth. Human skin microbiome based on the 16S rRNA encoding gene can reveal bacterial species diversity. Also bacterial species in the skin have diverse and unique composition between individuals. We thought that a bacterial fingerprint obtained from surfaces including computer keyboards aids forensic individual identification in case of evidence deficiency. Next generation sequencing was used to analyze the bacterial community on objects and fingertips to match the object to the individual. The 16S rRNA gene sequence was submitted to EMBL SRA with accession number PRJEB8760. Higher similarity of bacterial community between public computer keyboards and laboratory member's fingertips were evident than between other locations including doorknobs. Here we studied the challenges that bacterial fingerprint can be used as a human identification tool in forensic fields.

Keywords: Bacterial fingerprint; Human identification; Microbiome; Microbial forensics; Next generation sequencing

Introduction

Every individual harbors a large number of microorganisms. The human microbiome refers to the total microorganisms found in and on the human body.

Human identification is important in forensic science [1] and it will continue to do so. However, trace evidence like inadequate quality and low copy number of DNA cannot be detected with the stringency needed for prosecution of crimes including those involving violence. An intelligent offender can take steps to decrease contamination of the crime scene with blood, semen, and fingerprints, which can complicate offender detection.

Bacterial DNA is a novel avenue in forensic science. Bacterial DNA is more resistant to environmental factors than human DNA and so can persist longer on a surface than human DNA. The configuration of bacterial DNA is influenced by the surrounding environment [2] and the individual's microbiome. It is conceivable that the different bacterial patterns could discriminate individuals with different lifestyles.

We hypothesized that personal identification can be possible by analysis of the pattern of skin bacterial DNA. To assess this, we configured the differences among individuals and conducted a study to identify individuals. Skin bacteria left on surfaces that were touched were identified using next-generation sequencing (NGS).

NGS is a bioinformatic technique that uses molecular and computational approaches to generate and analyze DNA sequences [3]. NGS has an advantage of an economical production of large volumes of sequence data. Pyrosequencing is a NGS technology. In pyrosequencing is a technology that reads the sequence by detection of pyrophosphate generated when DNA polymerase attaches to the nucleotide monomer.

We hypothesized that bacterial DNA analyses could discriminate the differing bacterial profiles between individuals in a way that has forensic value. To explore this, we analyzed the bacterial signatures left by different individuals on surfaces including fingertips, a computer keyboard and a doorknob using pyrosequencing based on the 16S rRNA gene.

Materials and Methods

Sample collection and DNA extraction

We swabbed keyboards of a public computer and individual fingertips in the laboratory to explore the correspondence between the bacterial communities of individuals and the bacterial fingerprints recovered from the keyboard. For a precise comparison, we swabbed fingertips that had never touched the keyboard of the selected public computer. Fingertips and keyboards were sampled using autoclaved cotton-tipped swabs premoistened with normal saline.

The two individuals who participated were from the same laboratory. These individuals had not taken antibiotics. This was important as antibiotics, diet, and smoking can influence the microbiome composition [1].

To explore bacterial species diversity, a doorknob in the same laboratory was similarly sampled. Swabs were each inoculated in 10 ml Brain Heart Infusion broth (Difco BBL, USA) and then incubated for 24 h at 37 in a 5% CO_2 incubator (Thermo, CO_2 forma series2 incubator). Bacterial DNA was extracted from the pelleted cells using a QIAamp DNA minikit (Qiagen, Valencia, CA, USA). Tissue protocol was used according to the manufacturer's instructions.

PCR amplication and pyrosequencing

PCR amplication 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') and 518R (5'-CCATCTCATCCCTGCGTGTCTCCGAC-TCAG-X-AC-WTTACCGCGGCTGCTGG-3'(with the underlined sequences indicating the target regions and X indicates the unique barcode for each subject) (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 2% agarose gel electrophoresis with TAE buffer and the resolved species were visualized using a Gel Doc system (BioRad, Hercules, CA, USA). The amplified products were purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA).

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 NGS system (Roche, Branford, CT, USA) according to the manufacturer's instructions.

Pyrosequencing Data Analysis

The basic analysis was conducted as previously described [4-6]. 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 300bp 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 [7].

After removing chimera sequences, the taxonomic classification of each read was assigned against the EzTaxon-e database (http://eztaxon-e.ezbiocloud.net) [8], which contains 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 subsampling 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 [9]. To compare Operational Taxonomic Units (OTUs) between samples, shared OTUs were obtained with the XOR analysis of the CLcommunity program (Chunlab Inc., Seoul, Korea).

Results

Bacterial fingerprints were extracted from swabs and bacterial community composition was determined using the high-efficiency pyrosequencing protocol. An average of 8,000 bacterial 16S rRNA gene sequences per sample was obtained.

Pyrosequencing reads generated in this study are available at the EMBL SRA database under the study accession number PRJEB8760 (http://www.ebi.ac.uk/ena/data/view/ PRJEB8760). Both keyboard and doorknob samples displayed a rich and diverse taxonomic species composition (Figure 1). Following species groups are predominated at the doorknob and keyboard sample.

Doorknob sample has the *B. subtilis* group (*B. subtilis* subsp. *subtilis, B. subtilis* subsp. *spizizenii, B. subtilis* subsp. *inaquosorum, B. mojavensis, Brevibacterium halotolerans, B. tequilensis*), the *Streptococcus salivarius* group (*S. salivarius* subsp. *salivarius, S. salivarius* subsp. *thermophilus, S. vestibularis*), the *Lysinibacillus sphaericus* group (*L. sphaericus, L. fusiformis*). Keyboard sample has the *Bacillus anthracis* group (*B. anthracis, B. cereus, B. thurinqiensis, B. toyonensis*), *Staphylococcus aureus* group (*Staphylococcus aureus subsp. aureus, Staphylococcus aureus* subsp. *anaerobius*) (http://eztaxon-e.ezbiocloud.net/ezt_taxgroup).

The doorknob yielded a markedly more diverse species composition than the computer keyboard, likely reflecting the larger contact with the doorknob.

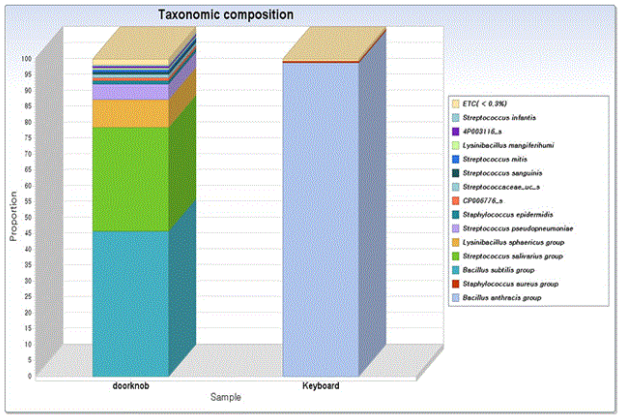

Figure 1: Three-dimensional depiction of species diversity of the computer keyboard and doorknob. The species denoted by each color is defined right the figure. The doorknob provided a more diverse result than keyboard. ETC is a collection of minor components whose portion is below the cutoff value.

The unweighted pair group method with arithmetic averages was used to cluster the community samples with unweighted UniFrac [10]. UniFrac distance metric [11] in the dendrogram is a widely-used measure for comparison of two or more microbial communities. Fast UniFrac [9] is a variant of the original UniFrac algorithm designed to handle larger datasets using taxonomic assignment to a phylogenetic tree.

Bacterial communities between the laboratory personnel and the computer keyboard were more similar than other bacterial communities, such as the control and doorknob (Figure 2).

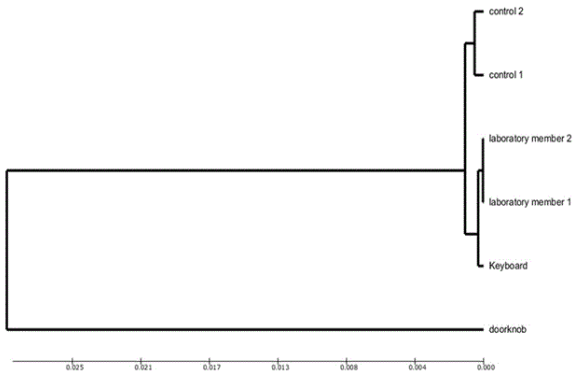

Figure 2: Unweighted pair group method with arithmetic mean dendrogram. This compared two or more microbial communities. If the UniFrac distance of X axis is large, two or more microbial communities will drift apart.

The taxonomic composition of the sample showed a statistical picture (Figure 3). The double pie chart depicts the taxonomic compositions of two different taxonomic ranks simultaneously. Species composition of the *B. mycoides* group (*B. mycoides*, *B. weihenstephasnesis*) of laboratory member 1 was identical with the keyboard species composition, with a variance of 0.01% in the double pie chart. At the time of swab collection, this participant was using the computer an average of 3 hours per day, which was the most use of any laboratory member. The result supports the view that the frequency of contact with a surface increases the likelihood of identity in bacterial fingerprinting of the individual.

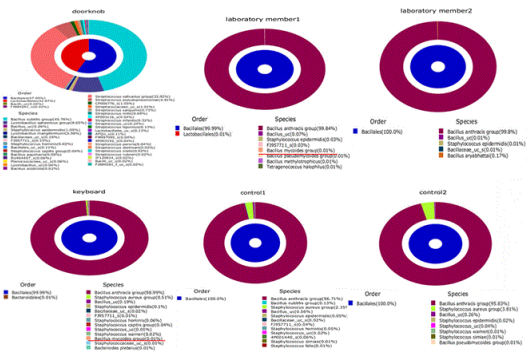

Figure 3: Average composition double pie chart presenting the summed composition of all samples. Underline denotes the same bacteria species between laboratory member 1 and the computer keyboard.

Discussion

The Human Microbiome Project has been designed to understand the microbial components in the human body and how they contribute to our body [12]. The project has focused on medical and environmental microbiology. The present study expands the focus to include the applicability of the skin microbiome in microbial forensics. Microbial forensics has become a useful adjunct to investigations of bioterrorism and biocrime [13] and its potential has been recognized as an alternative plan to overcome limitations of current forensic science, such as a deficiency in blood, tissue, semen, or saliva at the site of crime scene investigation. Bacterial DNA analysis will not replace standard DNA identification, but could become a complimentary technique for when standard DNA identification provides only limited information [1]. Bacterial components recovered from computer keyboards following use allows for adequate characterization and comparison of bacterial community, and surfaces that are touched can be linked to individual skin surface [14]. The report of a relationship between personal computer and the user bolstered the optimism of bacterial DNA analysis in forensic science. The present study provides further encouragement, with the finding that individuals can be discriminated based on their bacterial DNA fingerprint of the public computer.

The bacterial species diversity was not extensive, reflecting the limited capacity of bacterial growth in the medium. Yet, the same bacteria were consistently detected in samples from the most frequent user of the public computer keyboard. These results show the potential of identifying an individual based on the bacterial species composition of the analyzed surface.

The present skin bacterial fingerprint study should permit a valuable advancement in forensic identification. We plan to increase the number of samples and to use the metagenome method. The metagenome is the set of all microbial genomes present in any given environment [15]. Present efforts to use metagenome analysis were of limited effectiveness. Culturing on common media recovers only a limited variety of bacteria, estimated as 0.1~1% of the total available bacteria [16,17]. This hampers the forensic potential of the approach, as the gene profiles of the uncultured bacteria are excluded from the analysis. A metagenome approach would overcome this hurdle. Infuture research, we will extract DNA samples directly from non-cultured specimens.

Acknowledgment

This study was supported by Soonchunhyang University research fund, by Basic Science Research Program, through the National Research Foundation of Korea, funded by the Ministry of Education (NRF-2014R1A1A2A16055670) and by the Technological Innovation R&D Program (C0249241) funded by the Small and Medium Business Administration (SMBA, Korea).

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