Quantitative and Qualitative Estimation of Bacteria Contaminating Human Hairs

Aram Yun¹, Eun Jin Yang¹, Young Mi Lee¹, Seon Sook Chae¹, Ha Na Seo² and Doo Hyun Park*²

¹Department of Beauty Arts, ²Department of Biological Engineering, Seokyeong University, Seoul, Korea

Human hairs have been known to be easily contaminated with microorganisms. This study was performed in order to measure what bacterial species and how much microorganisms contaminate human hairs in specific place. Virgin human hairs were left at 6 positions in inside corner and beside window in a laboratory for 7 days. The number of viable bacterial cells, which were determined by most probable number method, contaminating the human hairs was measured at a maximum of 10⁶/g hair and a minimum of 10³/g hair in inside corner and maximum of 10⁶/g hair and a minimum of 10³/g hair beside window. The bacterial cells-contaminating human hairs were observed via fluorescence light microscopy after 4',6-diamino-2-phenylindole (DAPI) staining. The bacterial community contaminating human hairs was analyzed via the thermal gradient gel electrophoresis (TGGE) technique, based on the diversity of the 16S-rDNA variable region. In total, approximately 20 bacterial species were detected from 12 groups of hair samples. In this study, general experimental methods-fluorescence staining, TGGE and MPN-were combined to develop new method for observation and estimation of bacteria contaminating human hairs.

Key Words: Human hair, Bacterial community, Most Probable Number, DAPI-staining, TGGE

INTRODUCTION

Not all airborne microorganisms exert adverse effects on human health, but some of them may cause infectious diseases (1, 2), allergic or irritant responses (3, 4), respiratory problems (5, 6), or hypersensitivity reactions (7, 8). Airborne microorganisms have been identified and enumerated via a broad variety of aerobiological sampling methods (9, 10). Certain air sampling techniques may prove effective in the detection and identification of airborne microorganisms in a specific location, but may be not appropriate for the characterization of their relevant mediators and transfer routes. General human activities involve movement and transfer, either minimally from one local region to another, or maximally from one country to another country; during such activities, a variety of microscopic particles containing bacteria may migrate through these translocation routes.

Humans have been identified as the primary source of microbial contaminants within industrial clean rooms and hospital operating rooms (11, 12). The majority of airborne contaminants containing bacteria has been associated with the hair, skin, and respiratory tracts of humans (13). Microbial contaminants evaluated in hospital operating rooms have been associated largely with humans, rather than dust and soil particles (14, 15). Human hairs may function as an air-collecting agent for micro-contaminants, because the hairs are constantly exposed to air and can readily adsorb a variety of airborne particles via electrostatic attraction, grooved surfaces, thin and long structures, and biochemical affinity (16). The cultivation technique for the identification of airborne microorganisms is limited to cultivable microorganisms on a specific culture medium,
according to research data showing that less than 20% of bacteria in the environment were cultivable (17, 18).

The diameter of human hair ranges between 50 to 150 μm (circumference, 157 to 470 μm), and thus any bacterium could theoretically be attached to human hairs (19, 20).

Light microscopy is a very useful technique for the observation of microorganisms on slide glasses however, it is not possible to observe bacteria contaminating hair surfaces, because the human hair is too thick for light to pass through it from the lamp to the objective glass.

In this study, we have quantitatively estimated and taxonomically analyzed bacterial cells contaminating human hair via the most probable number (MPN) method and thermal gradient gel electrophoresis (TTGE) technique, respectively, and have applied the 4',6-diamino-2-phenylindole (DAPI) staining method to hair in an effort to observe microorganisms contaminating human hair. This research may not prove to be meaningful for environmental or clinical evaluations of bacterial contamination; however, our results may prove to be useful in estimates of the amount and species of micro-contaminants that are transferred and transmitted via human hairs.

Figure 1. Fixation of hair bundle in an opened square box, by which human hairs can be naturally moved and contact with air (A). Six of them were placed in inside corner and another six were placed beside window (B).

MATERIALS AND METHODS

Viable cell count

Virgin hairs (21) obtained from young woman (15 years old) were cleaned, rinsed and dried according to general hair cleaning habit. One end of 15 cm-long hair bundles (about 200 pieces) was fixed in an opened square box (200 × 150 × 100 mm) as shown in Figure 1A, by which other part of hairs was freely moved by air flow. The fixed hair bundles were put at 6 positions in inside corners or beside windows in our laboratory as is shown in Figure 1B, which is located on the 4th floor of the Department of Biological Engineering building for 7 days, from July 29th to August 5th of 2009. Four of outside windows (width 3 m × height 1.5 m) and 6 of corridor windows (width 2 m × height 0.6 m) were installed on each side of 15 m of window wall; however, no window was installed on 9 m of side wall. Distance from bottom to outside window and corridor window was 1.1 m and 2.2 m, respectively, as shown in Figure 1B. Eleven students were doing experiments about microbiology, biochemistry and molecular biology from 9 am to 6 pm. Outside windows were closed during daytime from 7 am to 8 pm under air-conditioning and opened after
work during night-time from 8 pm to 7 am; however, corridor windows were constantly closed and outside windows were closed conditionally during even night-time in rainy day. Hair bundles were disposed on laboratory tables (height 0.8 m) that were located in front of the outside windows. Distance from window to hair bundle was adjusted to 0.6 m and interval between hair bundles was controlled to be 2 m. Other 6 hair bundles were located on the bottom under corridor windows. Distance from wall to hair bundle was adjusted to 0.2 m and interval between hair bundles was controlled to be 1.5 m.

Some of the hairs were sampled for microscopic observation right after placed in specific positions for the control test. The laboratory temperature was maintained naturally from 25°C to 30°C, but moisture was uncontrolled. The hairs (0.1 g) cut from the hair bundles were put into test tubes containing 9 ml of a complex medium after slicing with autoclaved scissors under aseptic conditions. The hair-suspended medium was serially diluted with the identical medium via 10-fold dilution. The complex medium, which was optionally prepared based on the nutritional conditions required for aerobic bacterial growth, was composed of 1 g/L of glucose, 2 g/L of lactate, 3 g/L of acetate, 1 g/L of yeast extract, 1 g/L of peptone, 25 mM of phosphate buffer (pH 7.0) and 1 ml/L of trace mineral stock solution. The trace mineral stock solution contained 0.01 g/L of MnSO₄, 0.01 g/L of MgSO₄, 0.01 g/L of CaCl₂, 0.002 g/L of NiCl₂, 0.002 g/L of CoCl₂, 0.002 g/L of SeSO₄, 0.002 g/L of WSO₄, 0.002 g/L of ZnSO₄, 0.002 g/L of Al₂(SO₄)₃, 0.0001 g/L of TiCl₄, 0.002 g/L of MoSO₄, and 10 mM EDTA.

The number of viable cells was estimated on the basis of the MPN method according to standard protocols (22).

**Observation of microorganisms contaminating human hair**

The human hairs, which were left in inside corner and beside windows without any handling for 7 days, were fixed on a slide glass. Immersion oil was dropped on the dried hairs and covered with a cover glass. The oil immersion objective was assessed under a bright field (Zeiss, Axioskop 50, München, German).

**Fluorescent staining of bacteria contaminated hair surfaces**

DAPI solution (0.5 μg/mL) was dropped on the hairs for 60 min at room temperature. The fluorescence-stained hairs were rinsed with autoclaved double-distilled water and dried in a drying oven for 10 min at 60°C, and immersion oil was dropped onto the dried hairs and covered with a cover glass. The oil immersion objective of the fluorescence microscope (Zeiss, Axioskop 50) was used under UV light (23).

**Thermal gradient gel electrophoresis (TGGE)**

Ten grams of human hairs, which were placed into contact with airborne microorganisms for 7 days, were suspended in 100 ml of filtered distilled water with pre-sterilized membrane filters (pore size 0.22 μm, Millipore, Billerica, MA, USA) under aseptic conditions, and subsequently shaken at 200 rpm for 3 h at room temperature. The hair-suspended water was filtered with a pre-sterilized membrane filter and the membrane filter was utilized for DNA extraction. Total DNA was extracted from the bacterial cells attached to the membrane filters using an AccuPrep Genomic DNA Extraction Kit (Bioneer, Daedeon, Korea) according to the manufacturer's protocols. The 16S-rDNA amplified from chromosomal DNA was utilized as a template for the preparation of the TGGE samples (16S-rDNA variable region). A variable region of 16S-rDNA was amplified with the forward primer (eubacteria, V3 region) 341f 5'-CCTACGGGAGGCAGCAG-3' and reverse primer (universal, V3 region) 518r 5'-ATTACCGCGGCTGCTGG-3'. GC clamp (5'-CGCCCGCGGCGCGCGCGGGGCGGGGGCACGGGGGCCTACGGGGGAGGCAGCAG-3') was attached to the 5'-end of the GC341f primer (24). The procedures for PCR and DNA sequencing were identical to the 16S-rDNA amplification conditions except for the annealing temperature of 53°C. The TGGE system (Bio-Rad, Dcode™, Universal Mutation Detection System, Hercules, CA, USA) was operated as specified by the manufacturer. Aliquots (45 μl) of PCR products were
electrophoresed in gels containing 8% acrylamide, 8 M urea, and 20% formamide with a 1.5 × TAE buffer system at a constant voltage of 100 V for 12.5 h and then for 0.5 h at 40 V, with an applied thermal gradient of 39 to 52°C. Prior to electrophoresis, the gel was equilibrated to the temperature gradient for 30 to 45 min.

**RESULTS**

**MPN of viable bacteria contaminating hairs**

Number of bacterial cells contaminating human hairs determined by MPN was $10^3$ to $10^6$ in hair samples in inside corner and $10^2$ to $10^5$ in them beside window as shown in Table 1. Viable cell number was significantly variable according to the position. These results may be an indicator that human hair may be randomly contaminated by air-floating bacteria in any place and the air-floating bacterial cells may have chance to more stably contact to hair in inside corner than beside window.

**Observation of human hair by light microscopy**

Human hair surface is difficult to be observed with a light microscope under the bright field, as the optical density of hair structure is too high to transmit light. The contours of the sides of hairs can, however, be observed as shown in Figure 2A, 2B and 2C, in which fungal hyphae and conidia-contaminated hair surfaces could be visualized under bright field ($\times$ 400). Some spots (arrow marks) on hair surfaces (Fig. 2D) were noted with the hair cuticle grooves. However, these spots differed from morphology generated by microorganisms. Accordingly, bacterial cells contaminating hair surface cannot be visualized via bright-field light microscopy.

**Observation of bacterial cells by fluorescence light microscopy**

In order to obtain clear images of the bacterial cell-contaminated hair, the hair was stained with a fluorescent dye (DAPI), which is selectively bound to DNA but also bound to hair surface as shown in Figure 3. Hair cuticles were distinctly observed and some microorganisms were detected on the hair surface, even though the hairs were sampled for control test at the time when the hair bundle was placed in 12 positions. The microscopic image of cleaned hairs was significantly different from that of the contaminated hairs for 7 days. As shown in Figure 4A, clear images displaying bacterial cell-contaminated hair cuticles were obtained. The bigger fluorescent spots (marked with arrows) than the bacteria spots were thought to be developed by fungal cells contaminating hair as shown in Figure 4B. Some fluorescent clots were noted in the hair cuticle grooves as shown in Figure 4C. The clots may be developed by the mixture of various microorganisms grown in the cuticle grooves, which constitute compelling evidence that the grooves between hair cuticles may be an appropriate habitat.

<table>
<thead>
<tr>
<th>Disposition</th>
<th>Group</th>
<th>MPN/g hair</th>
</tr>
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<tbody>
<tr>
<td>Inside corner</td>
<td>I</td>
<td>$1.1 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$8.5 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$4.5 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>$5.5 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>V</td>
<td>$7.0 \times 10^3$</td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>$1.1 \times 10^6$</td>
</tr>
<tr>
<td>Beside window</td>
<td>I</td>
<td>$3.0 \times 10^5$</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>$1.0 \times 10^2$</td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>$1.3 \times 10^5$</td>
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<tr>
<td></td>
<td>IV</td>
<td>$8.5 \times 10^3$</td>
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<td></td>
<td>V</td>
<td>$1.0 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>VI</td>
<td>$1.1 \times 10^4$</td>
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Estimation of Bacteria Contaminating Hairs

for microorganisms and a good place for the collection of organic compounds.

Diversity of bacterial species contaminating hair surface

The variable region of 16S-rDNA, which was amplified with DNA extracted from bacterial cell-contaminated hair surfaces, was separated via TGGE as shown in Figure 5. The DNAs extracted from the TGGE band were more than 90% homologous with a variety of bacterial species that were more than 20 species. *Janthinobacterium* sp. (FJ605429) was recorded to be isolated from spider mite, *Bacillus* sp. (FJ941090) was recorded to be an endophytic bacterium, *Stenotrophomonas* sp. (FJ897489) isolated from industrial estate, *Erwinia* sp. (FJ816023) related to antibacterial immunity of *Anopheles gambiae* lysozyme, *Bacillus* sp. (AM292066) isolated from soil, *Oxalobacter* sp. (AV-
related to house mouse, *Naxibacter* sp. (FJ621304) isolated from rhizosphere, *Deinococcus* sp. (DQ223543) related to radiation resistant bacteria, *Pseudomonas* sp. (AB461633) related to nodulation-dependent communities, *Bacillus* sp. (FJ981907) related to marine environment, *Comamonas* sp. (AM711890) was recorded to grow in wetland, *Pantoea ananat* (AF491932) was recorded to be a soil bacterium, *Pantoea eucalypti* (GQ169378) related to soil environmental organism and *Actinobacterium* sp. (EU810962) was recorded to grow in cave in the GenBank database. These results demonstrate that various bacterial species can be floated freely through air flow and attached to hair without making direct contact with the carrier or vectors.

**DISCUSSION**

Human hair can be readily contaminated by a variety of chemical compounds, which is useful for the hair-monitoring of organic pollutants in the atmospheric environment (25–28); however, research data and techniques for the hair-biomonitoring of microorganisms have yet to be developed. Fish scale-like hair cuticles may develop into various sized gaps, which represent excellent sites for microorganismic contamination. However, what quantities of microorganisms and how many species of microorganisms contaminating human hair are issues that have yet to be thoroughly elucidated.

In this study, we first developed the DAPI staining technique in order to observe the surfaces of hair contaminated with bacteria under light microscopy. The bacterial cells stained with DAPI are discriminated from the hair, as DAPI binds selectively to DNA. The DAPI staining technique has proven useful for the detection and observation of bacterial cell-contaminated hairs; however, this is limited to structural display (29). The TGGE technique may be an excellent method for the estimation of diversity of bacterial cells contaminating hairs. A variety of airborne microorganisms can be readily attached to or detached from human hair by transfer or the normal activities of community living. Pathogenic contagion or infection may be mediated by the attachment of airborne microorganisms to hairs, or their detachment from hairs. Any species of bacteria can be attached to hair based on the diversity of bacterial communities separated from human hairs and analyzed via the TGGE technique. Anyone residing in a locality contaminated with a specific pathogen makes it very possible that pathogenic microorganisms will be transferred to another localities via the bacterial contamination of hair.

Humans basically use two types of mobile polymers: clothes and hairs. Clothes can be readily and frequently...
cleaned with detergents containing disinfectants or hot water, by which the bacterial cells attached to cloth fibers can be sterilized effectively; however, human hair cannot be cleaned with disinfectants. Some bacterial cells may be not cleaned completely using hair soap or shampoo by general cleaning habit (Fig. 3).

In conclusion, we have developed, and presented herein, a new technique by which microorganism-contaminated human hairs can be observed via light microscopy. Our results represent that microorganisms contaminate human hair may be observed and estimated effectively by combination of most probable number method, fluorescent staining and TGGE technique but do not represent any new discovery regarding the bacterial contamination of hair. We found no evidence to suggest that bacterial cells attached to human hair mediate the transfer of epidemic disease by contagion; however, surgeons generally cover their hair in surgical operating rooms for precisely this reason. Currently, we are analyzing and comparing the bacterially-contaminated hairs of individuals working in hospitals, subways, and agricultural areas.

REFERENCES


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