Comparative Ocular Microbial Communities in Humans with and without Blepharitis

Se Hee Lee, Doo Hwan Oh, Ji Young Jung, Jae Chan Kim, and Che Ok Jeon

PURPOSE. The aims of our study were to compare the ocular microbial communities of humans with and without blepharitis in an attempt to elucidate which microorganisms may cause blepharitis.

METHODS. Bacterial 16S rRNA genes of eyelash and tear samples from seven blepharitis patients and four healthy controls were sequenced using a pyrosequencing method, and their bacterial community structures were compared bioinformatically.

RESULTS. Phylotypic analysis demonstrated that eyelash and tear samples had highly diverse bacterial communities with many previously undescribed bacteria. Bacterial communities in eyelash samples from subjects with blepharitis were less diverse than those from healthy controls, while the bacterial communities of tear samples with blepharitis were more diverse than those of healthy subjects. Statistical analyses using UniFrac and a principle coordinate analysis showed that the bacterial communities of tear samples from subjects with blepharitis were well clustered, regardless of individual, while the bacterial communities of all eyelash samples and healthy tear samples were not well clustered due to high interpersonal variability. Bioinformatic analysis revealed that Propionibacterium, Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter were the common ocular bacteria. An increase of Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter, and a decrease of Propionibacterium were observed from blepharitis subjects, in terms of the relative abundances.

CONCLUSIONS. Higher abundances of Streptophyta, Corynebacterium, and Enhydrobacter in blepharitis subjects suggested that human blepharitis might be induced by the infestations of pollens, dusts, and soil particles. These results will provide valuable information for the prevention and treatment of human blepharitis based on ocular microbial flora. (Invest Ophthalmol Vis Sci. 2012;53:5585–5593) DOI:10.1167/iovs.12-9922

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METHODS
Sample Collection
Seven patients with blepharitis who visited a clinic (Chung-Ang University Hospital) for ophthalmic examinations between September 1 and November 30, 2010, and four controls without blepharitis were included in the study. Informed consent was obtained from all participants. This study was approved by the Chung-Ang University Hospital Institutional Review Board, and all methods adhered to the principles of the Declaration of Helsinki. The information from all participants is summarized in Table 1. All participants underwent a complete ophthalmic examination under a slit-lamp biomicroscope. Blepharitis was diagnosed based on clinical evidence of lid margin or tarsal conjunctival erythema, bulbar conjunctival hyperemia, telangiectasia, thickening, or irregularity of the eyelid margins, or meibomian gland orifice inclusions. Eyelash samples were obtained by epilation of four eyelashes (two eyelashes from each lower and upper lids), and the number of Demodex mites was counted with an optical microscope. We attempted to epilate as deeply as possible lashes with cylindrical danduff around the root of the lash. For tear sampling, a small amount of 0.9% (wt/vol) saline solution was dropped on the bulbar conjunctiva of both eyes, and then the participants blinked several times to spread the saline solution to the corner of the bulbar conjunctiva. Tear samples were collected from the corner of the bulbar conjunctiva using hematocrit-capillary tubes (Haematokrit-kapillaren, Eberstadt, Germany), and the presence of Demodex was determined using an optical microscope. The obtained eyelash and tear samples were transferred to 0.6 mL tubes and stored in a −80°C freezer until DNA extraction.

DNA Extraction and PCR Amplification for Barcoded Pyrosequencing
To extract total genomic DNA from eyelash and tear samples, 0.3 g of 0.1 mm zirconia/silica beads (Biospec, Bartlesville, OK) and 50 μL of 5% (wt/vol) Chelex-100 (BioRad, Hercules, CA) were added to the 0.6 mL tubes containing eyelash or tear samples, and the tubes then were vortexed vigorously for 2 minutes. After boiling for 10 minutes, the tubes were vortexed vigorously again for 2 minutes and centrifuged for 2 minutes at a maximum speed. The supernatants of the samples were used as templates for PCR amplification of bacterial 16S rRNA genes. For barcoded pyrosequencing, bacterial 16S rRNA genes containing hypervariable regions (V1–V3) were amplified using primer sets, Bac9F (5′-adapter BAC-GAG TTT GAT CMT GCC TCA G-3′) and Bac541R (5′-adapter A-XAC-WTT ACC GCG GCT GCT GAC-3′) where X denotes unique 7–11 barcode sequences inserted between the 454 Life Sciences adaptor A sequence and the common linker, AC (see Supplemental Table S1, http://www.iovs.orglookup/suppl/doi:10.1167/ iovs.12-9922/DCSupplemental). All PCR amplifications were done in a 50 μL C1000 thermal cycler (BioRad) containing 5 μL of template genomic DNA, 20 pmol of each primer, and a Taq polymerase mixture (Solgent, Daejeon, Korea), using a cycling regimen of 94°C for 5 minutes (1 cycle), 94°C for 45 seconds, 56°C for 45 seconds, 72°C for 1 minute (50 cycles), and 72°C for 10 minutes (1 cycle).

Pyrosequencing and Data Analysis
The PCR products were purified using a PCR purification kit (Solgent), and their concentrations were assayed carefully using an ELISA reader equipped with a Take3 multivolume plate (SynergyMx; BioTek, Winooski, VT). A composite DNA sample was prepared by pooling equal amounts of PCR products from each sample. Pyrosequencing of the composite DNA sample was performed on 1/8 plate two times by Macrogen (Seoul, Korea) using a 454 GS-FLX Titanium system (Roche, Branford, CT). Pyrosequencing data were processed and analyzed using the RDP pyrosequencing pipeline (available in the public domain at http://pyro.cme.msu.edu). The sequencing reads were assigned to specific samples based on their unique barcodes sequences, and then the barcodes were removed. The resulting sequencing reads were trimmed by removing beginning and ending bases with a quality score <20 (error rate 0.01), and only sequences >300 base pairs (bp) in length were chosen for further analyses using the Pipeline initial process. Unexpected or nonnatural reads were removed manually using the RDP classifier. Taxonomic assignments of the processed bacterial reads were performed using the RDP naive Bayesian RNA Classifier at an 80% confidence threshold. Operational taxonomic units (OTUs) and rarefaction curves were generated using the RDP pyrosequencing pipeline at a 3% dissimilarity level. The Shannon-Weaver and Chao1 biodiversity indices, and evenness were calculated by the RDP pyrosequencing pipeline. The bacterial community structures of eyelash and tear samples were compared using a UniFrac analysis based on the phylogenetic relationships of representative sequences derived from all reads of the individual samples. Briefly, the processed read sequences were clustered into OTUs using CD-HIT with an identity cutoff of 97%. The representative sequences from CD-HIT were aligned using NAST based on the greengenes database, with a minimum alignment length of 300 bp and a minimum identity of 75%. A phylogenetic tree was constructed using the PHYLIP software (ver. 3.6) with the Kimura two-parameter model and was used as an input file for the hierarchical clustering of bacterial communities in the weighted UniFrac analysis. To confirm the multiple community comparison from the UniFrac analysis, a principal coordinate analysis (PCoA) also was performed. The relative bacterial diversity was calculated by the RDP pyrosequencing pipeline to obtain the evenness among samples.

Table 1. Demographics of Blepharitis Patients and Healthy Controls for Eyelash and Tear Sampling

<table>
<thead>
<tr>
<th>Group</th>
<th>Subject No.</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Left</th>
<th>Right</th>
<th>Allergy†</th>
</tr>
</thead>
<tbody>
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<td>65</td>
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<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>66</td>
<td>Male</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>65</td>
<td>Male</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>66</td>
<td>Male</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>5</td>
<td>69</td>
<td>Male</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>64</td>
<td>Male</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>7</td>
<td>76</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Healthy controls (H)</td>
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<td>54</td>
<td>Male</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>2</td>
<td>76</td>
<td>Female</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25</td>
<td>Male</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27</td>
<td>Male</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

† Allergies were determined by symptoms (itching, feeling of irritation, or indisposition) and examination of eyes under a slit-lamp biomicroscope.
Acidobacteria
Proteobacteria
Rarefaction analysis of bacterial 16S rRNA gene sequences
Cyanobacteria
1. (0–2.61%),
Enhydrobacter
accounting for only 3.03% of
Proteobacteria
(0–0.45%), and
Actinobacteria
B (0–44.38%), or
(0.45%–99.67%),
Fusobacteria
Proteobacteria
Propioni-
Corynebacterium
TM7
(0–0.44%) also were found as minor groups.
Deinococcus
Firmicutes
Bacteroidetes
Actinobacteria
OP10
(0–0.25%)
unclassified by the RDP classifier and H3-T-R were not used in the analysis.
Matlab program (ver. 6.5; MathWorks, Inc., Natick, MA). Genera
communities and ocular sample type, shown in Figure 2B, were evaluated
samples. The correlations between relative abundance of microbial
(0.17%–84.23%),
which together accounted for 88.89% to 100% of
(0.06%–95.53%),
affiliated predominantly with five phyla:
Actinobacteria
(0.06%–95.53%),
Proteobacteria
(0.45%–99.67%),
Firmicutes
(0.17%–84.23%),
Cyanobacteria
(0–4.38%),
Bacteroidetes
(0–32.48%),
Thermus
(0–7.31%)
unclassified
(0.29%)
and
(13.42%)
(Table 1). Among a total of 44 subjects, seven tear samples did not produce sufficient 16S rRNA gene amplicons for pyrosequencing analysis. From the pyrosequencing of 37 successful PCR amplicons, a total of 96,151 sequencing reads was generated. After the removal of low quality or nonbacterial 16S rRNA sequencing reads, 79,085 high quality reads (82.25% of the total reads) with an average sequence length of approximately 486 bp and an average of >2157 reads for each sample were used for further analysis (Table 2). A rarefaction analysis using the culled 16S rRNA gene sequences was performed to assess the number of microbial communities in eyelash and tear samples were recovered from the pyrosequencing analysis (Fig. 3). Surprisingly, individual rarefaction curves of eyelash and tear samples demonstrated failures to approach asymptotes, which suggested that eyelash and tear samples had highly diverse bacterial communities, and that many unexploited OTUs still remained in the samples. The number of estimated OTUs in each subject by Chao1 richness estimator also was significantly higher than the number of observed OTUs (corresponding to 41.2%–90.7% of the estimated richness), indicating that more sequencing efforts may be required to obtain additional microbial community information. Although the number of OTUs estimated in a subject was a function of the number of pyrosequencing reads obtained, interestingly, Chao1 richness analysis demonstrated that the eyelash microbial communities of blepharitis subjects were relatively less diverse than those of healthy subjects, while the tear microbial communities of blepharitis subjects were relatively more diverse than those of healthy subjects (Table 2), which was supported more clearly by the rarefaction analysis (Fig. 3).

**Ocular Microbial Communities of Blepharitis and Healthy Subjects**
To compare the ocular bacterial taxa compositions of blepharitis and healthy subjects, the bacterial 16S rRNA sequencing reads of individual subjects were classified using the RDP naive Bayesian rRNA Classifier at both phylum and genus levels (Fig. 4). At a 80% confidence threshold in the RDP Classifier, the 16S rRNA gene sequencing reads of eyelash and tear samples were classified into 12 bacterial phyla, and most sequences were affiliated predominantly with five phyla: Actinobacteria (0.06%–95.53%), Proteobacteria (0.45%–99.67%), Firmicutes (0.17%–84.23%), Cyanobacteria (0–4.38%), or Bacteroidetes (0–32.48%), which together accounted for 88.89% to 100% of all sequencing reads (Fig. 4A). The relative abundances of the five prevalent phyla in each subject were significantly variable, depending on individual and sample type. Interestingly, some subjects were predominated by a single phylum. For example, subject B4-E-L was predominated by Proteobacteria, with 99.67% abundance of total sequencing reads, while subject B3-E-L was predominated by Actinobacteria, with 95.5% abundance, and Proteobacteria accounting for only 3.03% of the total sequencing reads in the same sample. Eyelash samples had higher variability than tear samples in terms of the relative abundances of the prevalent phyla in each sample, possibly because eyelids have less consistent conditions due to environmental exposures, as compared to the bulbar conjunctiva. The bacterial reads belonging to Fusobacteria (0–2.61%), Planctomycetes (0–0.61%), Acidobacteria (0–1.02%), OP10 (0–0.69%), TM7 (0–0.25%), Deinococcus-Thermus (0–0.45%), and Spirochaetes (0–0.44%) also were found as minor groups.

At the genus level, most 16S rRNA gene sequencing reads from eyelash and tear samples were categorized into 24 bacterial genera (Fig. 4A). Among these, five genera, Propionibacterium, Staphylococcus, Streptococcus, Corynebacterium, and Enhydrobacter, were identified as common ocular bacteria in most subjects; however, B4-E-L (0.29%) and B4-E-R (13.42%)

**RESULTS**

**Sampling and Sequencing Analysis of 16S rRNA Genes**
To analyze the ocular microbial communities of humans with and without blepharitis, 22 eyelash and 22 tear samples were collected from the left and right eyes of 11 participants (seven blepharitis patients and four healthy controls), respectively

**Nucleotide Sequence Accession Numbers**
The pyrosequencing data of the 16S rRNA genes are available publicly in the NCBI Short Read Archive (http://www.ncbi.nlm.nih.gov/sra/) under accession No. SRA050907.

**Figures**
Figure 1. Rarefaction analysis of bacterial 16S rRNA gene sequences from eyelash (A) and tear (B) samples of blepharitis patients and healthy controls. OTUs were calculated by the RDP pipeline with a 97% sequence similarity cut-off value. B, blepharitis subjects; H, healthy subjects; E, eyelash; T, tear; L, left eye; R, right eye.
Relative bacterial compositions of eyelash and tear samples from blepharitis patients and healthy controls. Partial 16S rRNA gene sequences were classified into phylum (A) and genus (B) levels using the RDP naive Bayesian rRNA Classifier based on the RDP 16S rRNA gene database at an 80% confidence threshold. Others in panel (B) are composed of the genera, each showing a percentage of reads <3.0% of the total reads in all of the subjects.
had very low overall abundances of the five major genera because these subjects contained high proportions of the previously unclassified genera. The relative abundances of the prevalent genera and unclassified bacterial phylotypes also varied significantly depending on individual and sample type. For example, subject B4-E-L was predominated by only a single genus, *Propionibacterium*, with 94.48% abundance of total reads, while trace sequencing reads belonging to *Staphylococcus* and *Corynebacterium* were detected from the same subject (<0.017%). Also, the genus *Propionibacterium* accounted for only 0.29% in subject B5-E-R, but *Staphylococcus* and *Corynebacterium* represented 52.24% and 45.87% of the total reads in the same subject, respectively.

### Statistical Comparisons of Ocular Microbial Communities of Blepharitis and Healthy Subjects

The bacterial compositions of eyelash and tear samples from humans with and without blepharitis were assessed statistically using a phylogeny-based metric, UniFrac based on representa-

tive sequences derived from all culled 16S rRNA gene sequences of the individual subjects. As shown in Figure 5, although there were some exceptions, intrapersonal bacterial communities of eyelash subjects were relatively well clustered compared to the interpersonal bacterial communities, which was consistent with previous results that interpersonal variability was high, whereas individuals exhibited less internal variability.22 Bacterial communities of tear samples from blepharitis subjects were well clustered from those of eyelash samples or those of healthy tear subjects. PCoA also demonstrated that tear samples from blepharitis subjects were distinguished clearly from those of other subjects, which suggested that some genera or microbiota representing human blepharitis may be present in the conjunctiva in these patients.

However, eyelash samples from subjects with blepharitis were not clearly statistically different (Fig. 6), which might be explained by the fact that microbial communities of eyelashes can be influenced easily by external factors.

### Table 2. Summary of the Pyrosequencing and Statistical Data of Bacterial Communities of Eyelash and Tear Samples from Blepharitis Patients and Healthy Controls

<table>
<thead>
<tr>
<th>Subject</th>
<th>No. of Reads</th>
<th>No. of High Quality Reads</th>
<th>Average Read Length (bp)</th>
<th>OTUs†</th>
<th>Shannon-Weaver Index (H')†</th>
<th>Chao1†</th>
<th>Evenness (E)†</th>
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<td>B1-E-L</td>
<td>4784</td>
<td>4488</td>
<td>482</td>
<td>102</td>
<td>2.47</td>
<td>168.23</td>
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<td>B1-E-R</td>
<td>2109</td>
<td>2010</td>
<td>477</td>
<td>49</td>
<td>1.86</td>
<td>70.11</td>
<td>0.48</td>
</tr>
<tr>
<td>B1-T-L</td>
<td>2161</td>
<td>1706</td>
<td>484</td>
<td>127</td>
<td>3.11</td>
<td>183.89</td>
<td>0.64</td>
</tr>
<tr>
<td>B1-T-R</td>
<td>2785</td>
<td>2454</td>
<td>487</td>
<td>140</td>
<td>5.07</td>
<td>179.06</td>
<td>0.62</td>
</tr>
<tr>
<td>B2-E-L</td>
<td>7147</td>
<td>6532</td>
<td>485</td>
<td>74</td>
<td>1.78</td>
<td>101.00</td>
<td>0.41</td>
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<tr>
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<tr>
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OTUs were calculated by the RDP pipeline with a 97% OTU cutoff of the 16S rRNA gene sequences.

* B, blepharitis subjects; H, healthy subjects; E, eyelash; T, tear; L, left eye; R, right eye.

† Diversity indices of the microbial communities were calculated using the RDP pyrosequencing pipeline based on the 16S rRNA gene sequences.

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personal variability to Pseudomonas than healthy subjects (Fig. 1B). The RDA results also were identified as one of the major populations was excluded Corynebacterium, and Chryseobacterium Acinetobacter, and Enhydrobacter and Propionibacterium in tear samples from patients with blepharitis were clearly higher than those in tear samples from healthy controls. Surprisingly, the relative proportions of Cyanobacteria, whose source may be plant material, such as pollen, in eyelash and tear samples from blepharitis patients were clearly higher than those in tear samples from healthy controls. Interestingly, tear samples of healthy controls had a significantly higher proportion of Bacteroidetes than did other tear and eyelash samples, which suggests that Bacteroidetes might be important as a resident commensal microbiota and may contribute to the prevention of blepharitis. The genus level analysis demonstrated that Propionibacterium, Staphylococcus, Streptophyta, Corynebacterium, and Enhydrobacter were common ocular bacteria in all eyelash and tear samples, regardless of the presence of blepharitis (Fig. 1B); these results differed slightly from prior results that Pseudomonas, Bradyrhizobium, Propionibacterium, Acinetobacter, Corynebacterium, and Staphylococcus were dominant in healthy human conjunctiva. This discrepancy may be caused by differences of individuals, sampling methods, and sample types.

Subjects with blepharitis had lower proportions of Propionibacterium than those of healthy subject, whereas the relative proportions of Streptophyta, Corynebacterium, and Enhydrobacter in eyelash and tear samples from blepharitis patients were higher than those of healthy controls. The relative proportions of Staphylococcus in eyelash samples from blepharitis patients and healthy controls were similar, while the relative proportions of Staphylococcus in tear samples from blepharitis patients clearly were higher than those in tear samples from healthy controls. Figure 1B shows that Chryseobacterium was identified as one of the major populations from tear samples of healthy controls (6.5%). However, because its high proportion in healthy tear samples was found in only one tear sample (H5-T-R; Fig. 4B), the high proportion of Chryseobacterium was not considered to be normal flora of the ocular microbiome, and Chryseobacterium was excluded from the following discussion. The correlation between sample type and microbial community was confirmed by redundancy analysis (Fig. 2). The distributions of subject type and microbial community in the ordination space, as determined by RDA, clearly highlighted that subjects with blepharitis had more abundant Streptophyta, Corynebacterium, and Enhydrobacter than healthy subjects (Fig. 1B). The RDA results also highlighted the uniqueness of the tear samples, mainly due to the abundance of the genus Propionibacterium (Fig. 2).

**DISCUSSION**

An understanding of the ocular microbial community is essential for the prevention and treatment of blepharitis, as the ocular microbiota contributes to infection and prevention of
Many researchers have analyzed ocular microbial communities, and members of the genera *Propionibacterium*, *Staphylococcus*, *Acinetobacter*, and *Corynebacterium* have been identified as major microbiota from eye conjunctivas or lids with blepharitis. Comparative community analysis using culture-based approaches found that the ocular microbial communities of patients with and without blepharitis could differ in terms of their relative abundance proportions, which suggested that the differences in ocular microbial communities, especially in the relative abundance of *Propionibacterium* and *Staphylococcus*, might contribute to the occurrence of blepharitis. However, culture-based approaches have many limitations in terms of the culturability of microorganisms. Recently, a culture-independent approach based on pyrosequencing demonstrated that *Pseudomonas*, *Propionibacterium*, *Bradyrhizobium*, *Corynebacterium*, *Acinetobacter*, *Brevundimonas*, and *Staphylococcus*, as well as many other previously undescribed bacteria, were identified from healthy human conjunctiva. Therefore, in our current study, we applied a massively parallel pyrosequencing strategy to compare the ocular microbial communities of humans with and without blepharitis, which potentially will be very helpful in understanding the occurrence and treatment of human blepharitis on the basis of ocular microbial flora. Groden et al. demonstrated that members of *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Acinetobacter* were identified as the most common isolates from all lids, but that normal skin bacteria, such as *Staphylococcus* and *Propionibacterium*, were isolated in greater quantities from lids with blepharitis, which potentially will be very helpful in understanding the occurrence and treatment of human blepharitis on the basis of ocular microbial flora. Groden et al. demonstrated that members of *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, and *Acinetobacter* were identified as the most common isolates from all lids, but that normal skin bacteria, such as *Staphylococcus* and *Propionibacterium*, were isolated in greater quantities from lids with blepharitis. These previous reports suggest that skin microbial flora can be a cause of human blepharitis, which supports previous results that found that elevated levels of skin microbial flora, such as *Staphylococcus*, in the eye can be a cause of human blepharitis. The relative proportions of *Staphylococcus*, *Streptophyta*, *Corynebacterium*, and *Enhydrobacter* were higher in subjects with blepharitis than in healthy subjects, especially in tear samples; however, surprisingly, the proportions of *Propionibacterium* clearly were lower in subjects with blepharitis than in healthy subjects (Fig. 1B), which suggests that *Propionibacterium* might be important as a resident commensal microbiota for the prevention of blepharitis. The relative proportion of *Staphylococcus* was clearly higher especially in tear samples from subjects with blepharitis than in healthy tear samples, which supports previous results that found that elevated levels of skin microbial flora, such as *Staphylococcus*, in the eye can be a cause of human blepharitis.

**Figure 5.** Relative bacterial mean abundances in eyelash and tear samples from blepharitis patients and healthy controls at phylum (A) and genus (B) levels. The relative bacterial mean abundances were calculated by the mean values of relative phylotypic compositions of respective eyelash and tear samples. Others in panel (B) are composed of the genera each showing a percentage of reads <3.0% of the total reads in all subjects.

**Figure 6.** Ordination biplot of RDA showing correlations between subject types and microbial communities of Figure 5B. Subject types are represented by circles and rectangles (closed or open), and genera are represented by inverted triangles. Genera unclassified by the RDP classifier were not used in the analysis. Arrow: directions point toward maximal abundance, and their lengths are proportional to the maximal rate of change between subject types.
phytotypes and statistical redundancy analyses demonstrated clearly that the relative abundances of Streptophyta, Corynebacterium, and Enhydrobacterium were higher in tear samples from subjects with blepharitis than in healthy tear subjects (Figs. 1, 2), which is supported by a previous report that Corynebacterium elicited human blepharitis by immunoreactivity. These results suggested that human blepharitis might be induced by infections of mixed skin microbial flora, as well as plant pollens, dusts, and soil particles, because pollens, dusts, and soil are the main sources of the genera Streptophyta, Corynebacterium, and Enhydrobacterium. In previous studies, Pseudomonas aerguina was cultured from blepharitis subjects and Pseudomonas represented one of the major genera in healthy conjunctiva; however, our analysis showed that Pseudomonas was detected in minor abundance in all subjects, regardless of the occurrence of blepharitis (Figs. 4B, 1B).

Demodex mites are the most common permanent ectoparasites in human skin. They are easily found, especially from infundibular portions of pilose follicles of the eyelash, small hair sebaceous glands, meibomian glands, face, and external otic tract, where active sebum excretion provides a favorable habitat for breeding. Some prior studies have reported that Demodex or Demodex-related Bacillus might contribute to the occurrence of blepharitis. However, in our analysis, the incidence of Demodex mites did not demonstrate a clear correlation with ocular microbial community, although sample sizes were too small to allow for a statistical comparison. Despite the finding that Demodex mites are found more frequently in blepharitis patients, there is controversy as to whether Demodex is a cause of blepharitis, since blepharitis symptoms often are found in humans not associated with blepharitis. Therefore, the frequent discovery of Demodex mites from blepharitis patients may not reflect the cause of blepharitis occurrence, but rather a result of blepharitis because active sebum excretion caused by blepharitis can provide favorable conditions for Demodex mites. Although Demodex mites may worsen blepharitis symptoms, additional investigations are required to clarify these hypotheses. In our current study, we compared ocular microbial communities of humans with and without blepharitis using pyrosequencing and suggested that some ocular microbiota can contribute to infection and prevention of human blepharitis. Our analysis demonstrated that many bacteria known as ocular surface pathogens were identified with high abundance in ocular samples, regardless of the occurrence of blepharitis, as reported previously, but that their compositions were different depending on the occurrence of blepharitis. These results suggested that the balance or the commensal growth between ocular microbiota might be important for the prevention of blepharitis, because ocular health and blepharitis may depend on the interplay between the eye and the ocular microbial community. However, further studies at species or strain levels will be required to test the validity of this hypothesis. Continued investigations of ocular microbial communities are required to add valuable information for the prevention and treatment of human blepharitis, because the roles of the ocular microbial community in humans with and without blepharitis are unknown.

References


