

Identification and visualization of a distinct microbiome in ocular surface conjunctival tissue

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INTRODUCTION

Numerous regions of the body contain infolded regions which act as unique habitats for microbial communities [1,2]. Knowledge of whether microorganisms are able to reside in protected niches of the conjunctiva is potentially significant in terms of minimising risks of contact lens inflammation/infection and endophthalmitis.

AIM

In this study, we aimed to define if and how microbial communities of limbal and forniceal crypts differ from those on the conjunctival surface.

METHOD

- Human limbal and fornix conjunctival tissue was obtained from 23 patients undergoing pterygium surgery and surface swabs of conjunctival surface were obtained from 45 individuals.
- Negative controls consisted of sample controls (section of sterile filter paper without exposure to tissue samples) and blank extractions.
- Microbial communities were analysed by extracting total DNA from tissue samples and surface swabs and sequencing the 16S rRNA gene using the Illumina MiSeq platform.
- Sequences were quality filtered, clustered into operational taxonomic units (OTUs) at 97% similarity using USEARCH and then taxonomically classified using SILVA database.
- Fluorescent *in situ* hybridization (FISH) was performed on cryosections (7 μ m) of limbal and conjunctival forniceal tissue
 - Universal bacterial probe EUB338 [3]
 - Pseudomonas* genus specific probe PSE1284 [4]
- Slides were examined with a Nikon A1 Spectral confocal microscope

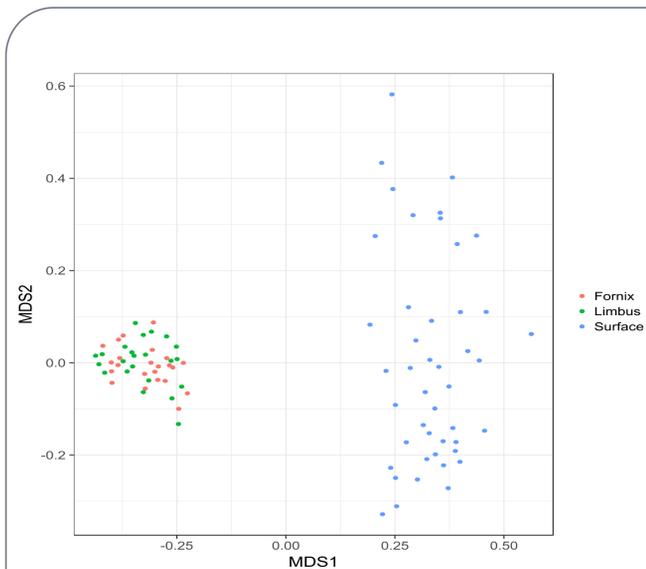


Figure 1: Non-metric multidimensional scaling ordination of the limbus & fornix tissue microbiome compared to surface conjunctiva microbiome using Bray-Curtis dissimilarity of transformed OTU data (stress 0.17)

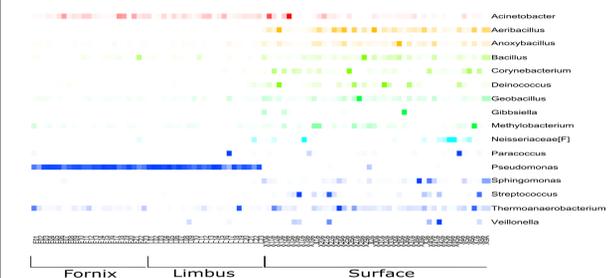


Figure 4: Relative abundance (>1%) at the genera-level for the microbial communities of the fornix, limbus, and conjunctival surface.

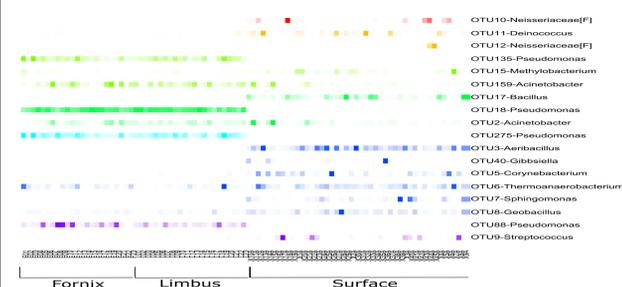


Figure 5. Relative abundance (>1%) at the OTU-level for the microbial communities of the fornix, limbus, and conjunctival surface.

RESULTS

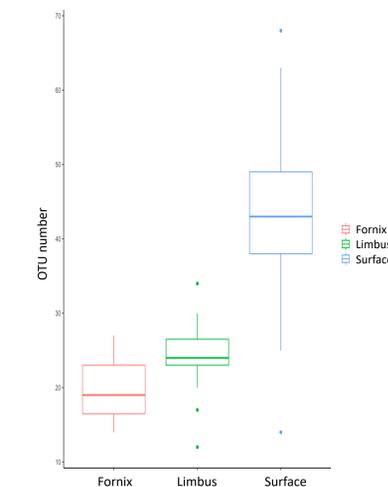


Figure 2: Microbial richness at the OTU level across sampled regions - fornix, limbus and ocular surface (P < 0.001)

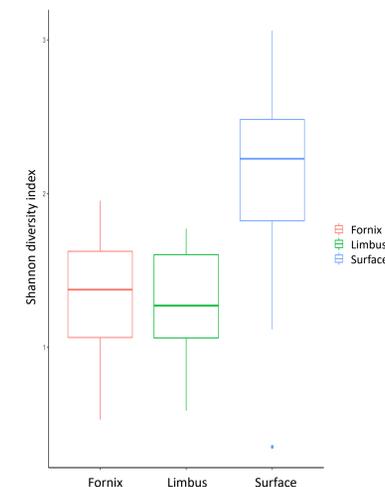


Figure 3: Shannon diversity index at the OTU level across sampled regions - fornix, limbus and ocular surface (P < 0.001)

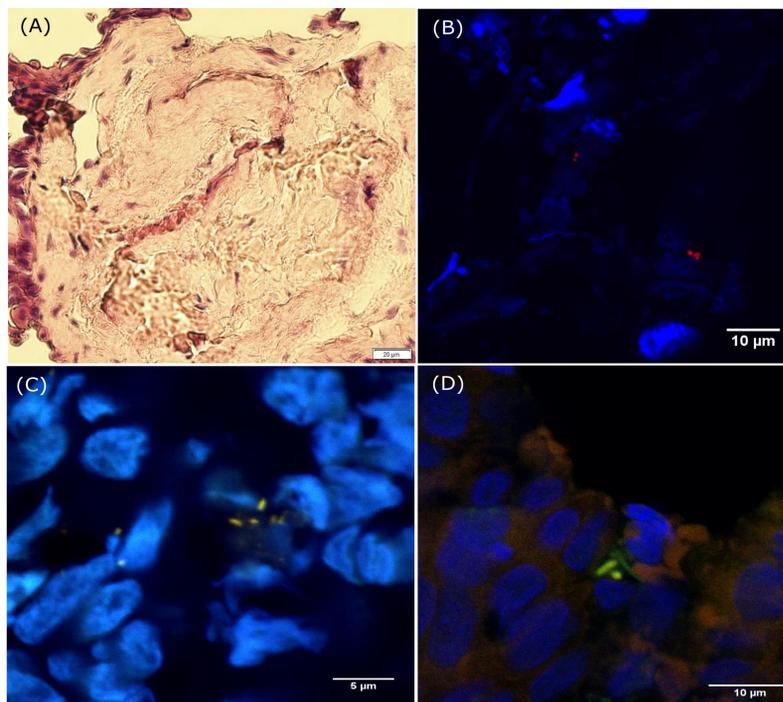


Figure 6: (A) Hematoxylin-eosin staining of a representative fornix tissue section; Fluorescence micrographs of section of conjunctival forniceal tissue with fluorescence signal of (B) universal bacterial probe EUB338 (red signal [Cy3]; FISH) and, (C - D) *Pseudomonas* specific oligonucleotide probe PSE1284 (green signal [6-Pham]; FISH) within tissue; DAPI stained eukaryotic cellular DNA (blue signal).

STATISTICAL ANALYSIS

- Indicspecies* R package was used to identify OTU's associated with sample and blank extraction negative controls. These OTUs were removed prior to analysis
- Vegan* R package was used for microbial alpha- and beta-diversities.

RESULTS

- There was a significant difference in bacterial community structure between the conjunctival surface and the fornix (P=0.001) and the limbus (P=0.001) tissue. No difference was found in bacterial community structure between the limbus and fornix (P=0.764, Fig. 1).
- Pairwise comparison showed a significant variation in richness and Shannon diversity index between the limbus-surface and fornix-surface comparisons (both P < 0.001, Fig. 2 & 3).
- Fornix and limbal samples were dominated by OTUs classified to the genus *Pseudomonas* (relative abundance 79.9%), which were only found in low relative abundances on conjunctival surfaces (6.3%) (Fig. 4 & 5)
- Although rare, as indicated by sparse signals from the universal bacterial probe (Fig. 6B), rod shaped bacteria of 1 μ m length and 0.5 μ m width hybridized to the *Pseudomonas* probe and were embedded in fornix tissue (Fig. 6C-D).

CONCLUSION

- The results showed the presence of a discrete tissue-associated microbiomes in freshly collected human limbal and fornix tissue.
- There was a clear distinction between the microbial community structure and composition of the conjunctival tissue to the ocular surface microbiome previously analysed.

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