

Heterogeneity of Vaginal Bacterial Communities within Individuals: Impact of Anatomical Site and Sampling Method on Microbial Detection

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Abstract

Recent bacterial 16S rDNA sequence-based studies have revealed that the healthy human vaginal ecosystem has greater bacterial diversity than previously known. However, variation in detection of bacteria from different vaginal locations and by different sampling methods has not been investigated. In this study, we analyzed the bacterial content of samples from three vaginal locations and by three sample collection methods. 16S rDNA libraries were constructed from vaginal samples collected from eight clinically healthy women. The results showed, for the first time, that the vaginal microbiota is not homogeneous, but differs significantly with regard to location and sampling method. These results corroborate the complexity of vaginal microbial communities and suggest an important role for sampling technique for the prediction of vaginal health.

Materials and Methods

Eight premenopausal healthy women (mean age = 26.5 yr) were recruited for this study. For molecular analysis, a total of six vaginal samples were obtained from each subject. Three swab samples were collected from the cervix, fornix and lower region of the vaginal canal. An ectocervical lavage sample was also collected. Then, two vaginal scrapings were obtained from the upper and lower region of the vaginal canal using sterile vaginal spatulas. Genomic DNA was extracted from the samples and 16S rDNA was amplified using PCR with species-specific 27f forward primers and the 1492r universal reverse primer. The 16S rDNA were cloned and sequenced. The sequences were then grouped into representative type strains for analysis. Statistical analysis was performed using diverse statistical programs to analyze bacterial communities.

Results

Overall structure of vaginal bacterial community

The 16S rDNA libraries generated 5,340 well-characterized bacterial 16S rDNA sequences using the SEQMATCH program in the Ribosomal Database Project (RDP) II. The characterized sequences were grouped into 67 representative type strains. The 67

type strains showed that the vaginal bacteria were comprised of five phyla: *Proteobacteria*, *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, and *Actinobacteria*.

Variation of bacterial composition between subjects

Bacterial composition for each subject was calculated and displayed using histograms at the phylum level (Figure 1). A dendrogram comparing eight subjects were also generated using Bray-Curtis distances of subjects.

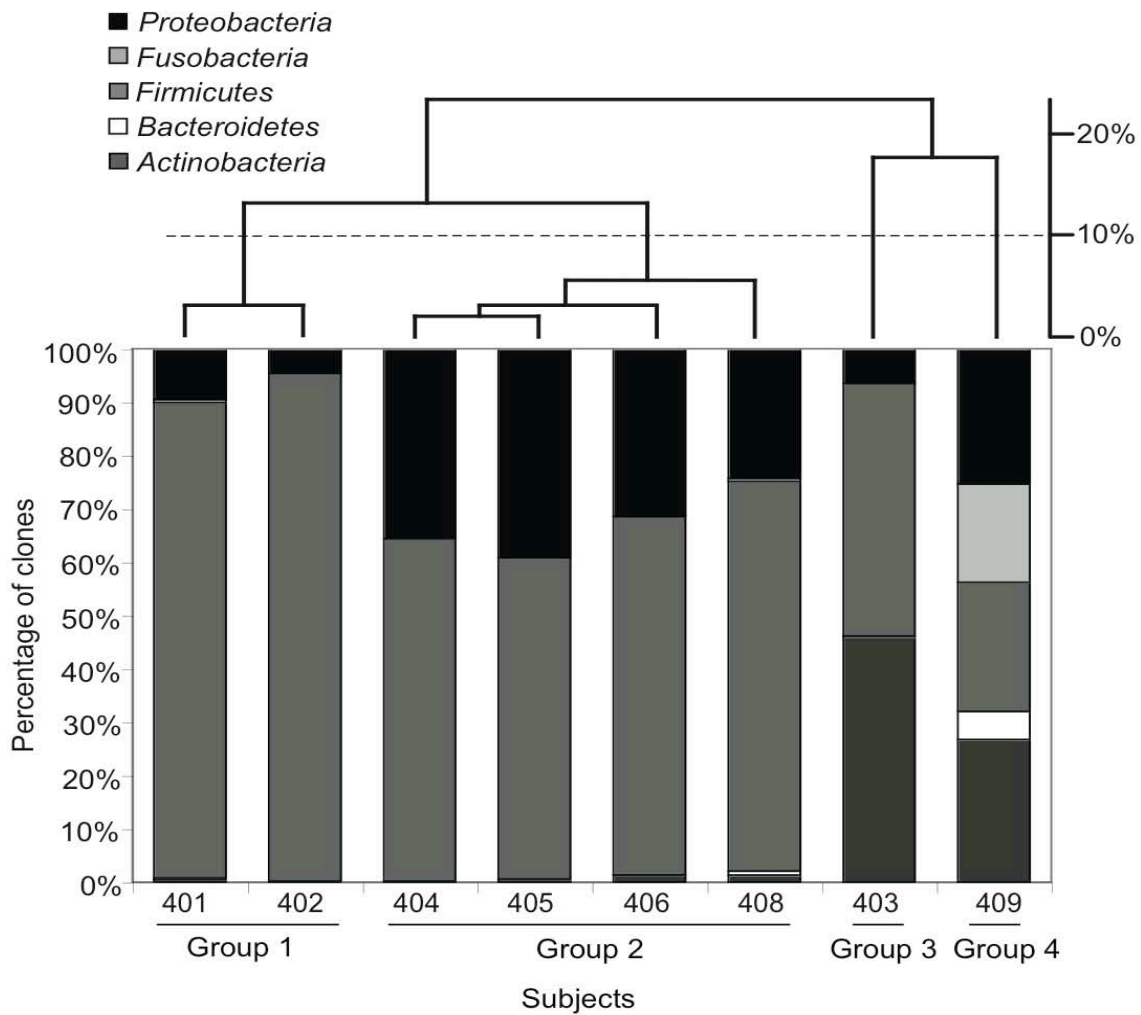


Figure 1. Grouping of subjects by bacterial composition at the phylum level. The histogram (lower portion) shows the phylum-level bacterial composition of each subject and the dendrogram (upper portion) displays the clustering of the eight subjects. Four groups were identified using a cut-off distance value of 20%.

Variation of bacterial composition by sampling site within subjects

Heterogeneity of bacterial content within subjects was shown by illustrating bacterial composition by location and sampling method (Figure 2). Differences among sampling location and method were greater in subjects with more diverse bacterial compositions. Lavage samples harbored the least bacterial diversity compared to the other locations and also contained no *Proteobacteria* representatives in any subject.

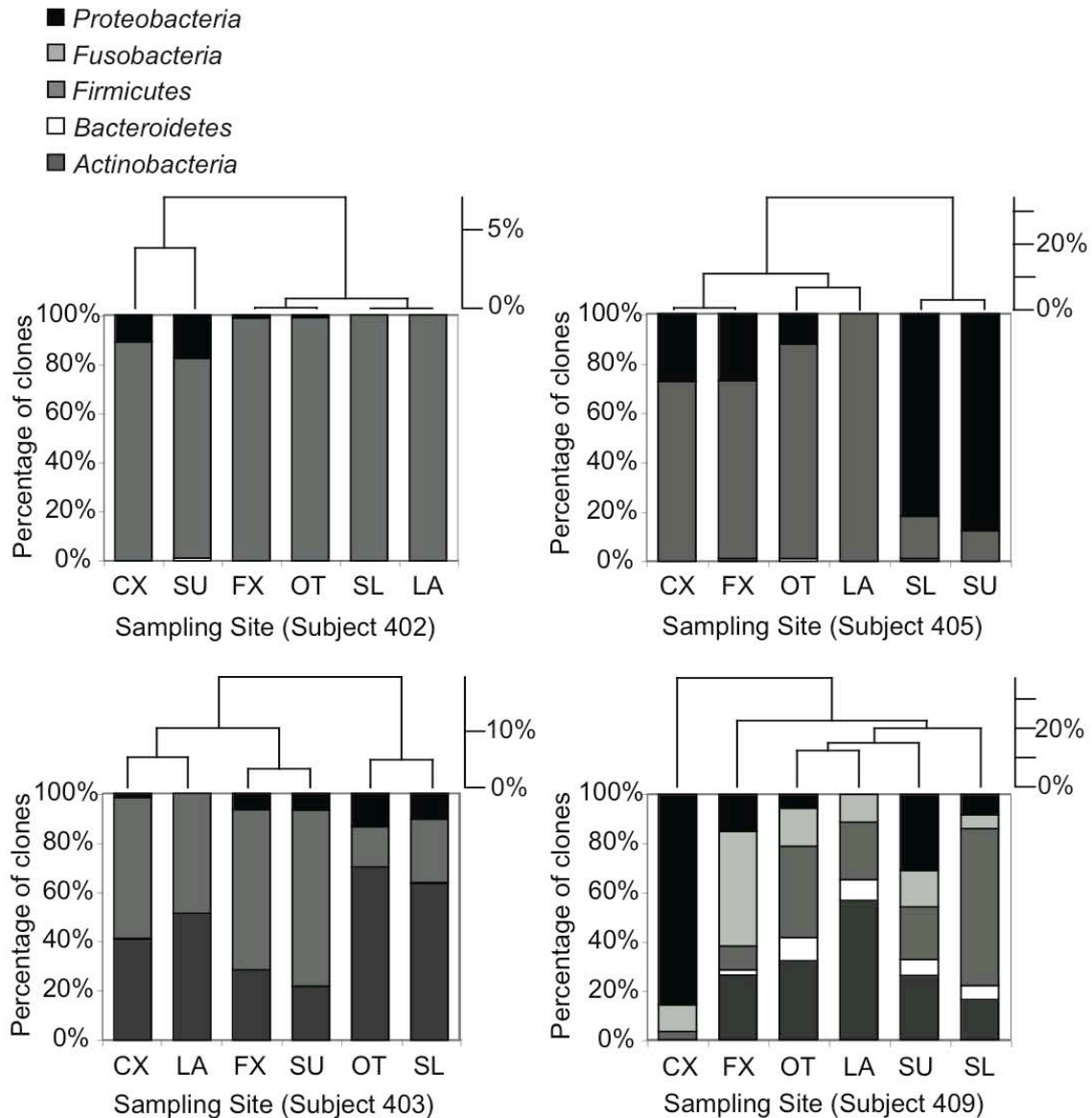


Figure 2. Clustering of vaginal samples within individuals based on microbial composition. Clustering of the samples from different sampling sites within each subject based on bacterial composition is shown by the corresponding dendrograms.