Metagenomic analysis of the human vaginal microbiome with a vaginal self-collection swab & Microbiome Vaginal Kit

Introduction

The **vaginal microbiome** is a specific compartment of the human microbiome. Unique conditions of the vagina are characterized by a few microbial species, usually lactobacilli.

The cervicovaginal ecosystem is made up of diverse microorganisms coexisting in a dynamic balance and establishing complex connections with each other and with the host. In healthy reproductive-aged women, the vaginal microbiome, generally, shows a predominance of *Lactobacillus* genus, and most women display the prevalence of one species among *L. crispatus*, *L. iners*, *L. jensenii* and *L. gasseri*. Lactobacilli promote the maintenance of the vaginal homeostasis and prevent the colonization and growth of adverse microorganisms, including those responsible for sexually transmitted infections (STI). The composition of the vaginal microbiome depends on age, menstruations, hormonal fluctuations, sexual behaviors, and also the use of drugs such as probiotics and antibiotics causing its imbalance.

Material and Methods

Sample Collection

Vaginal samples were taken using our **Vaginal Self-Collection Swab** (Danagen) a new solution for home self-sampling, collection, shipping and easy processing in the laboratory from 4 women, they were not pregnant, of reproductive age, ranging from 20 to 45 years and regularly menstruating.

Whole-Genomic DNA Extraction from Vaginal Swabs

Swab samples were stored for 1 week to room temperature, then preserved vaginal samples were processed following the **DANAGENE MICROBIOME Vaginal DNA kit** protocol (Danagen).

Targeted Library Preparations, Sequencing and Bioinformatics Analysis

The extracted DNA was quantified using Quant-IT PicoGreen (Invitrogen). The sequencing libraries

Are prepared according to the Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 region. The input gDNA 2ng was PCR amplified with 5x reaction buffer, 1mM of dNTP mix, 500nM each of the universal F/R PCR primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The cycle condition for 1st PCR was 3 min at 95°C for heat activation, and 25 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, followed by a 5-min final extension at 72°C. The universal primer pair with Illumina adapter overhang sequences used for the first amplifications were as follows:

V3-F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3'
V4-R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3'

The 1st PCR product was purified with AMPure beads (Agencourt Bioscience, Beverly, MA). Following purification, the 2ul of 1st PCR product was PCR amplified for final library construction containing the index using NexteraXT Indexed Primer. The cycle condition for 2nd PCR was same as the 1st PCR condition except for 10 cycles. The PCR product was purified with AMPure beads. The final purified product is then quantified using qPCR according to the qPCR Quantification Protocol Guide(KAPA Library Quantificatoin kits for IlluminaSequecing platforms) and qualified using the TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). The paired-end (2×300 bp) sequencing was performed by the Macrogen using the MiSeq™ platform (Illumina, San Diego, USA).

Sequencing reads were analysed with the cloud metagenomics software GAIA (Paytuví *et al.*, 2019)

(https://metagenomics.sequentiabiotech.com) to obtain OTU tables at different taxonomic levels with their corresponding Shannon alpha-diversity and Bray-Curtis beta-diversity values.

Results

Taxonomic composition

The analysis was performed on four samples, two of them showed profile extremely rich in *Lactobacilli* while the other two samples showed a profile that could be classified as a Community State Type (CST) IV, rich in anaerobic bacteria with a very low abundance of the *Lactobacillus* genus (figure 1). Results show that the taxonomic composition observed in the samples is consistent with the current knowledge about the expected genera and abundance in the vaginal microbiome.

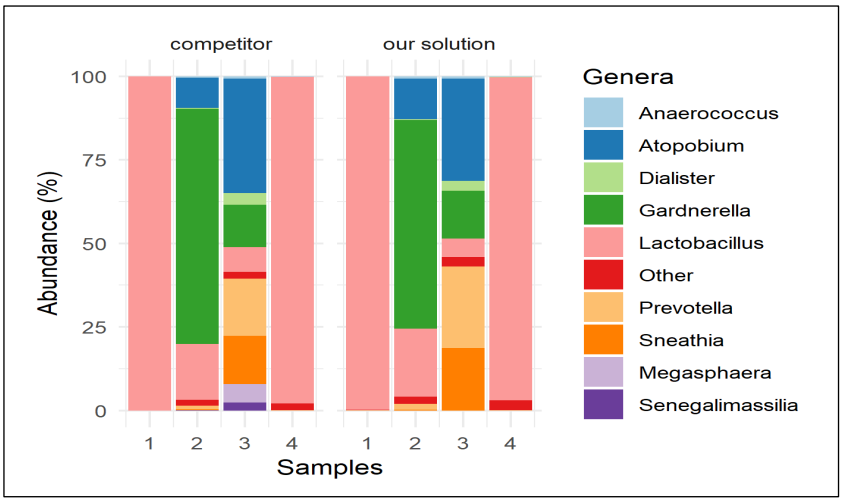

Figure 1. Barplot showing the taxonomic composition, excluding unclassified reads, of our analysis and the analysis performed on the same samples by a competitor.

Competitors

The same samples were sent to our competitor in order to compare the taxonomic composition of the samples. The taxonomic profile was visually very similar to the one observed with our solution. In terms of Bray-Curtis dissimilarities, the average value between samples was 0.064, suggesting that the taxonomic profile is very similar, thus confirming that our solution is suitable for the analysis of the vaginal microbiome.

Conclusion

When building a house, any good metagenomic analysis is founded in a proper starting material, efficient and reproducible DNA isolation method and bioinformatic analysis to give you the maximum information on your sample.

In this study it has been demonstrated that our system for sampling, DNA isolation and bioinformatic analysis of the vaginal microbiome can be used for an efficient characterization of the vaginal microbiome of asymptomatic and sexually active women.

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