High-throughput generation of pure microbial cultures using image-based single-cell isolation: Proof of concept on vaginal swabs

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Context and objectives:

Vaginal microbiome plays significant roles in women's health, modulating their fertility, their immunity or their susceptibility to some cancer types. However, while meta-omics allowed the elaboration of a plethora of microbial ecology theories, culture-based approaches are rising back with the need to test these theories and elucidate underlying mechanisms. Classical methods for isolate library generation are tedious and time-consuming, therefore increase in throughput is now needed.

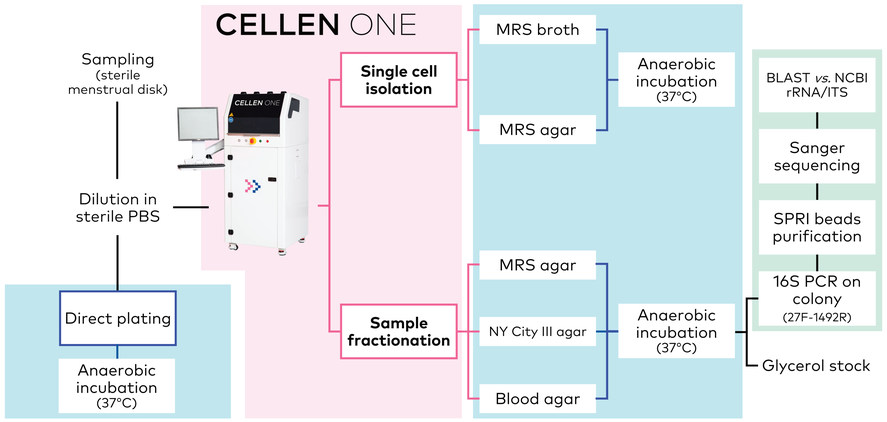
The objectives of this study were:

To evaluate the ability of a single cell isolation instrument, the cellenONE, to detect and isolate bacterial cells from vaginal microbiota with low-effort sample preparation.

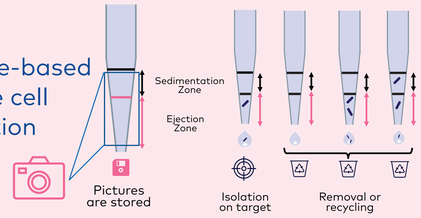
To test the suitability of single bacteria isolation for high-throughput generation of diverse culture libraries.

Materials and methods

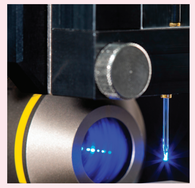
After collection, vaginal fluid was diluted in sterile PBS before cellenONE experiments, which are summarized in the workflow diagram below. Direct plating was systematically conducted in parallel for comparison of cultivability. For isolate identification, 66 colonies belonging to 8 different colony morphotypes were identified by 16S sequencing.



The cellenONE Technology



1. Cell suspension is loaded in a glass capillary
2. Capillary tip moves to the optical system and is automatically mapped into "Ejection zone" = what will be in the next generated droplet, and "Sedimentation zone" = safety zone considering possible sedimentation
3. Cell is isolated if, and only if, it is single, and a picture is recorded



Sample fractionation:

1. Image-based determination of cell concentration with cellenONE
2. Concentration adjustment for optimal probability of getting 1, and only 1, CFU per droplet
3. Ultra-high throughput and highly reproducible (CV<0.2%) generation of picodroplets (150-600 pl)

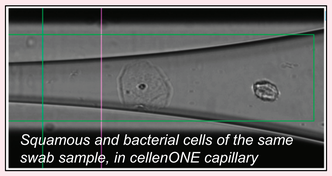
Sample preparation and cell detection

Very good detection... of both host and microbial cells!

- The cellenONE software detects and can differentially isolate host and microbiota cells, based on size parameter settings, when equipped with new microLIFE add-on, dedicated to <5-µm particles.

Recorded images allow monoclonality QC

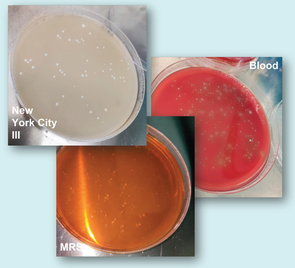
- The microLIFE software allows verification of monoclonality for each cell dispensed, and in the case of non-monoclonal events, to remove them from further analyses



Isolate generation throughput

Isolated cells remain highly cultivable after cellenONE isolation

- 14.7% outgrowth in average after single cell isolation (MRS agar, cultivation in anaerobic conditions), not significantly different in liquid and agar media, and not significantly different from direct plating cultivability.



Colonies on agar media inoculated with cellenONE (8 colony morphotypes for that sample)

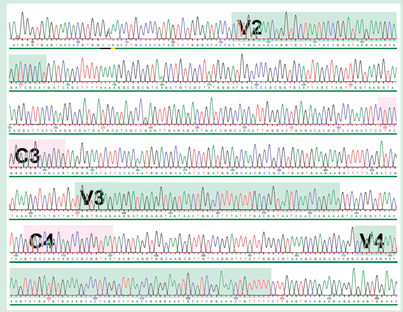
Hundreds of isolates generated in minutes

- <10 min for isolating 100 single cells.
- In sample fractionation mode, dispense of 2,048 droplets (0.18 cells /droplet) for each medium in <28 min total, generating 679 colonie

Isolate Identification

High purity of isolates indicated by ultra-clean electropherogram without cloning step

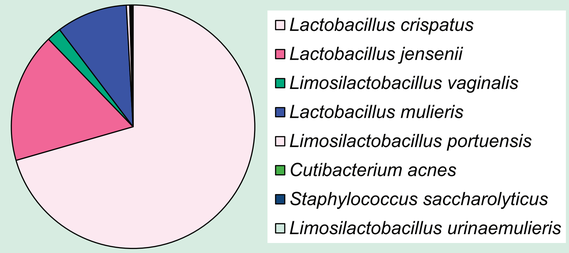
- >95% of isolate 16S sequences have less than 0.5% unspecified bases (N) in 1 kbp sequences, incl. in hypervariable regions



Representative example: Electropherogram of isolate #1.3.9 16S PCR product: 100% identity with L. crispatus (strain DSM 20584), 0 unspecified base (N).

Typical diversity for a unique healthy individual

- All pure isolates were identified as typical vaginal bacteria with >99.2% 16S identity, and community was largely dominated by *Lactobacillaceae*.
- Cultivable richness compares very well with literature for 1 unique sample.



Taxonomic classification of closest NCBI referenced representatives based on 16S rRNA gene amplicon sequence BLAST for 66 isolates.

Conclusions and perspectives

- The cellenONE instrument accurately detected both microbial and host cells, in the same sample, from vaginal swab with low sample preparation effort.
- cellenONE had no impact on the ability of isolated cells to form growing cultures, in both liquid and solid media, and generated diverse isolate libraries in a few minutes per sample.
- Direct single-cell isolation in liquid cultures enables direct storage or further experiments, without streaking steps, thanks to monoclonality QC on recorded images.

The cellenONE single cell isolation instrument increases the throughput of culture generation from host microbiota samples, opening unprecedented opportunities in microbiome research.