Screening of filamentous fungi using droplet-based microfluidics

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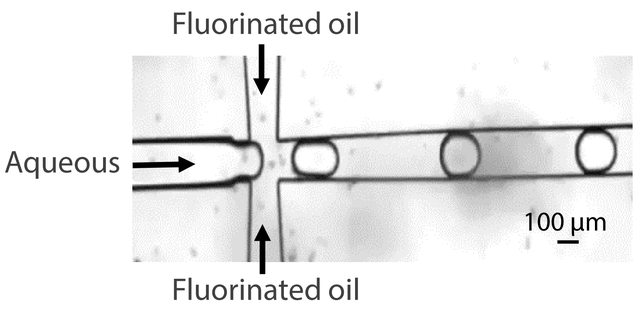
Introduction

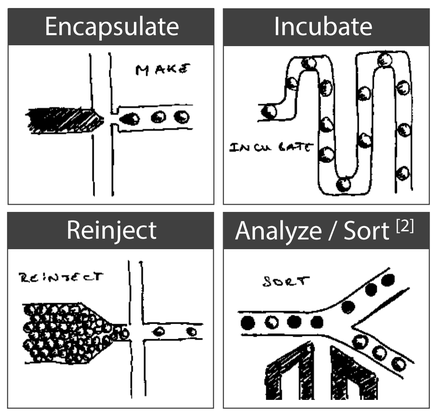
Filamentous fungi are widely used in the fermentation industry because of their natural ability to secrete large amounts of hydrolytic enzymes involved in the degradation of biomass. Directed evolution or metabolic engineering strategies are used to discover industrial relevant fungi having improved secretion yields. However, such strategies are limited due to limited throughput and high cost and space footprints of high throughput screening (HTS) technologies.

We developed a droplet-based microfluidics platform for the HTS of filamentous fungi for secreted enzymatic activities. We used this tool to screen libraries of *Aspergillus niger* mutants for α-amylase production. It allowed the fast enrichment of libraries in active fungi associated with significantly reduced cost, time and space footprints.

1. Droplet-based microfluidics

Droplet-based microfluidics consists in controlling aqueous droplets dispersed in an oil phase at the single droplet level and very high frequencies[1].



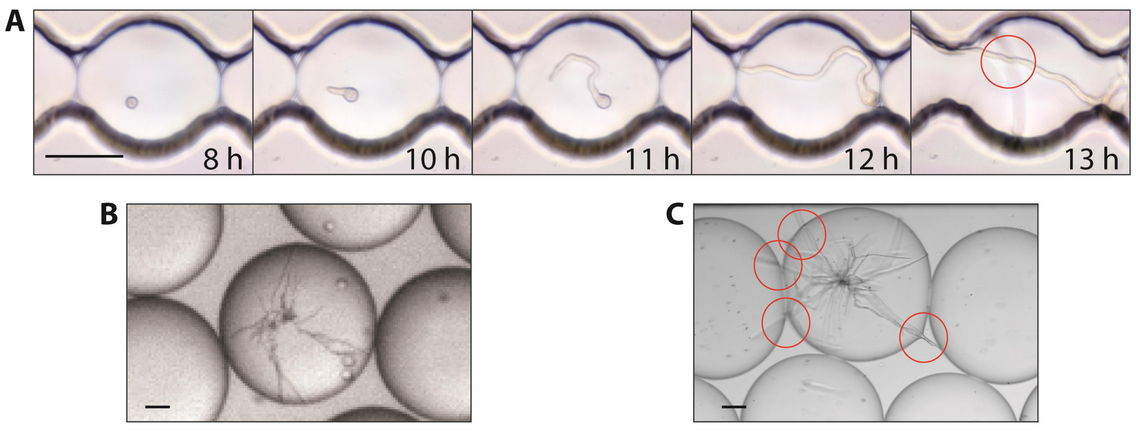


Each droplet is used as an independent microreactor to perform chemical or biological assays. This technology can be used for the analysis of enzymatic activities produced by microorganisms and the selection of the most interesting ones[3, 4].

Droplet-based microfluidics HTS		Robotized microplate HTS	
Volume	Throughput	Volume	Throughput
pL to nL	10 to 1000 Hz	µL to mL	up to 1 Hz

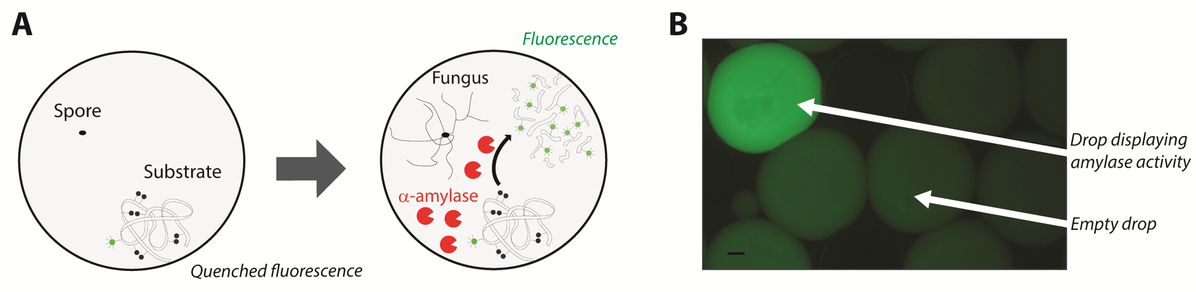
2. Growth of *A. niger* in droplets

Nanolitre droplets have to be used to cultivate filamentous fungi as hyphal tips rapidly exit picoliter droplets after germination. *A. niger* can be grown in 18 nL droplets for at least 24h.



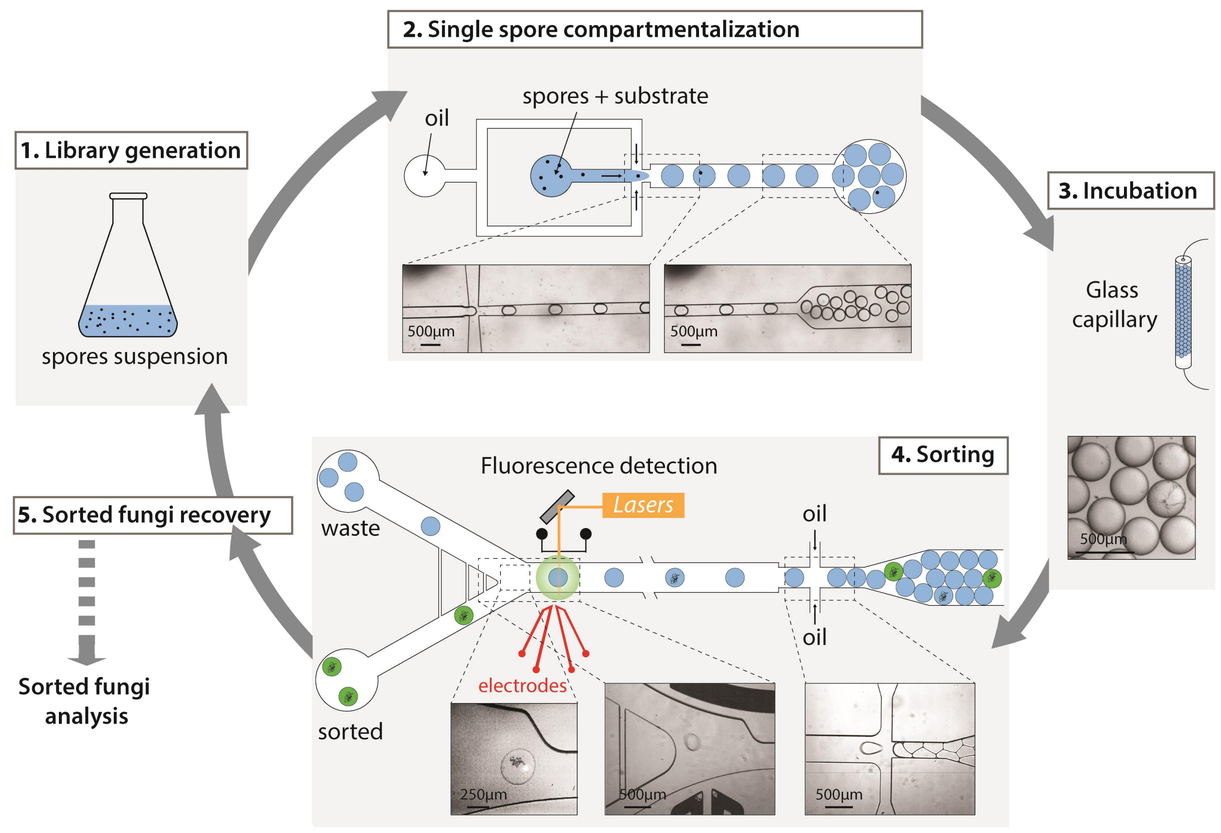
(A) Image sequence (40x) over 13 h of a 250 pL droplet containing one single spore and incubated at 30°C. **(B)** Microscope image (15x) of 18 nL droplets after single spore encapsulation and 24 h incubation at 30°C. **(C)** Microscope image (15x) of 18 nL droplet after single spore encapsulation and 32 h incubation at 30°C. Red circles highlights the hyphal tips exiting the droplet. Scale bar is 50 µm.

3. α-amylase fluorogenic assay



(A) The substrate consists of a starch backbone with multiple quenched BODIPY fluorophores. α-amylase hydrolyzes the starch backbone to unquench the fluorophores and induce fluorescence. **(B)** Epifluorescence image (10x) of 18 nL droplet emulsion after single spore encapsulation with a fluorogenic substrate and 24 h incubation at 30°C: the secreted α-amylase activity is inducing green fluorescence within droplets containing fungi.

4. Screening platform

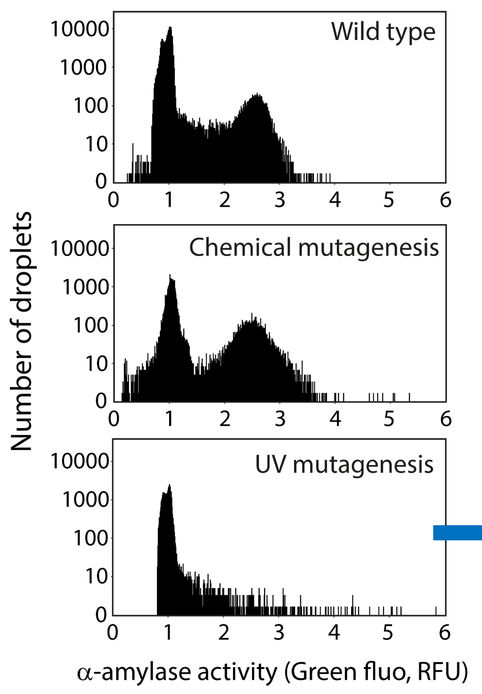


1. Generation of a whole-genome mutated fungi library. **2.** The spores are encapsulated in 18 nL droplets with the substrate using an appropriate spore to droplet ratio allowing single spore compartmentalization. **3.** The emulsion is incubated 24 h off-chip at 30°C in a glass capillary to allow fungi development, enzyme secretion and substrate digestion within the droplets. **4.** Droplets are reloaded in a sorting device to sort them based on α-amylase activity according to fluorescence intensity. Active droplets are sorted at 30 Hz and collected. **5.** Sorted fungi are recovered from sorted droplets and could be either characterized or engaged in another round of mutagenesis and/or selection.

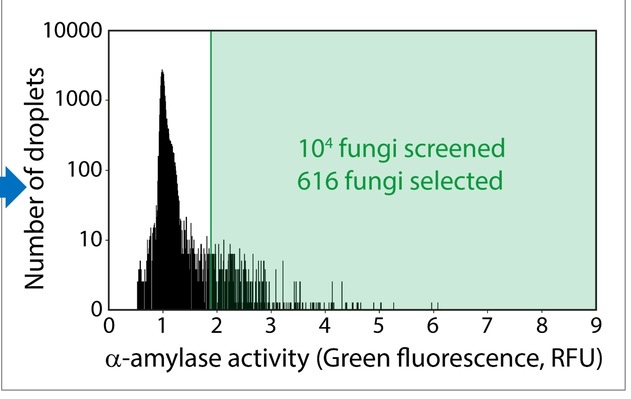
5. Screening of whole-genome mutated *A. niger* libraries

Tuning the library construction

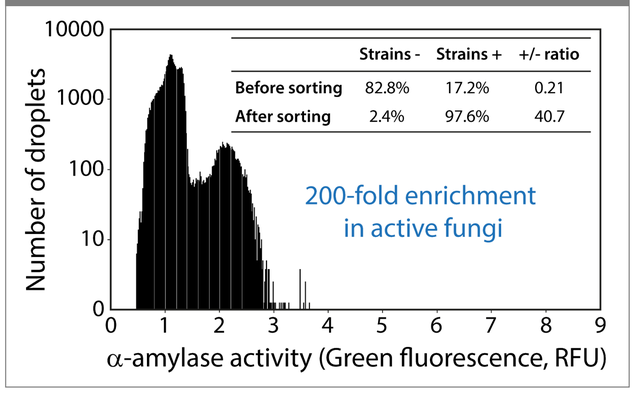
Access to the activity distribution of a library at the single member level



Screening of a UV-mutagenesis library

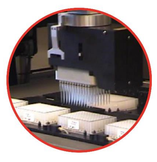


Analysis of the selected population



6. Microplate screening of the population enriched in droplets

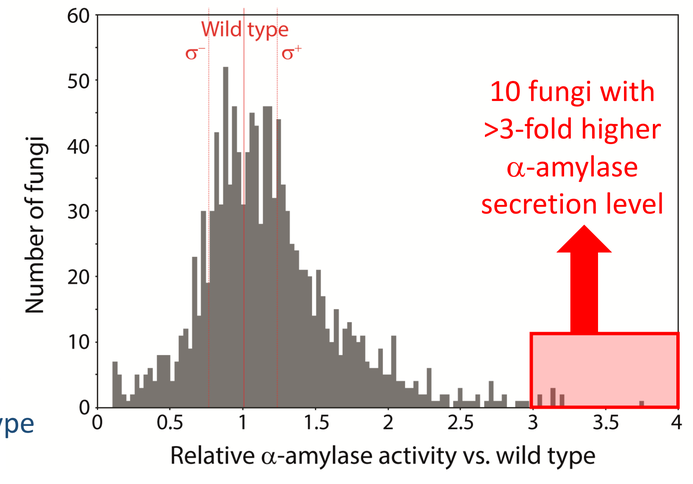
Robotized picking of 1296 fungi



Robotized α-amylase activity titration

555 fungi (44%) as active as wild type

517 fungi (41%) more active than wild type



References

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[3] Najah et al., 2014, Chem. Biol., Accepted
[4] Beneyton et al., 2014, Analyst, 139, 3314-3323

Conclusion

Those microfluidics HTS tools give access to the activity distribution of large fungi populations at the single individual level and allows the fast enrichment in active fungi. Screening time and reagent volume are significantly reduced compared to plate format, while screening costs are proportionally reduced.

		Droplets	Microplates	Gain
Reagent	Growth medium	2 mL	2 L	10^4
	Fluorogenic substrate	100 µg	5 mg	10^3
Time	Compartmentalization	1 h	24 h	10^2
	Culture	24 h	7 days	10^2
	Screening	90 min	48 h	10^2