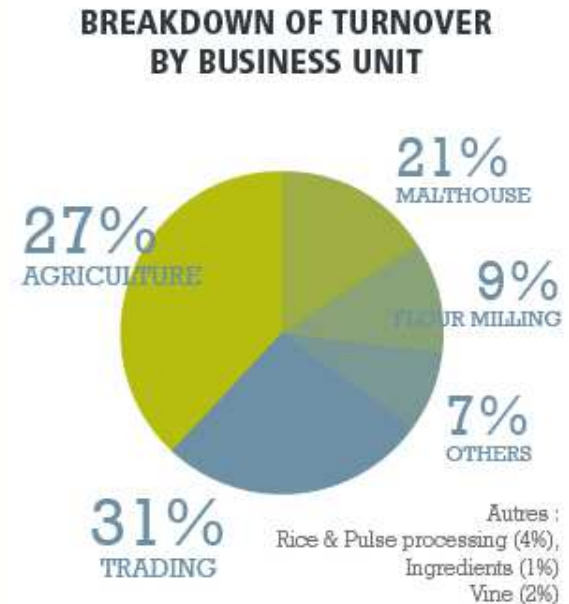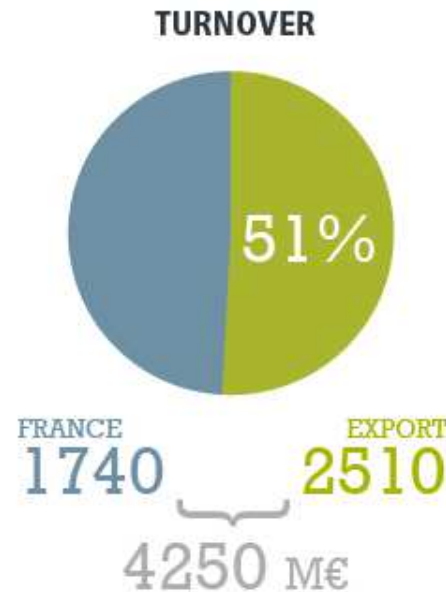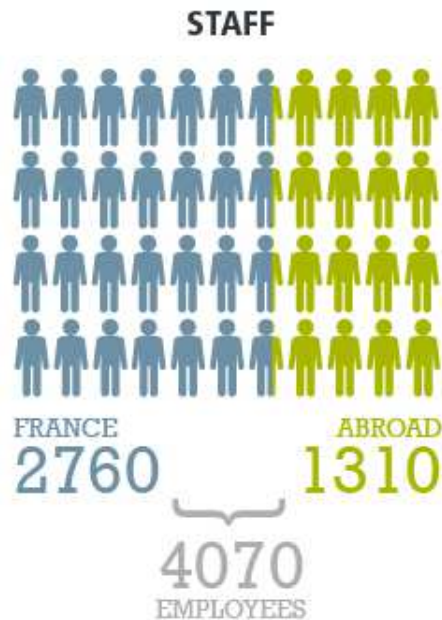# Screening of industrial enzymes and microbes using droplet-based microfluidics

Antoine Drevelle, PhD - Director of Research

Biotechnologies Division – Ets. J. Soufflet

WHO IS SOUFFLET GROUP ?

A key player in the wheat and barley industries in Europe and the world

STAFF

FRANCE
2760

ABROAD
1310

4070
EMPLOYEES

TURNOVER

51%

FRANCE
1740

EXPORT
2510

4250 M€

BREAKDOWN OF TURNOVER BY BUSINESS UNIT

21% MALTHOUSE

9% FLOUR MILLING

7% OTHERS

27% AGRICULTURE

31% TRADING

Autres :
Rice & Pulse processing (4%),
Ingredients (1%)
Vine (2%)

THE BIOTECHNOLOGIES DIVISION

European Expert for Solid State Fermentation

Enzyme Producer

Research for innovative products and biocatalysts from agro-supplies

14 M€ Turnover

126 Staff

1 Industrial site in France

1,400 m² Research Laboratory
200 m² technology Hall

C.R.I.S.
Soufflet Research and Innovation Center
1,400 m² OF LABORATORIES

H.T.R.
Technology Research Hall
2,000 m²

R&D FOCUS

◆ Enzyme production by solid state fermentation

Strain

Biodiversity screening
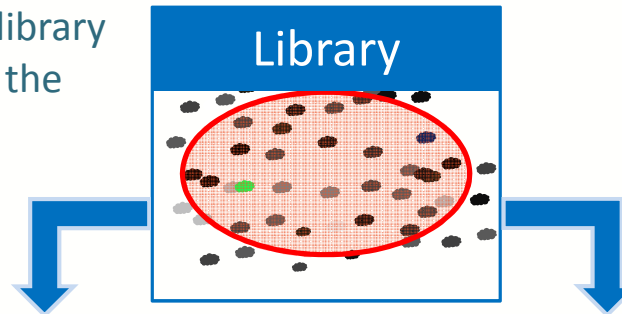Mutant library screening

3S

Substrate

Agro-ressources & by-products
Analysis & characterisation
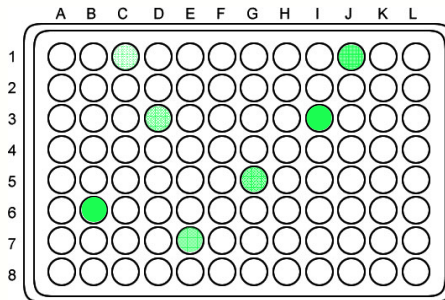Pre-treatment optimisation

System

Solid state fermentors
Optimisation of fermentation
Lab scale & Pilot scale

SCREENING TECHNOLOGIES

- Screening a larger number of library members invariably increases the probability of identifying hits
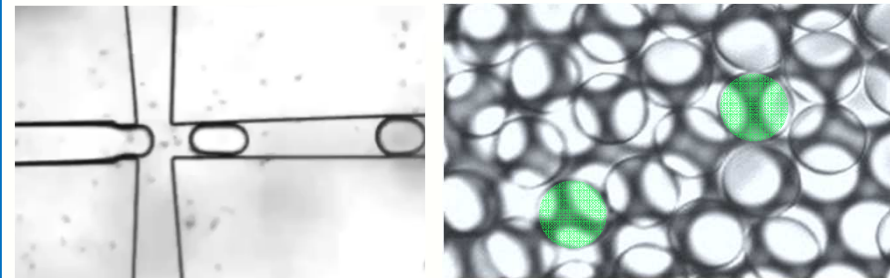
Library

Robotized microtiterplates HTS

- Spatial confinement (Genotype/Phenotype)
- Wide choice of enzymatic assays
- ID tracking of the sample
- Volume (µl – mL)
- Limited throughput (up to 1 Hz)
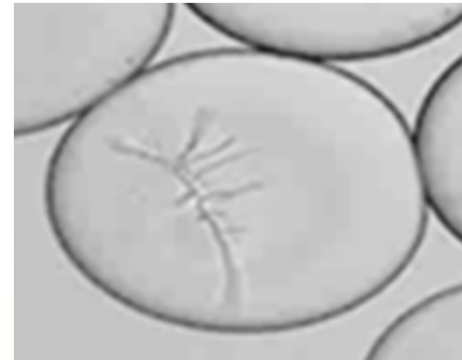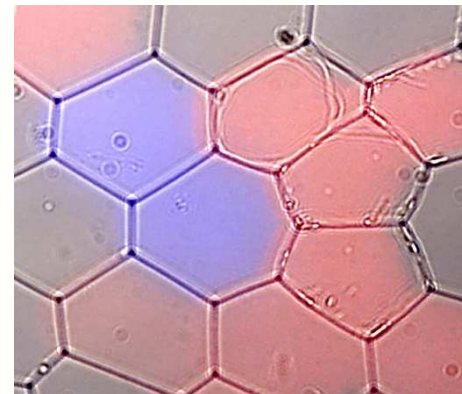- High cost and space footprints

Droplet-based microfluidics HTS

- Spatial confinement (Genotype/Phenotype)
- Smaller volume (pL – nL)
- Higher throughput (100 to 1000 kHz)
- Small cost and space footprints
- Adapt the enzymatic assay
- No ID tracking of the sample

TODAY TOPICS
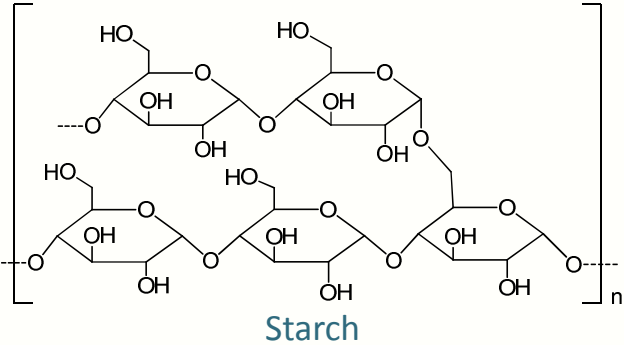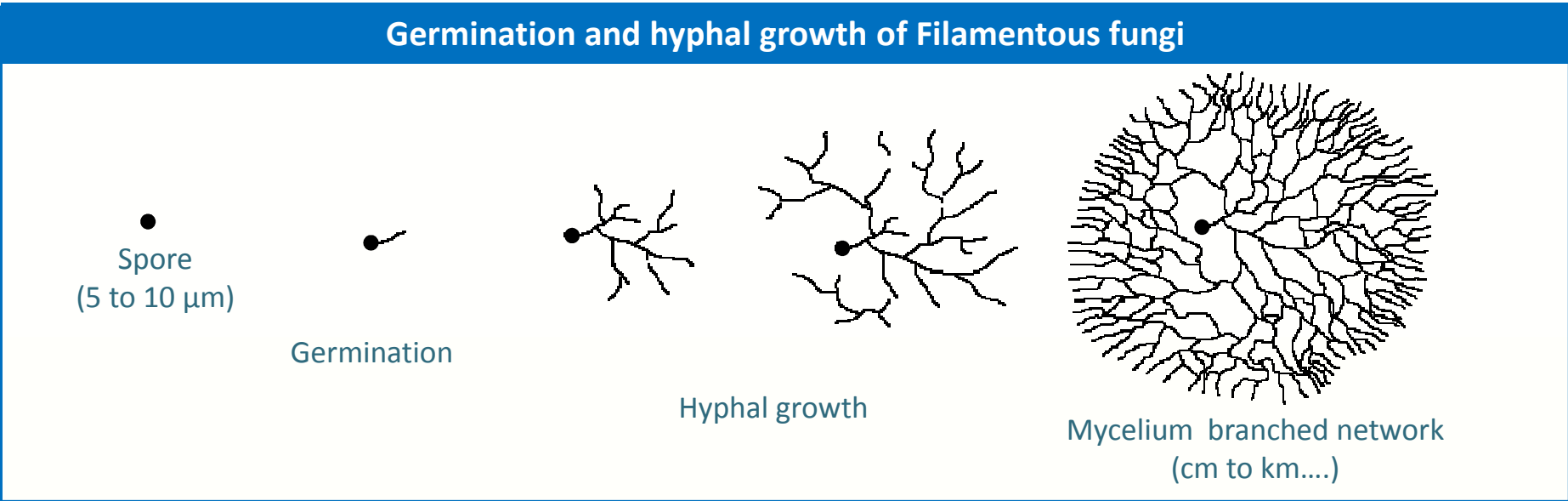
1. Screening of fungi library

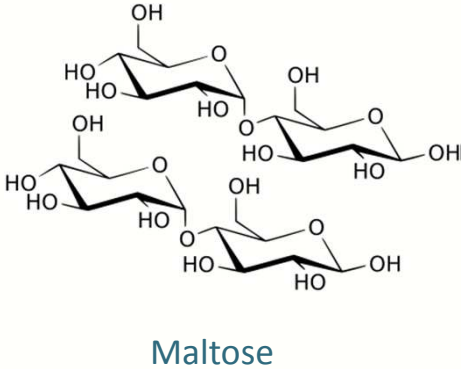2. Screening of bacterial biodiversity

SCREENING OF FUNGI LIBRARY

- Screening of *Aspergillus niger* library for acidic α-amylase secretion

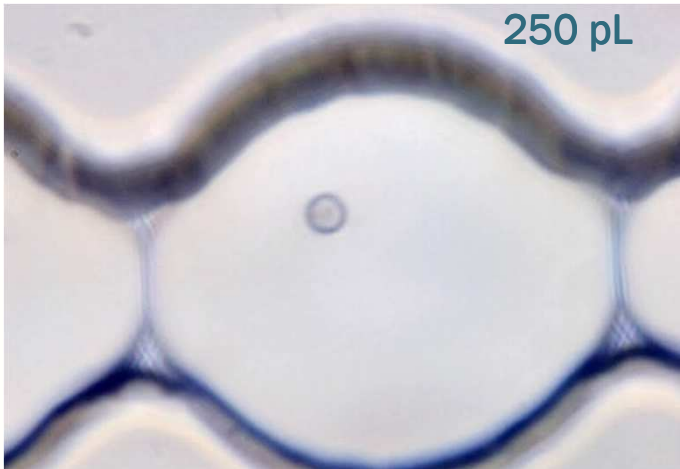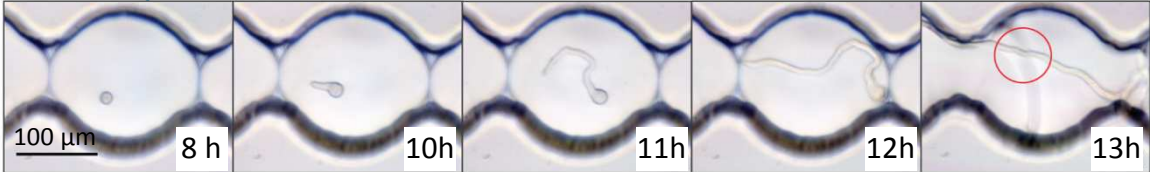**Germination and hyphal growth of Filamentous fungi**

Spore
(5 to 10 μm)

Germination

Hyphal growth

Mycelium branched network
(cm to km….)

Starch

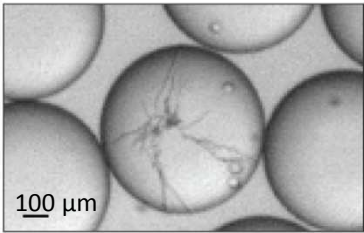$\xrightarrow{\text{A. Niger}}$

α-amylase

Maltose

SCREENING OF FUNGI LIBRARY

- Screening of *Aspergillus niger* library for acidic α-amylase secretion
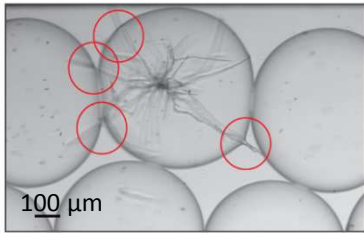
  - Growing *A. niger* in droplets
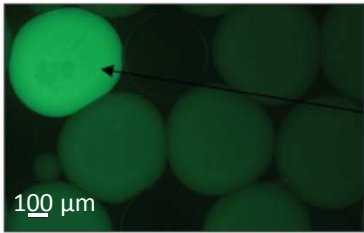

A 250 pL
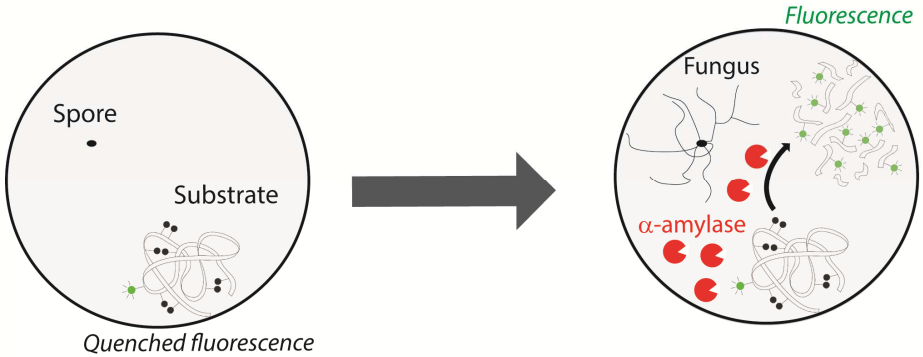100 µm | 8 h | 10h | 11h | 12h | 13h

B 18 nL - 24h
100 µm

C 18 nL - 32h
100 µm

D 18 nL - 24h
100 µm

250 pL

  - α-amylase fluorogenic assay

Fluorescence
Fungus
Spore
Substrate
α-amylase
Quenched fluorescence

SCREENING OF FUNGI LIBRARY

▪ Screening of *Aspergillus niger* library for acidic α-amylase secretion

18 nL droplets
Sorting at 40 Hz

Beneyton *et al*
(in preparation)

Enzinov - 27/10/14

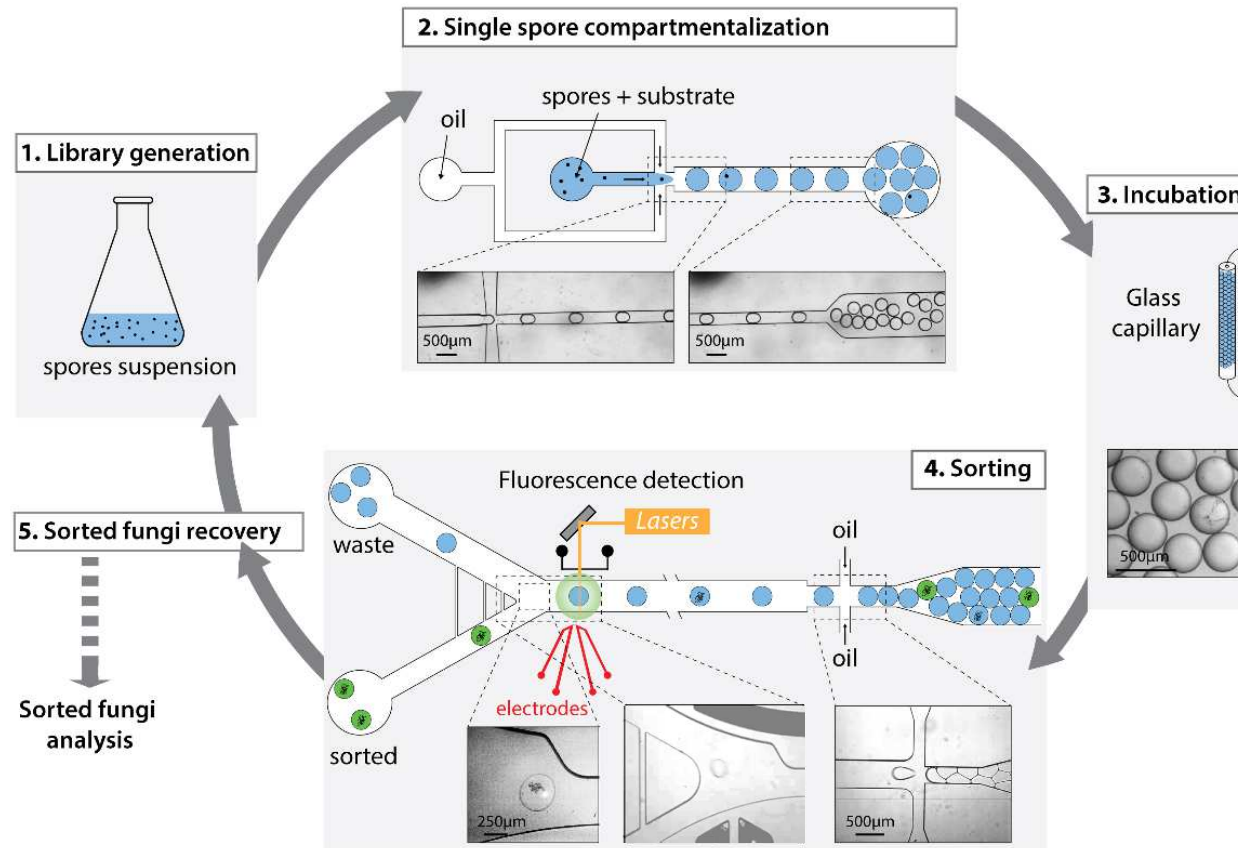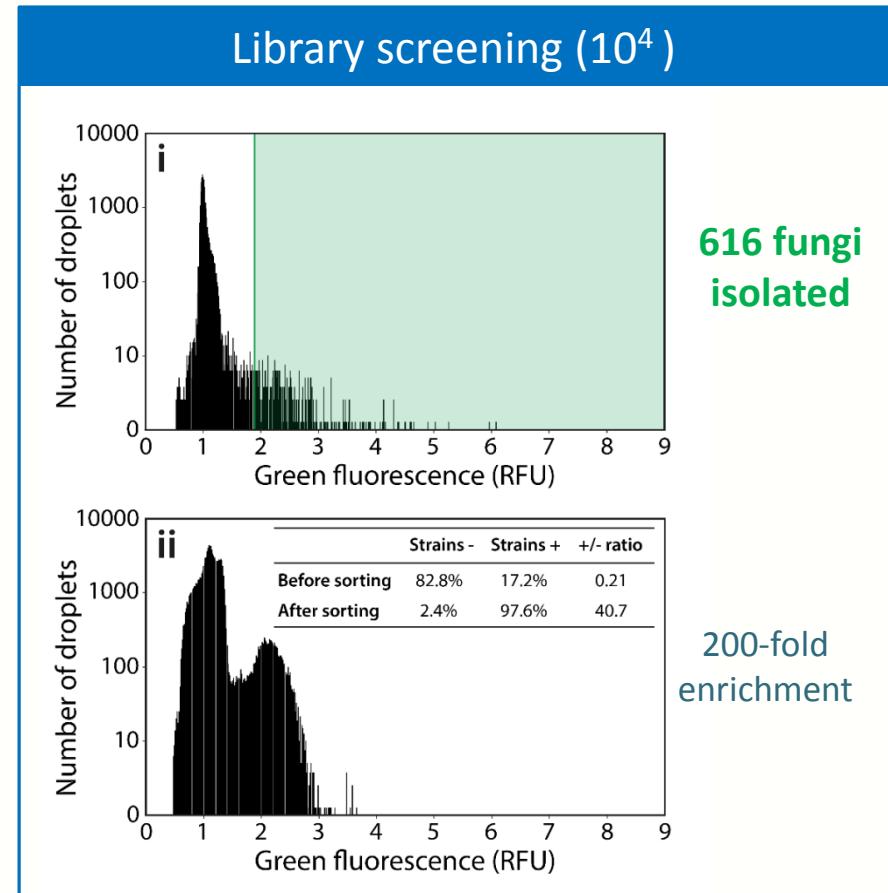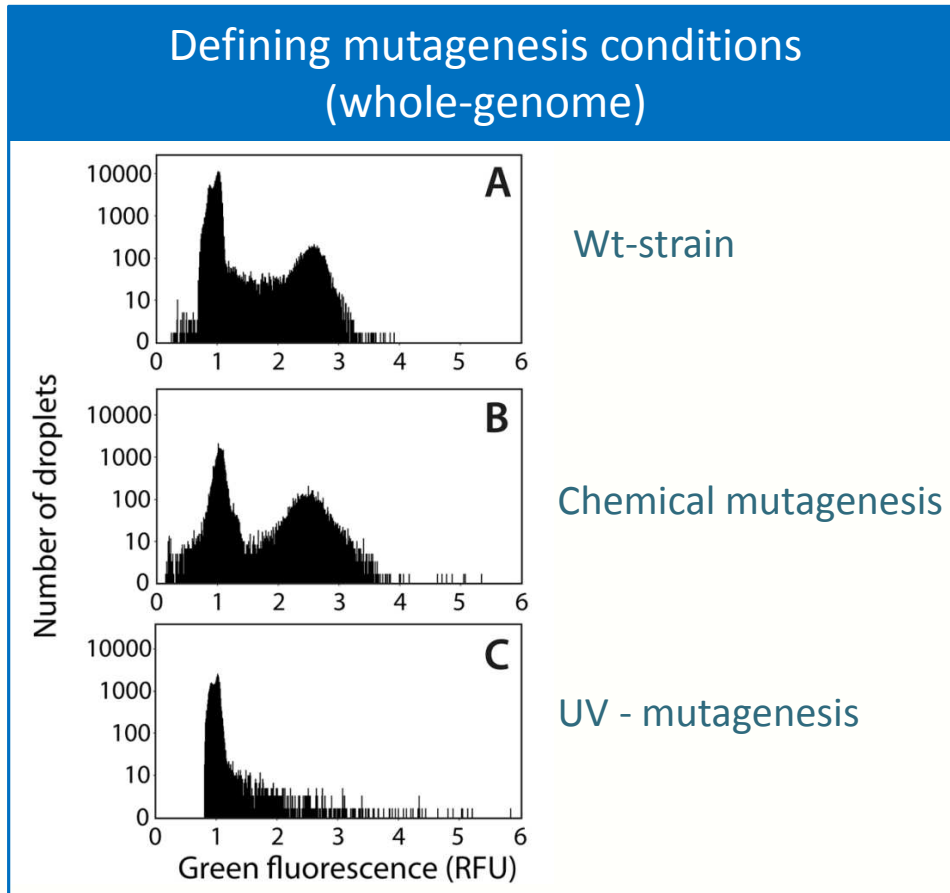# SCREENING OF FUNGI LIBRARY

■ Screening of *Aspergillus niger* library for acidic α-amylase secretion
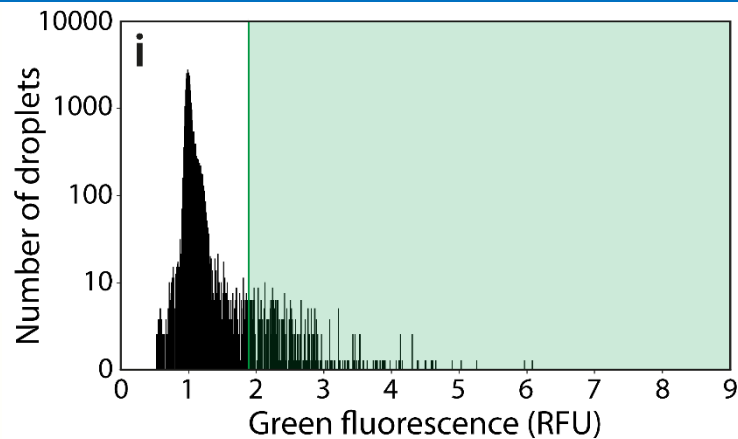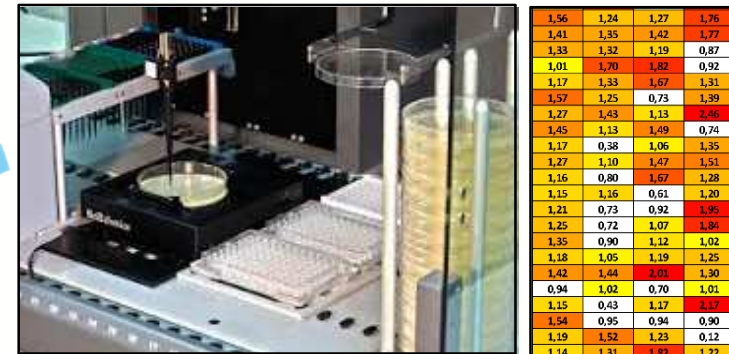
SCREENING OF FUNGI LIBRARY

- Screening of *Aspergillus niger* library for acidic α-amylase secretion

Droplet-based screening

616 fungi

Microtiter plate screening

Primary screening
10⁴ fungi

Culture over 24h + 90 min of assay/sorting

Sorting of 616 active mutants

Secondary screening
1 262 picked fungi

Culture over 7-10 days + half day of assay

85% of fungi as active as wild type
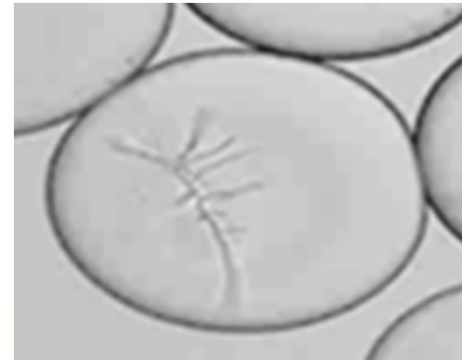
41% of fungi more active than wild type

10 mutants > 3-fold activity (up to 3,75-fold)

**Focus your powerful (but costly) robotic screening
on active and relevant genetic diversity**

Beneyton *et al*
(in preparation)

GROUPE soufflet

Enzinov - 27/10/14

ESPCI ParisTech   lyven   osiris

TODAY TOPICS

1. Screening of fungi library

2. Screening of bacterial biodiversity

SCREENING OF BACTERIAL BIODIVERSITY

- Screening of bacteria isolated from wheat stubble for cellulolytic activity

Cellulose → Cellobiohydrolase / Endoglucanase → Cellobiose → β-glucosidase → Glucose
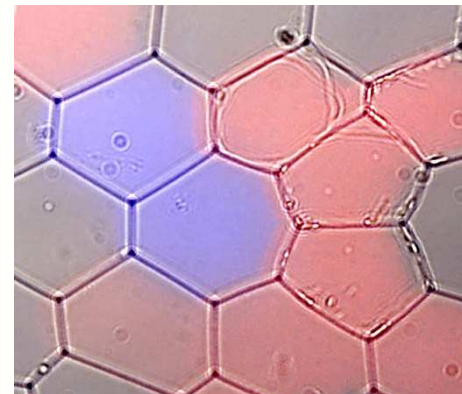
New fluorogenic assay for glycosidases in droplet
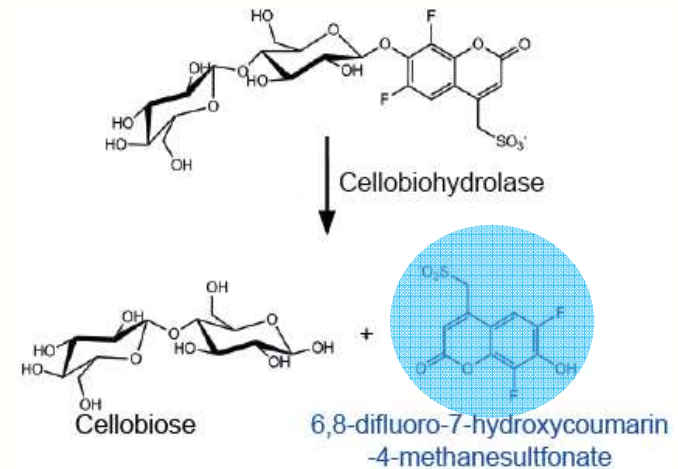
Patent WO/2012/038614 | Najah *et al* Anal. Chem (2013)

Cellobiohydrolase fluorogenic assay

Cellobiohydrolase

Cellobiose + 6,8-difluoro-7-hydroxycoumarin-4-methanesultfonate

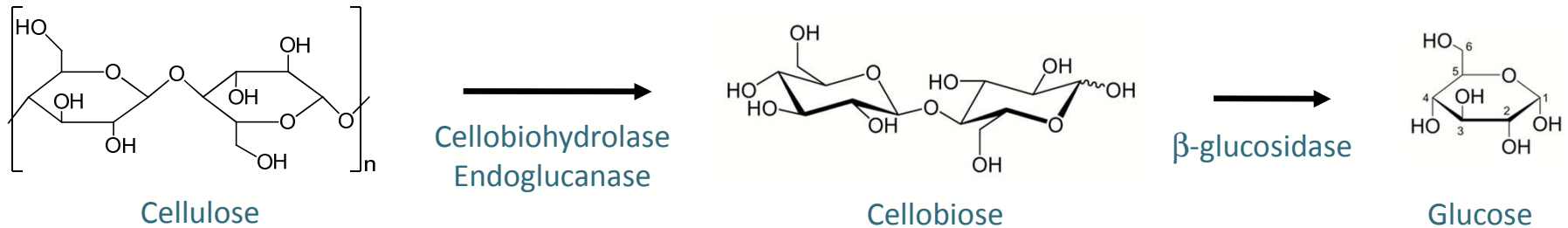# SCREENING OF BACTERIAL BIODIVERSITY

■ Screening of bacteria isolated from wheat stubble for cellulolytic activity

Wheat stubble field → Soil sample → Extract bacteria

oil
cells
collection outlet

Cellobiohydrolase fluorogenic assay

Cellobiohydrolase

Cellobiose + 6,8-difluoro-7-hydroxycoumarin-4-methanesultfonate

Fluorescence detection
Lasers
waste
sorted
electrodes

20 pL droplets
Sorting at 300 Hz

oil
oil

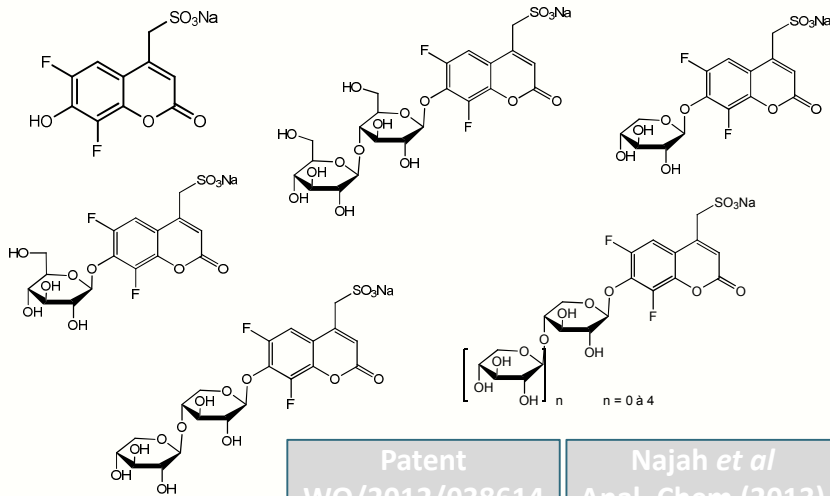Najah *et al*
Chem.Bio. (2014)

GROUPE soufflet

ESPCI ParisTech   lyven   osiris

SCREENING OF BACTERIAL BIODIVERSITY

■ Screening of bacteria isolated from wheat stubble for cellulolytic activity

Wheat stubble field   Soil sample   Extract bacteria

oil

cells

collection outlet

100 000 bacteria screened in 20 min
Sorting of the 2 000 most active bacteria

Fluorescence detection

Lasers

20 pL droplets
Sorting at 300 Hz

waste

oil

sorted

oil

electrodes

Soil sample   EcBL21   Bs2439

2%

■ 1 droplet
■ 10 droplets
■ 100 droplets
■ 1000 droplets

Cellobiohydrolase activity (Blue fluorescence, RFU)

Optical coding (Red fluorescence, RFU)

Comparison of selection based on activity or growth :
Phenotypic analysis : activity measurement in droplet and microtiterplate
Genotypic analysis : metagenomic sequencing

Najah *et al*
Chem.Bio. (2014)

Enzinov - 27/10/14

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SCREENING OF BACTERIAL BIODIVERSITY

- Screening of bacteria isolated from wheat stubble for cellulolytic activity
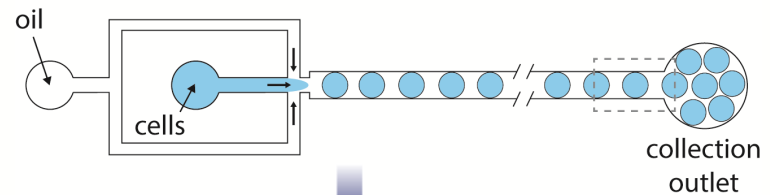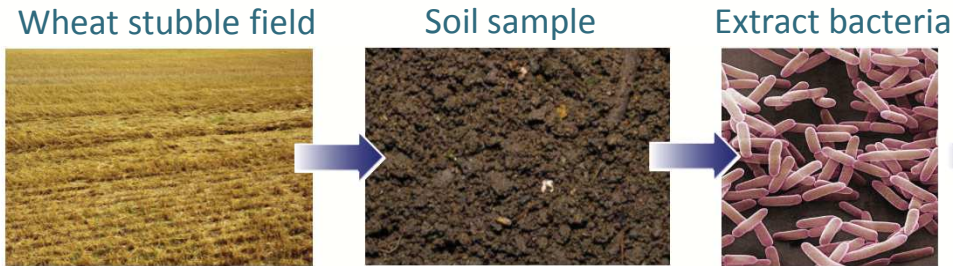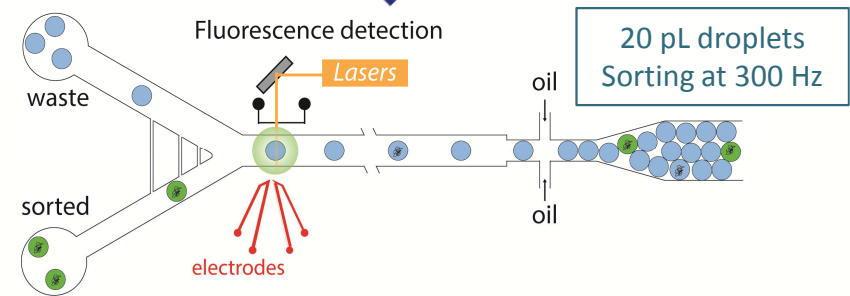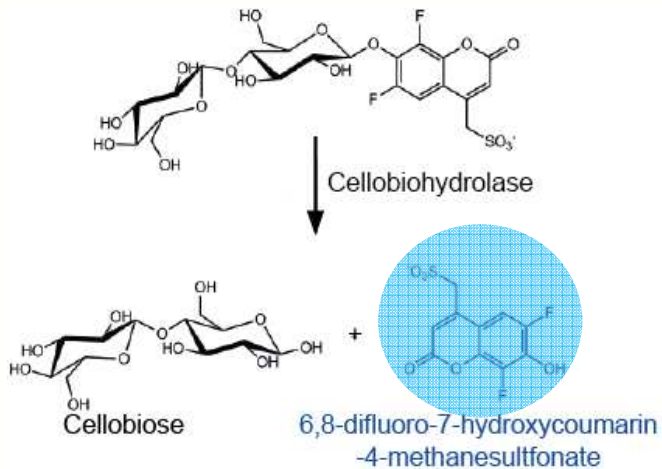

Phenotypic analysis in droplet
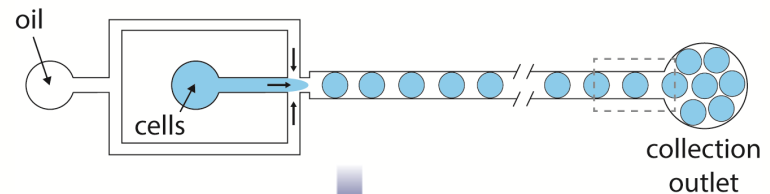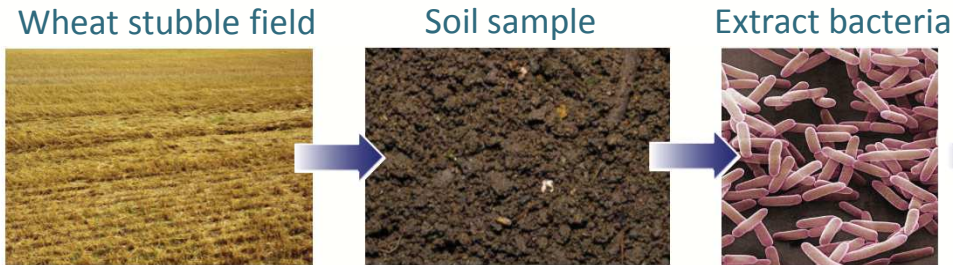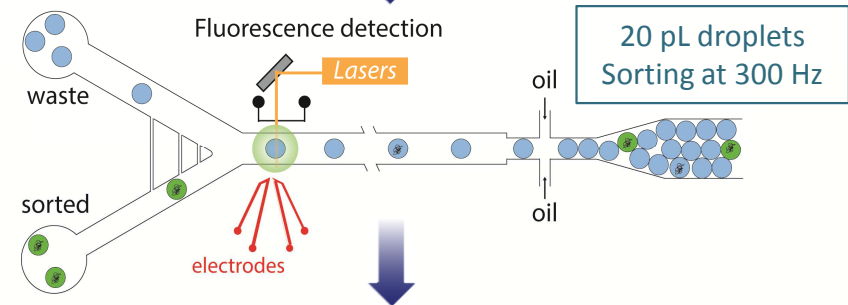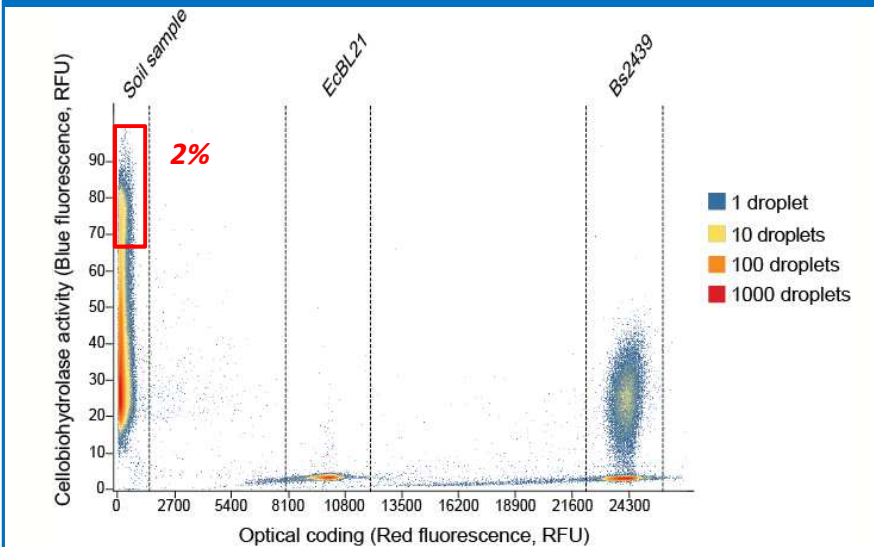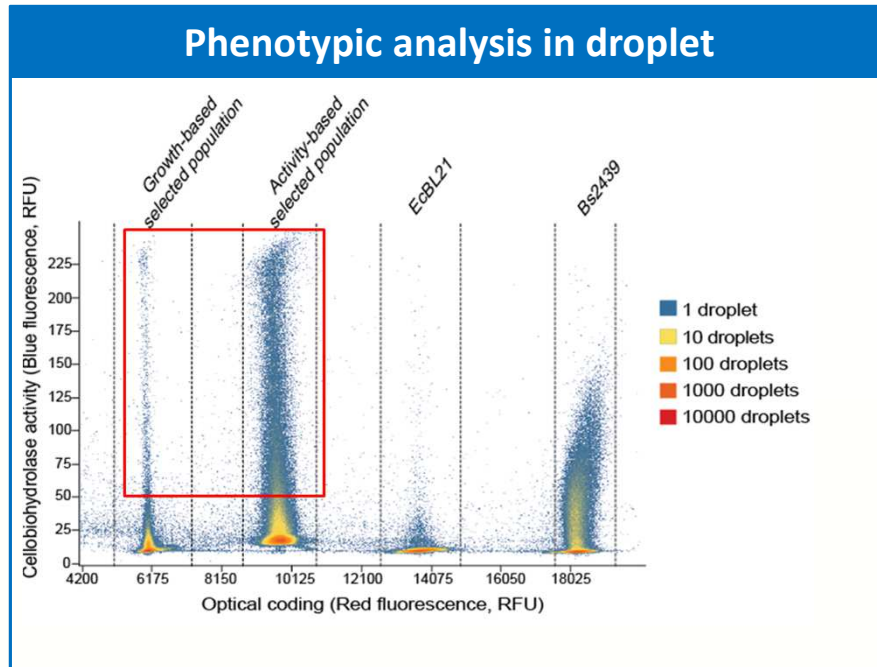
Activity-based vs growth-based selection :
- 17-fold higher cellobiohydrolase activity
- 9-fold higher endogluconase activity

Genotypic analysis (metagenomic)
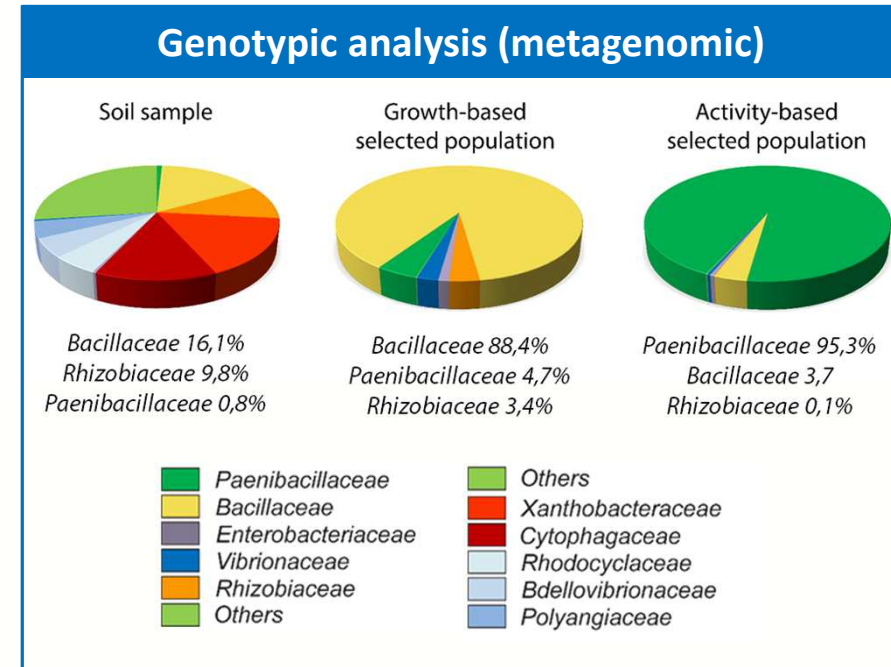
Activity based selection lead to **a very different taxonomic** diversityt than growth-based selection,

No need of prior amplification of the genetic diversity
→ Screen the whole genetic diversity of your sample

TAKE HOME MESSAGES

- Higher throughput, lower volume and lower cost

Throughput	Volume	Cost
10 to 100-fold >	10^6-fold <	10^4 to 10^6-fold <

- High-throughput analysis tool:
  - Give access to the activity distribution of a library at the single member level
  - Tune the library creation method and optimise the library to be sorted
- High-throughput screening tool:
  - Screen large library before/without robotic screening
  - Avoid any prior amplification of natural samples

Library size

$10^4 - 10^7$	$10^2 - 10^4$	10	HIT
Droplet microfluidics	Microtiter plate	Flask	

THANK YOU!

- Thomas Beneyton
- Majdi Najah
- Prexilia Postros
- I Putu Mahendra-Wijaya
- Pascal Leblond
- Estelle Mayot
- Raphaël Calbrix
- Andrew Griffiths