Biomass hydrolyzing enzymes identified by functional screening of a metagenomic library from algal biofilms

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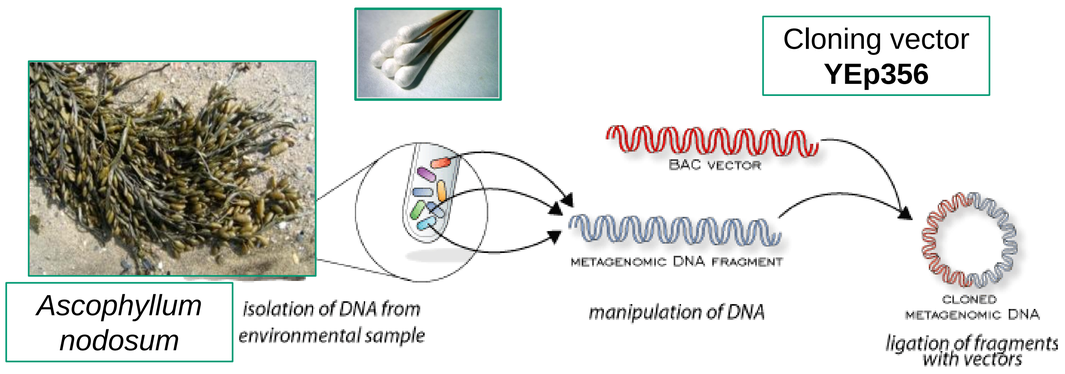
Microorganisms on algae : an unexplored reservoir of biomass hydrolyzing enzymes

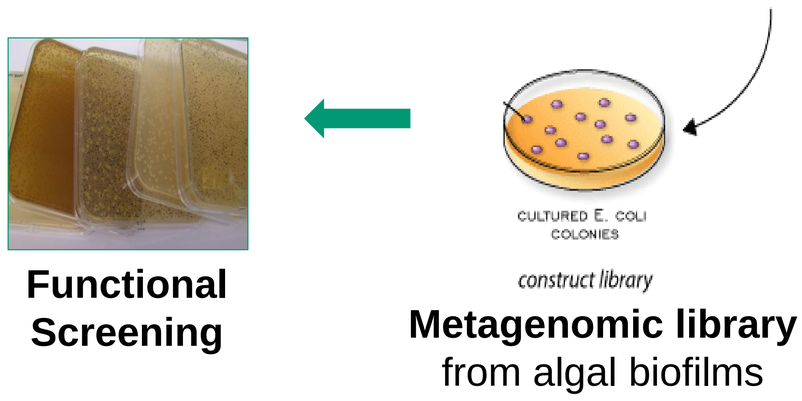
Microorganisms living on algae are in constant interaction with algal biomass, making them an interesting reservoir of biomass hydrolyzing enzymes. To access those interesting enzymes, metagenomic DNA libraries from algal biofilms (from winter and summer samples) were constructed and functionally screened on solid media.

1. Algae surfaces are swabbed with coton tips to recover microbial cells for **total microbial DNA extraction**



2. Extracted microbial DNA is restricted and **ligated in a cloning vector**

3. Ligated DNA are used for **transformation in *Escherchia coli***



Metagenomic library from algal biofilms

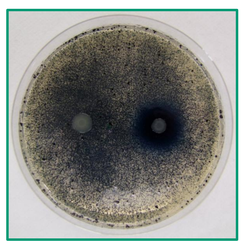
4. DNA is expressed in *E.coli* and **screened for enzymatic activities** on specific enzymes substrates (triglycerides, cellulose, starch, agars, carrageenans,…)

Functional Screening

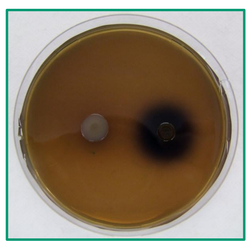
New hydrolyzing enzymes identified by functional screening of metagenomic libraries from algal biofilms

"Winter" and "Summer" DNA libraries were screened, on solid media, for biomass hydrolyzing enzymes commonly used in bioindustries (lipases, β-glucosidases, α-amylases, proteases, cellulases, xylanases and arabinanases) and are currently being screened for enzymes hydrolyzing specific algal polysaccharides (agarases, kapppa and iota carrageenases, alginate lyases, laminarinases,…).

Lipolytic enzymes



Cellulase



Beta-glucosidase

The "Winter" screening revealed
- ✓12 new lipolytic enzymes
- ✓1 new cellulase
- ✓1 new β-glucosidase.

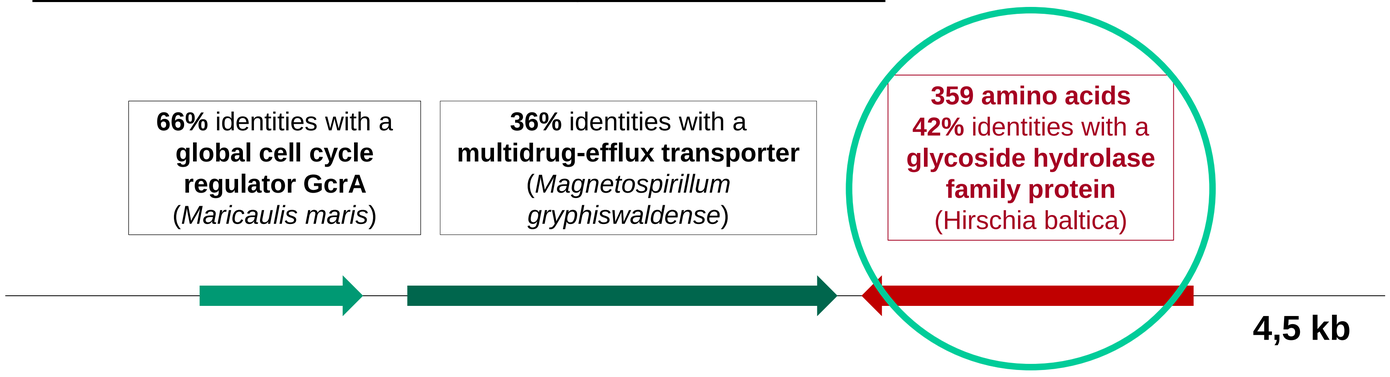
The "Summer" screening revealed
- ✓3 new lipolytic enzymes
- ✓1 new β-glucosidase.

DNA sequences of those identified enzymes are far different (<50% identities) from known enzyme sequences

First cellulase identified by marine metagenomics is purified and is being characterized

Even if cellulases were already identified in cultivable marine bacteria, until now, no marine cellulase was identified by metagenomics. Cellulases are one of the most used enzymes in bioindustries (pulp and paper, textile, bioethanol, wine and brewery, food processing, detergent applications,…). Furthermore, as processing cellulosic biomass become the crux of future research, new cellulases are increasingly searched for further application.

DNA insert of our transformant exhibiting the cellulase activity :

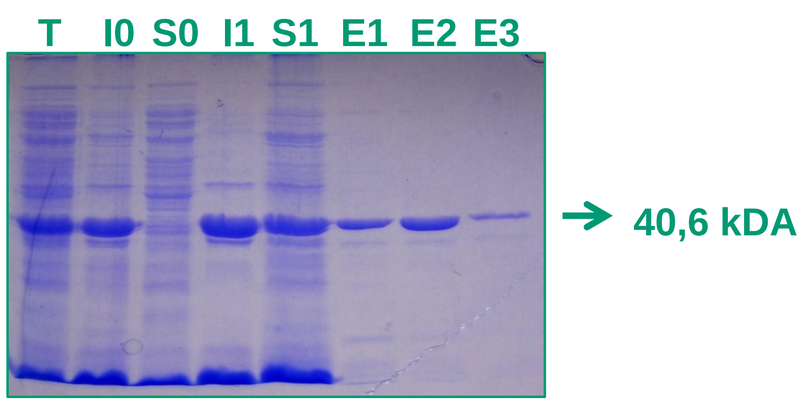


CAZyme classification GH5_26 :only 1 enzyme of this subfamily has been characterizd yet

This ORF was inserted in the pET30b expression vector and expressed in Rosetta2 *E.coli* strain for purification

< 50% identities with known cellulase sequences: a new cellulase, distantly related to known cellulases

Protein purification gel of our purified cellulase :



T : Total extract
I0 : First Insoluble fraction
S0 : First Soluble Fraction
I1 : Second insoluble fraction
S1: Second soluble fraction
E1, E2, E3 : Eluates

To our knowledge, this is the first functional screening that was realized on a metagenomic library from algal biofilms and this is the first marine cellulase identified by metagenomics.

Acknowledgments

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