Identification of New Microbial Enzymes from Forest and Marine Ecosystems by Functional Metagenomics

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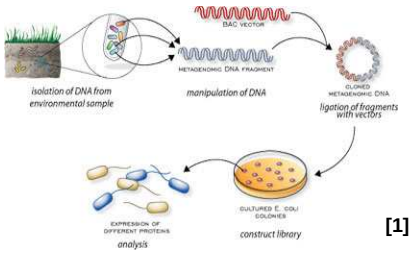
Metagenomics provides acces to the genome of *viable but non culturable* micro-organisms

More than 99% of micro-organisms living in an environnement are unknown and unculturable. By studying the microbial metagenome of an environmental sample we have access to information about those unknowns, bypassing any cultivation step.



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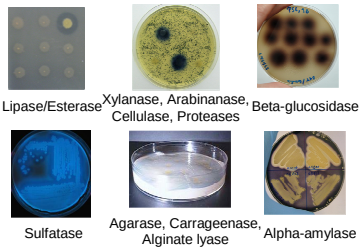
Metagenomic libraries are plated on media containing specific substrates to identify new (families of) microbial enzymes



Advantages of Functional Metagenomic Screening on Solid Media :

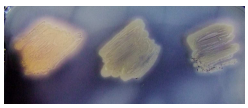
- **Direct observation of the searched activity**
- **No initial comparison with annotated sequences**
  → **Functional identification of NEW (families of) microbial enzymes**
- **Less data than while doing massive sequencing → more focused work**

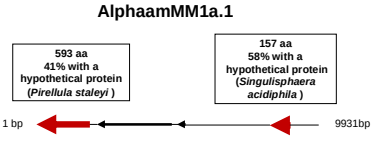
Metagenomic libraries of terrestrial and marine ecosystems were constructed and screened for activities on solid media

Terrestrial ecosystem : Six enzymatic activities were identified by screening a metagenomic library from a forest soil sample

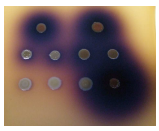
160Mb were screened for lipase/esterase, xylanase, arabinanase, cellulase, protease, beta-glucosidase and alpha-amylase activities

One Alpha-amylase activity

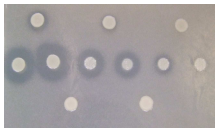


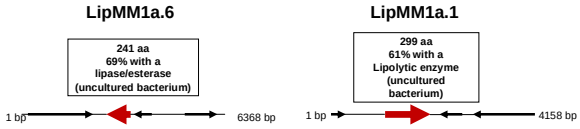


Two Beta-glucosidase activities



Three Lipolytic activities





- **No sequence similarities with known microbial alpha-amylases**
- **2 ORF sequences close to « hypothetical proteins » unique to Planctomyces, may be responsible for the observed alpha-amylase activity.**

- **No sequence similarities with known microbial beta-glucosidases**
- **A sequence close to a « hypothetical protein » of a *Lingbya* species seems to encode for a completely new beta-glucosidase family**

- **ORF's with > 60% of sequence identity with known esterases**
- **LipMM1a.1 and LipMM1a.4 were classified in the family IV and LipMM1a.6 in the family V of the lipase and esterase classification by Arpigny *et al* [2]**

Marine ecosystems : Metagenomic libraries from algal biofilms are screened for algal cell wall degradation activities

Agar
Porphyran
Carrageenan

Alginic acid
Fucans

Specific cell wall polysaccharide composition of *Chondrus crispus* (red algae) and *Ascophyllum nodosum* (brown algae)

Bacterial DNA was also extracted from micro-organisms living on the surface (biofilms) of a red algae and a brown algae

Marine metagenomic libraries with the microbial gDNA are constructed and the screening has been started for :

- Carrageenase activities
- Agarase activities
- Alginate lyase activities
- Sulfatase activities
- **Cellulase activities**
- **Beta-glucosidase activities (100Mb screened)**
- **Alpha-amylase activities**

A new beta-glucosidase with 56% of sequence identity with a glucan 1,4- Beta-glucosidase of *Xanthomonas campestris* has already been identified in the microbial DNA library from *Ascophyllum nodosum*

References
[1] K. J. Shelswell, 2004, Metagenomics : the science of biological diversity, http://www.scq.ubc.ca, May 2012
[2] Arpigny J-L, Jaeger K-E, 1999, Bacterial lipolytic enzymes: classification and properties, Biochem. J., 343, 177-183

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