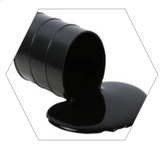
Using metagenomics approach to identify Polysaccharide Utilization Loci (PULs) and produce new carbohydrate active enzymes

Marie Bertucci[1,2], Magdalena Calusinska[1], Patrick A. Gerin[2], Philippe Delfosse[1]

1. Luxembourg Institute of Science and Technology — 41 rue du Brill, L-4422 Belvaux, Luxembourg
2. Université catholique de Louvain - Place de l'Université 1, 1348 Louvain-la-Neuve, Belgique

Introduction



Sources of energy and materials

Fossil carbon

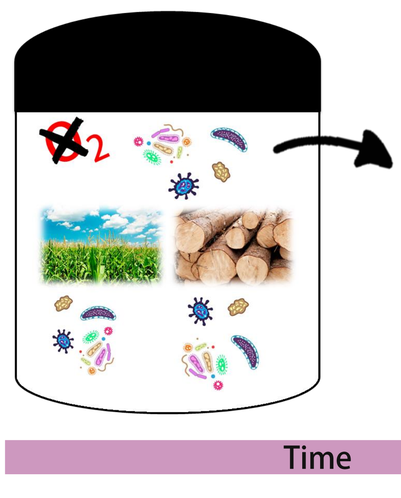
- Negative environmental impact, i.e. global warming[1]
- Non renewable = quickly diminishing resources

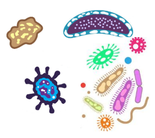
Renewable carbon

- Large availability as lignocellulose
- Highly resistant to bioconversions

➔ Need to be efficiently deconstructed and converted

Anaerobic digestion process





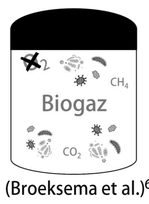
Bacteroidetes
Firmicutes

Most dominant Bacteria in anaerobic digesters[2,3]

Polysaccharide Utilization Loci (PUL)[4,5]

- Cluster of colocalized genes coding for different enzymes
- Targeting specific substrate(s)
- Used for the detection, binding, digestion and transport of carbohydrates

Workflow developed



(Broeksema et al.)[6]

DNA

Extraction
Sequencing
Bioanalysis

1 → PULs identification

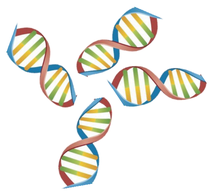
2 → Carbohydrate Active Enzymes selection

3

4

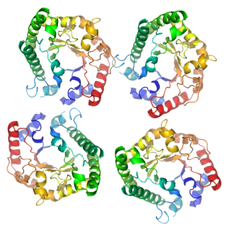
DNA

Amplification
Cloning



5

Protein expression



6 → Detection purification and identification

7 → Activity assays

Results

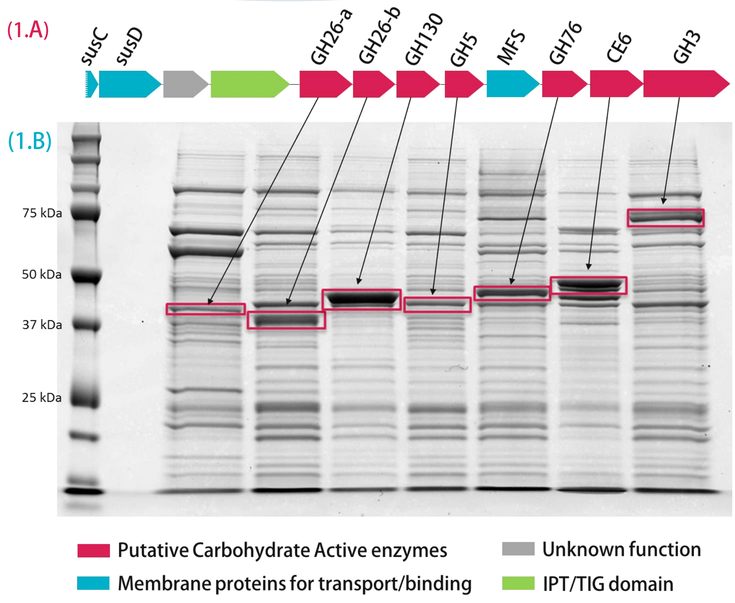


Fig. 1 : (A) Presentation of the selected PUL probably involved in cellulose-mannan deconstruction and (B) the heterologously expressed CAZymes in E. *coli* evidenced by SDS-PAGE

(2)

	Substrates	GH26-a	GH26-b	GH130	GH5	GH76	CE6	GH3
4-Nitrophenyl	α-D-mannopyranoside							
	β-D-glucopyranoside							
	β-D-xylopyranoside							
	β-D-mannopyranoside							
	α-L-arabinofuranoside							
	β-D-cellobioside							
	α-D-galactopyranoside							
	acetate							
	α-D-glucopyranoside							
Xylan								
Galactomannan								
Arabinoxylan								
Carboxymethyl cellulose								

Endo-β-mannanases
No activity detected
β-1,4-mannooligosaccharide phosphorylase ?
Endoglucanase
Esterase
No activity detected
Endo-α-mannanase ?
β-glucosidase

Fig. 2 : Activity of the different enzymes based on the results from activity assays of the purified enzymes towards various substrates

Conclusions

Use of metagenomics was efficient to identify …

- New PUL
- New Carbohydrate Active Enzymes

Using the developed workflow

- 7/7 genes successfully cloned and expressed in E. *coli*
- 5/7 enzymes showed activity against tested substrates
- 2/7 enzymes were not tested against predicted substrate or no activity

Diversity of the enzymes (mannanases, esterase, cellulase, etc. ...) ➔ PUL dedicated to specific substrates ?

References
1. Appels L, Lauwers J, Degrve J, et al (2011) Anaerobic digestion in global bio-energy production: Potential and research challenges. Renew Sustain Energy Rev 15:4295—4301.
2. Goux X, Calusinska M, Lemaigre S, et al (2015) Microbial community dynamics in replicate anaerobic digesters exposed sequentially to increasing organic loading rate, acidosis, and process recovery. Biotechnol Biofuels 8.
3. Sun L, Pope PB, Eijsink VGH, Schn ü rer A (2015) Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure. Microb Biotechnol 8:815—827.
4. Bjursell MK, Martens EC, Gordon JI (2006) Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, Bacteroides thetaiotaomicron, to the suckling period. Journal of Biological Chemistry 281:36269—36279.
5. Hemsworth GR, Dejean G, Davies GJ, Brumer H (2016) Learning from microbial strategies for polysaccharide degradation. Biochemical Society Transactions 44:94—108.
6. Broeksema B, Calusinska M, McGee F, et al (2017) ICoVeR — an interactive visualization tool for verification and refinement of metagenomic bins. BMC Bioinformatics 18:233.

Luxembourg Institute of Science and Technology
5, avenue des Hauts-Fourneaux
L-4362 Esch/Alzette
phone: (+352) 275 888 - 1
fax: (+352) 275 885
marie.bertucci@list.lu
LIST.lu

Cellulosomes, Cellulases & Other Carbohydrate Active Enzyme
Proctor Academy, Andover NH United States
23rd to 28th of July 2017