CRAZY POLYSACCHARIDES

CRiblage d'Activité enZYmatique sur une collection de polysaccharides de structures connues et inconnues

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Polysaccharides are the most abundant and the most diverse renewable materials found on land and in the oceans.

Terrestrial plants

Cellulose
Starch
Hemicelluloses
Pectines
Gums (guars,…)
…

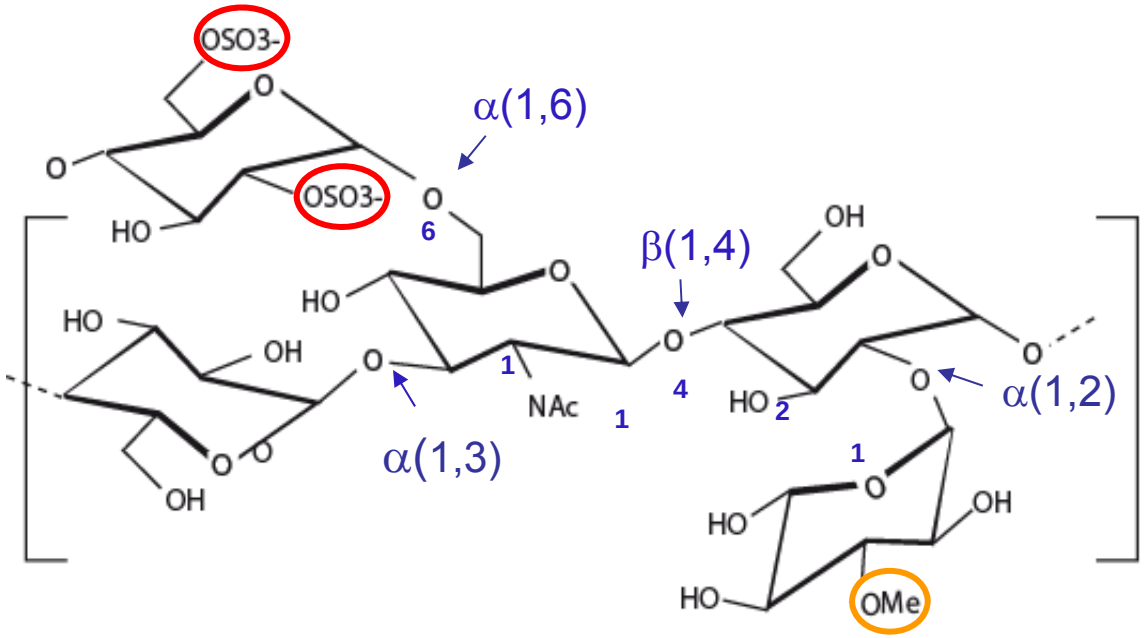
Marine algae

Agars
Carrageenans
Alginates
Ulvans
Xylans
…

Micro-organisms

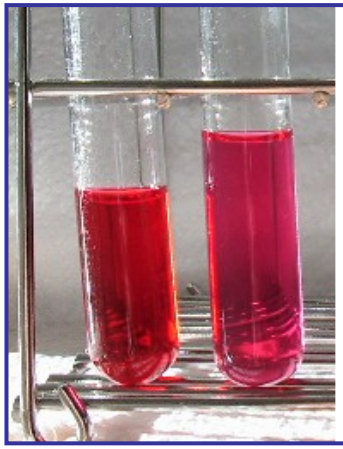
Fungi (chitosan, β(1,3)glucans…)
Animals (GAGs, glycogen, chitin)
Microalgae (?)

Structural diversity of polysaccharides



- Stereochemical variability of monosaccharides
- Numerous possibilities of linkage
- Copolymer – hybrid – structures
- Often mixture of polysaccharides (cell wall,…)

Structural analysis of polysaccharides



Chemical methods:

- Chemical depolymerization
- Chemical derivatization

⇨ Averaged composition and structure

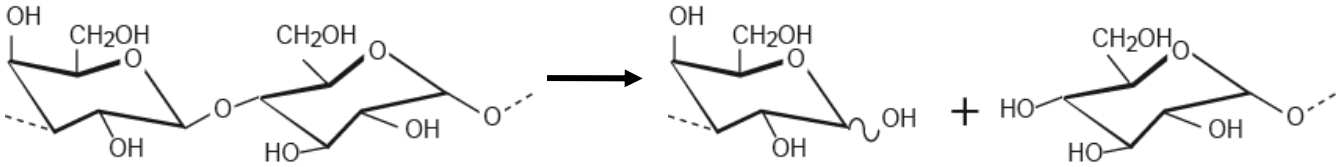
Enzymatic methods:

- Soft depolymerization
- Analysis of complex polysaccharides
- Give insight on the distribution of the moieties

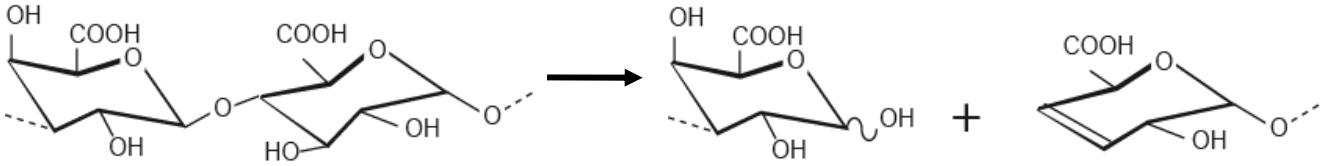
⇨ Low number of enzymes available

Diversity of polysaccharide degrading enzymes

Glycoside hydrolases (hydrolysis)



Polysaccharide lyases (β-elimination)

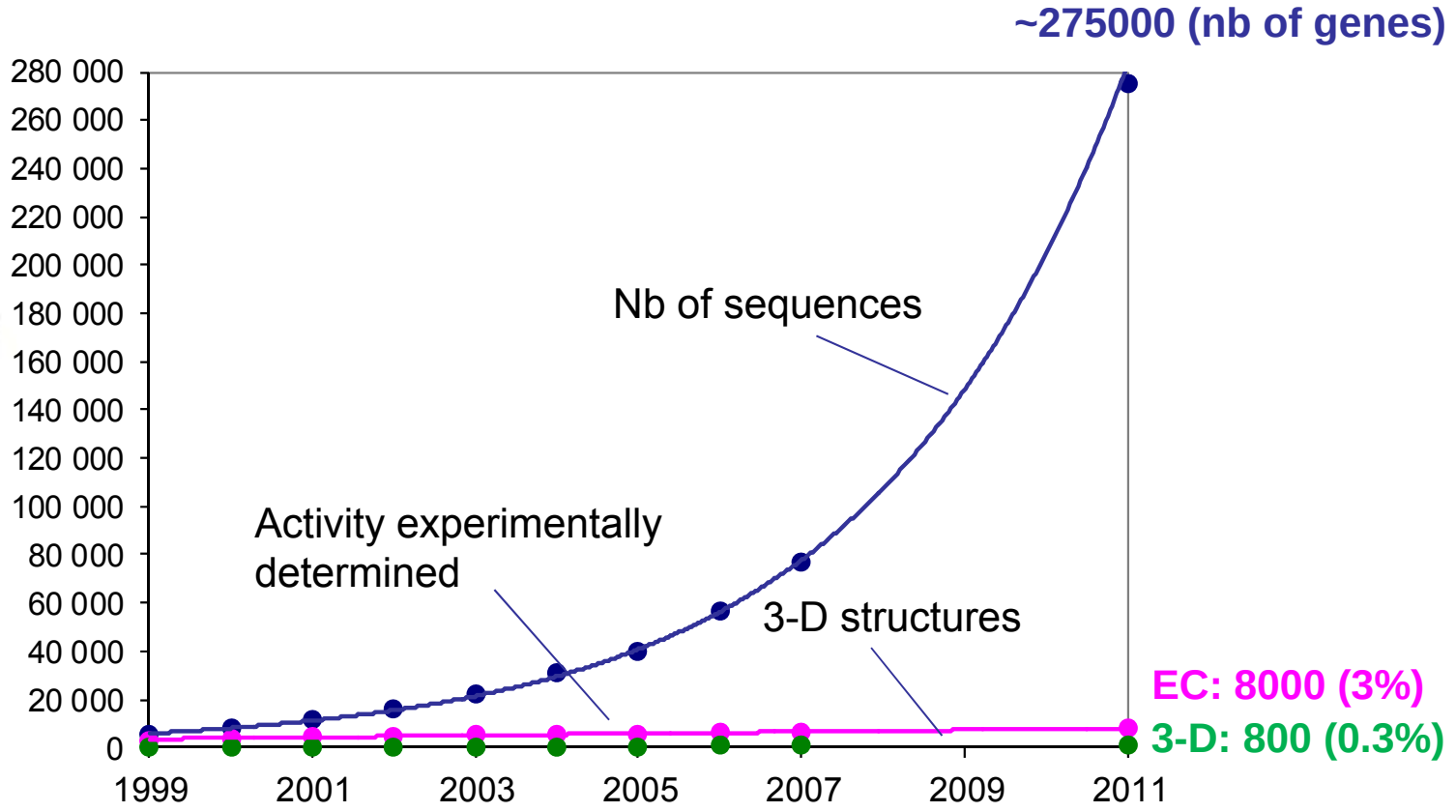


Classification

- IUBMB (enzymatic activity): 160 E.C. numbers
  International Union of Biochemistry and Molecular Biology

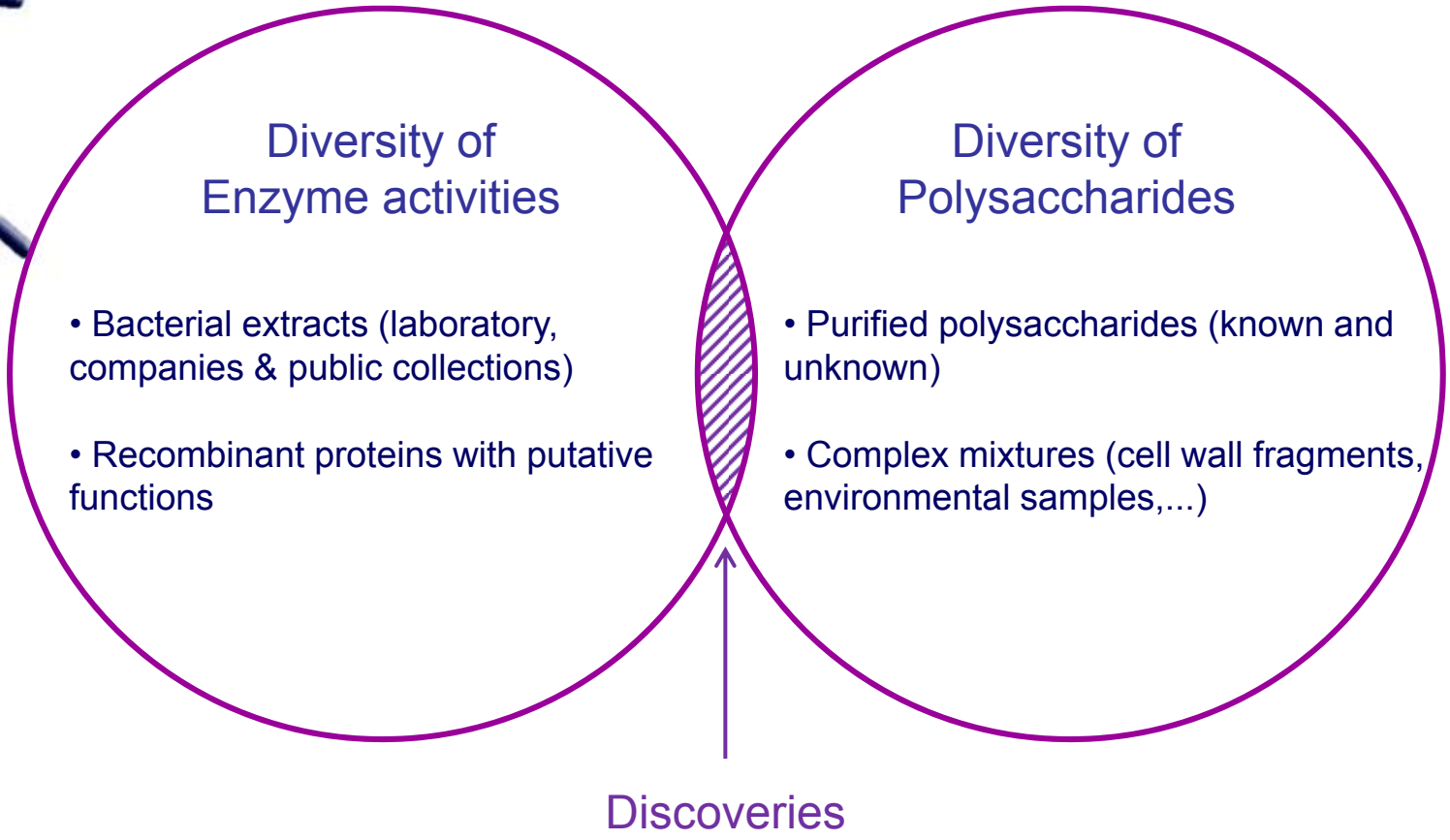
- CAZY (sequence homology): 118 GH and 21 PL families
  http://www.cazy.org

Rate of carbohydrate-active enzymes discoveries



Since 1995, constant increase of the number GH and PL families (5/an) !

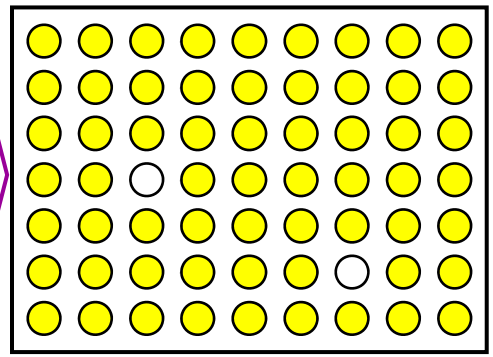
Strategy


Diversity of
Enzyme activities
• Bacterial extracts (laboratory, companies & public collections)
• Recombinant proteins with putative functions
Diversity of
Polysaccharides
• Purified polysaccharides (known and unknown)
• Complex mixtures (cell wall fragments, environmental samples,...)
Discoveries

Strategy

Collection of polysaccharides

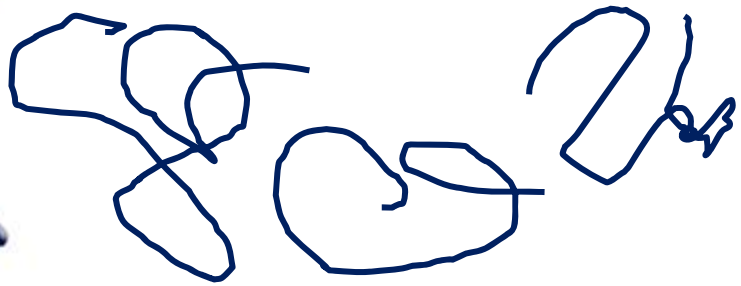
Bacterial extracts,
Recombinant proteins,
…



Colorimetric assay

Mass spectrometry
(MALDI)

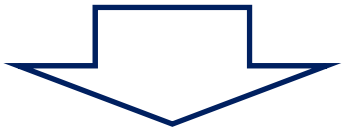
Rational of the method



+

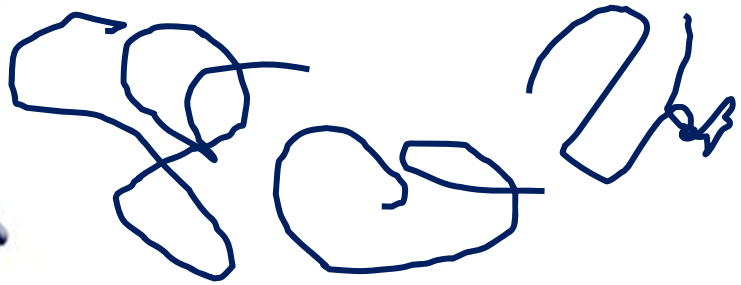
Polysaccharides

Enzymes



Oligosaccharides

Rational of the method



+

Enzymes

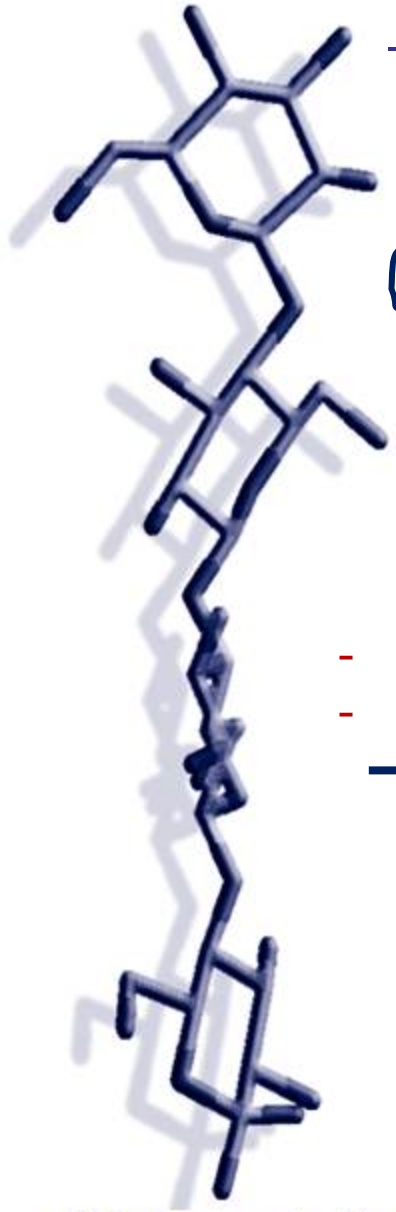
Polysaccharides

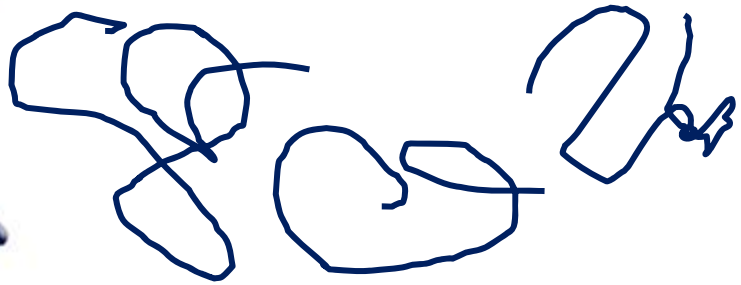
- High molecular weight
- Low number of chain ends

Oligosaccharides

- Low molecular weight = Mass spectrometry (Maldi)
- High number of chain end = Colorimetric assay (reducing ends)

Rational of the method





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Polysaccharides

- High molecular weight
- Low number of chain ends

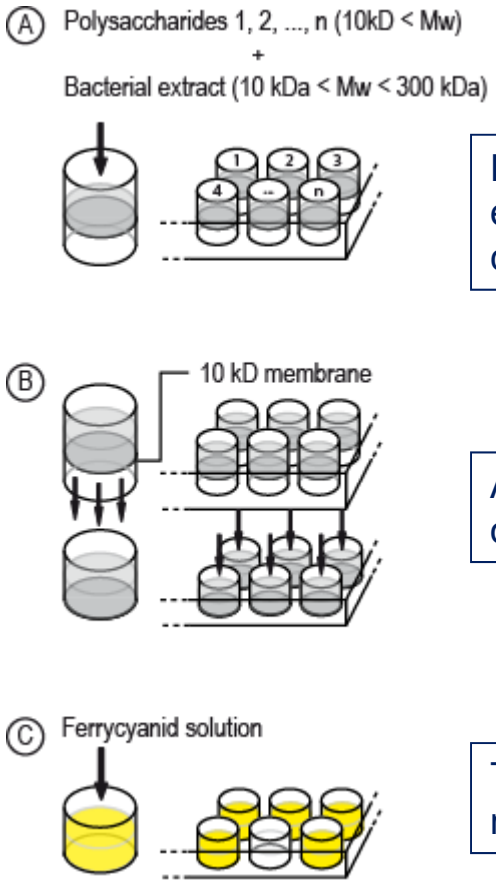
Enzymes

Filtration membrane

Oligosaccharides

- Low molecular weight = colorimetric assay (reducing ends)
- High number of chain end = Mass spectrometry (Maldi)

Protocol of screening



Filtration of all the polysaccharides and bacterial extracts in order to eliminate low molecular weight carbohydrates (10kDa).

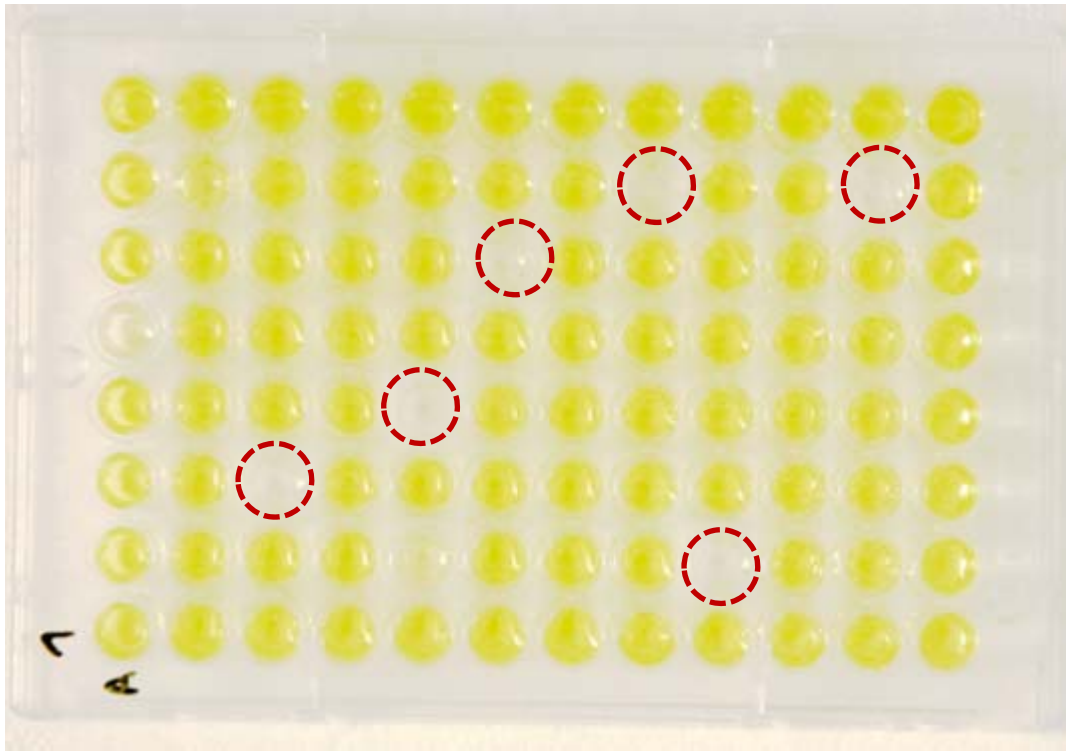
After incubation, low molecular weight oligosaccharides are recovered by filtration.

The reducing ends are assayed by the ferricyanid method.

SBR (Roscoff)
Maude Fer (CDD)
Aurélie Préchoux
William Helbert

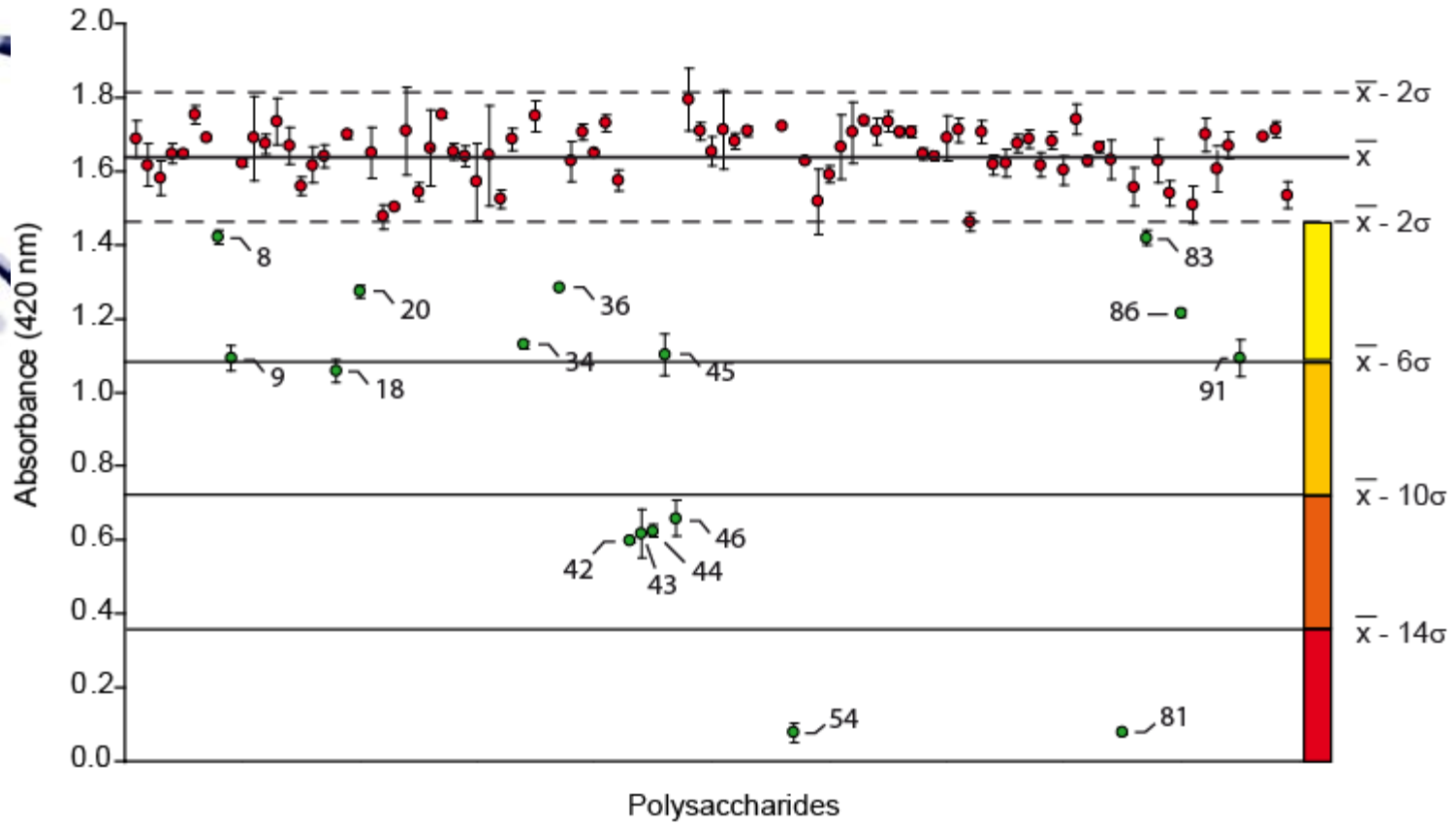
Fer et al. (2011) *J. Microbiol. Meth*. (2012) 89, 222-229

Example of screening



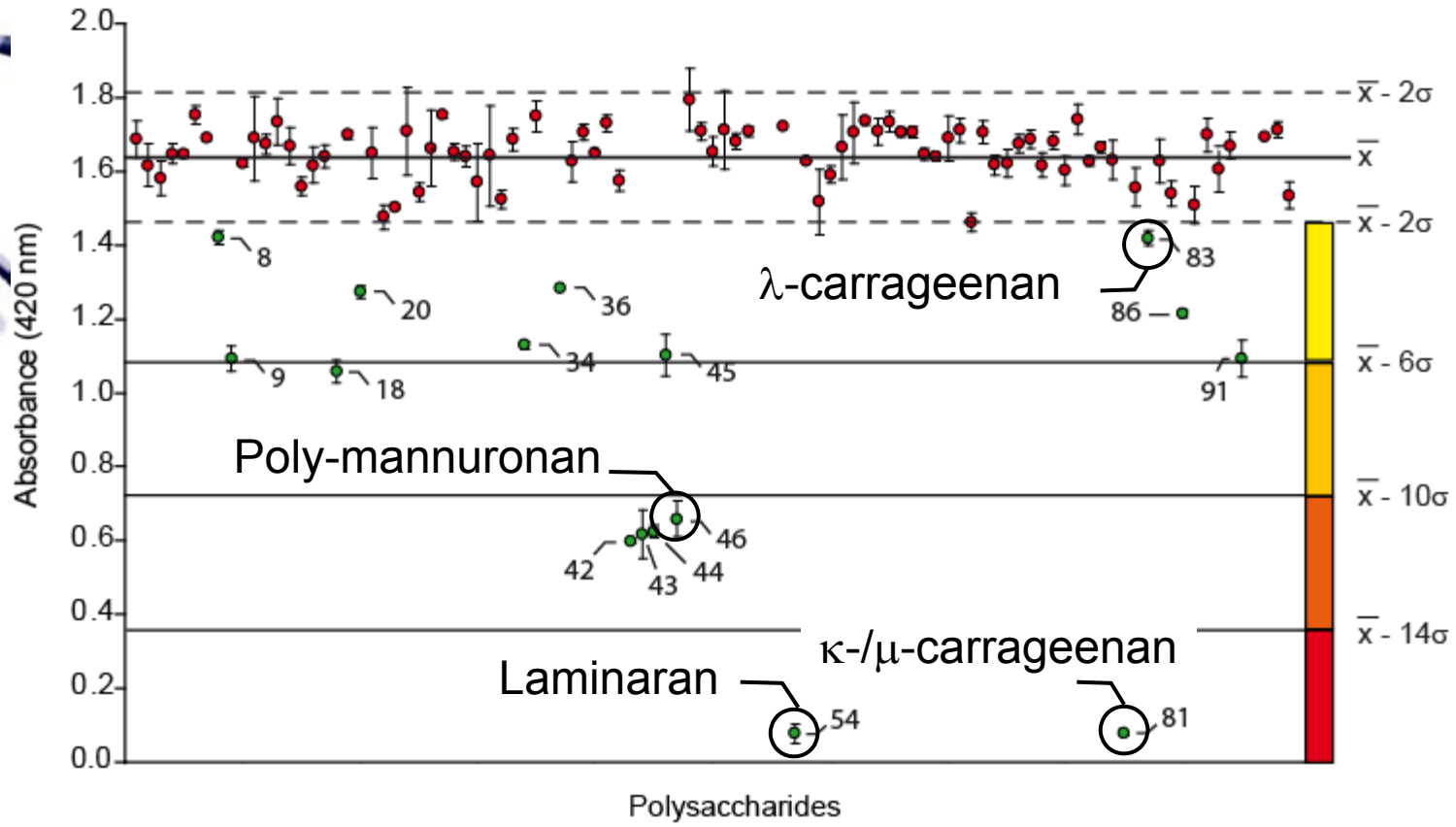
Detection of reducing ends using ferricyanid solution

Example of screening



Criblage du surnageant de culture de *P. carrageenovora*

Example of screening



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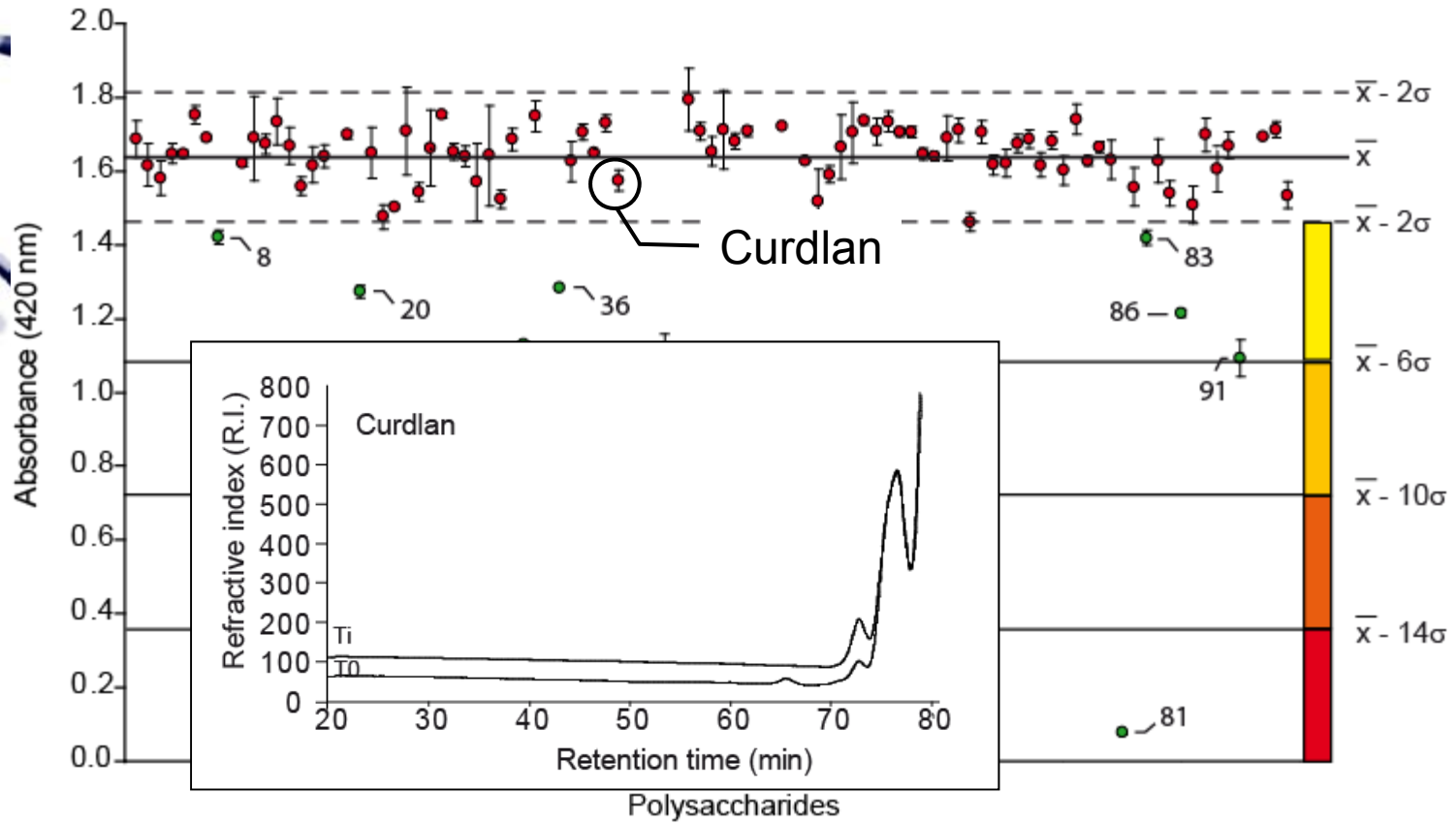

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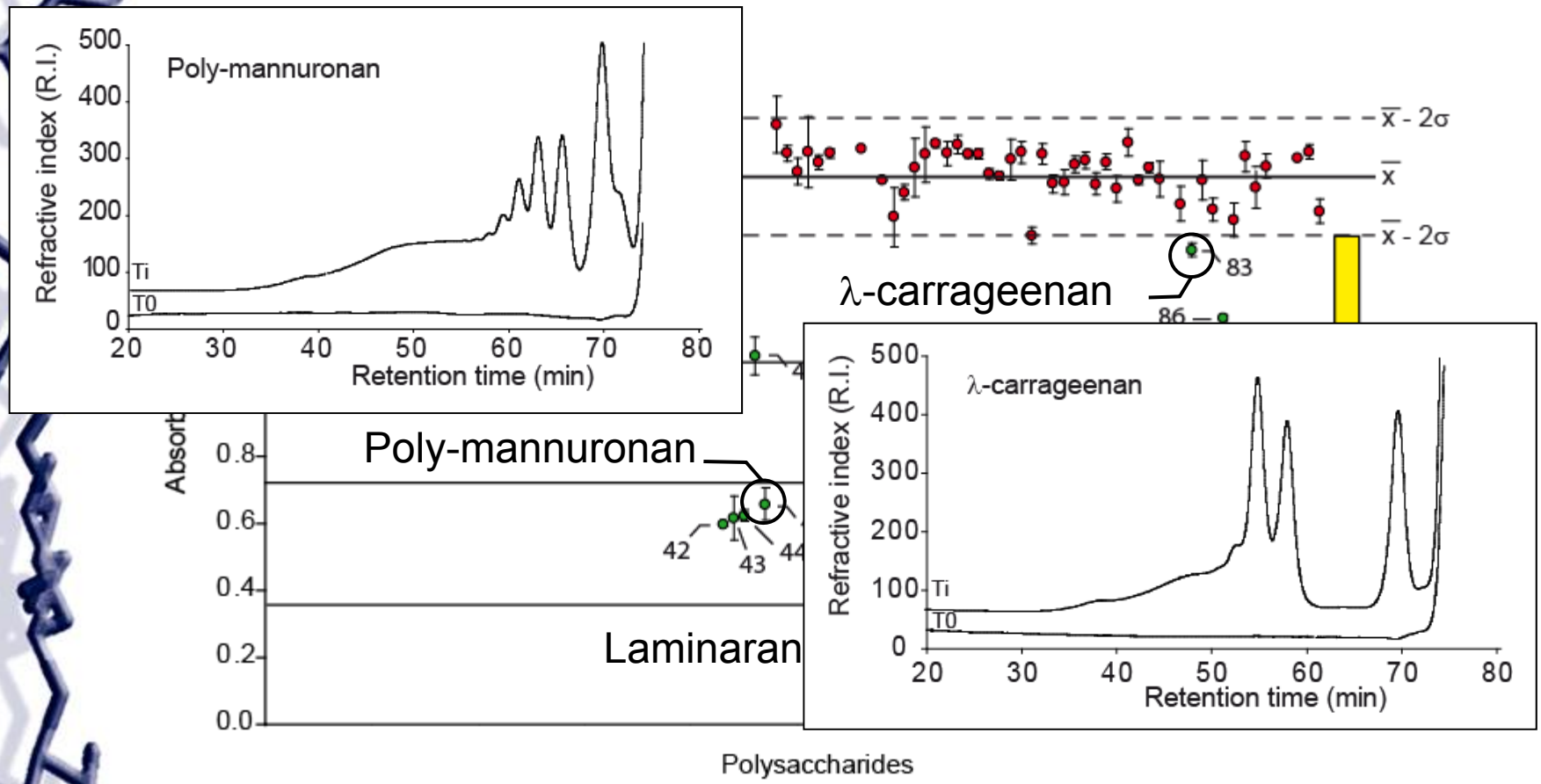
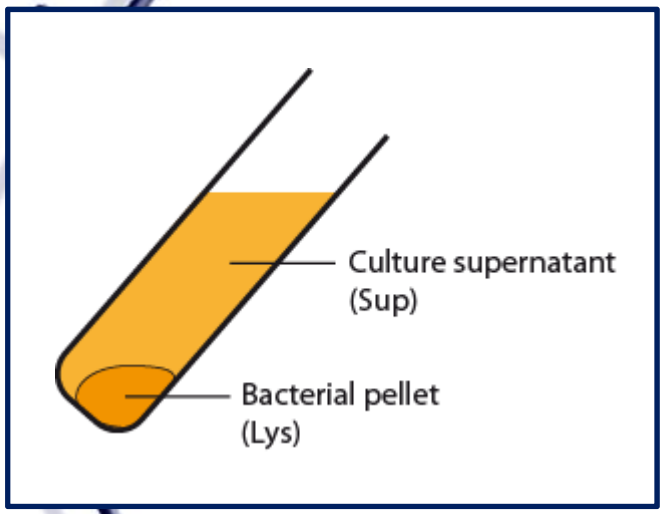


Criblage du surnageant de culture de *P. carrageenovora*

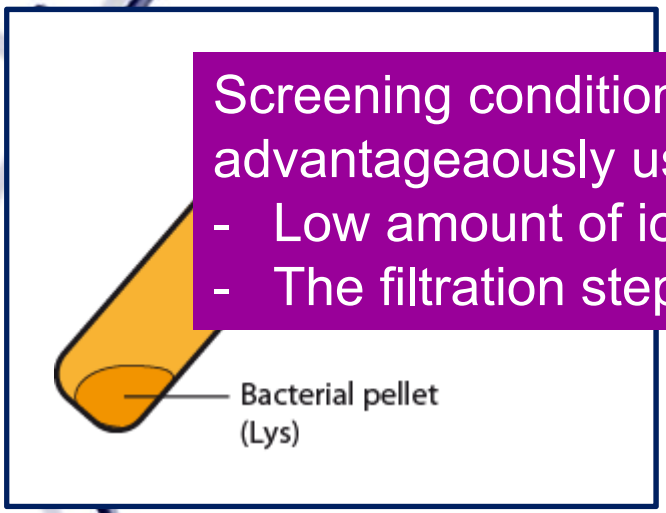
Phenotyping of Pseudoalteromonas carrageenovora

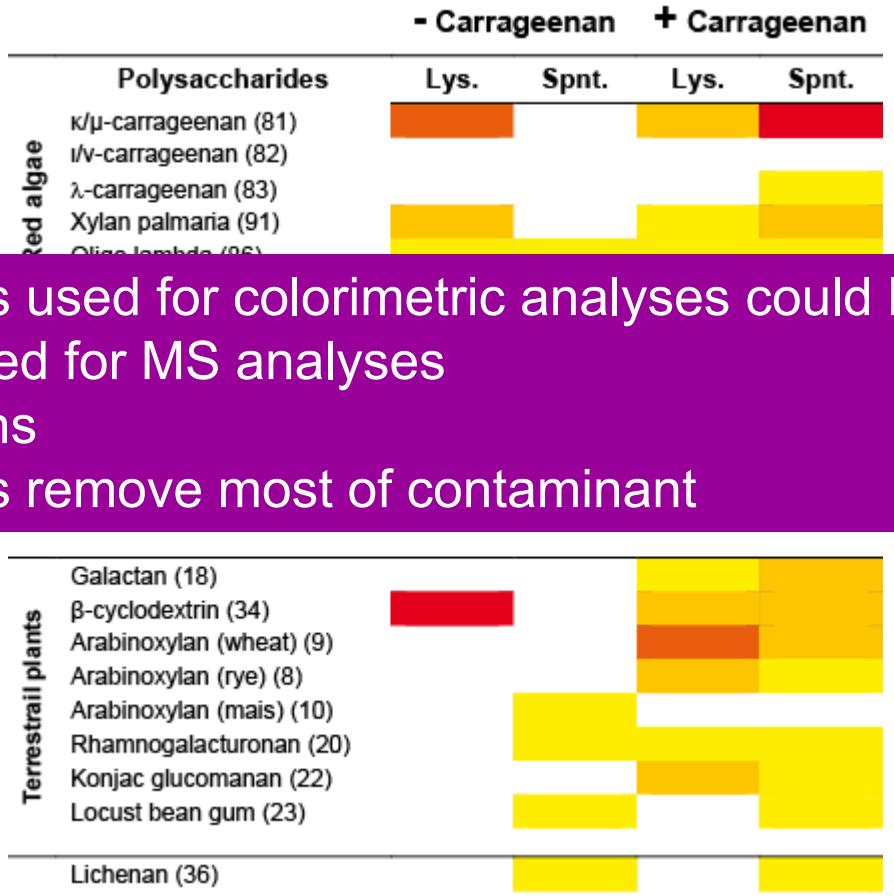


		- Carrageenan		+ Carrageenan	
	Polysaccharides	Lys.	Spnt.	Lys.	Spnt.
Red algae	κ/μ-carrageenan (81)				
	ι/ν-carrageenan (82)				
	λ-carrageenan (83)				
	Xylan palmaria (91)				
	Oligo lambda (86)				
Brown algae	Sodium alginate (LN) (43)				
	Sodium alginate (MP) (42)				
	Sodium alginate (AS) (44)				
	Propylen glycol alginate (45)				
	Mannuronan (46)				
	Laminaran (54)				
Terrestrail plants	Galactan (18)				
	β-cyclodextrin (34)				
	Arabinoxylan (wheat) (9)				
	Arabinoxylan (rye) (8)				
	Arabinoxylan (mais) (10)				
	Rhamnogalacturonan (20)				
	Konjac glucomanan (22)				
	Locust bean gum (23)				
	Lichenan (36)				

***P. carrageenovora* likes red and brown algae**

Phenotyping of Pseudoalteromonas carrageenovora





Screening conditions used for colorimetric analyses could be advantageaously used for MS analyses

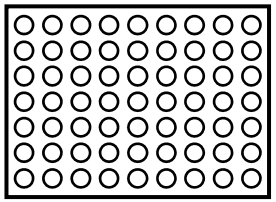
- Low amount of ions
- The filtration steps remove most of contaminant

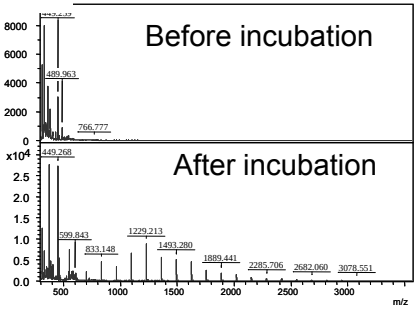
***P. carrageenovora* likes red and brown algae**

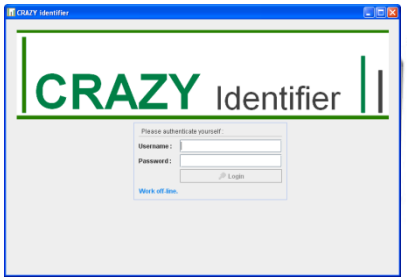
Pipeline for Mass spectrometry analyses

BIA (Nantes)
Marc Lahaye
Hélène Rogniaux
David Ropartz
Pierre.-Edouard Bodet (CDD)
Henry De Soyres (CDD)

LAMBE (Evry)
Régis Daniel
Florence Gonnet
Cedric Przybylski







Screening microtiter plate

Robotized MALDI deposition

Automatized analysis of MALDI TOF spectra

"Crazy identifier" software

Comparison with data bank of oligosaccharides structure

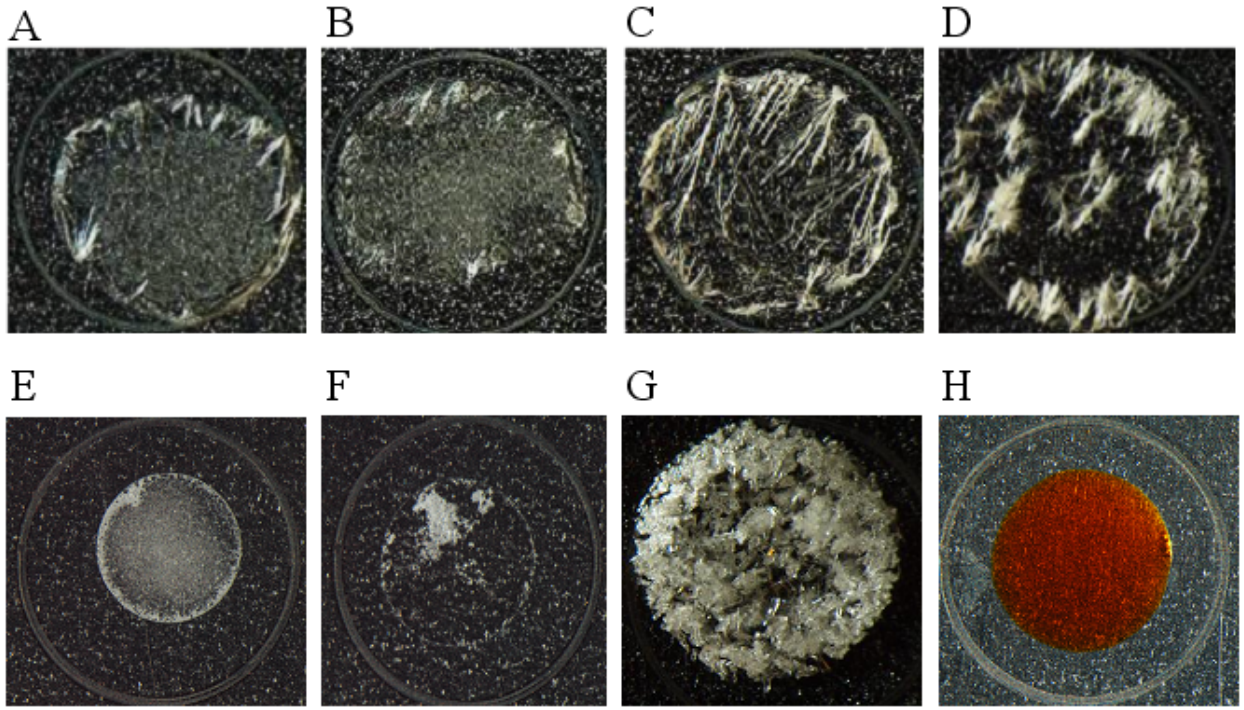
In depth analysis for new degradation products (i.e. new enzymes)

D. Ropartz, et al. (2011) *Rapid Communications in Mass Spectrometry* (2011) 25, 2059-2070

Matrix selection

- 40 matrices were selected based on littérature
- Assayed with neutral, anionic (sulfate, carboxyl acid) and cationic oligosaccharides
- Analyzed in positive and negative modes

Criteria 1: Deposit must be homogenous
Criteria 2: Signal Maldi must be recorded



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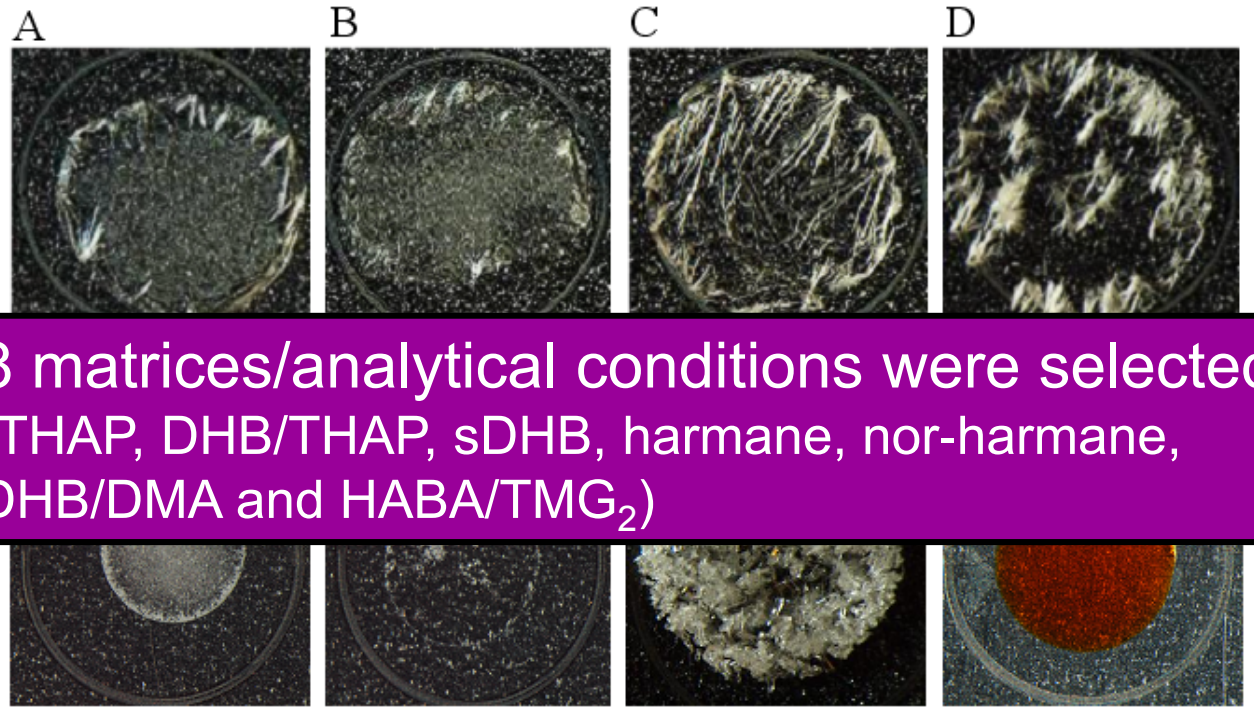


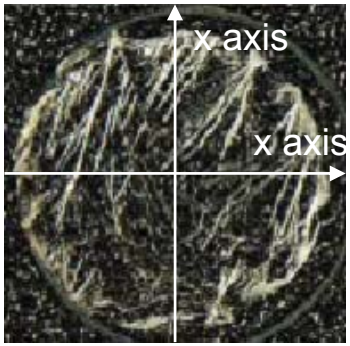
8 matrices/analytical conditions were selected
(THAP, DHB/THAP, sDHB, harmane, nor-harmane, DHB/DMA and HABA/TMG_2)

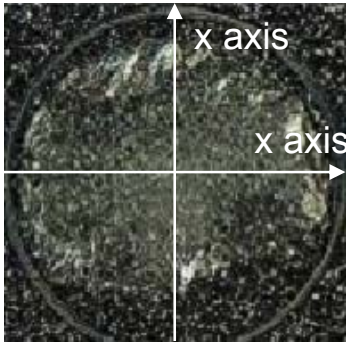
Statistical analyses of deposit

100 spectra MALDI-TOF were recorded on 1 spot by varying coordinates

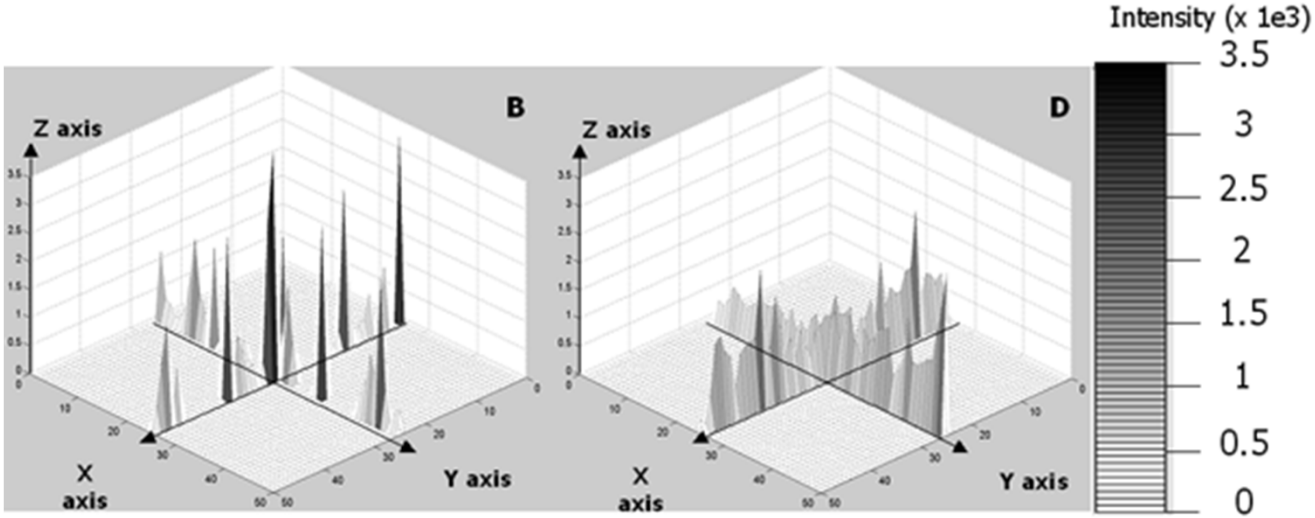
Criteria 3: Reproducibility of mass determination

DHB





DMA/DHB



Statistical analyses of deposit

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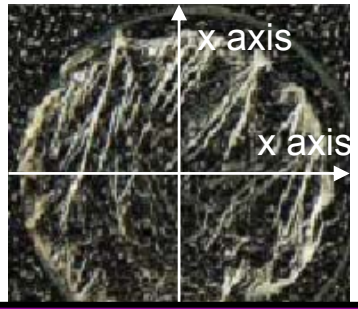
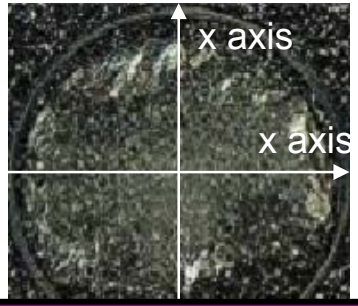
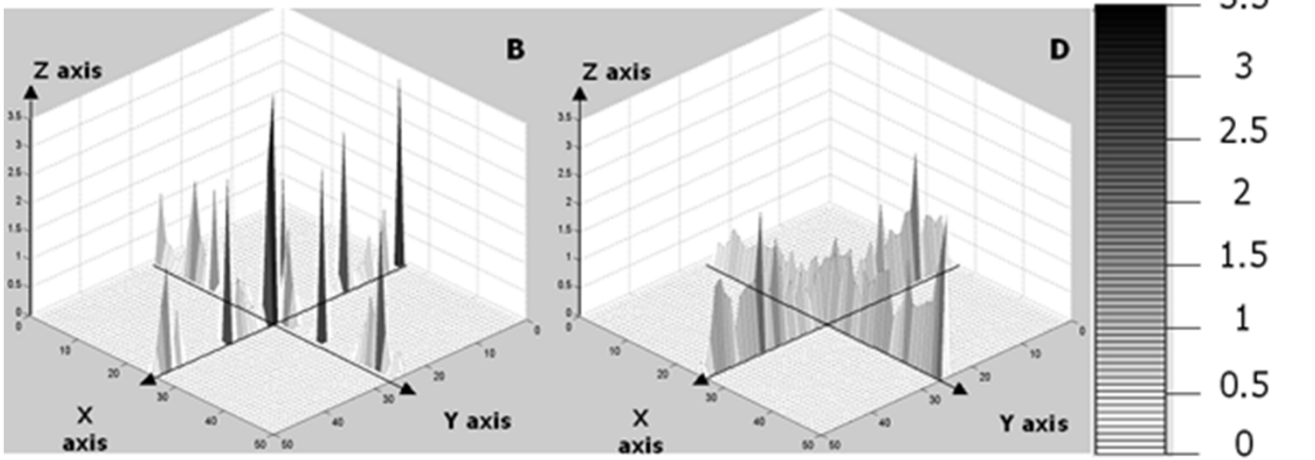


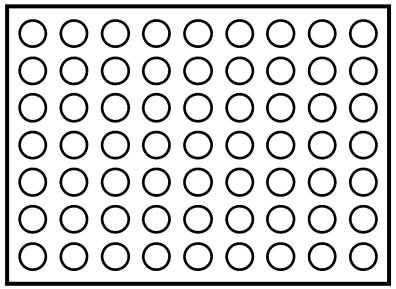


DHB DMA/DHB

4 matrices selected for automatic analyses



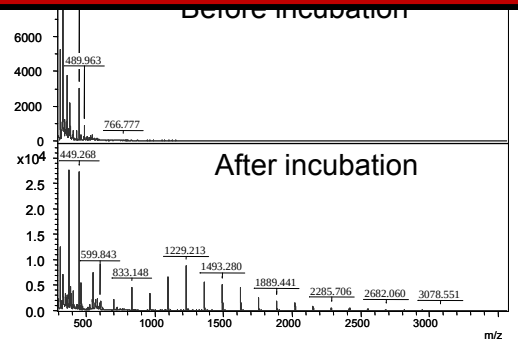
Assayed with incubated microtiter-plate



Microtiter-plate incubation

DMA/DHB (N,N-Dimethylaniline /2,5-dihydroxybenzoic acid) matrix was found the most adapted

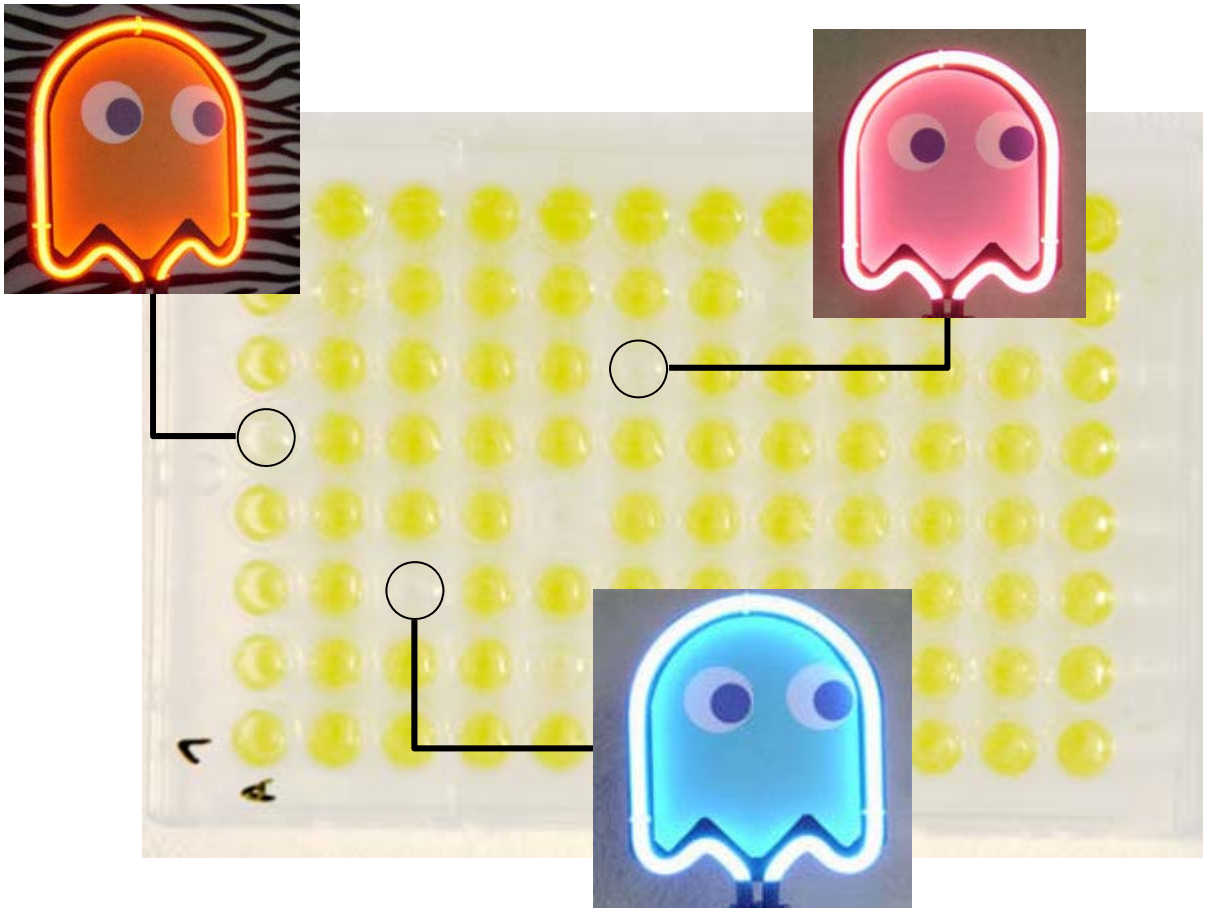
- Reproducible
- Positive and negative modes
- Adapted to the diversity of oligosaccharides



Automatized analysis of MALDI TOF spectra

D. Ropartz, et al. (2011) *Rapid Communications in Mass Spectrometry* (2011) 25, 2059-2070

Input of Mass spectrometry in the discovery of new enzyme activity



Comparison of predicted GH and PL in genome *vs* observed

http://www.cazy.org

HOME | ENZYME CLASSES | ASSOCIATED MODULES | GENOMES

Foreword Archaea **Bacteria** Eukaryota Viruses

Pseudoalteromonas atlantica T6c

Taxonomy ID : 342610

Lineage : cellular organisms ;Bacteria ;Proteobacteria ;Gammaproteobacteria ;Alteromonadales ;Pseudoalteromonadaceae ;Pseudoalteromonas ;Pseudoalteromonas atlantica

Glycoside Hydrolase Family	2	3	5	8	10	13	16	23	24	29	31	32	36	37	42	43	50	63	73	77	85	86	92	94	103	117
Number of sequences	5	4	1	1	1	11	4	4	1	2	2	1	1	1	1	3	3	1	2	1	1	3	4	1	3	2

GlycosylTransferase Family	1	2	4	5	19	26	28	30	35	51	81	83
Number of sequences	1	16	15	1	1	1	1	1	1	2	1	1

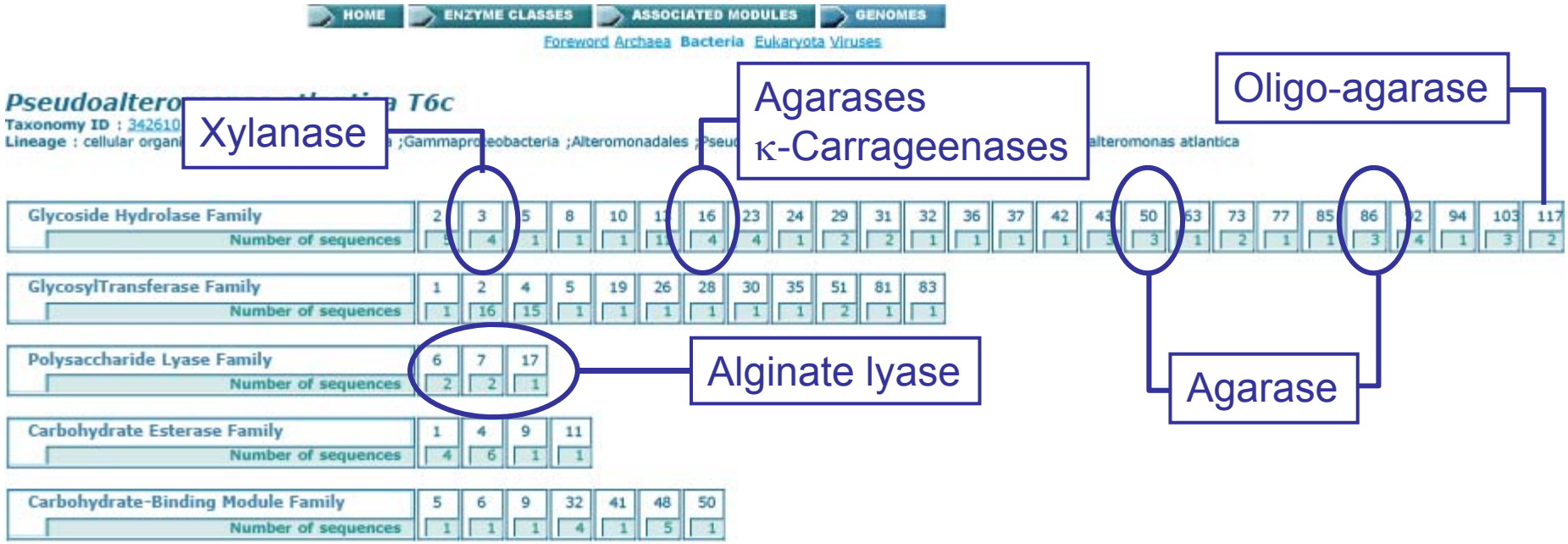
Polysaccharide Lyase Family	6	7	17
Number of sequences	2	2	1

Carbohydrate Esterase Family	1	4	9	11
Number of sequences	4	6	1	1

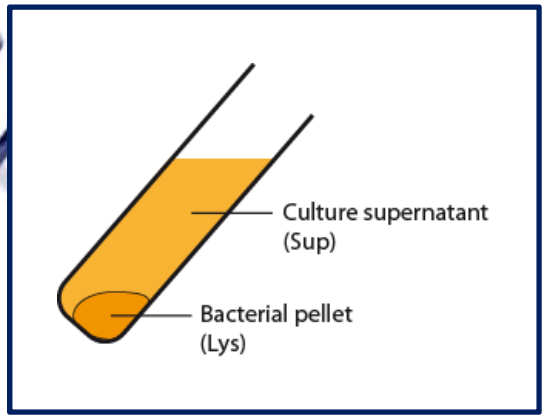
Carbohydrate-Binding Module Family	5	6	9	32	41	48	50
Number of sequences	1	1	1	4	1	5	1

Comparison of predicted GH and PL in genome *vs* observed

http://www.cazy.org



Screening results of *P. atlantica* (pellet)



	Polysaccharides	ZoBell medium	Glucose	Alginate	Pectin	Carrageenan
Red algae	Xylan (Palmaria sp.)					
	Porphyran					
	Agarose					
	Iota-carrageenan					
	Oligo-agarose					
	Oligo-iota-carrageenan					
Terrestrial plants	α,α-trehalose					
	Xylan (beechwood)					
	Arabinoxylan (rye)					
	Arabinoxylan (wheat)					
	Arabinogalactan					
	Amylose					
	CM-cellulose					
	Lichenan					
	Peptidoglycan					
Bacteria	Welan gum					
	Industrial n°1					
	Industrial n°2					
	EPS 657					
	EPS P. atlantica					
Others	Pullulan					
	Fucogalactan					
	Oligo-ulvans					
pNP	β-D-galactopyranoside					
	β-D-fucopyranoside					
	β-D-xylopyranoside					
	β-Dmannopyranoside					
	β-L-arabinopyranoside					
	β-D-glucopyranoside					
	2-acetamido-2-deoxy-β-D-glucopyranoside					
	α-D galactopyranoside					
	α-L-arabinopyranoside					
	α-L-arabinofuranoside					
	α-D-maltopyranoside					
	α-D-glucopyranoside					
	α-Dmannopyranoside					
	α-D-xylopyranoside					

Screening results of *P. atlantica*

Detected activities	Expected activities	nb Atl	Family
Porphyran	Porphryranase	3	GH16
agarose	Endo agarase	1	GH16
	exo agarase	3	GH50
	agarase	3	GH86
Oligo agarose	α-1,3-L-neoagarooligosaccharide hydrolase	2	GH117
iota nu , oligos iota	iotase	1	GH86
Xylan palmaria, xylan beechwood	endo-1,4-βxylanase	1	GH8
arabinoxylan (wheat, rye, mais)		1	GH10
α,α-trehalose	α,α-trehalase	1	GH37
CM-cellulose, acetate de cellulose	cellulase	1	GH5
Lichenan	glucanase	1	GH8
pullulan	pullulanase	1	GH13
amylose	amylase	7	GH13
peptidoglycan, pNP-GlcNac	β-N-acetylhexosaminidase lytic transglycosylase	2,1,4,2,1,3	GH3, GH224,GH23, GH73, GH85,GH103
b-D glactopyranoside	β-galactosidase	4	GH2
		1	GH42
b-D-mannopyranoside	β-mannosidase	1	GH2
b-D-xylopyranoside b-D-glucopyranoside	β-glucosidase/xylanase	2	GH3
a-D galactopyranoside	α-galactosidase	1	GH36
a-L-arabinofuranoside	α-L-arabinofuranosidase	1	GH43
a-D-maltopyranoside	malto-oligosyltrehalose trehalohydrolase	7	GH13
a-D-glucopyranoside	α-glucosidase/xylosidase	2	GH31
a-D-xylopyranoside	xylosidase	1	GH43
a-D-mannopyranoside	α-1,2-mannosidase	4	GH92

Screening results of *P. atlantica*

Detected activities	Expected activities	nb Atl	Family
Porphyran	Porphryranase	3	GH16
	Endo agarase	1	GH16
agarose	exo agarase	3	GH50
Xylan arab			
CM-c			
pe			GH23, H103
a-D-glucopyranoside	α-glucosidase/xylosidase	2	GH31
a-D-xylopyranoside	xylosidase	1	GH43
a-D-mannopyranoside	α-1,2-mannosidase	4	GH92

Summary of the screening

1) ~ 80 % of the predicted activities were detected

2) Some predicted enzymes have new modes of action (ι-carrageenase, Me-porphyranase)

3) Some predicted enzymes were not observed (5 alginate lyases)

4) Unpredicted enzymes

Conclusion

The screening method is now implemented

Profile of GH and PL in complex extracts (physiology, strains, microbiomes, …)

Comparison between predicted from genome and observed GH/PL

Ascribe function to putative polysaccharides degrading enzymes

Discovery of new enzyme activities

Production of new oligosaccharides series

New technological challenge

Improve polysaccharides collection require partnerships with academic but with companies

- Discoveries are correlated to the richness of the collection
- We must challenge polysaccharides with high interest

Next technical challenges: more integrated screening

- One site for screening *(from incubation to MS analysis)*
- Pursue the development of « CRAZY Identifier »
  (improve the beta-version, integrate in MS software)
- Improve automation of the micro-plate preparation

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Performance evaluation on a wide set of matrix-assisted laser desorption ionization matrices for the detection of oligosaccharides in a high-throughput mass spectrometric screening of carbohydrate depolymerizing enzymes

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Medium-throughput profiling method for screening polysaccharide-degrading enzymes in complex bacterial extracts

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ABSTRACT

Polysaccharides are the most abundant and the most diverse renewable materials found on earth. Due to the stereochemical variability of carbohydrates, polysaccharide-degrading enzymes – *i.e.* glycoside hydrolases and polysaccharide lyases – are essential tools for resolving the structure of these complex macromolecules. The exponential increase of genomic and metagenomic data contrasts sharply with the low number of