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INRAE – AMU

BBF lab, Luminy, Marseille, France



Enzyme-based valorization of agricultural by products for biotechnological applications



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Higher education



www2.isep.ipp.pt/agrima

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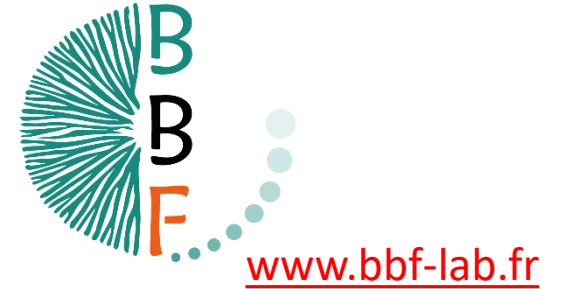
BIODIVERSITÉ ET BIOTECHNOLOGIE FONGIQUES (BBF)

INRAE



amU

Aix Marseille Université



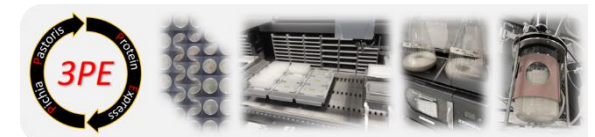
23 Permanent staff

12 Scientists/MCF/Prof.

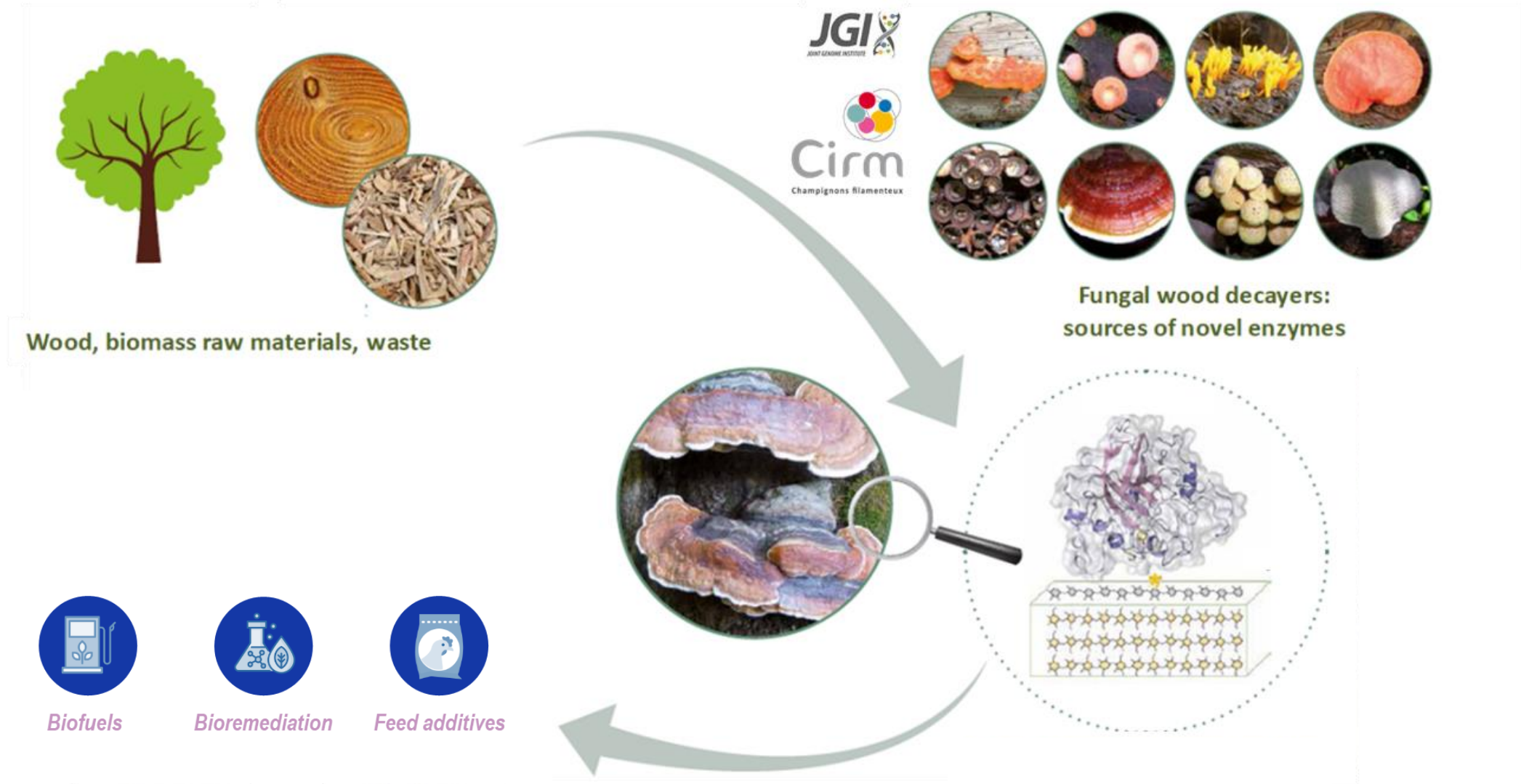
11 Technicians/engineers

25 PhD, Post-docs, CDD

2 platforms



Valorization of lignocellulosic biomass



Agricultural by-products: composition and structure

Agricultural and forest industry by-products, dedicated crops



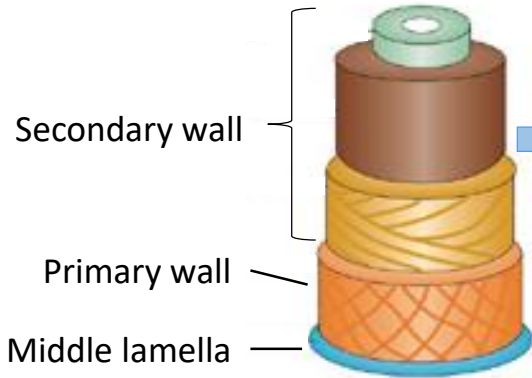
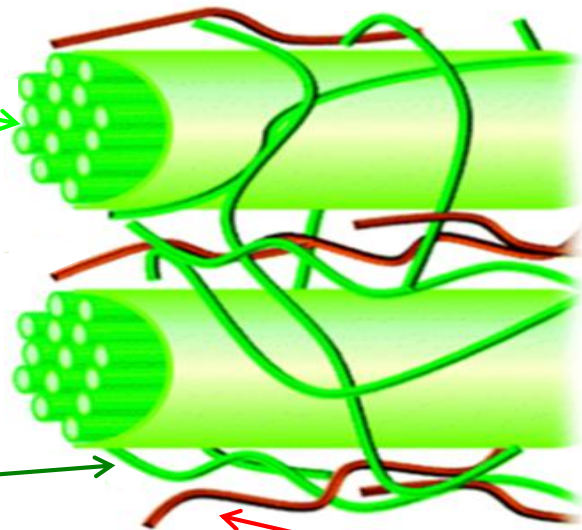
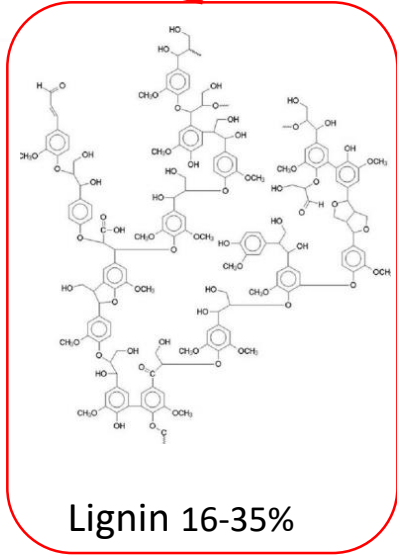
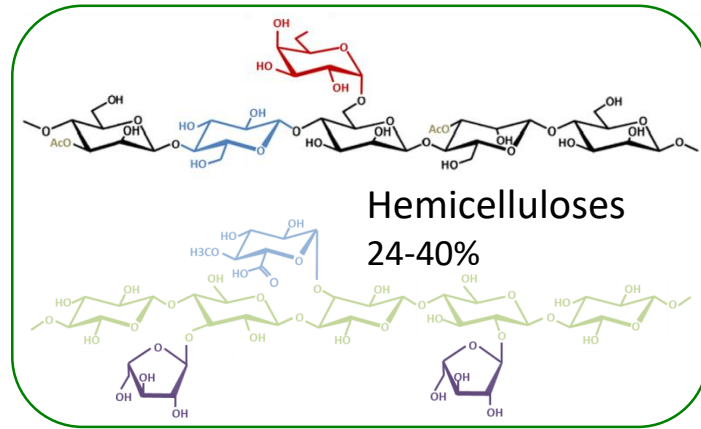
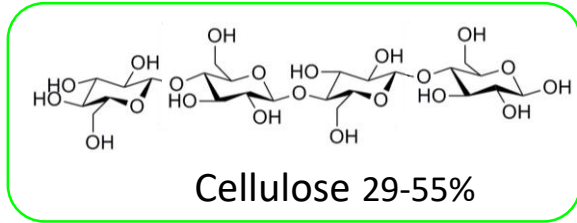
Short rotation coppice



Miscanthus

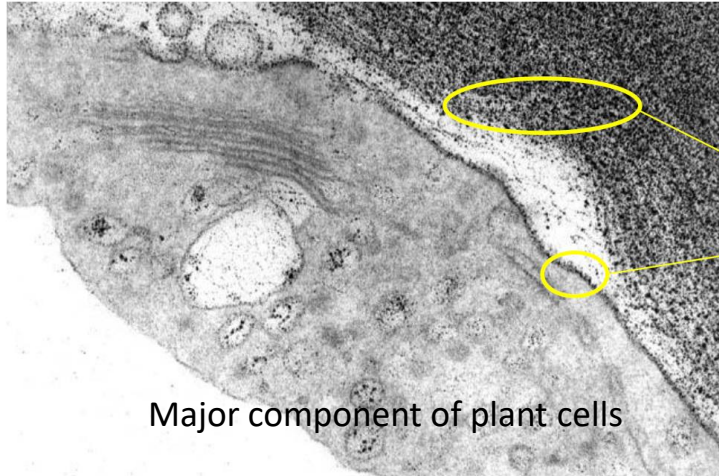


Cereal by-products



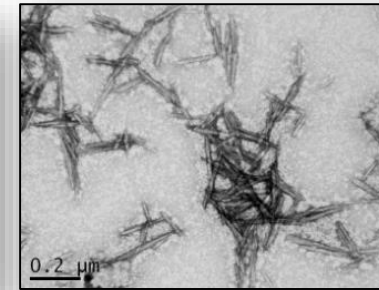
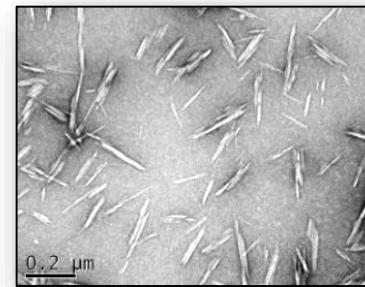
Cellulose

- Most abundant organic resource on earth : 50-100 billions of tons /year



Cellulose

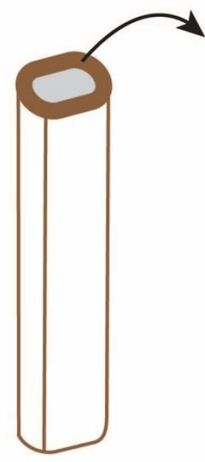
- Resistant to degradation



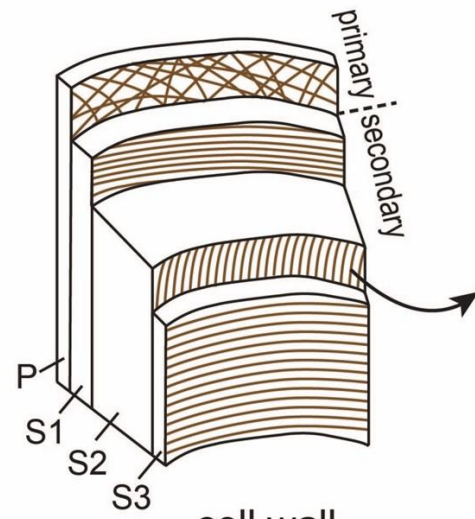
Cellulose Structure



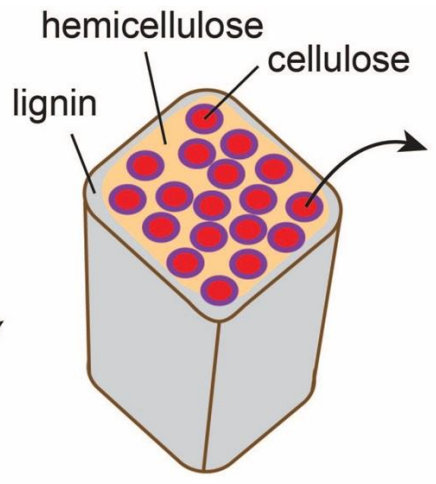
tree



longitudinal cell



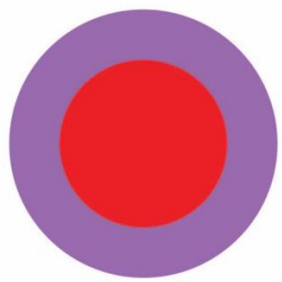
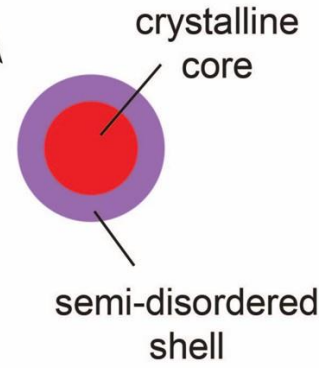
cell wall



macrofibril
(20-40 nm)

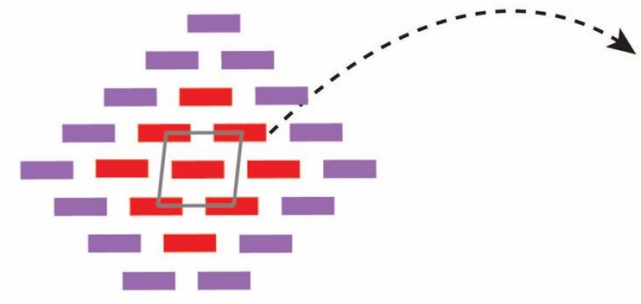


cellulose microfibril
(~3 nm)

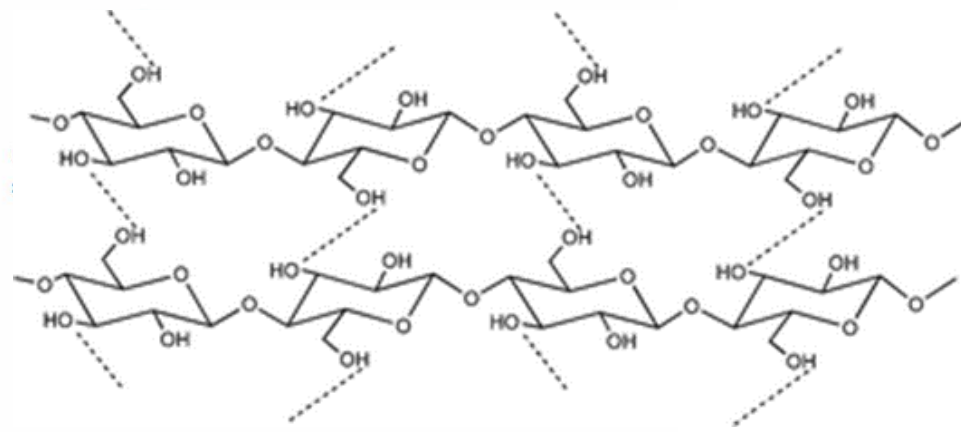


cellulose microfibril

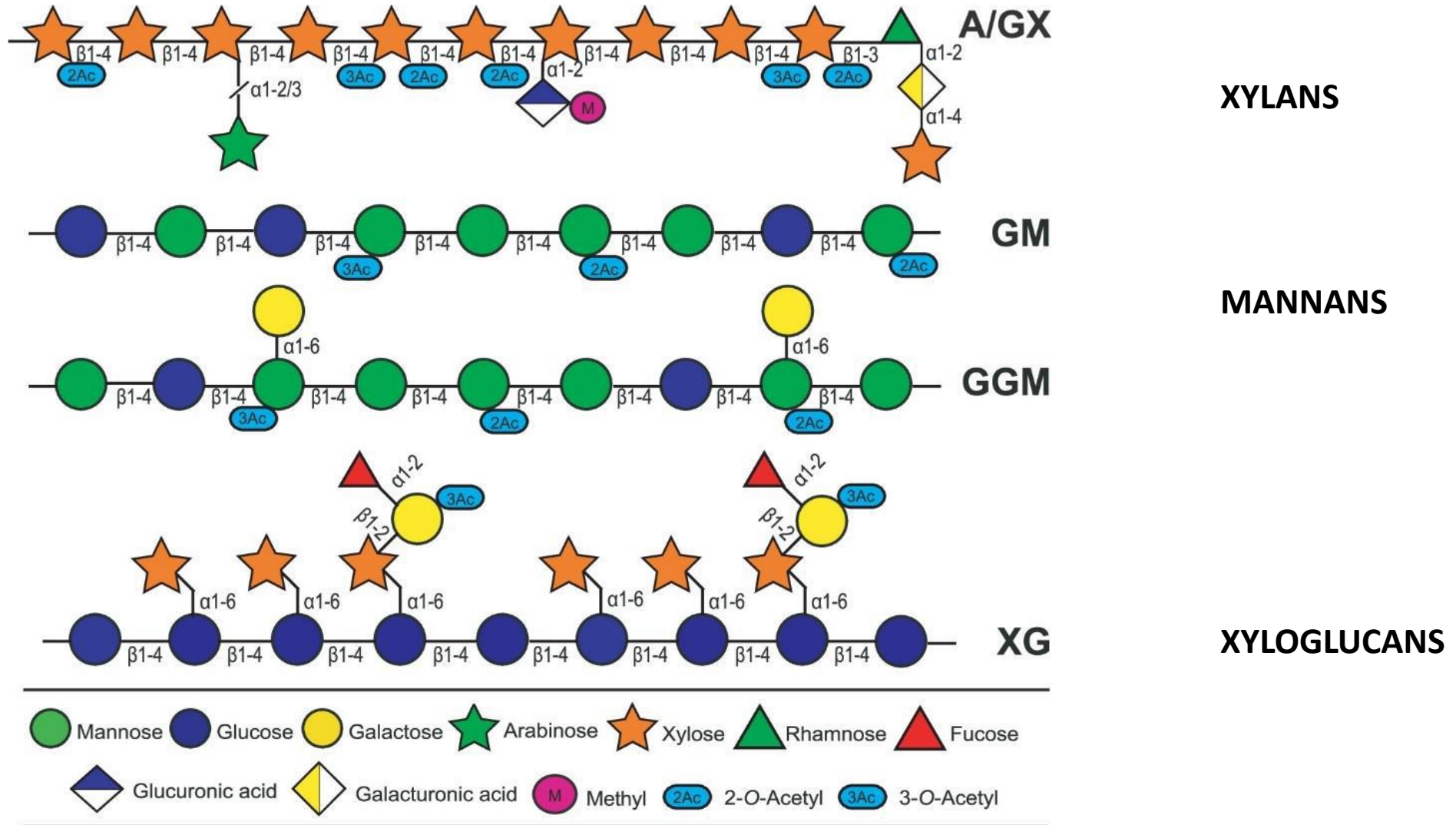
24 chains



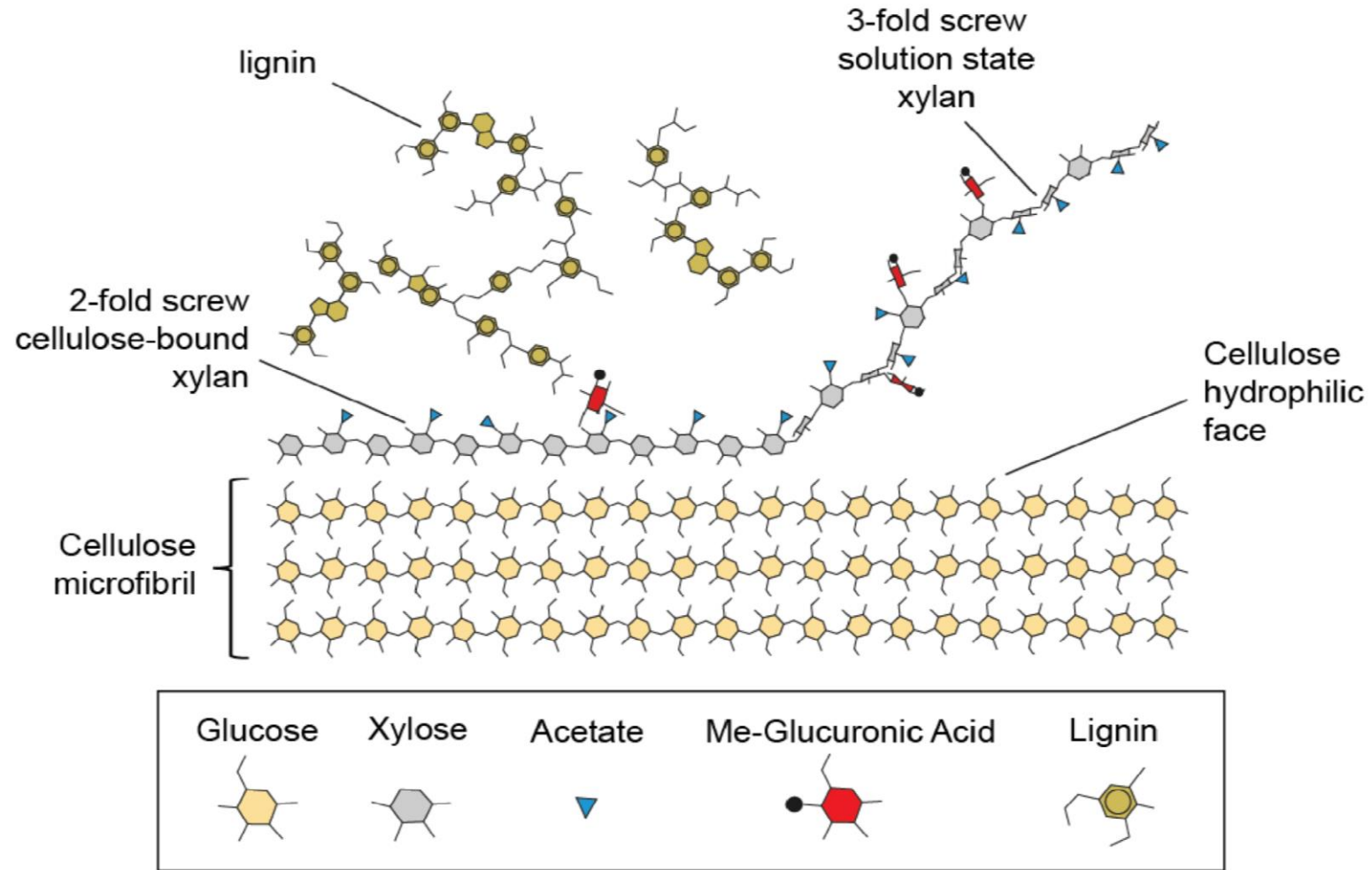
crystalline chain ■
semi-disordered chain ■



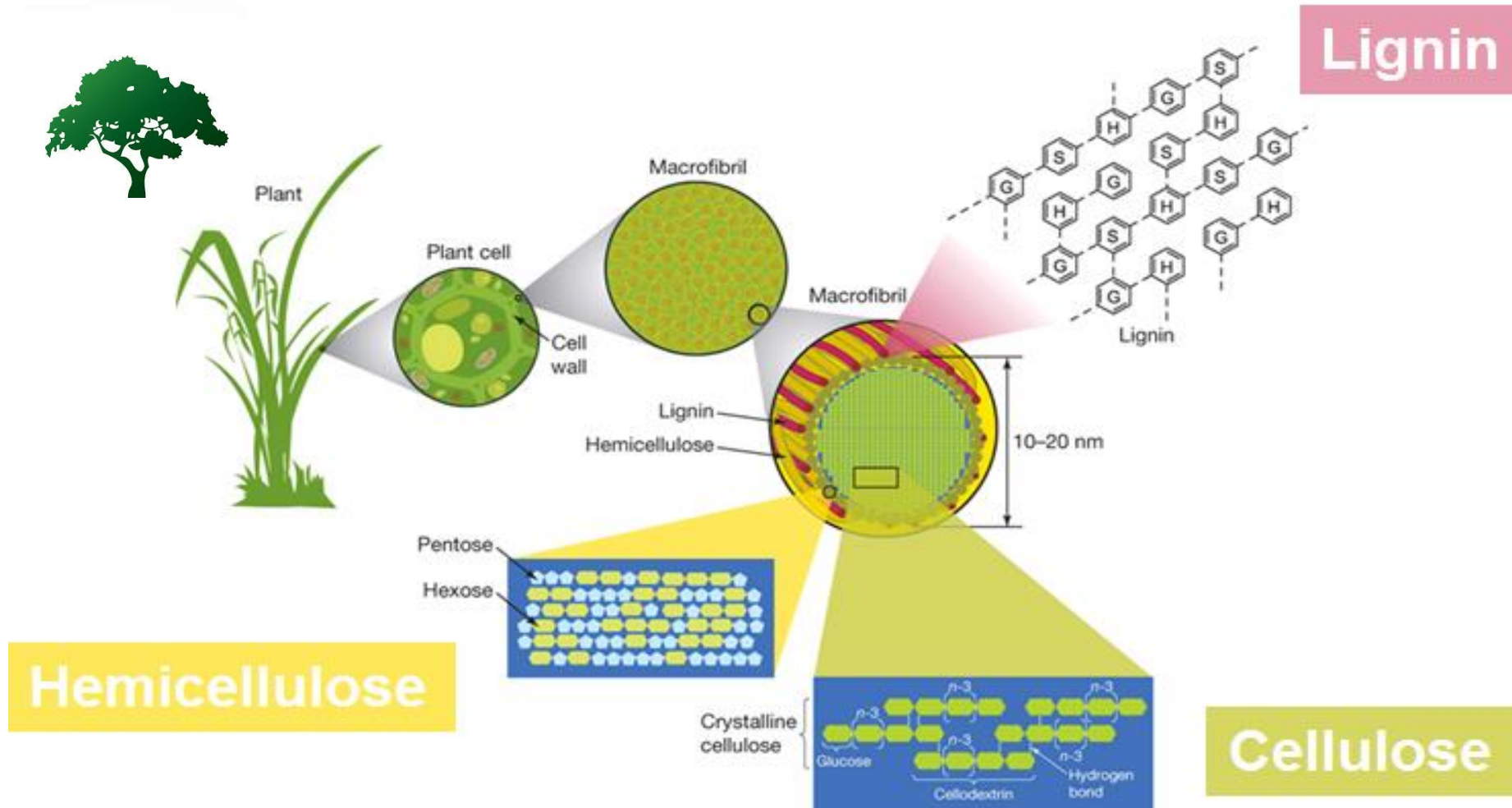
Hemicelluloses



Cellulose/Hemicelluloses interaction



Lignocelluloses are highly recalcitrant to degradation



In nature, fungi are potent degraders of biomass



Hypocrea jecorina
(an. *Trichoderma reesei*)



P. chrysosporium on wood
white rot fungi



Ustilago maydis
parasitizing maize

Fungi:
Large diversity in
enzymes



Aspergillus sporulating
on sorghum



Fusarium-infected corn cob

Plant cell-walls:
Large diversity in
composition

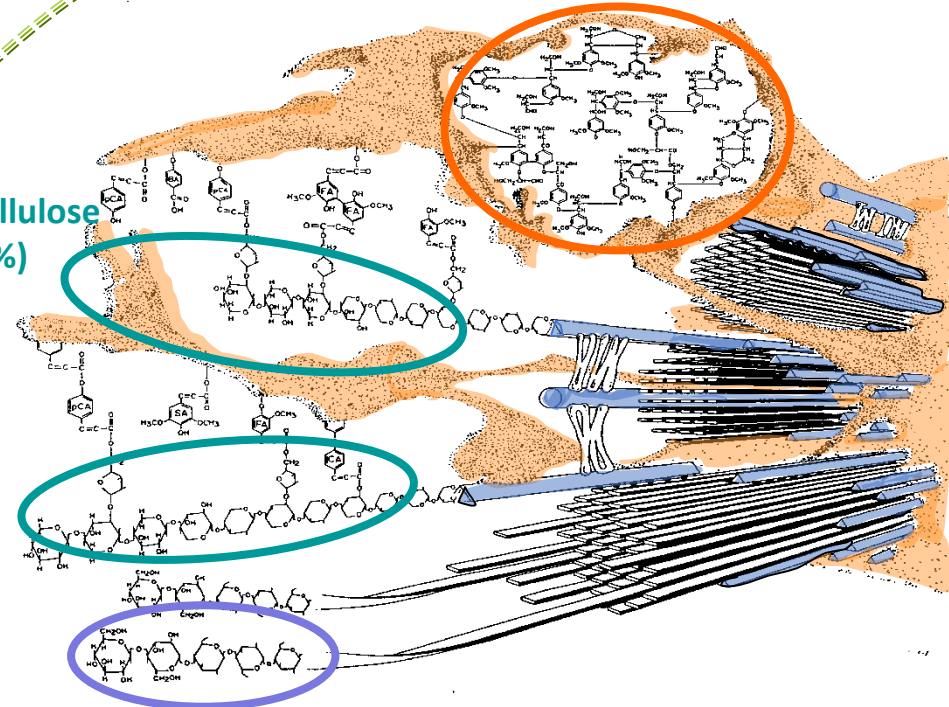


Fusarium-infected corn stalk

Hemicellulose
(23-32 %)

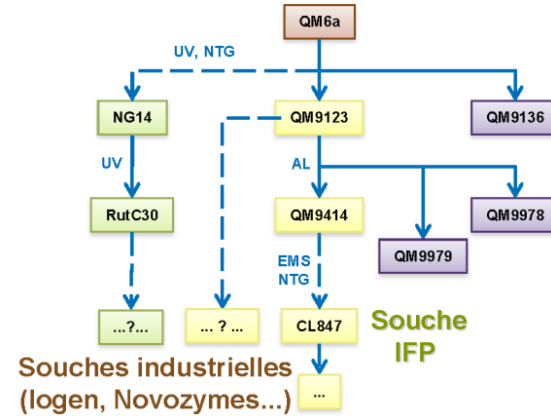
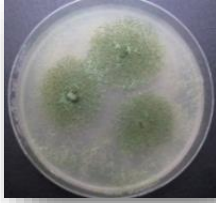
Lignin (15-25 %)

Cellulose
(38-50 %)

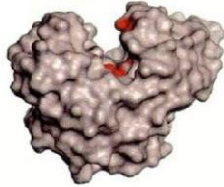


Trichoderma reesei for biomass degradation

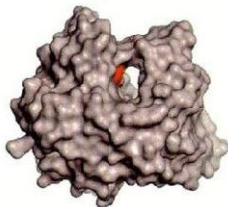
- *Trichoderma reesei*: cellulase producer (100 g/l)



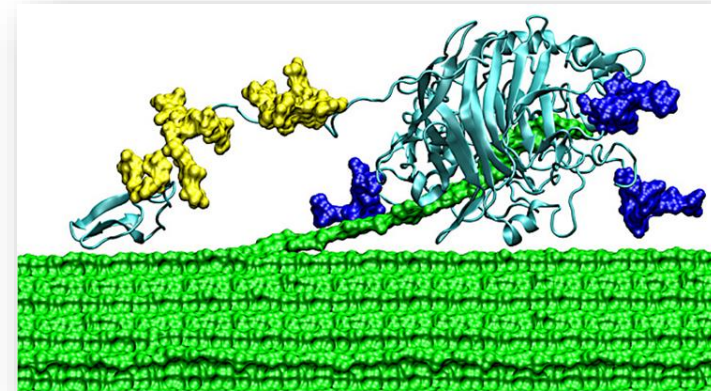
- Rich in cellulases



7 endoglucanases

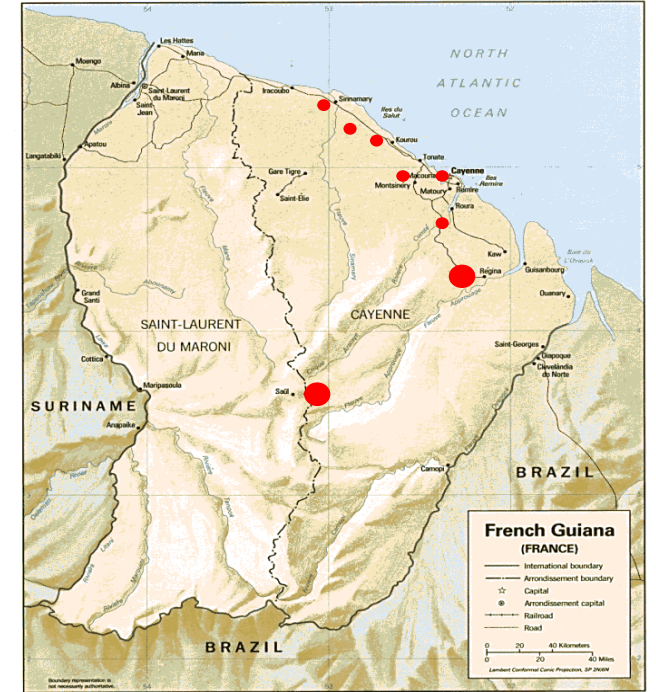


2 cellobiohydrolases



Cel7A (NREL)

INRAE Fungal collection



Lignocellulolytic filamentous fungi (> 3500 strains)



Fomitopsis pinicola



Pycnoporus sanguineus



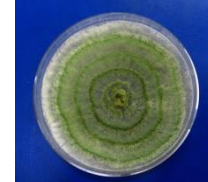
Leiotrametes sp.



Trichoderma lycogaloides



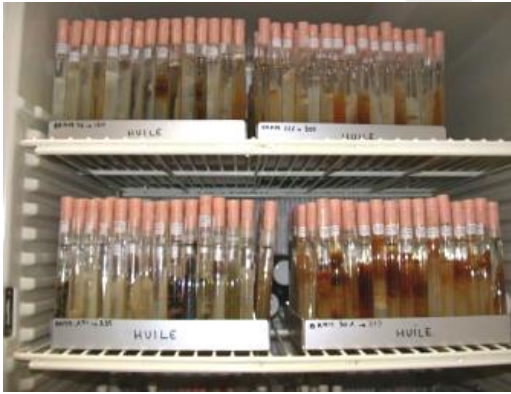
Xylaria sp.



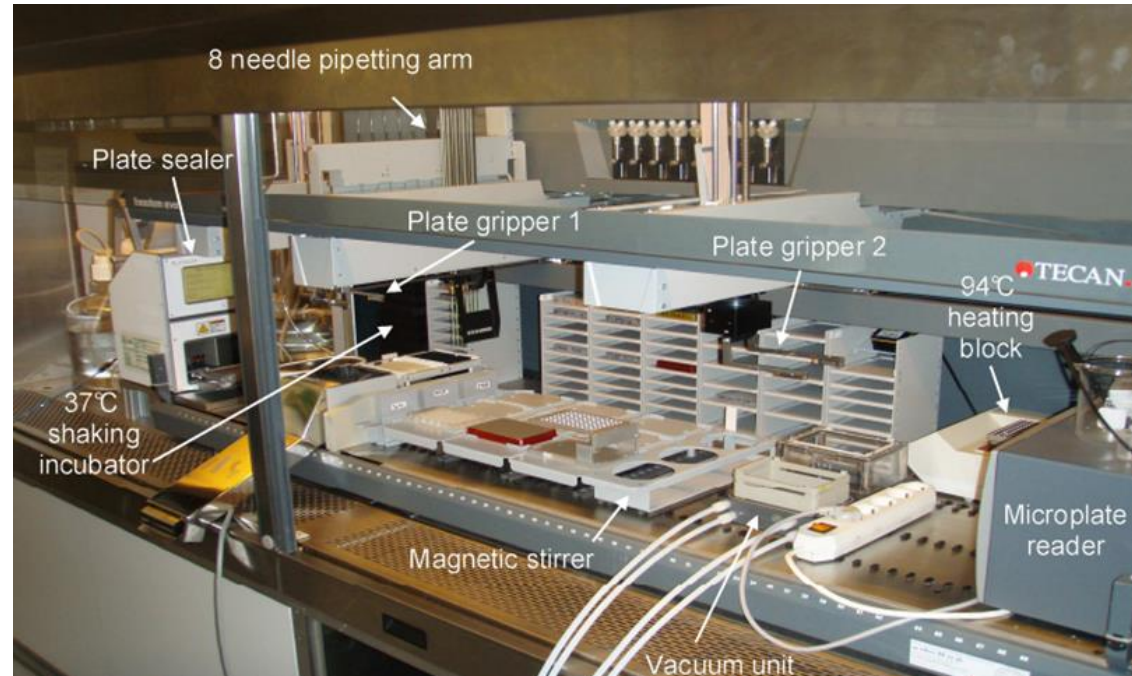
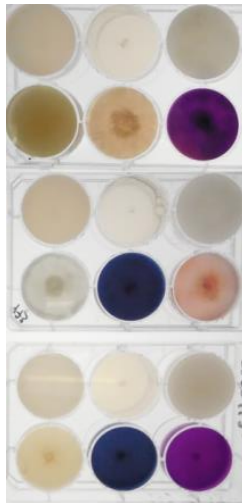
Trichoderma reesei



Exploring fungal biodiversity



- Biomass
- Recalcitrant polysaccharides
- Lignin
- Dyes
- Plastic
- ...



Pipeline of enzyme discovery

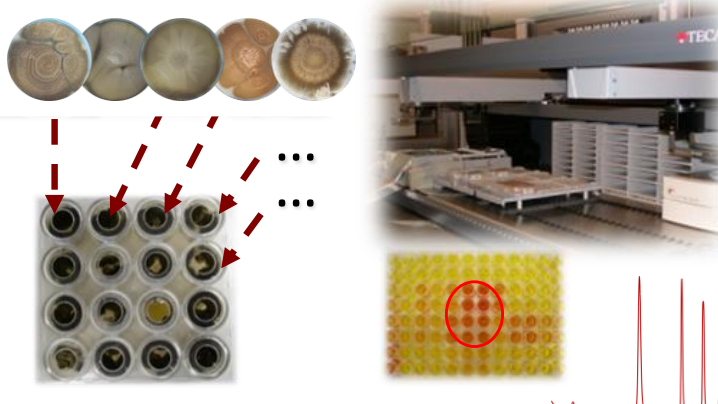


INRAE

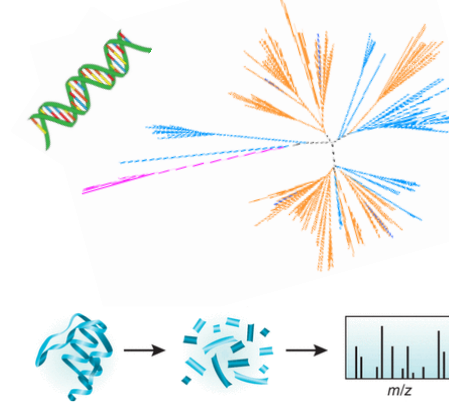
CIRM-CF
Champignons Filamenteux

> 3,000 fungal strains

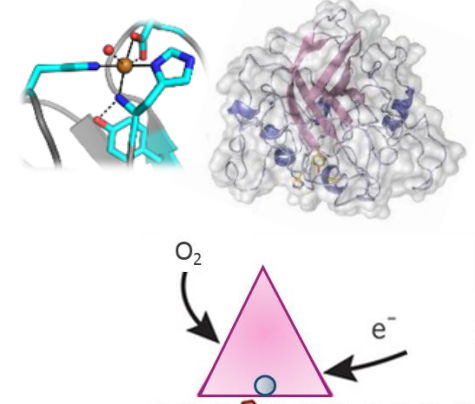
CAZY
Carbohydrate-Active Zymes



Development of high-throughput methods



Fungal -omics



Enzyme discovery

+ Bioinformatics
CAZy team



Classification of Carbohydrate-active enzymes

CAZy (www.cazy.org)



HOME ENZYME CLASSES ASSOCIATED MODULES GENOMES

Glycoside Hydrolases GlycosylTransferases Polysaccharide Lyases Carbohydrate Esterases Auxiliary Activities

GH1 Go Family Go

Glycoside Hydrolase family classification

Tables for Direct Access

► GH Family Number

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120
121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136																								

[Non-Classified Sequences](#)



HOME ENZYME CLASSES ASSOCIATED MODULES GENOMES

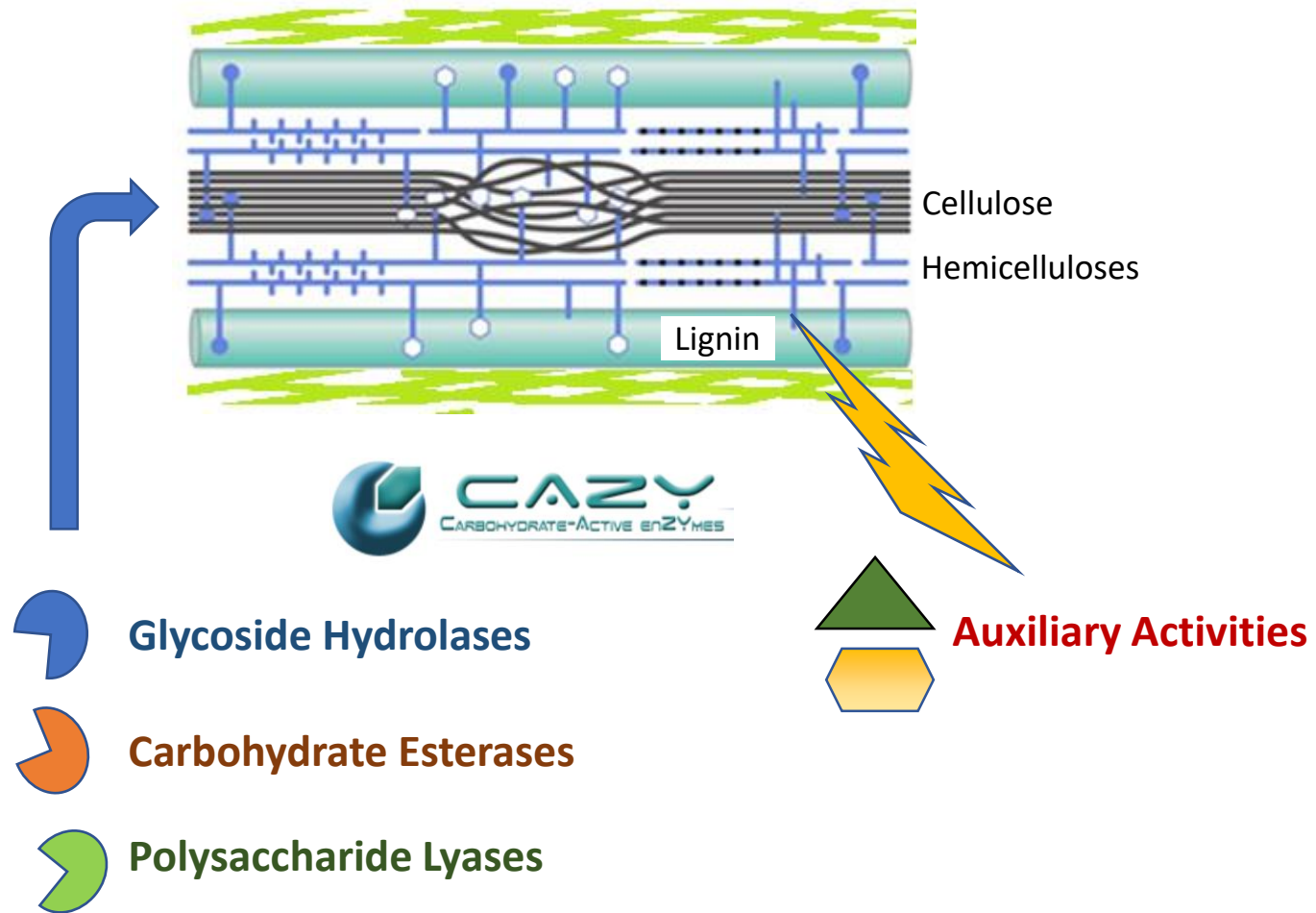
Glycoside Hydrolases GlycosylTransferases Polysaccharide Lyases Carbohydrate Esterases Auxiliary Activities

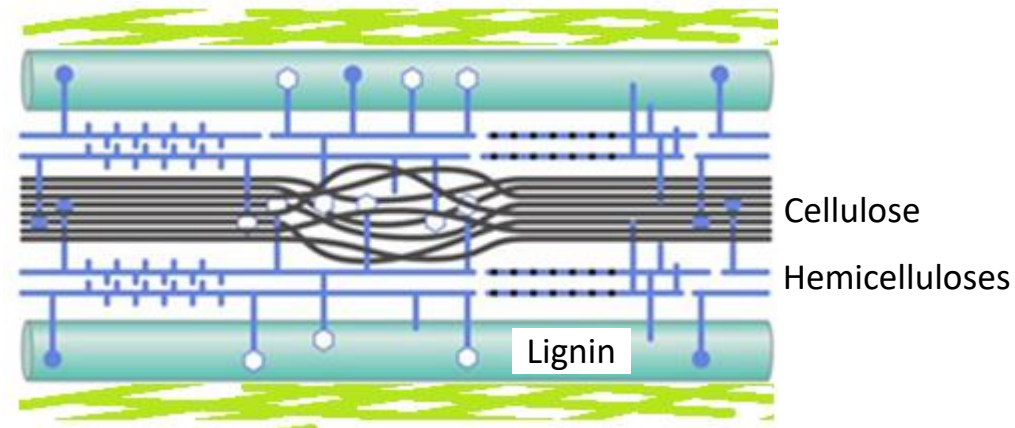
GH7 Go Family Go

Glycoside Hydrolase Family 7

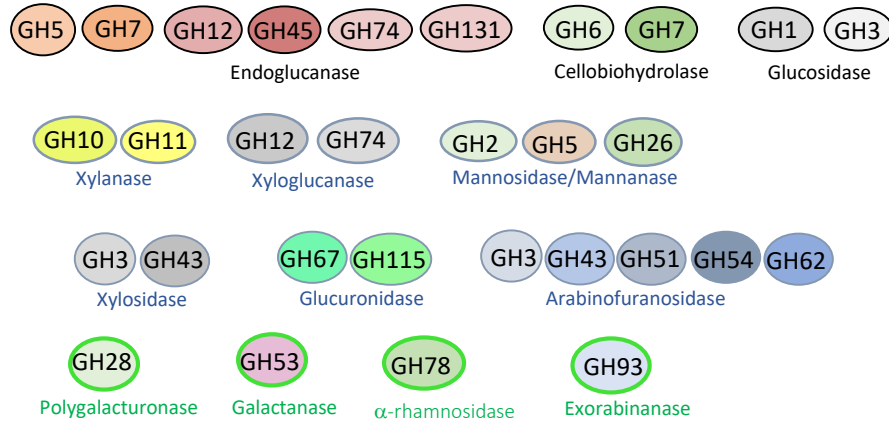
Known Activities	endo- β -1,4-glucanase (EC 3.2.1.4); reducing end-acting cellobiohydrolase (EC 3.2.1.176); chitosanase (EC 3.2.1.132); endo- β -1,3-1,4-glucanase (EC 3.2.1.73)
Mechanism	Retaining
Clan	GH-B
3D Structure Status	β -jelly roll
Catalytic Nucleophile/Base	Glu (experimental)
Catalytic Proton Donor	Glu (experimental)
Note	formerly known as cellulase family C. The cellobiohydrolases of this family act processively from the reducing ends of cellulose chains to generate cellobiose. This is markedly different from the IUBMB definition of cellobiohydrolases (EC 3.2.1.91), which act from the non-reducing ends of cellulose.
External resources	CAZypedia ; HOMSTRAD ; PRINTS ;
Commercial Enzyme Provider(s)	MEGAZYME ;
Statistics	GenBank accession (10547); Uniprot accession (528); PDB accession (83); 3D entries (17); cryst (0)
Summary	All (5082) Bacteria (10) Eukaryota (5068) unclassified (4) Structure (17) Characterized (84)

Fungal deconstruction of lignocellulose

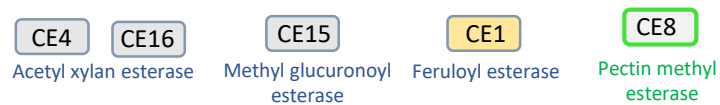




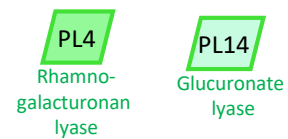
Glycoside Hydrolases



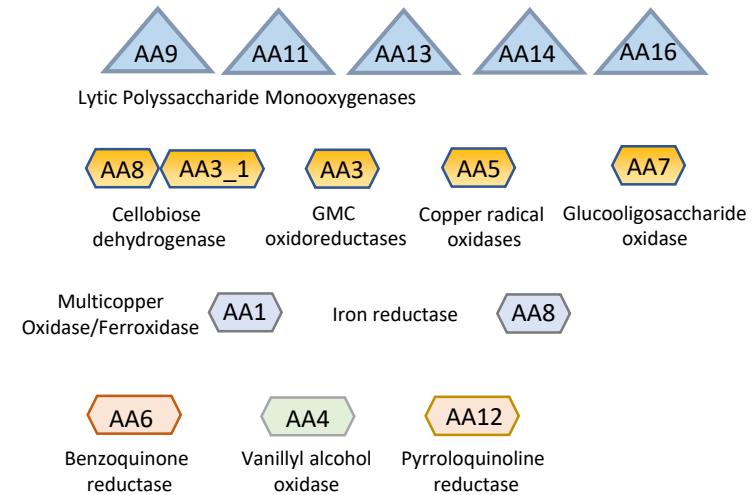
Carbohydrate Esterases



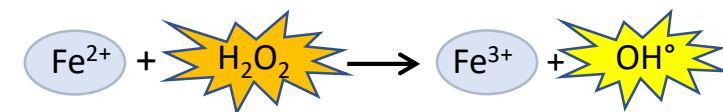
Polysaccharide lyases

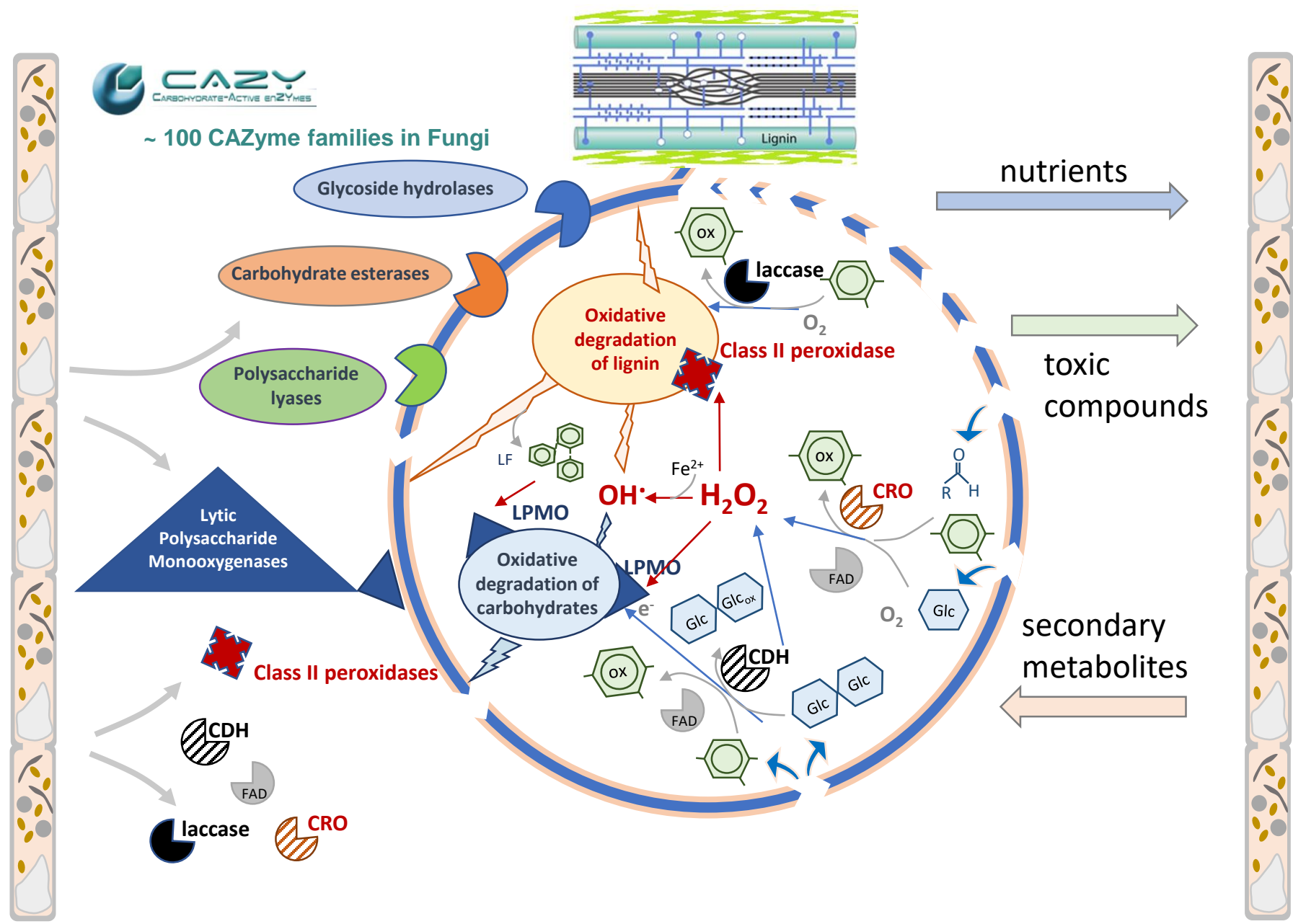


Auxiliary Activity Enzymes



Fenton reaction

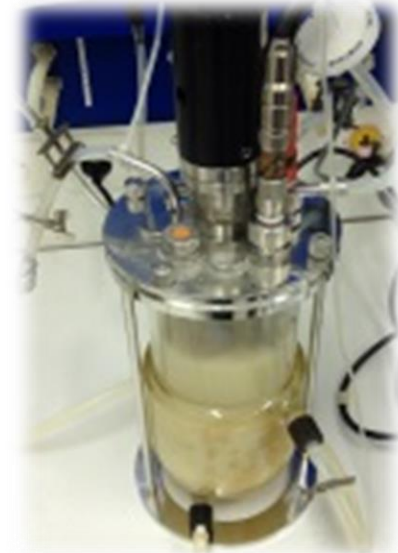
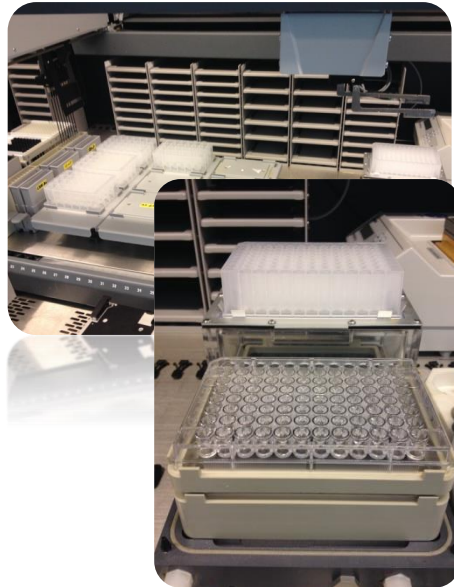
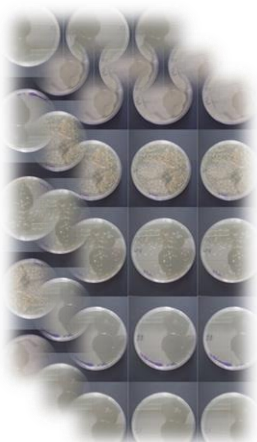
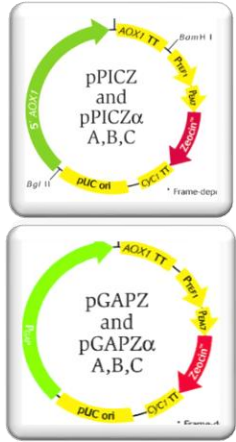




Recombinant production of Fungal enzymes



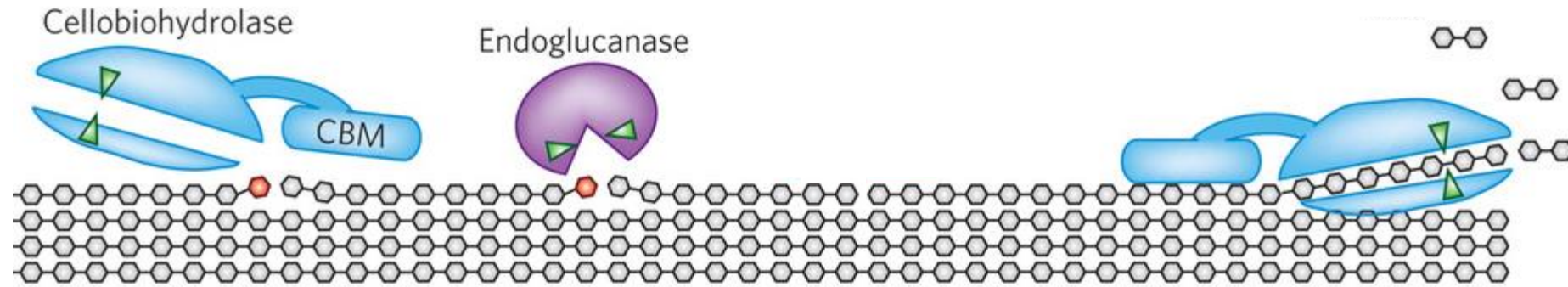
Pichia pastoris



Enzyme production at different scales: up to several grams in bioreactors

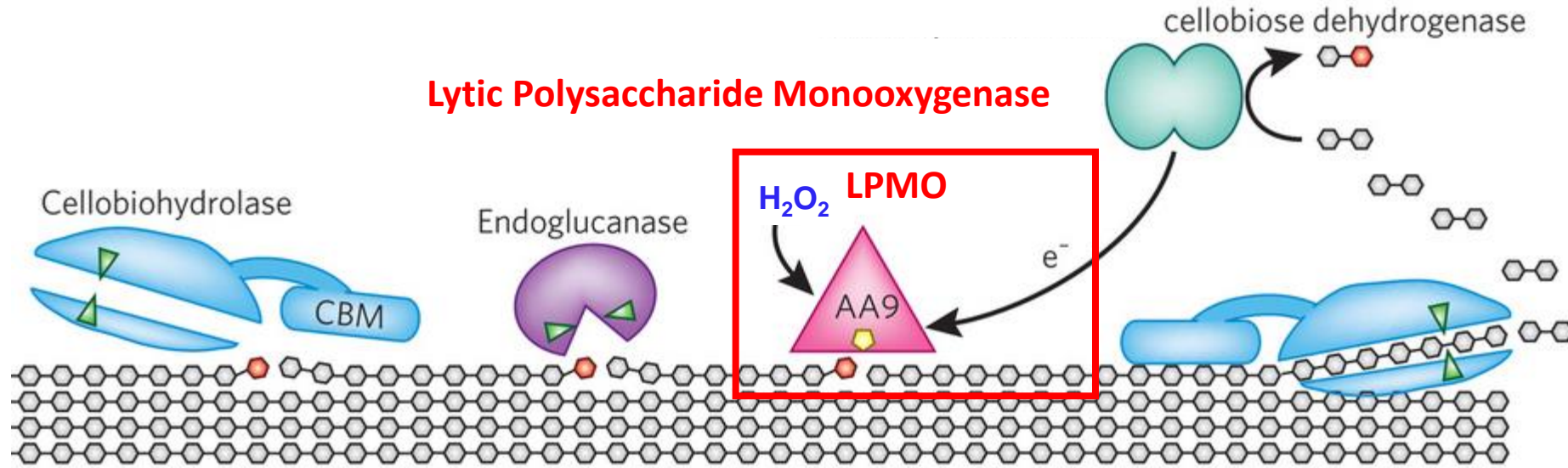


Enzymatic degradation of cellulose (before 2010)

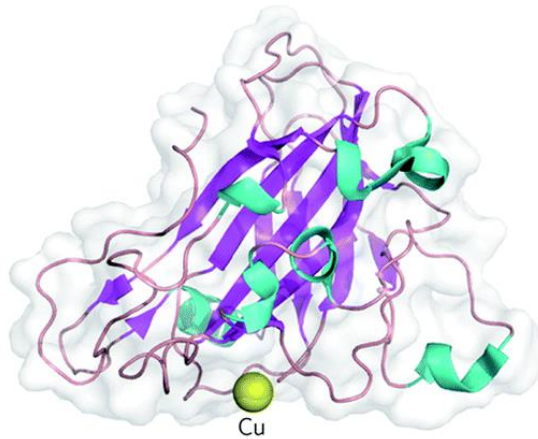


Fushinobu, Nature Chemical Biology (2014)

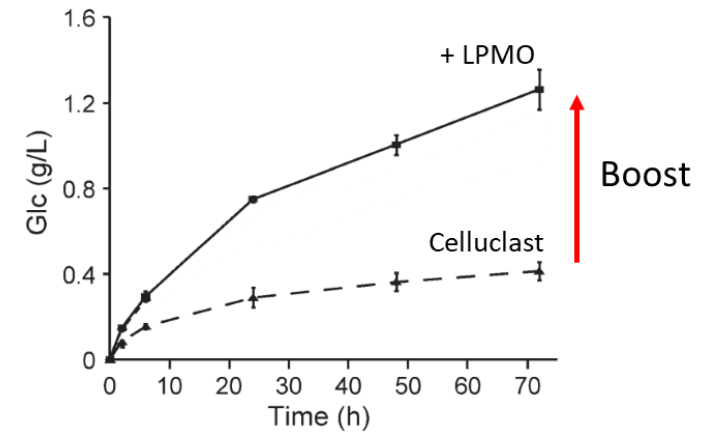
Enzymatic degradation of cellulose (after 2010)



Fushinobu, Nature Chemical Biology (2014)

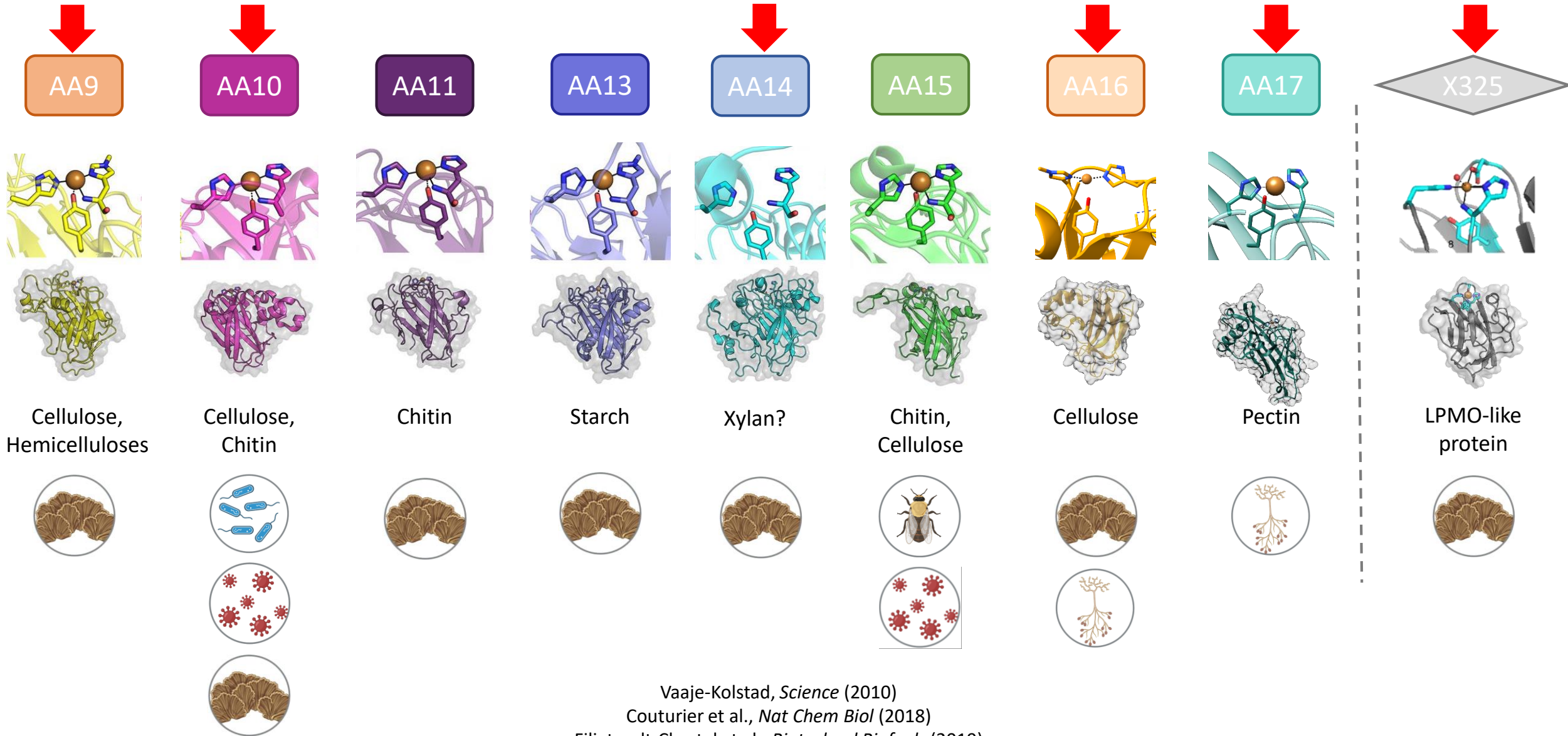


SACCHARIFICATION BOOST



Harris et al., Biochemistry (2010)
Vaaje-Kolstad et al., Science (2010)
Quinlan et al., PNAS (2011)

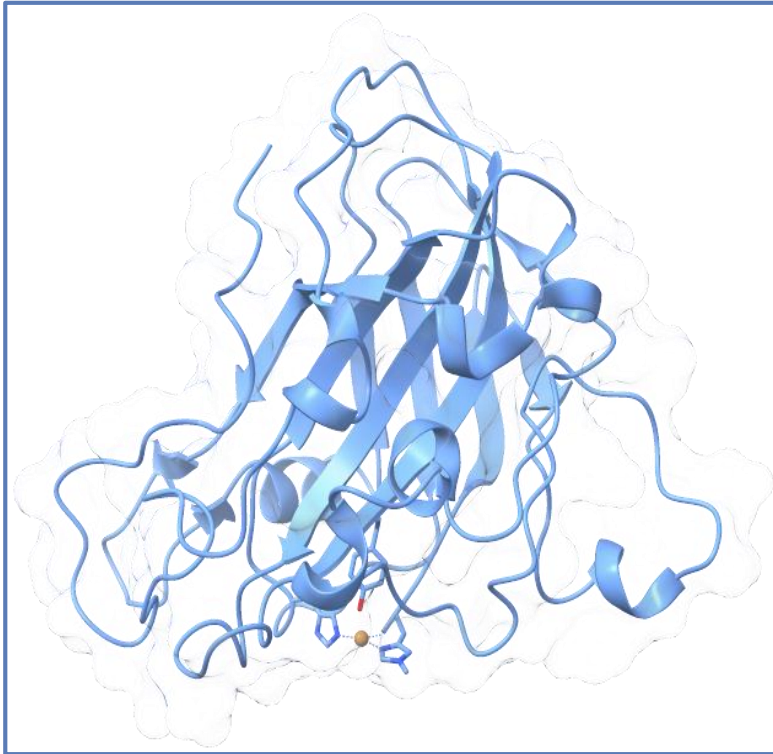
Lytic polysaccharide Monooxygenases (LPMOs)



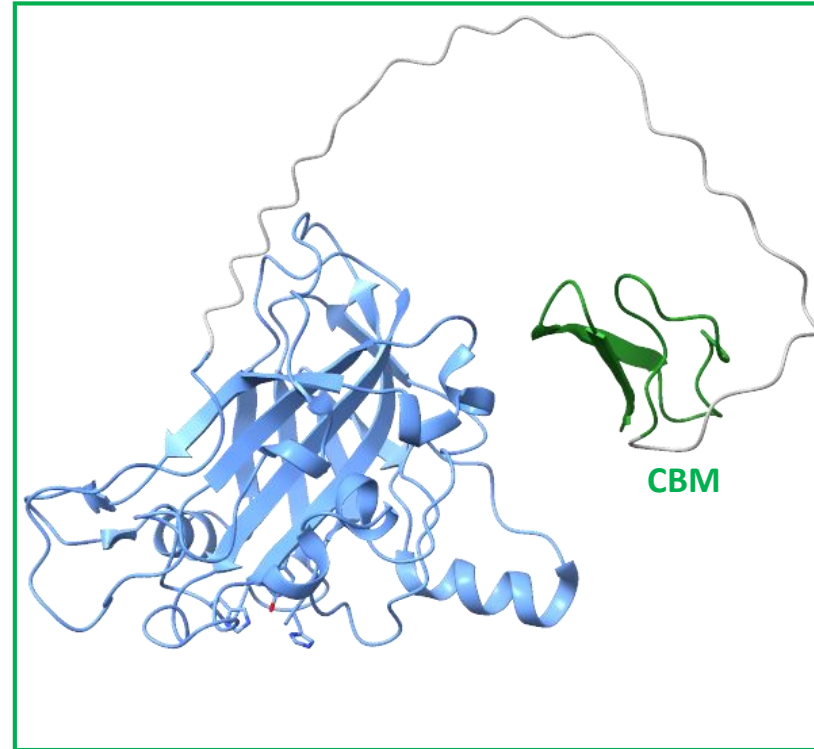
Vaaje-Kolstad, *Science* (2010)
 Couturier et al., *Nat Chem Biol* (2018)
 Filiatrault-Chastel et al., *Biotechnol Biofuels* (2019)
 Labourel et al., *Nat Chem Biol* (2020)
 Sabbadin et al., *Science* (2021)

Architecture of LPMOs

Catalytic Domain

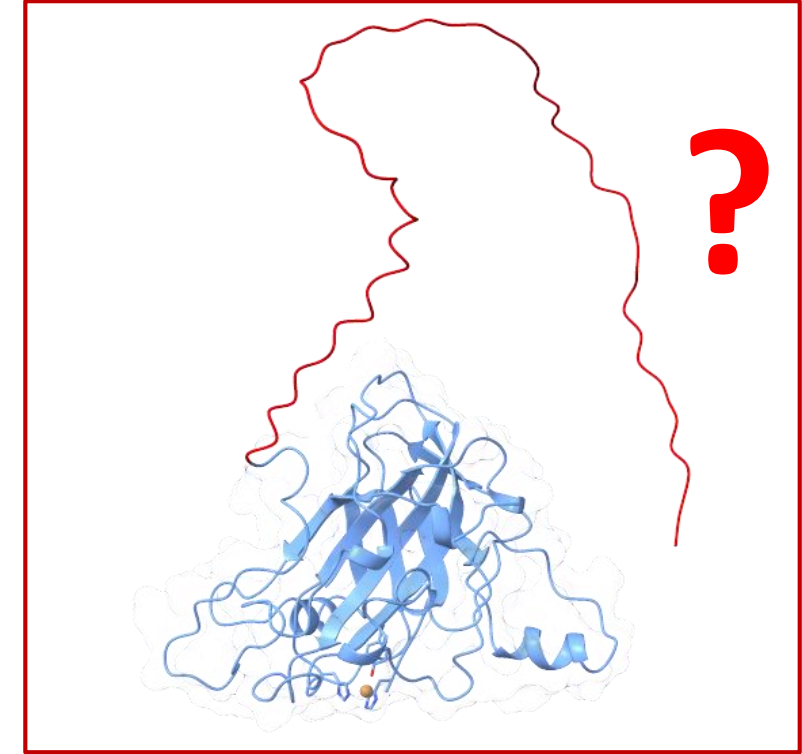


Carbohydrate Binding Modules



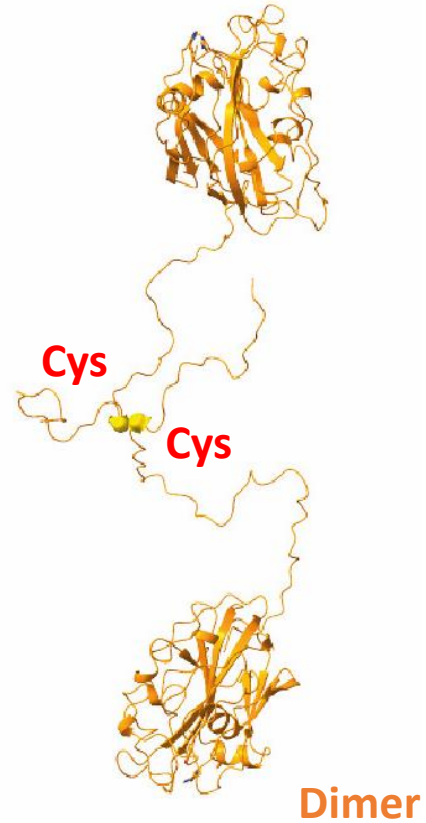
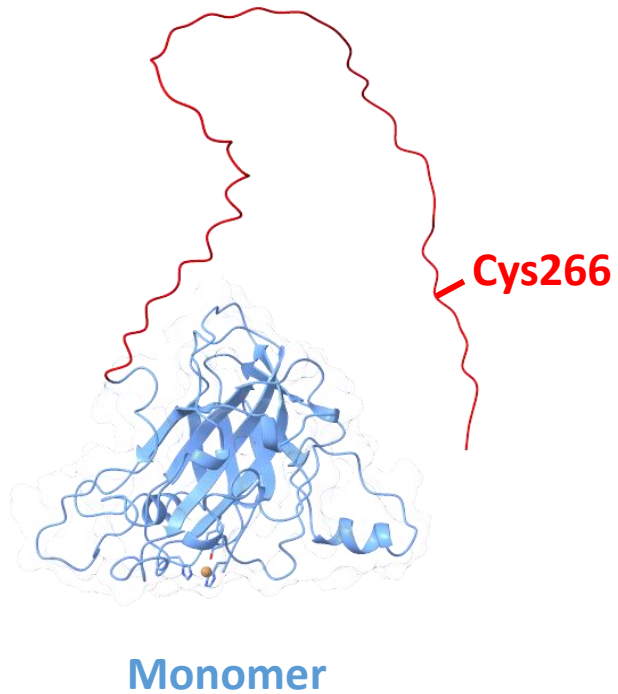
- Substrate binding

Unknown C-term regions

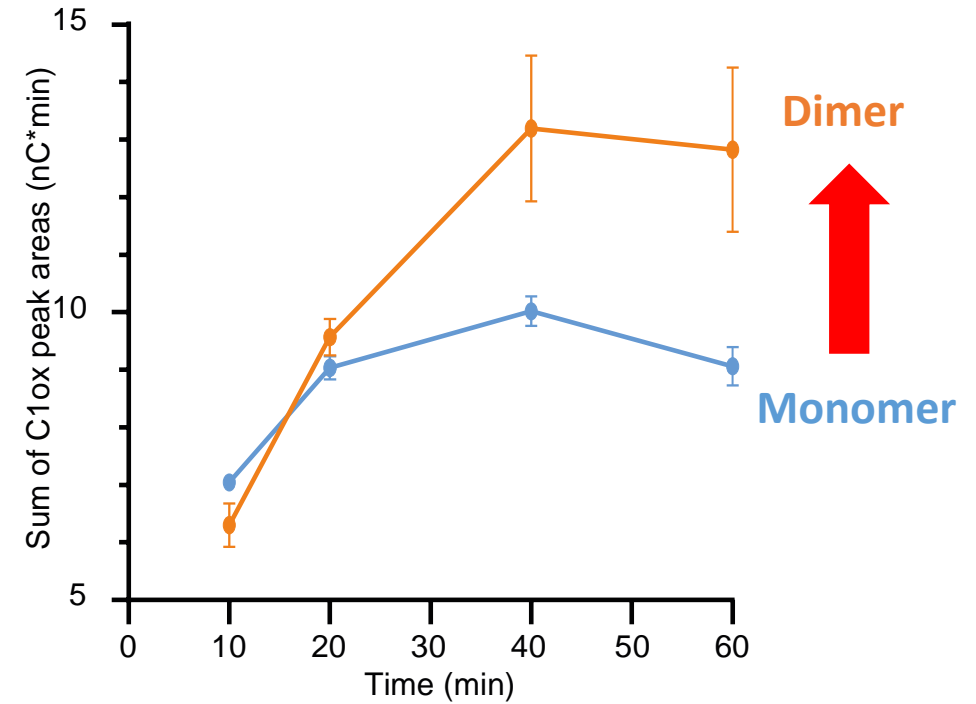


- Poor sequence similarity
- Usually removed

Dimerization of LPMOs

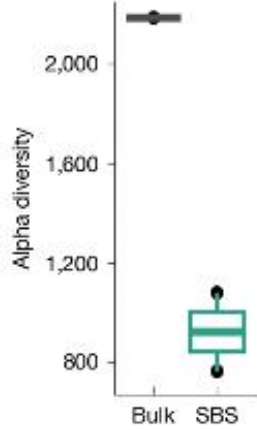
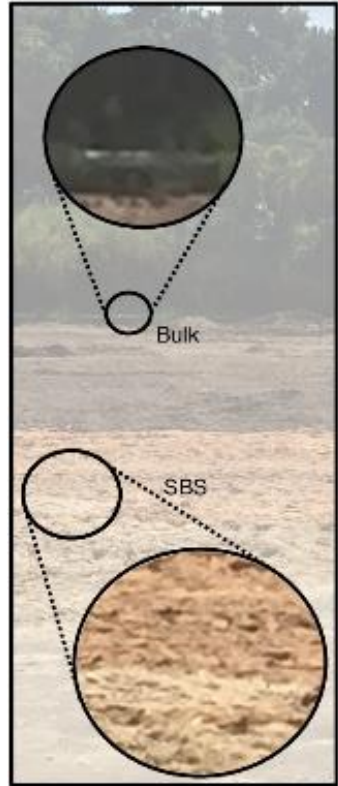


=> Higher activity!



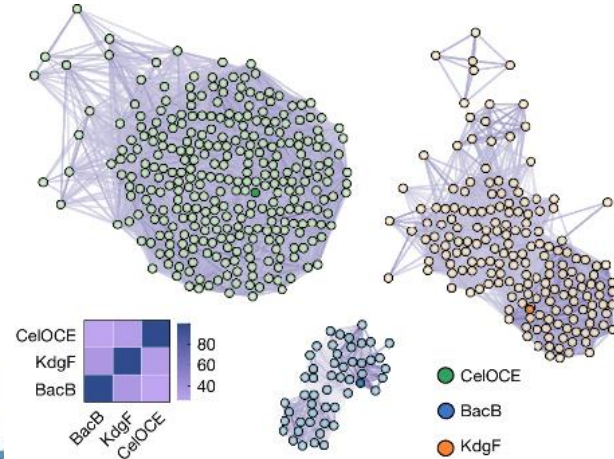
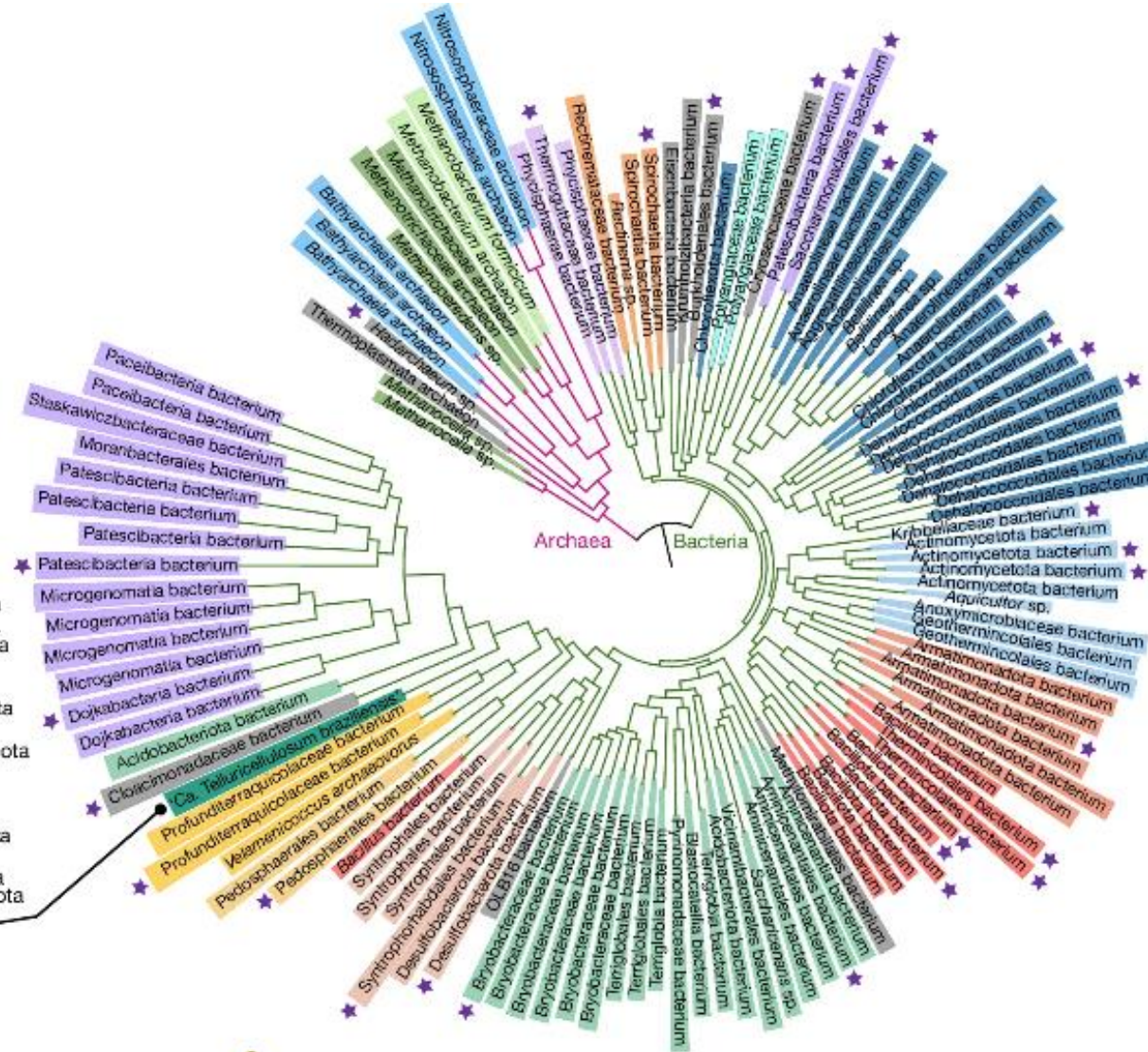
**Can we find other novel interesting enzymes
from microbial diversity?**

Exploring microbial diversity using -omic approaches

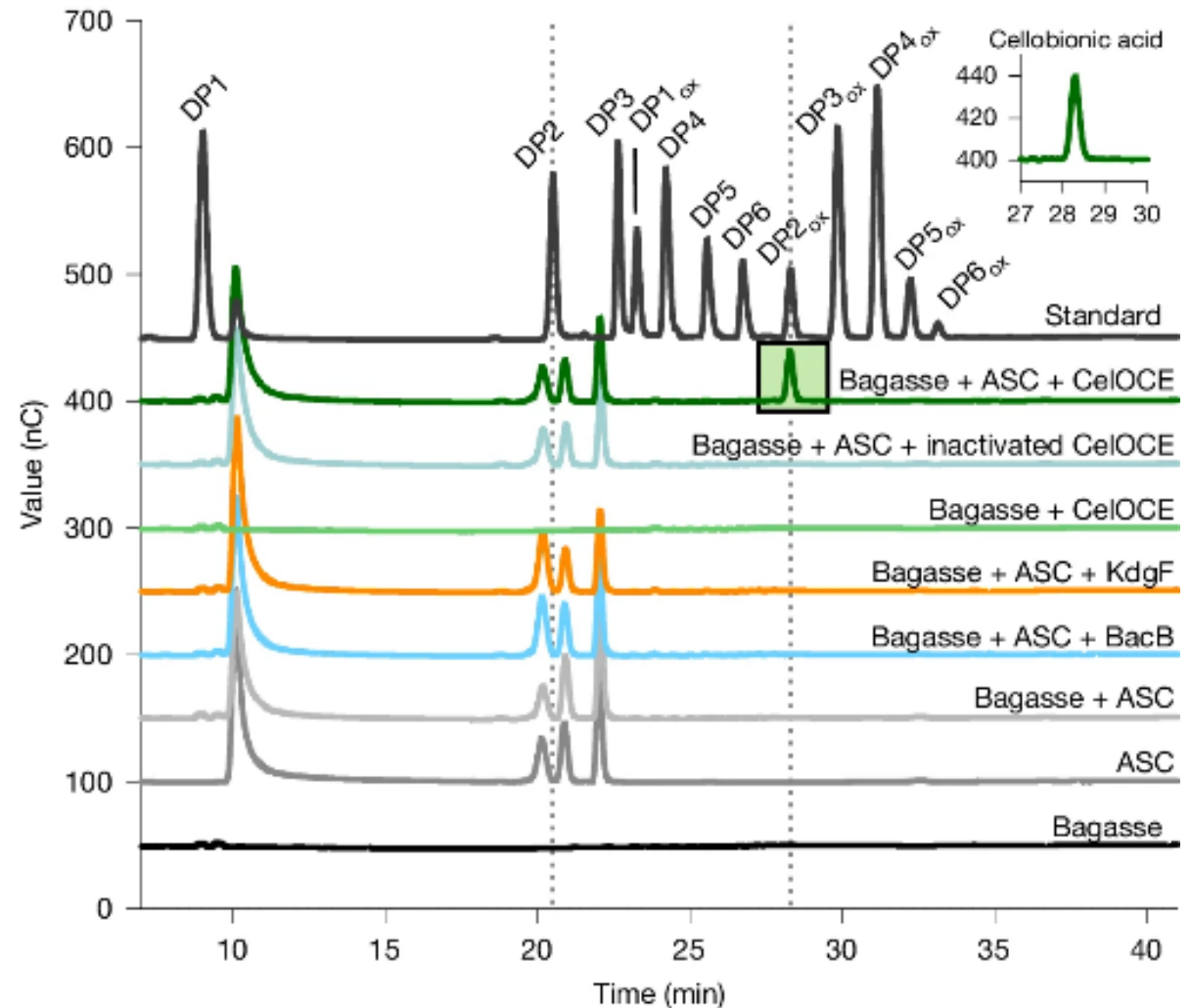
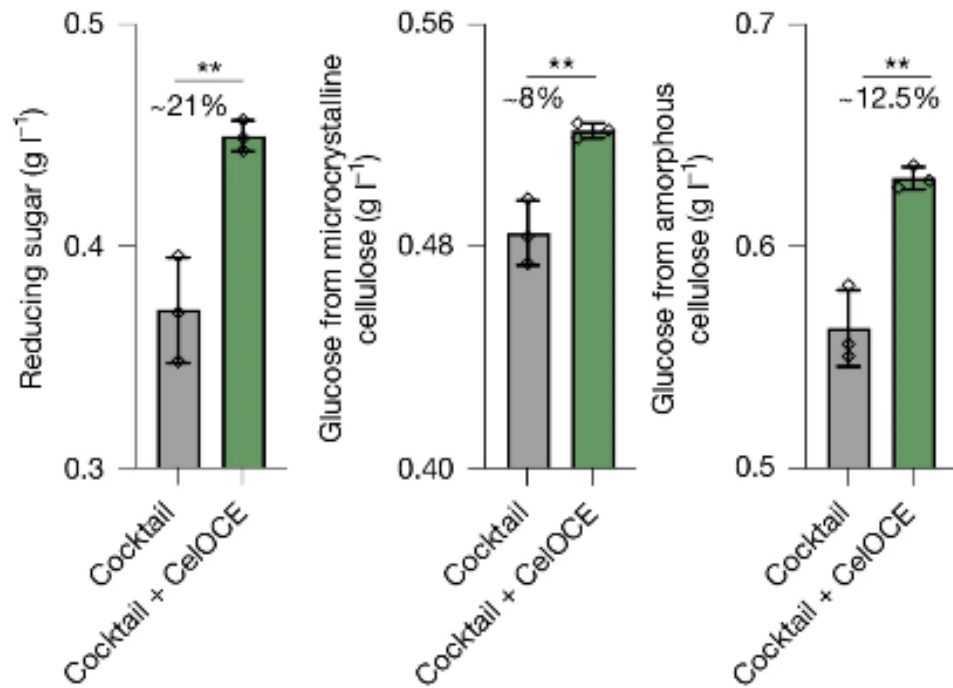


SBS, sugarcane bagasse-covered soil

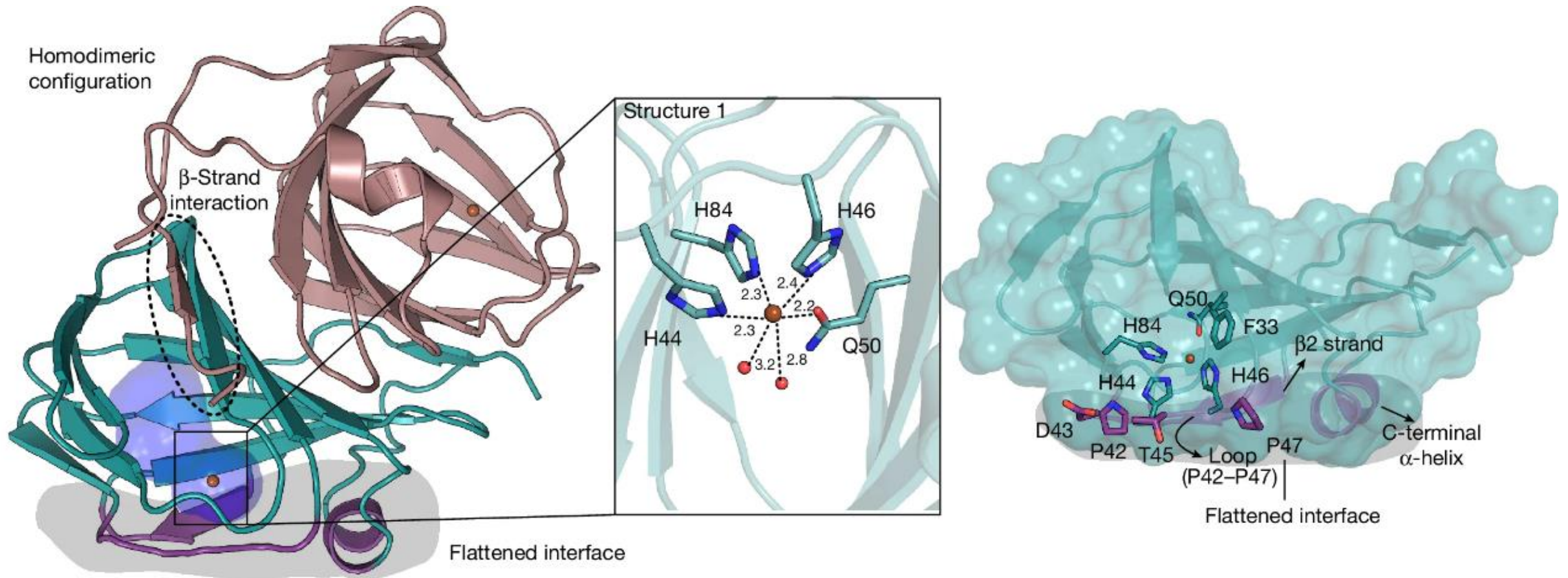
- Acidobacteriota
- Actinomycetota
- Armatimonadota
- Bacillota
- Chloroflexota
- Desulfobacterota
- Halobacteriota
- Methanobacteriota
- Myxococcota
- Omnitrophota
- Patescibacteria
- Planctomycetota
- Spirochaetota
- Thermoproteota
- Verrucomicrobiota
- Other
- UBP4



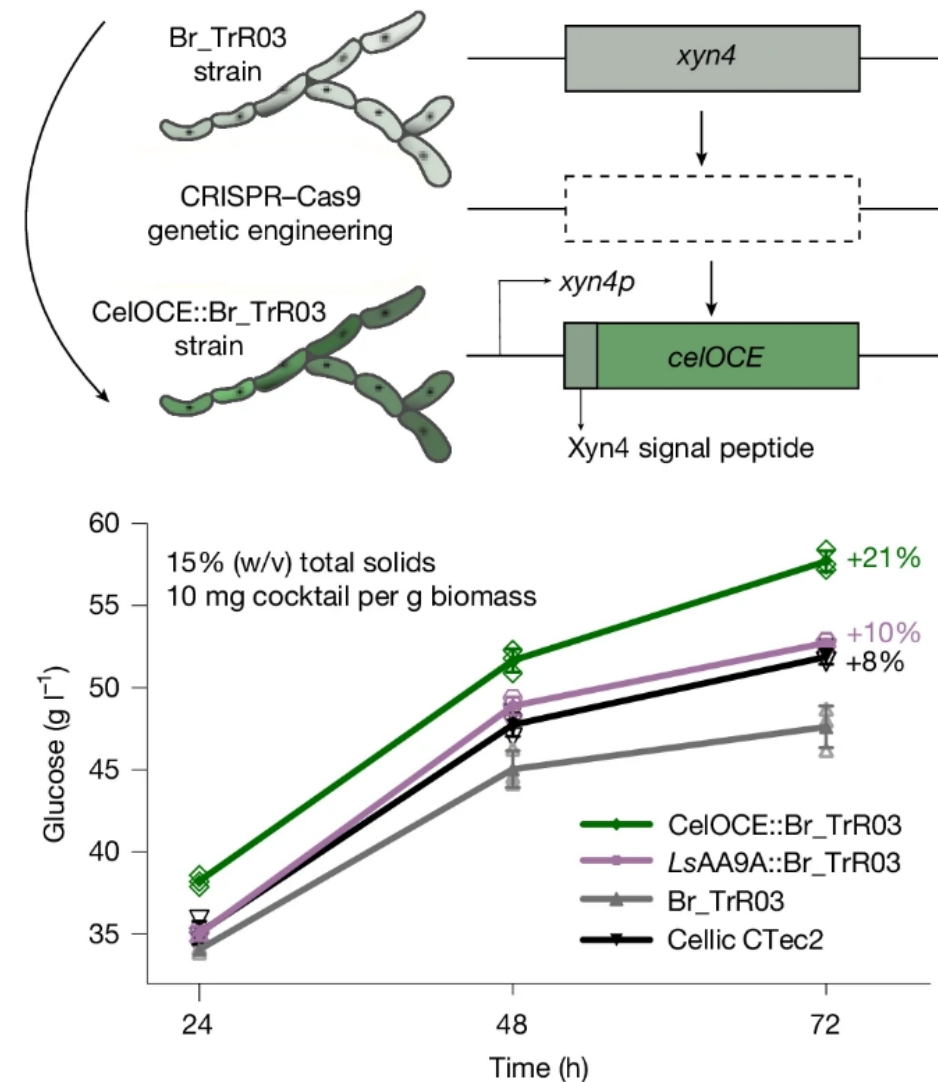
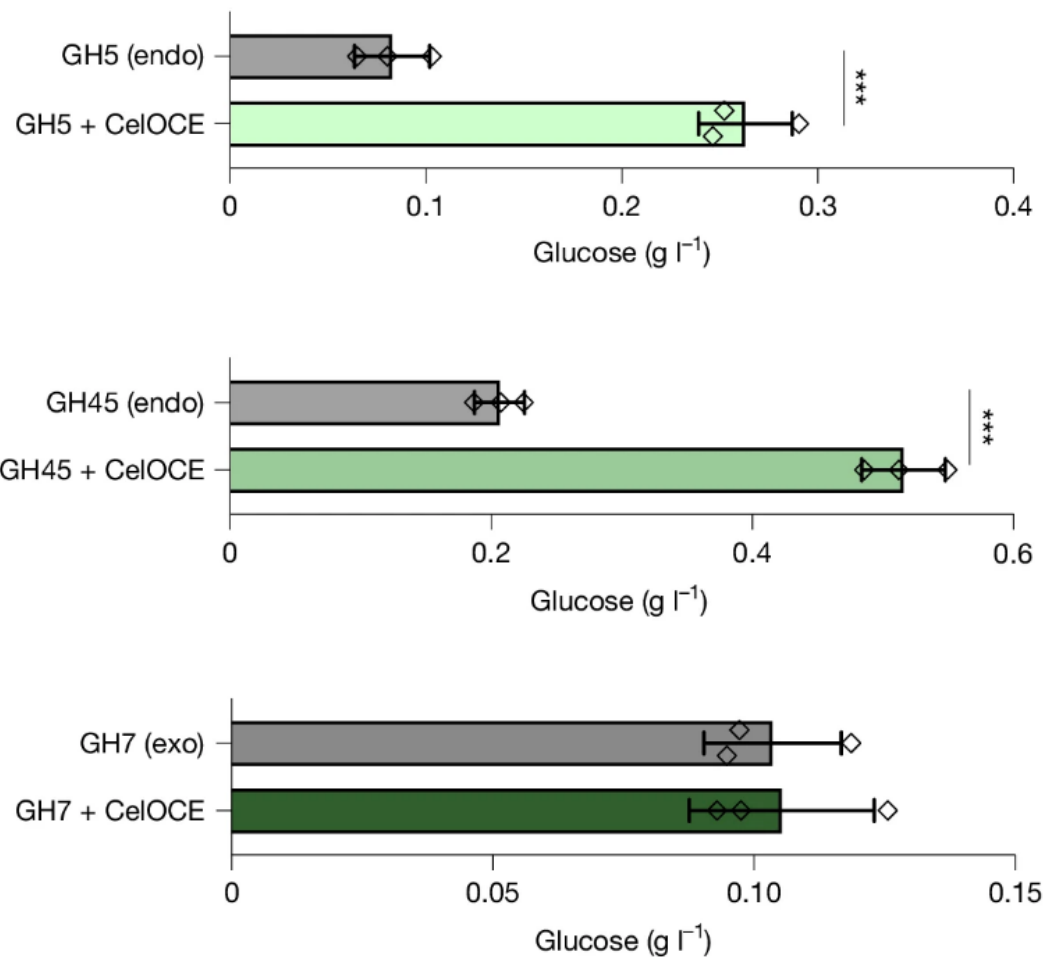
Identification of an enzyme that BOOST biomass degradation



Unique fold and features

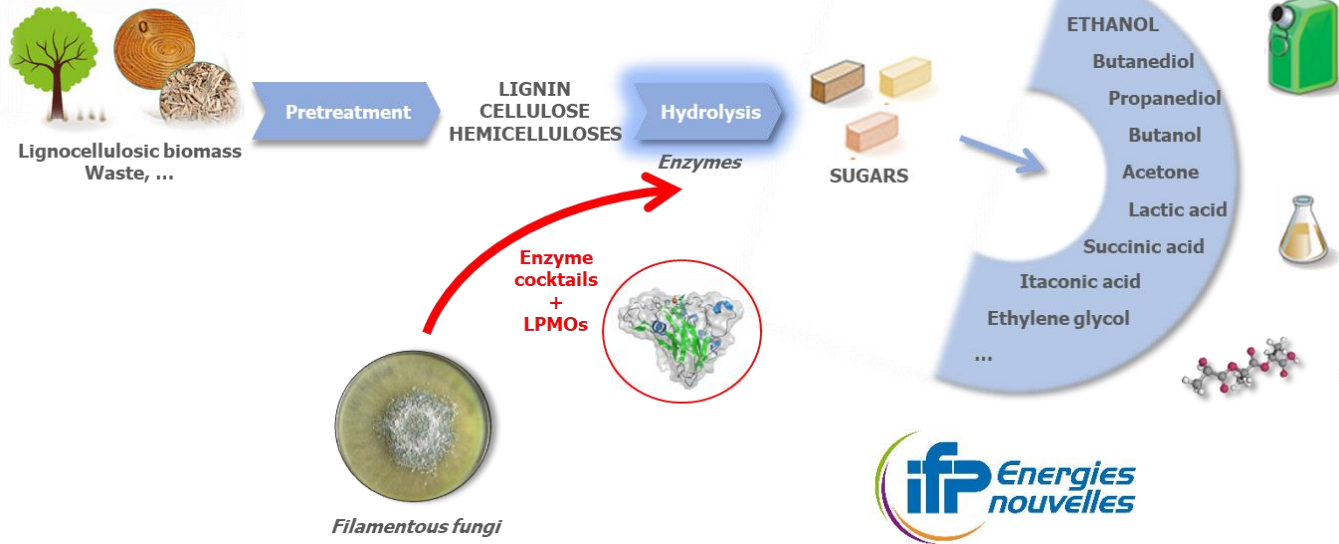


Validation of the BOOST at pilot scale

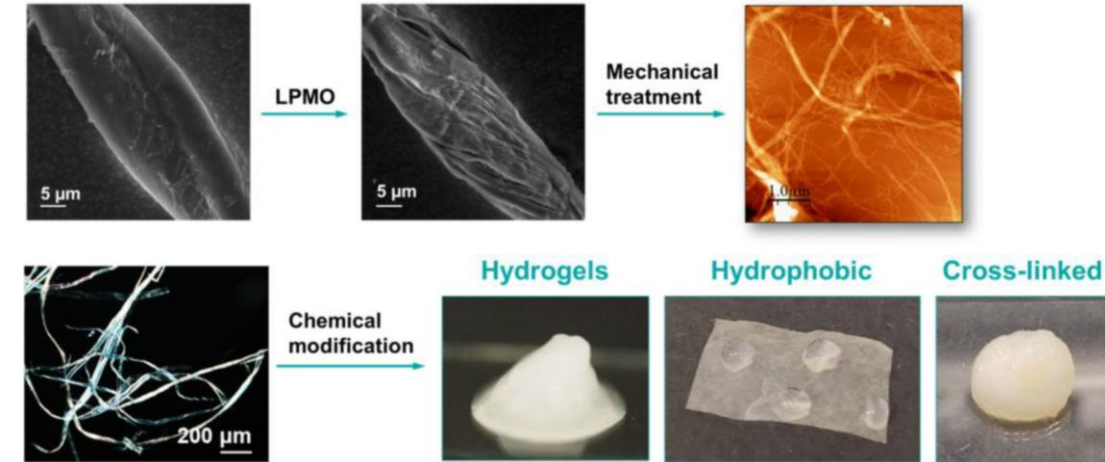


Enzymes in Biorefinery

Bioproducts



BioBased Materials



Action of LPMOs on cellulose :

- Release tension in twists
- Facilitates fibrillation

➔ **Ideal tools for nanocellulose production**

Villares et al., Scientific reports (2017)

Moreau et al., Biotech. Biofuels (2019)

Chemin et al., Biomacromolecules (2023)

Patents US2020/0157591 and US2018/0142084



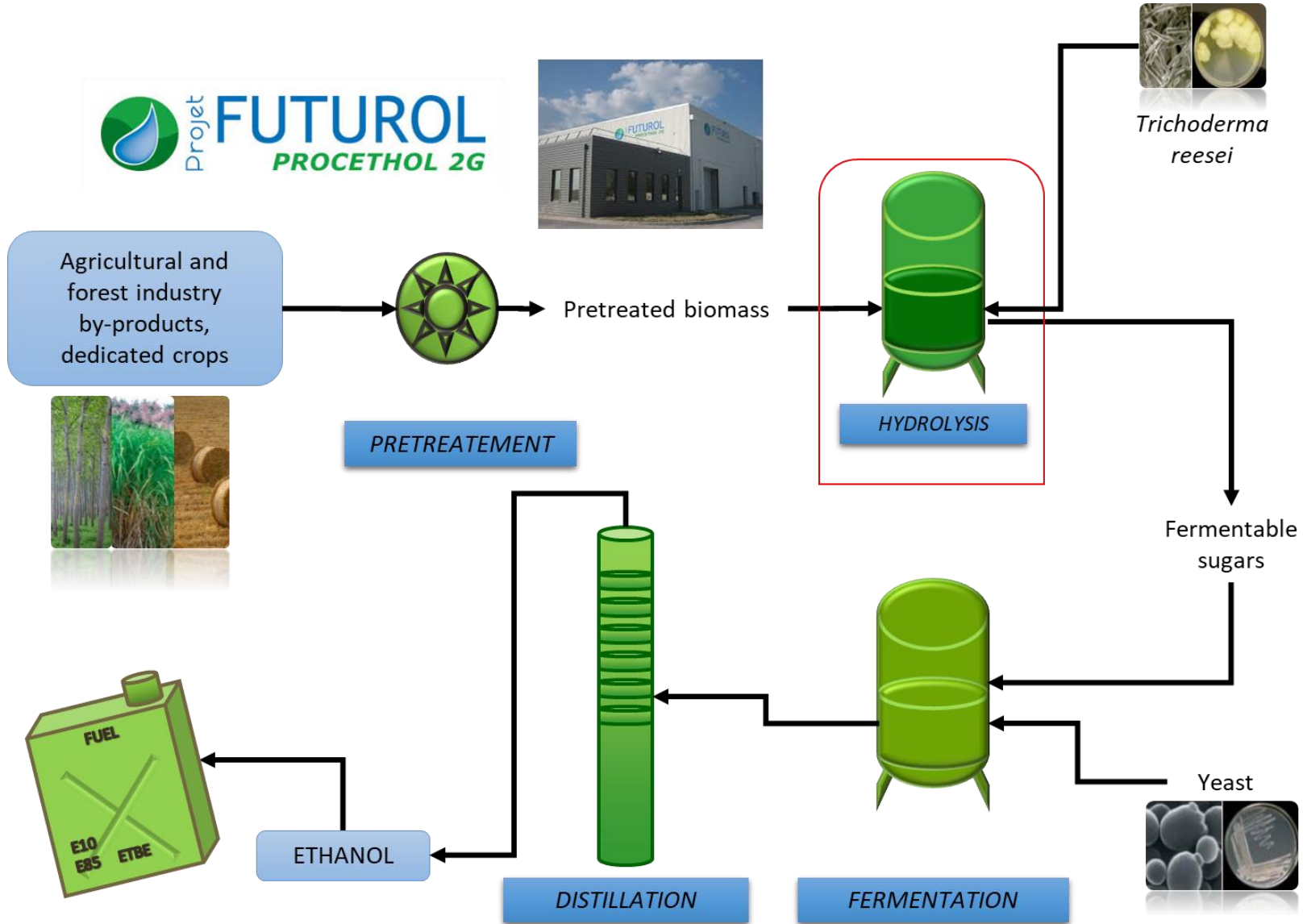
BOOST of hydrolysis

Reduction of enzyme loading

Filiatrault-Chastel et al. 2021, Biotech. Advances

Patents US20240254520A1; US20240043814A1; US20210171993A1





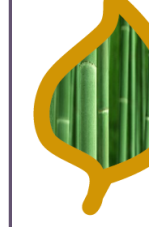

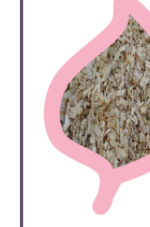


Second generation bioethanol process in France



BIOMASS RELATED CHALLENGES

NEW ENERGIES

Futurol™ : Biomass Portfolio Expansion for Maximum Flexibility

Wheat Straw	Rice Straw	Miscanthus	Sugarcane bagasse & leaves	Bamboo	Hardwood	Softwood	Corn cob	Corn stalks & stover
								



Proven **performance guarantees** on a wide range of biomasses

Futurol™: Extensive Experience in Woody Feedstocks

- Wood is one of the key representative biomasses in Futurol™
- Experience in various tree species
- Experience in a wide range of wood fractions
- Well proven and optimized pre-treatment technology
- Microorganisms with high robustness and resistance
- Typical biomass need \approx 60-300 kta dry basis

Saw
Mill
Chips



Forest
Residues
Chips



Whole
Tree
Chips



Sawdust



Delimbed
Stem
Wood
Chips



Woody
Mixtures



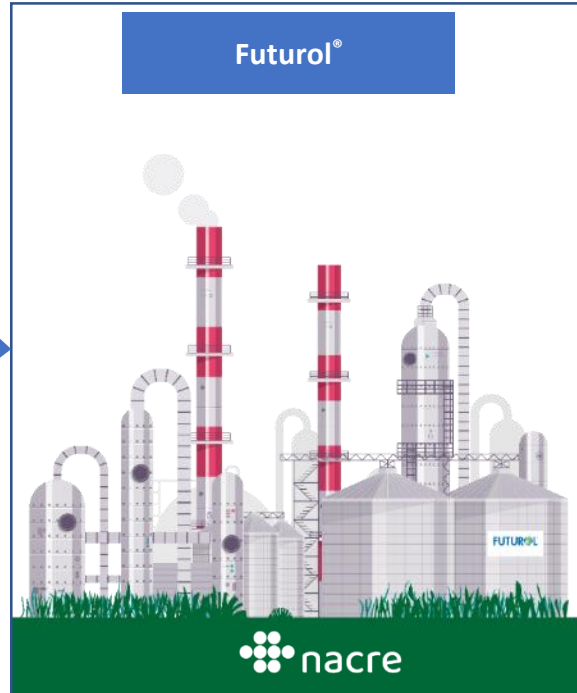
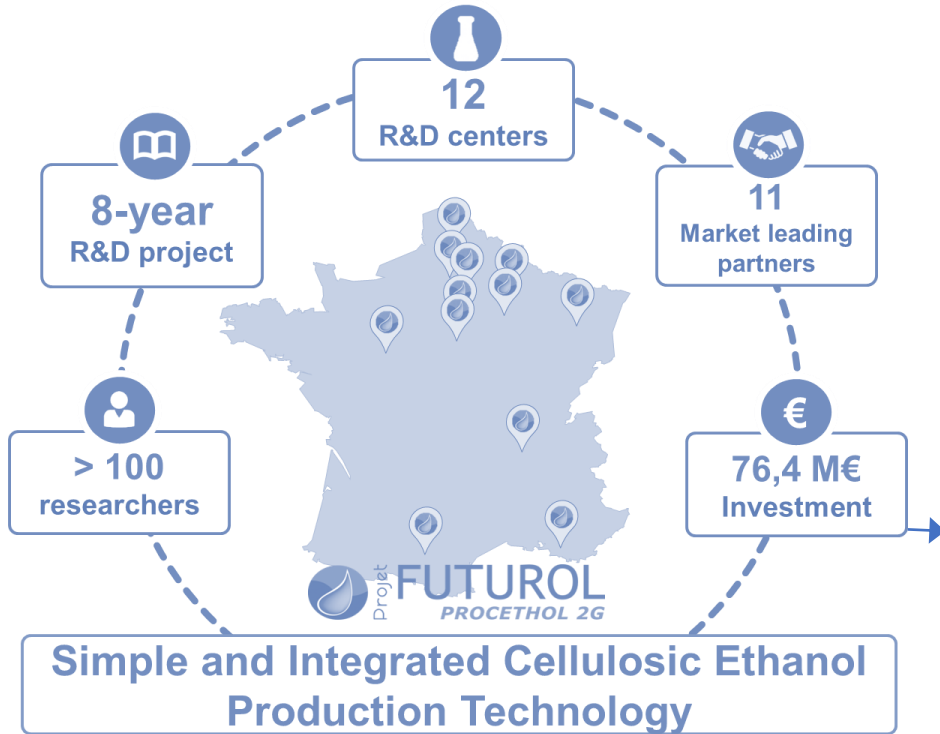
Futuro!™ Biomass Pretreatment Plant: a Real Outlook to Industrial Reality



› Extensive operation feedback on pretreatment unit in figures:

> 1,650 tons of Poplar	> 30 operating conditions tested
> 2,350 tons of Wheat Straw	> 9,000 analytical results performed
	equivalent to 1,000,000 liters of Ethanol produced

PILOT PLANTS



Financing	
Industry	



INA

Axens SOLUTIONS

DE SMET ENGINEERS & CONTRACTORS

Sisak Biorefinery :

- 300 M€ project,
- Use of agricultural residues (straw) and energy crop (miscanthus),
- Redevelopment of disused farmlands, social and economic benefits,
- A leading-edge energy transition project in EU.

INA, Croatia

55 kty 2G ethanol

Basic Engineering completed

Ecole Centrale, Marseille, November 2021

NEW ENERGIES

INRAE

amU
Aix Marseille Université



MICKAEL LAFOND
BASTIEN BISSARO
KETTY TAMBURRINI
ROSELINE YAO
DAVID RIBEAUCOURT
MIREILLE HAON
SACHA GRISEL
DAVID NAVARRO
+ ALUMINI
& THE BBF TEAM



BERNARD HENRISSAT
NICOLAS TERRAPON
SONIA LONGHI
& THE CAZY TEAM



SIMON ARRAGAIN
ANTOINE MARGEOT



MARIO MURAKAMI

THANK YOU



Take a minute to evaluate lecture #10

AGRIMA / WP2 - Innovation in
teaching and learning: waste
valorisation



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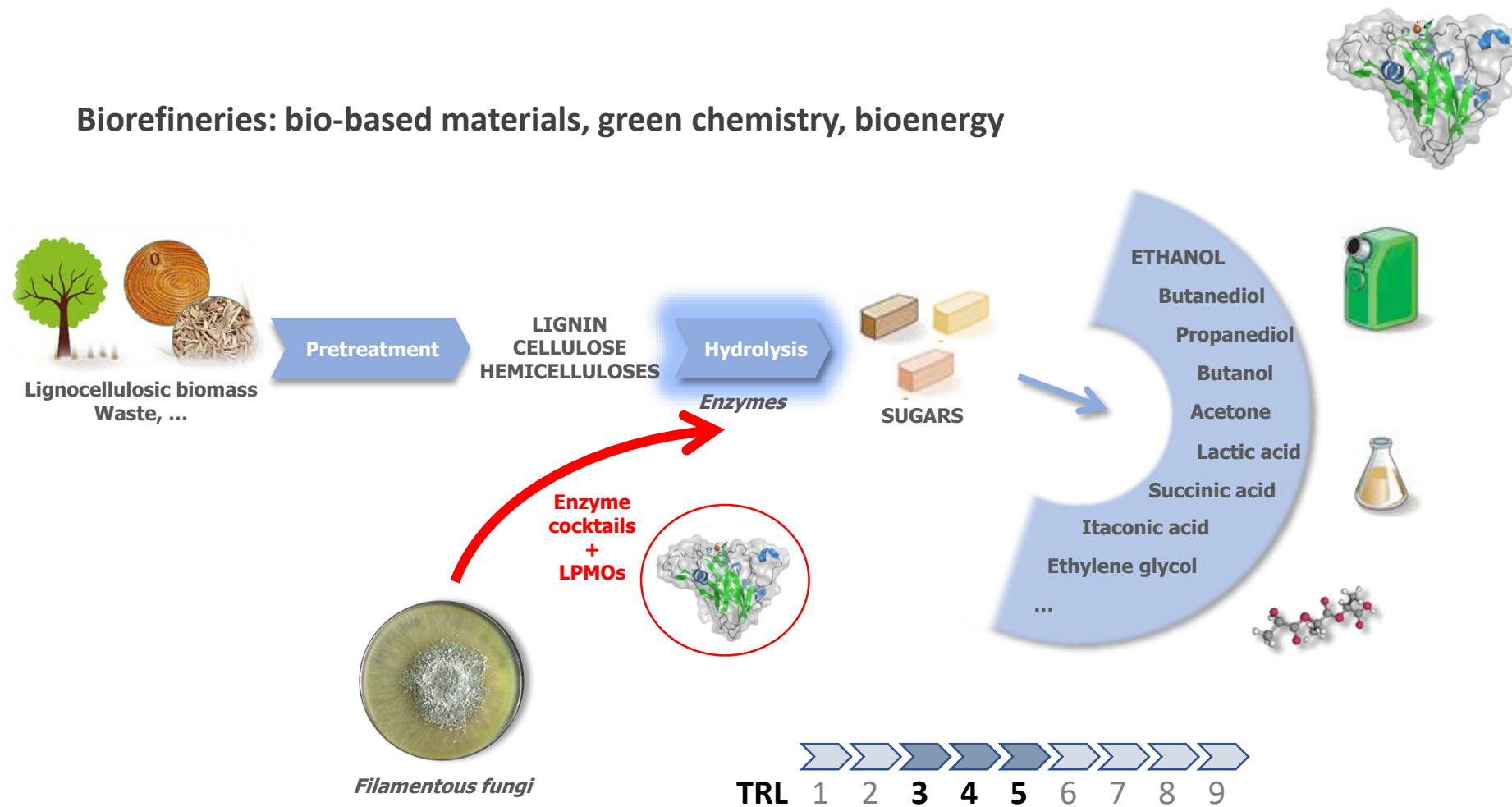
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erasmus+
PORTUGAL EDUCAÇÃO E FORMAÇÃO



Fungal enzymes in biorefinery and bioproduct production

Biorefineries: bio-based materials, green chemistry, bioenergy



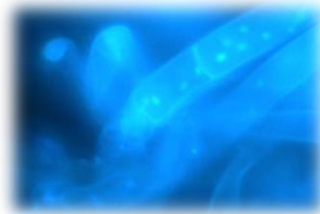
Filiatrault-Chastel et al. 2021, Biotech. advances

Fungi and their enzymes @ BBF

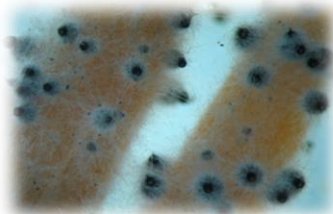
Identification of enzymes from natural biodiversity



Pycnoporus coccineus

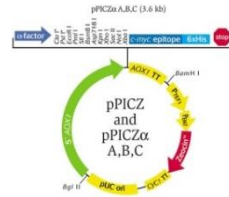


Laetisaria arvalis



Podospira anserina

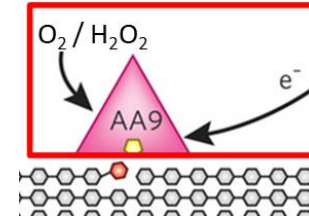
Lab scale heterologous production



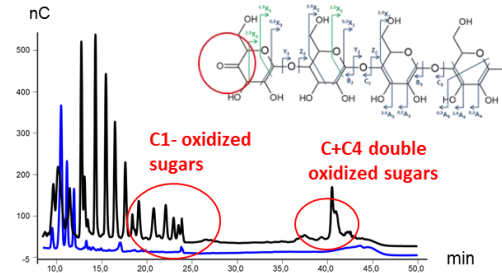
www.platform3pe.com



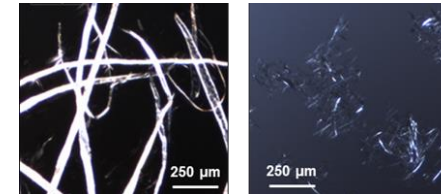
Enzyme assays



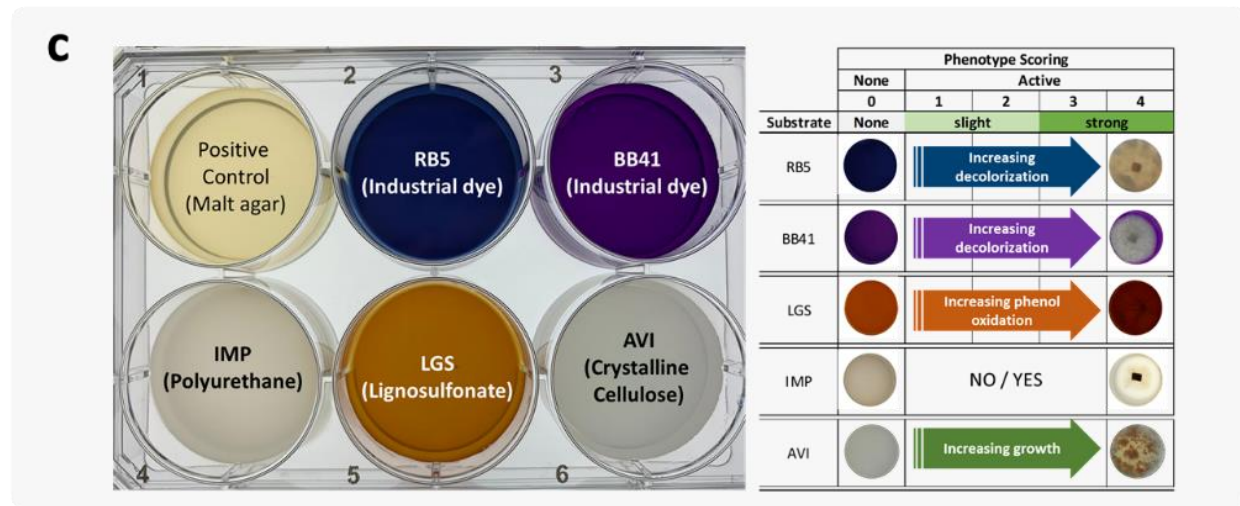
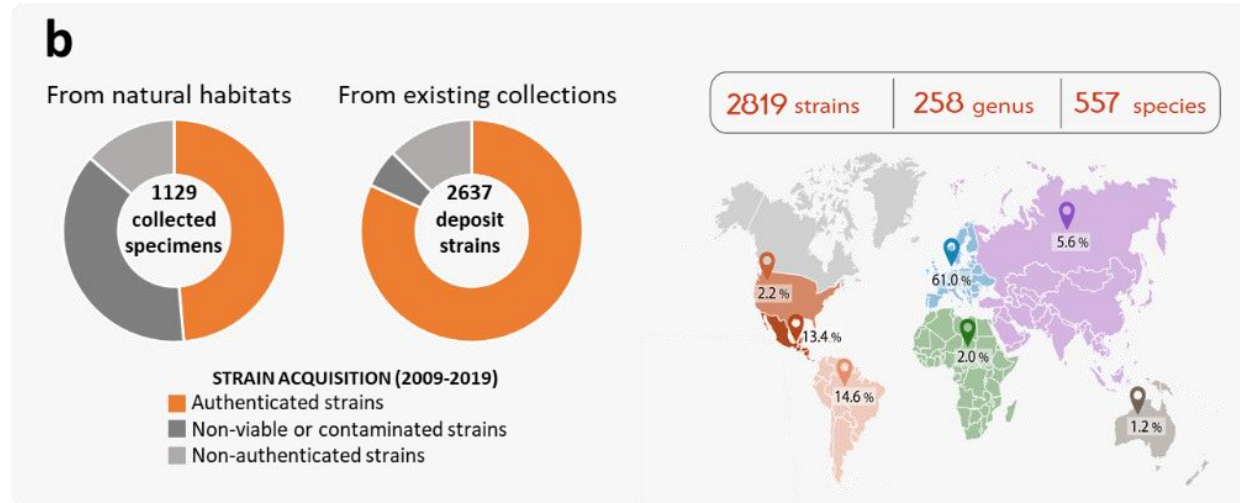
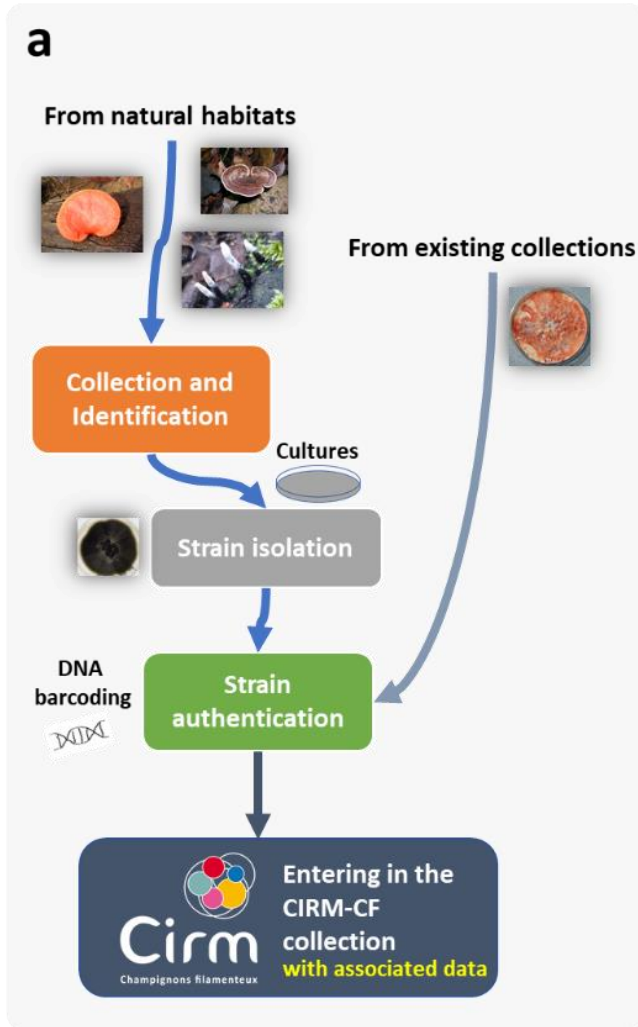
Soluble fraction



Insoluble fraction

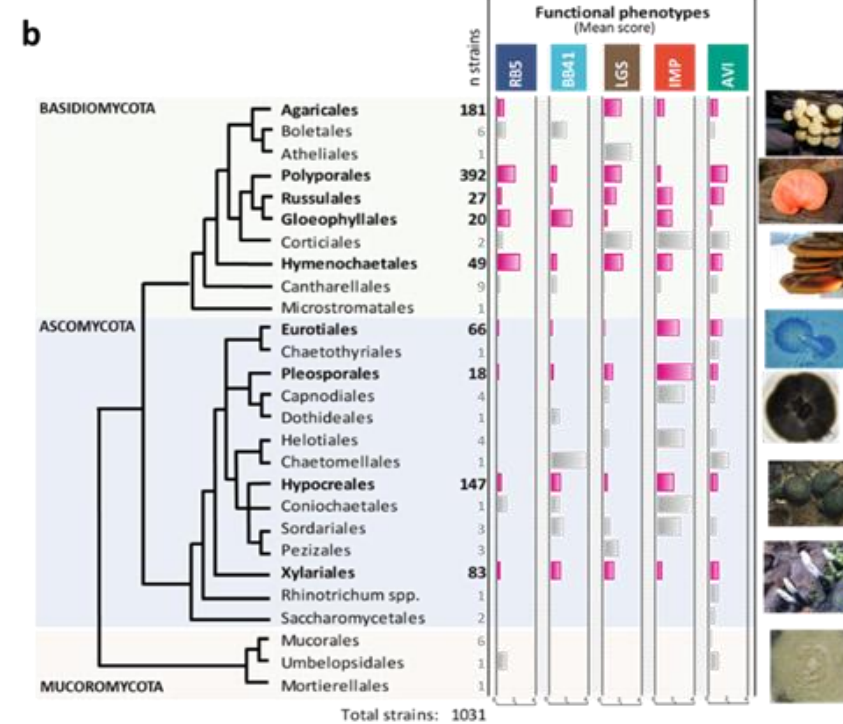
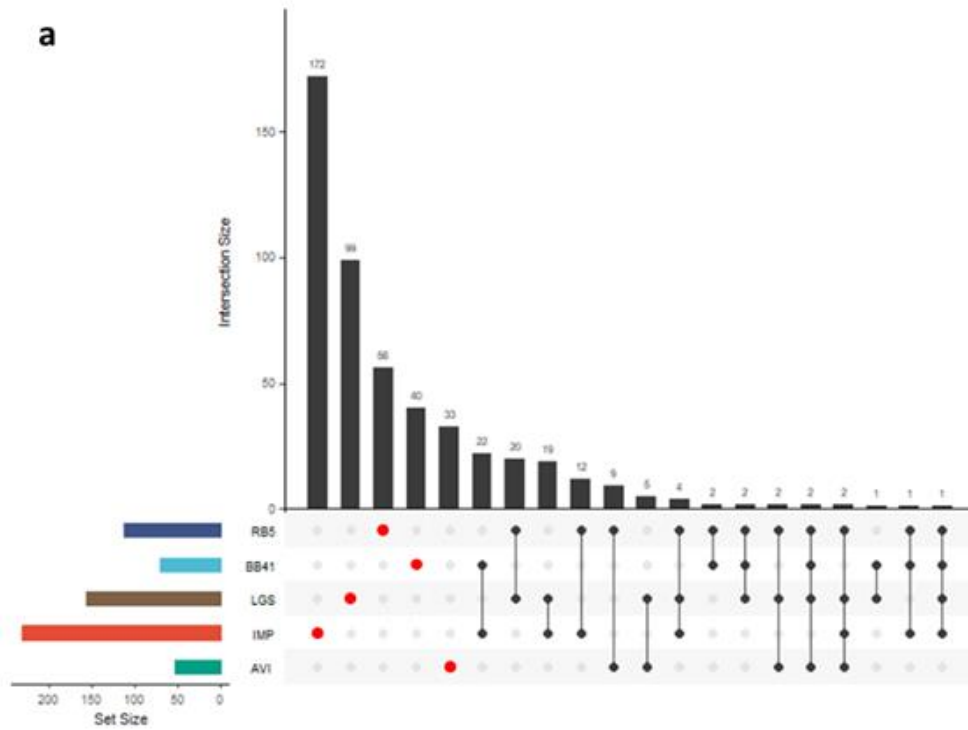


Example of Phenotyping



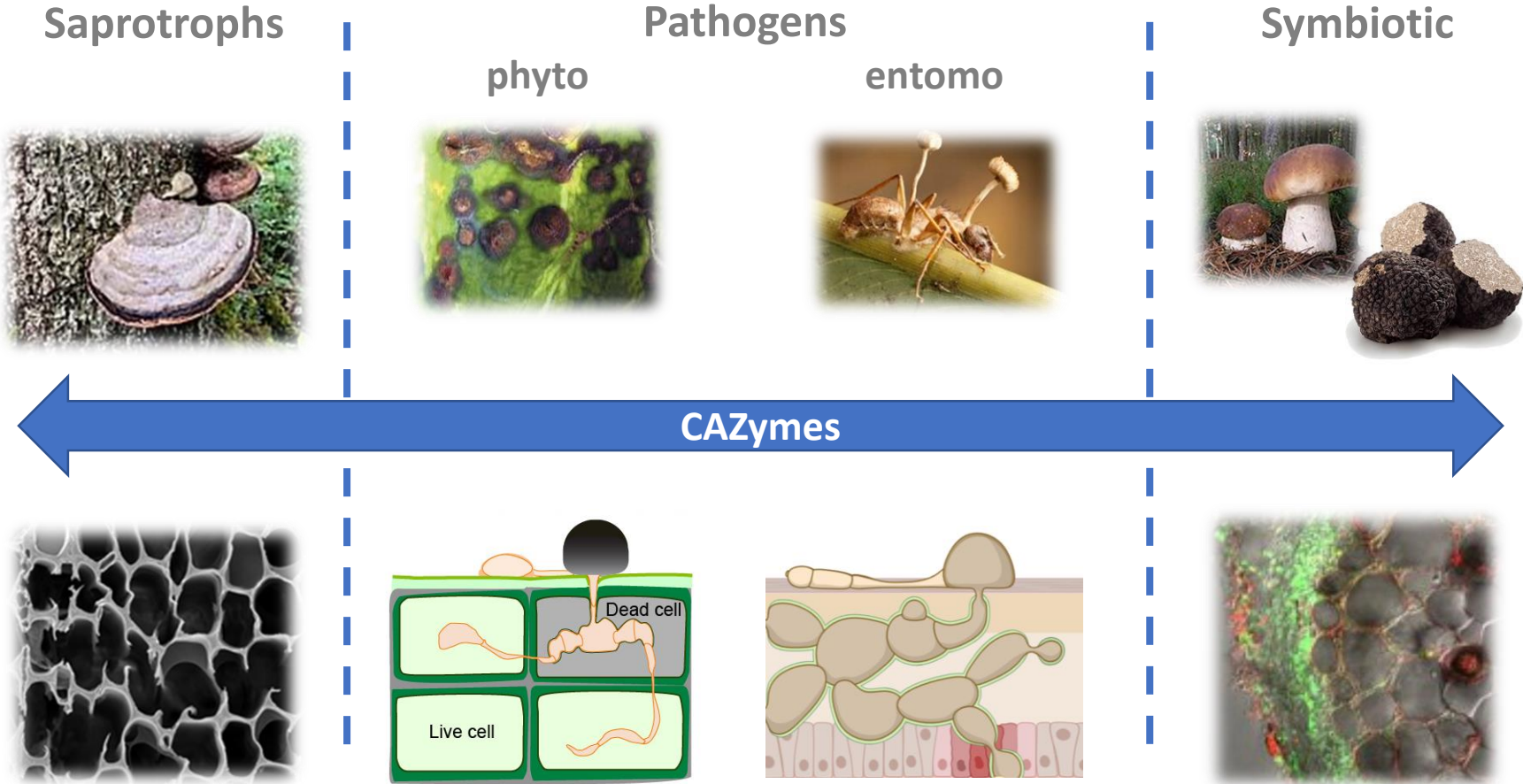
Navarro et al., Communications Biology, 2021

Functional screening scores of 1031 natural strains, according to (a) target substrates and (b) phylogenetic distribution

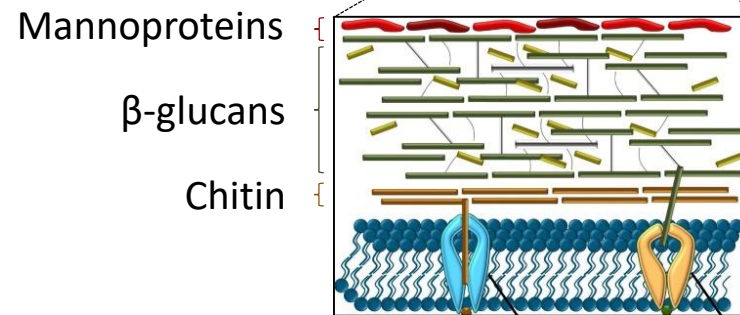
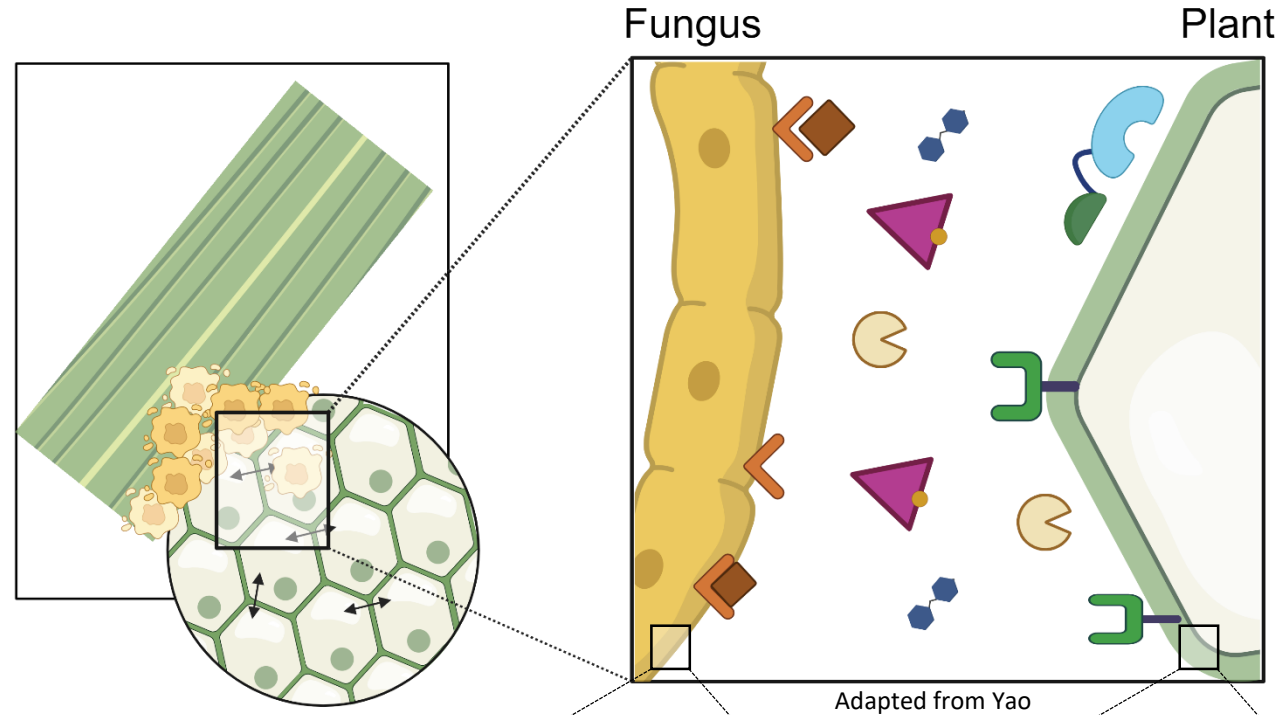


Navarro et al., Communications Biology, 2021

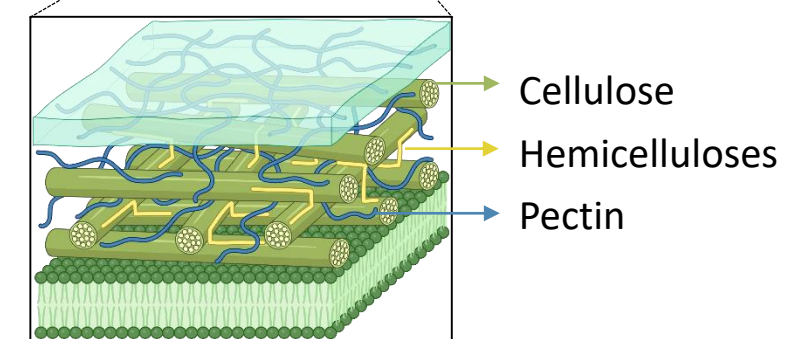
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CELL WALL ACTIVE ENZYMES



Fungal Cell Wall



Plant Cell Wall