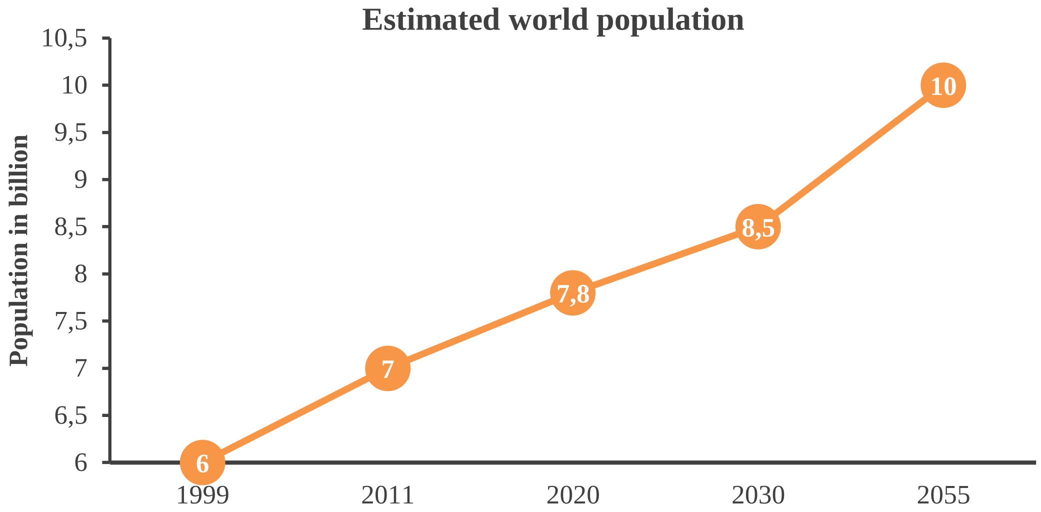
ENVIRONMENTAL AND SYNTHETIC MICROBIAL COMMUNITIES FOR THE VALORIZATION OF LIGNOCELLULOSE

Associate Pr. Ludovic Besaury
University Reims Champagne Ardennes
UMR FARE Fractionation Agroresources Environment / AFERE Chair

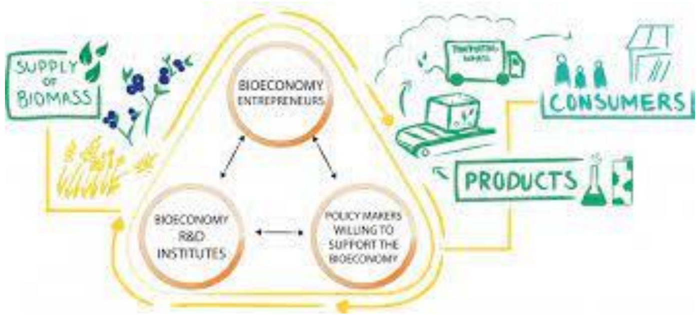
ludovic.besaury@univ-reims.fr

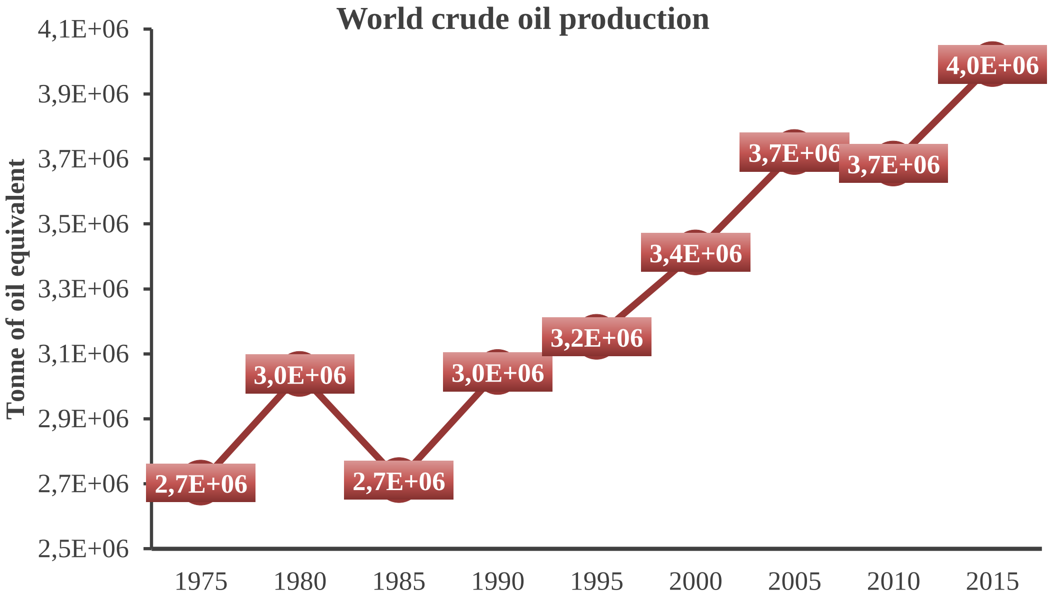
Context

- **Current Issues:**
  - Decrease in carbon resources
  - Increase in consumption
  - Increase in population
  - Increased pollution (global warming, plastic waste...)



- **Alternatives to be found:**
  - Policies
  - Techno-scientific
  - Renewable energies (solar, hydraulic, lignocellulosic biomass)
  - Bioeconomy





Biorefinery and lignocellulosic biomass

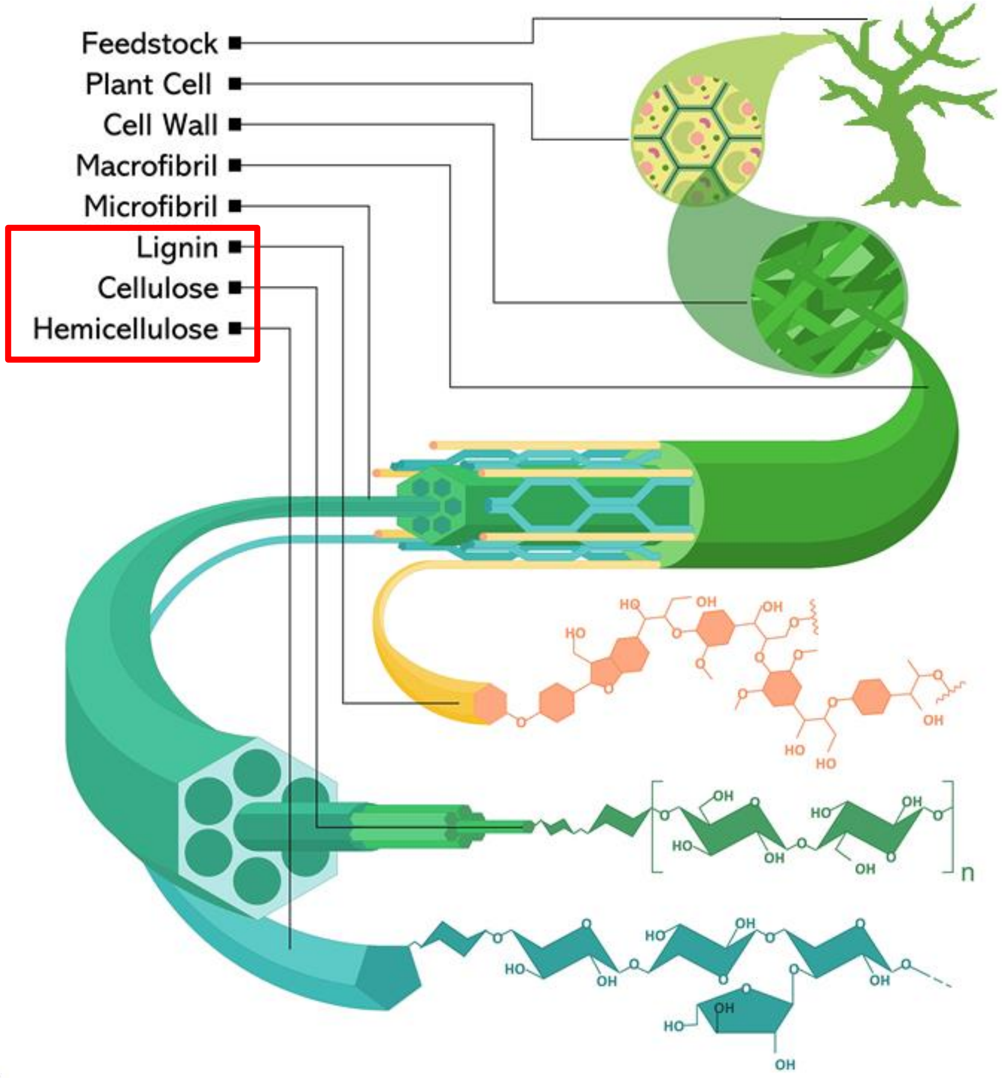
Lignocellulosic biomass = renewable resource

Biorefinery ← Agricultural/agroforestry co-products

Biofuel
Biosourced molecules
Natural fibers

Recovery processes :

- Physical-chemical
- Mechanical
- Biological (Bacteria, fungi)



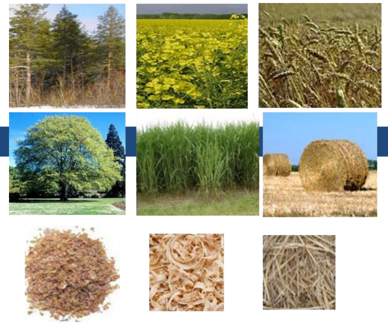
Structure of lignocellulosic biomas(Magalhães *et al.* 2019)

Enzymes and molecules productions from lignocelluloses

- **Enzymatic fractionation**
- **Microbial bioproduction**

Microorganisms of interest

Diversity of lignocellulosic biomasses and lignocellulosic components



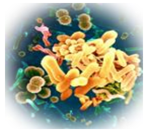
Environments of interest

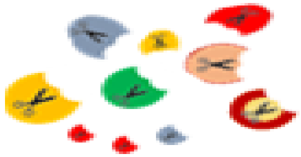
Microbial strategies and physiology

Bottom-up

Top-down

Axis 1. Identification of efficient/active microbial populations and characterization of efficient enzymes





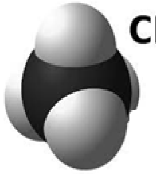
- Culturable and omics approaches

- GH, CE, OX
- pure enzymes; enzymatic cocktails

Axis 2. Lignocellulose bioconversion

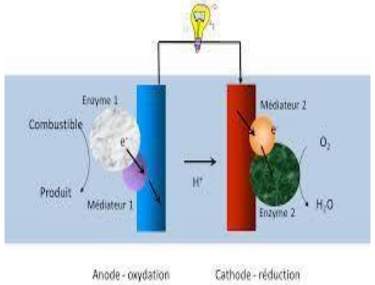
Bioplastics

Pigments



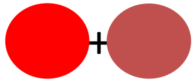
CH_4

Methane

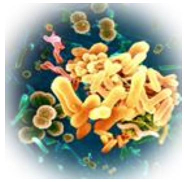


Bioelectrochemistry

Top-down: Phyllospheric microbiota



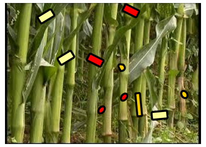
Top-down



Wheat bran

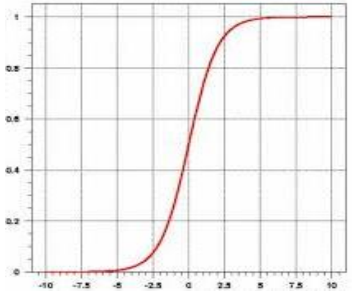
M3 media

Wheat straw



M3 media

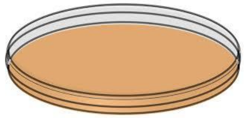
0 to 48 hours



Objectives and expected results :

Growth **dynamic** and **enzymatic activities (intra and extra)** of the microbial consortia

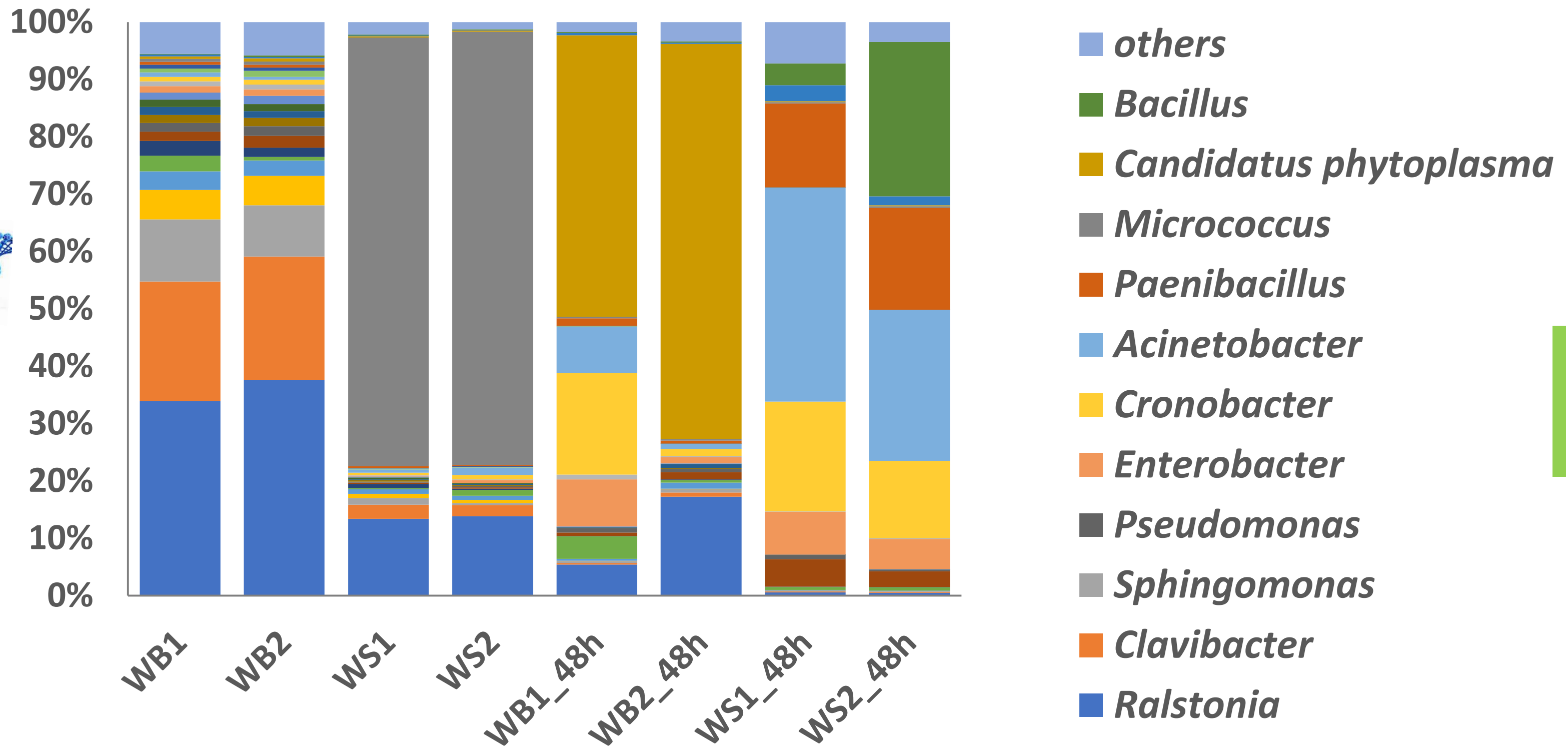
Presence of **new lignocellulolytic strains** ?



Metagenomics of the microbial consortia : Who is there and what might they do ?

Besaury et al., 2022

Top-down: Phyllospheric microbiota

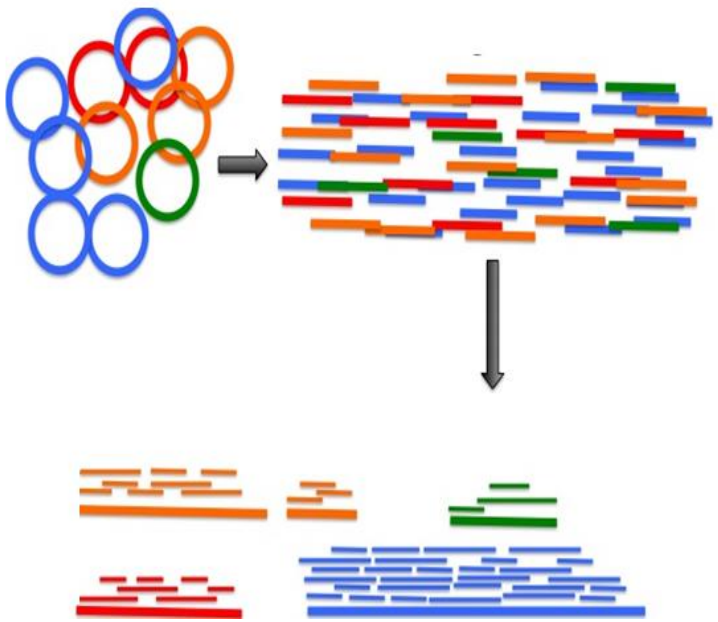


New taxa involved in lignocellulose degradation

- The final degrading microbial consortia for WB and WS was dominated by ***Candidatus phytoplasma*** and ***Cronobacter***/***Acinetobacter*** respectively.
- The presence of ***Candidatus phytoplasma** and **Cronobacter*** has never been detected in lignocellulose degrading microbial consortia and represents a new candidate for white biotechnology.

Besaury et al., 2022

Top-down: Phyllospheric microbiota



	Acinetobacter WB bin01	*Pseudomonas WB bin04*	*Bacillus WS bin02*
Size	1.615.226	6.962.632	1.656.097
CDS	1639	6710	1565
Number of CAZYmes	7	80	23

- *Acinetobacter WB bin01* harbours carbohydrate esterases CE2, CE4 and CE11 which have never identified in the *Acinetobacter baumanii* core genome
- The *Pseudomonas WB bin04* several auxiliary activities (AA2; AA3; AA6 and AA7) which were not detected in other *Pseudomonas* genomes

Novel metabolic pathways not previously described in specific genera of biomass degradation

Besaury et al., 2022

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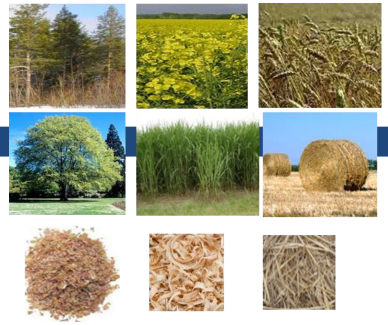

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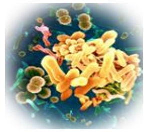

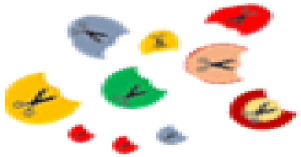

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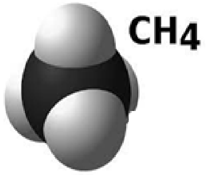

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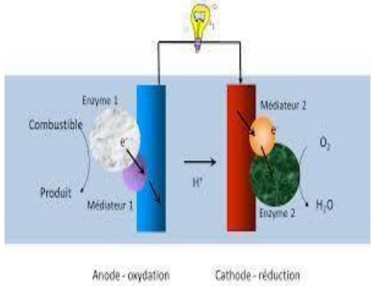
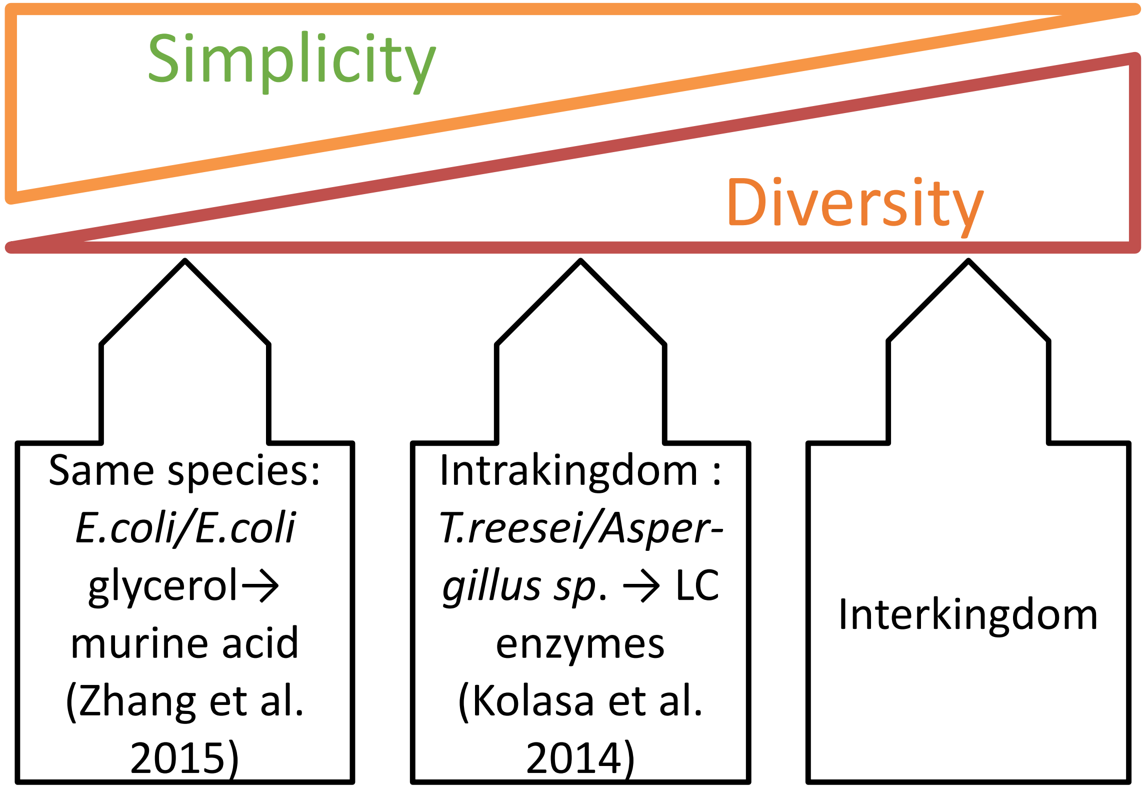


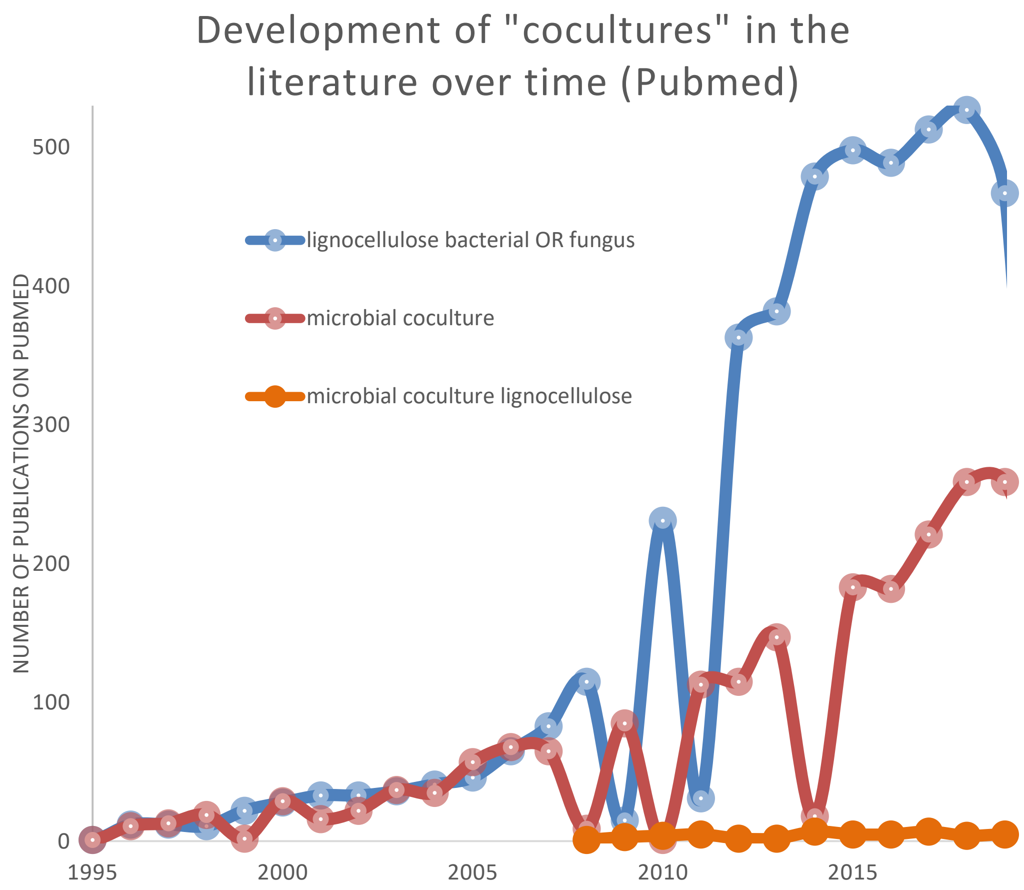
Bioelectrochemistry

Biological processes: cocultures

- In nature = syntrophic process

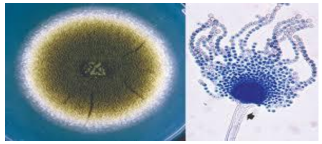
- Coculture = Culture with n ≥ 2 µorganisms





Advantages:
- Distribution of the different functions performed to specific and optimized populations
- Ability to prevent nutritional deficiency due to the diversity of metabolic pathways present

Lignocellulolytic enzymatic mechanisms present in *Aspergillus*



Nova seq,
2*150bp

Aspergillus niger

Genome size = 37,5 Mbp

Glycoside Hydrolases (GHs) : hydrolysis and/or rearrangement of glycosidic bonds

GlycosylTransferases (GTs) : formation of glycosidic bonds

Polysaccharide Lyases (PLs) : non-hydrolytic cleavage of glycosidic bonds

Carbohydrate Esterases (CEs) : hydrolysis of carbohydrate esters

Auxiliary Activities (AAs) : redox enzymes that act in conjunction with CAZymes

Carbohydrate-Binding Modules (CBMs) : adhesion to carbohydrates

Carbohydrate Active Enzymes (CAZymes)

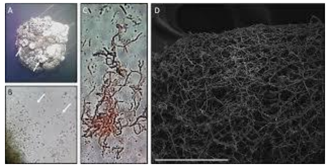
CAZypedia
carbohydrate-active ENZYMES

Strain	Length (MBp)	Single-coding proteins	CAZymes number	CAZymes (%)	AA	CBM	CE	GH	GT	PL
DSM 1957	35.6	10798	581	5.38	106	17	88	262	99	10
An76	34.6	10373	551	5.31	97	15	86	246	97	10
ATCC 1015	34.9	10950	565	5.16	104	17	81	256	97	10
ATCC 13496	35.7	12194	576	4.72	103	16	86	261	101	9
CBS 513.88	34	10609	576	5.43	104	15	83	249	107	18
CBS 101883	35.9	13359	581	4.35	106	15	88	262	100	10
ATCC 64974	35.5	11236	581	5.17	105	17	87	259	99	14

- **Highest content of CAZymes, AA, CE and GH compared to other Aspergillus niger strains**
- **Very high diversity of families within the AA, GH groups**

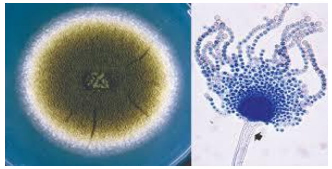
Detain et al., 2022

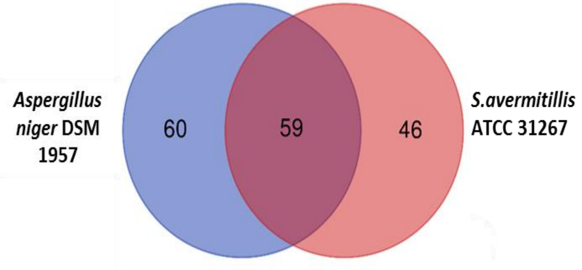
Gene pool diversity in co-culture

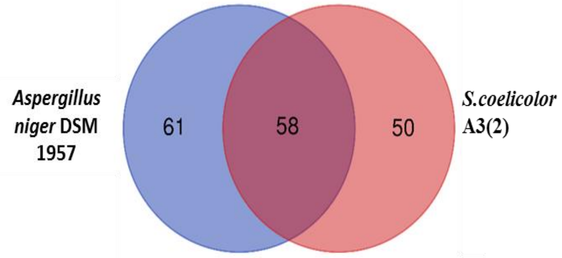


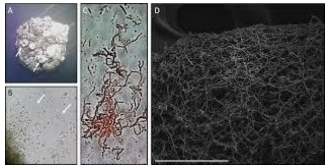
	Length (MBp)	Single-coding proteins	CAZymes number	CAZymes (%)	AA	CBM	CE	GH	GT	PL
***S.avermitillis* ATCC 31267**	10.5	10003	335	3,35	20	53	32	149	69	12
***S.coelicolor* A3(2)**	9.1	8128	336	4,13	15	57	37	156	59	12
S.griseorubens	7.7	6841	255	3,73	15	34	34	115	52	5

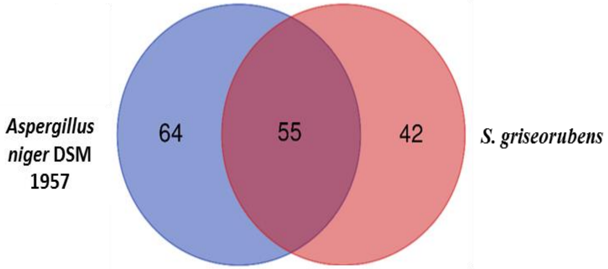
CAZypedia
carbohydrate-active ENZYMES







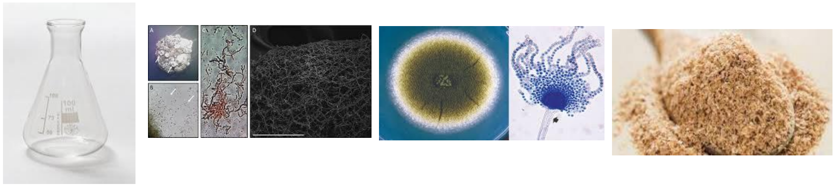




- **Increased number of enzymes related to biomass degradation**
- **Greater diversity of CAZyme families in the co-culture**
- **Specific enrichment in glycoside hydrolase**

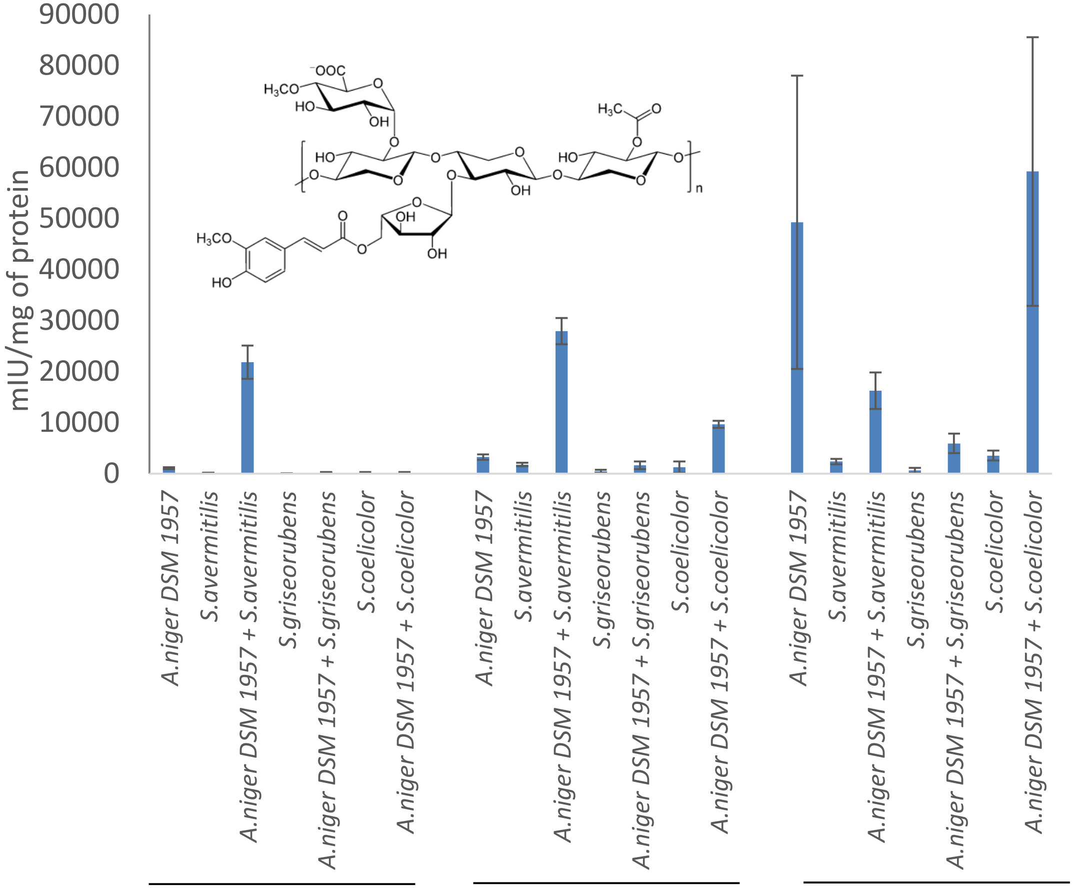
Detain et al., 2022

Elicitation of lignocellulolytic enzymatic activities

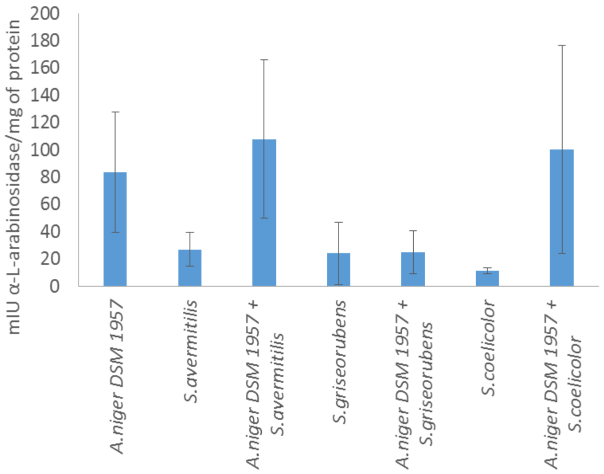


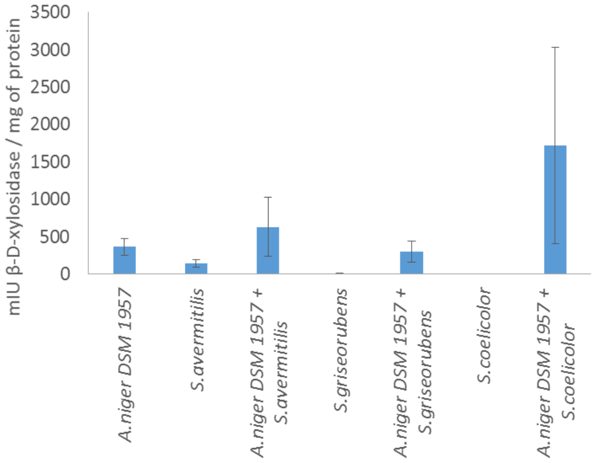
Wheat bran 5g/L

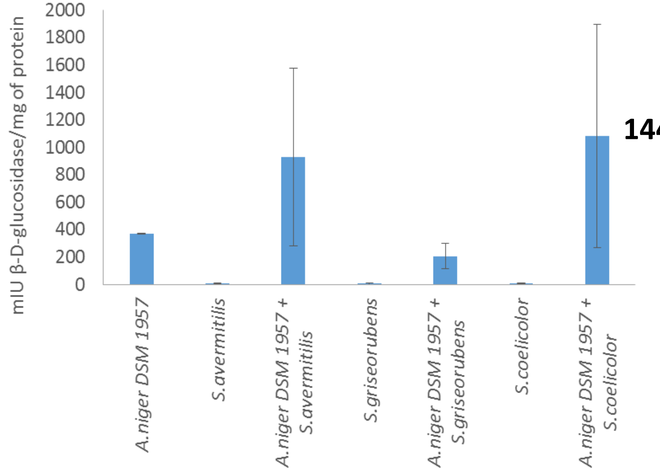
Xylanase



Debranching activities





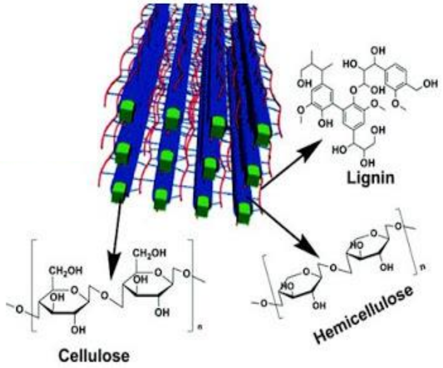


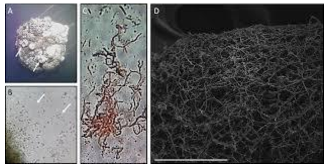
144 hours

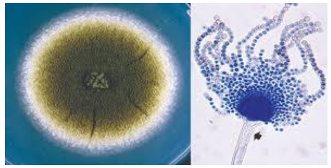
Elicitation of LC lytic enzymatic activities (up to 8 times)
Enhancement of biomass deconstruction
Elicitation of LC lytic activities of the fungus is *Streptomyces*-strain-dependent

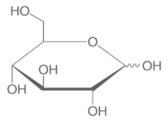
Detain et al., 2022

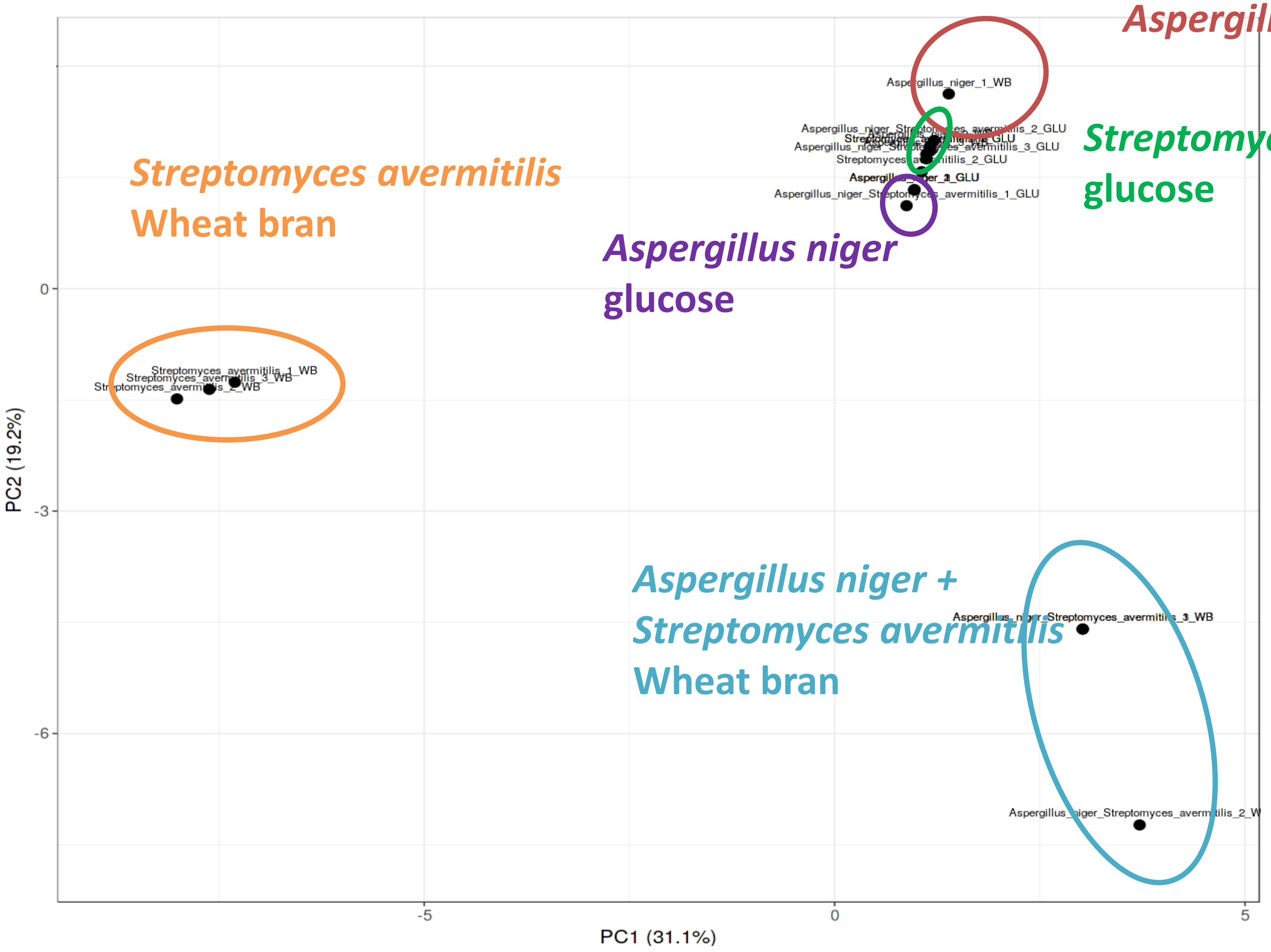
Elicitation of secondary metabolites production







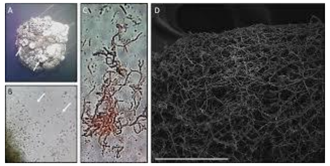




Activation of different biosynthetic cluster genes in wheat bran co-cultures involving *Aspergillus* and *Streptomyces* related to the diversity of carbonaceous molecules in wheat bran

Detain et al., 2022

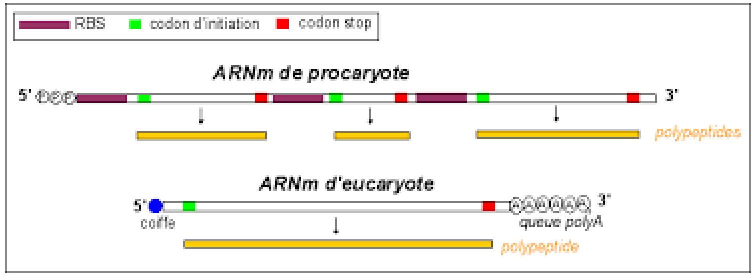
Molecular processes involved in co-culture



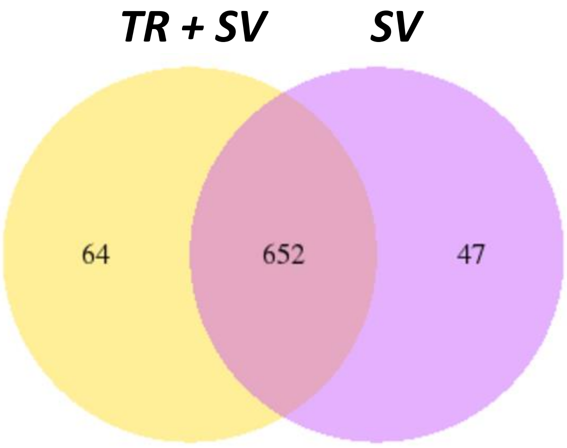
***Streptomyces violaceoruber* (SV)**

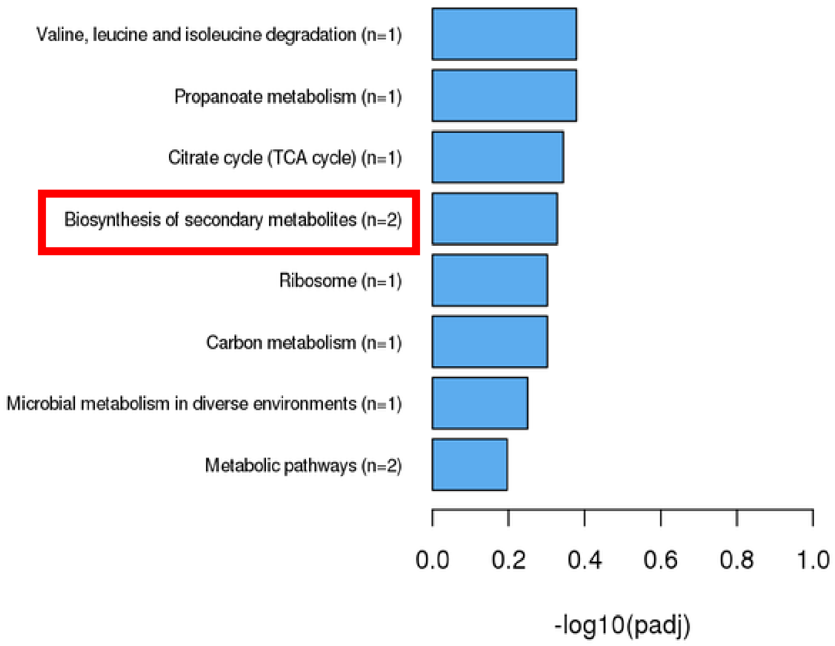
***Trichoderma reesei* (TR)**

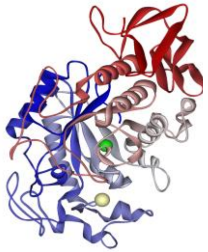
RNA extraction

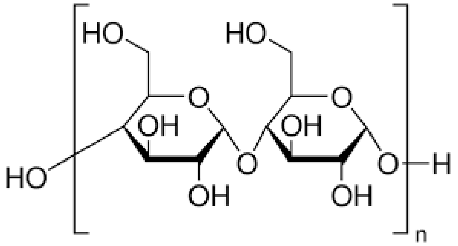


Dual RNA-seq





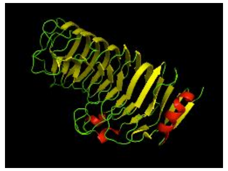




GH13

Amylase

Starch



polygalacturonase

GH28

Common core expressed but presence of 64 transcripts produced by SV due to the presence of fungi

- SV overexpression of genes involved in secondary metabolite production.
- Elicitation of new metabolites?

Overexpression of transcripts produced by SV involved in biomass degradation

Amrani et al., in preparation

14

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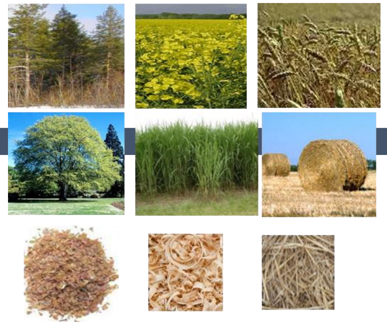

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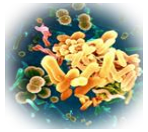

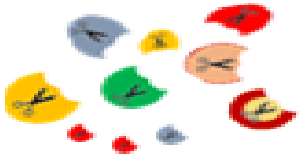

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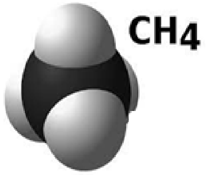

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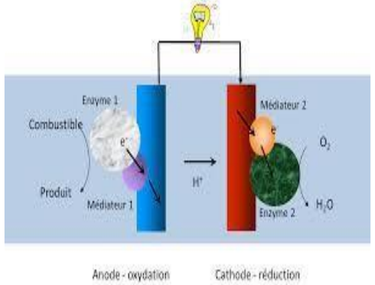
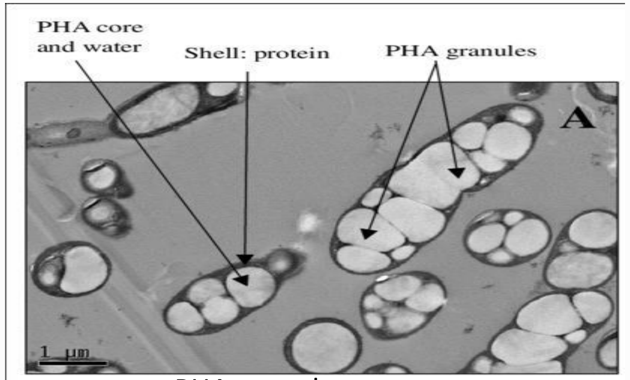


Bioelectrochemistry

Polyhydroxybutyrate (PHB)



PHA granules

Bioplastic

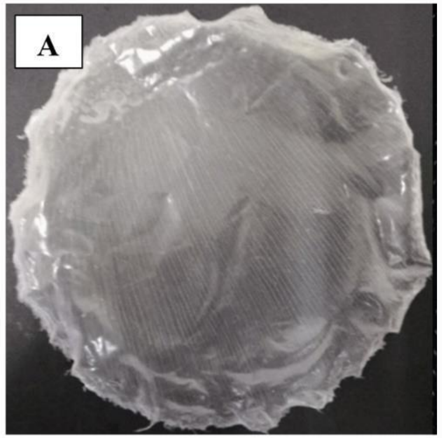
Properties similar to polypropylene (PP) (Daris et al. 2020)

Interesting properties

Biocompatible, biodegradable, biosourced (Chen et al. 2016)

Numerous applications

Encapsulating agent, packaging, bioactive tissue patches... (Arrieta et al. 2017) (Usurelu et al. 2022)



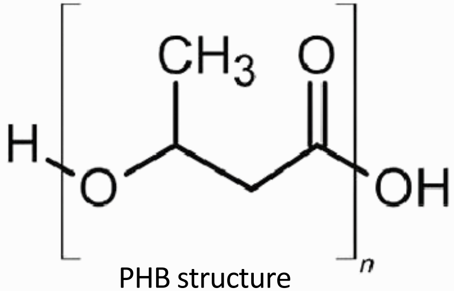
Film de PHB

Bacterial biosynthesis

Under stress conditions (Nitrogen, phosphorus...)
Storage: (granules) in inclusion bodies Genes: (Pathway I)phaA, phaB, phaC (Chen et al. 2016)

Linear polymer

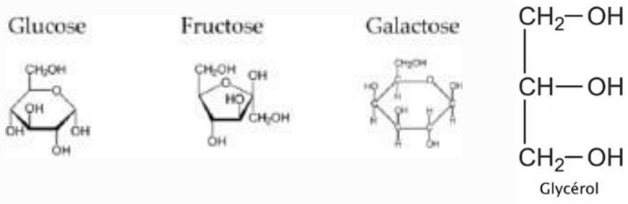
Monomer of 3-hydroxybutyryl (Chen *et al.*2016)



PHB structure

PHB production

-> Substrates: Sugars / glycerol / biomass hydrolysates



Costly and polluting pretreatments (release of fermentable sugars)(Wang et al.2021)

Costly substrates

Increase in production costs and final product

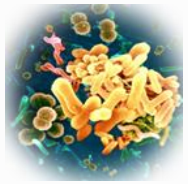
Decline in competitiveness (Atiwesh et al.2022)

PHB cost : 3,64$/kg
PP cost: 1,17$/kg
(Akkoyunlu *et al.* 2024)

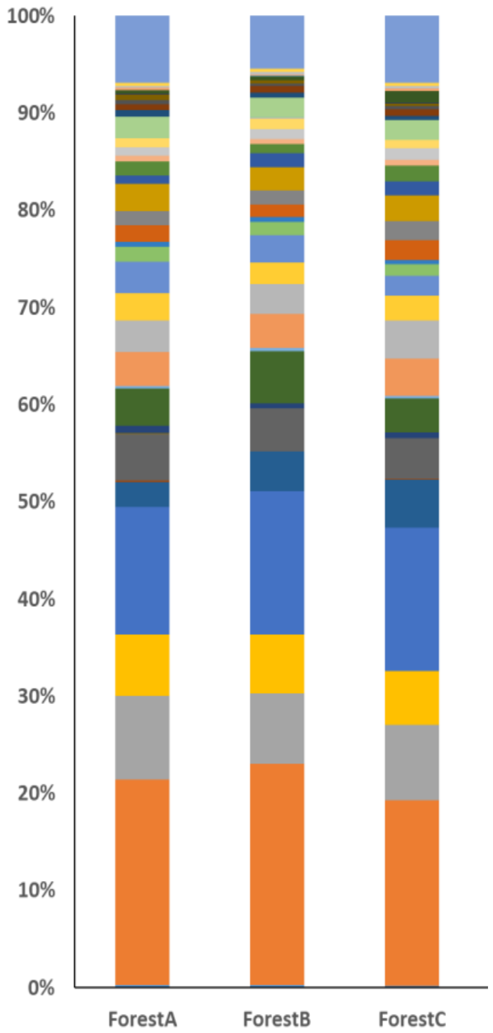
BLC as a substrate?

Glucose cost (Ton) : 600 $
Biomass cost (Ton) : 200 $

Need to find PHB-producing organisms/communities capable of simultaneously degrading biomass

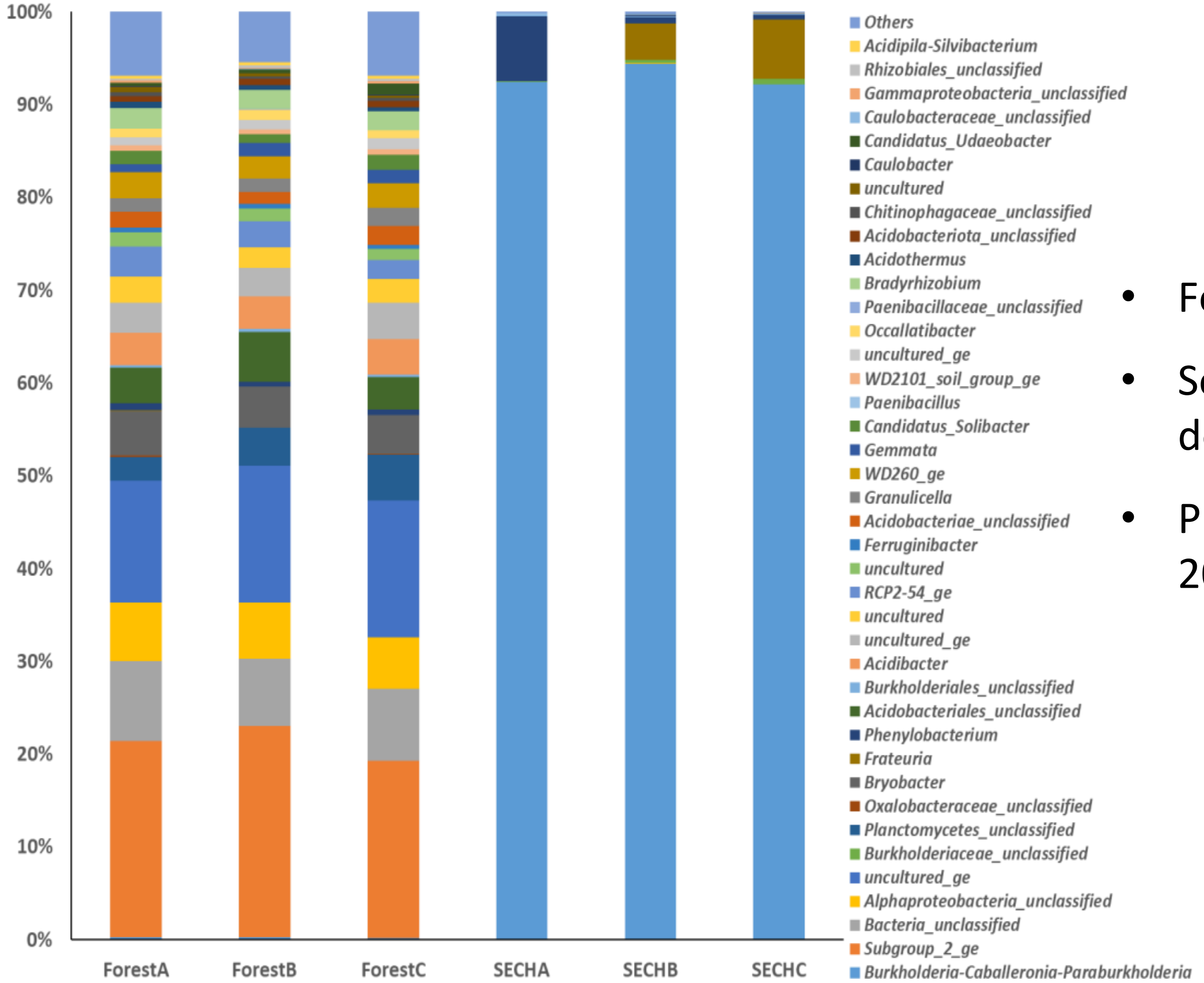


Relative abundance of the main genera present in biomass-degrading microbial communities



Ivaldi et al., LAM (2024)

Relative abundance of the main genera present in biomass-degrading microbial communities



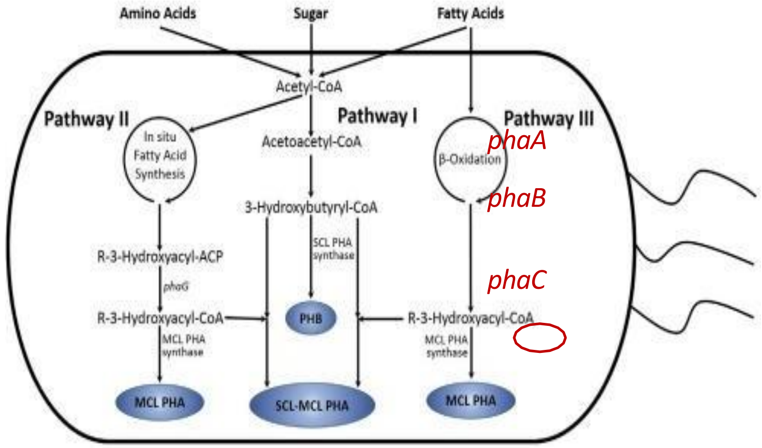
- Forest soil = complex diversity of microorganisms
- Soil enriched with sugar mixture (SECH) = One dominant genus = *Paraburkholderia*
- PHB production (Li & Wilkins, 2021) (Li et al., 2019)

Ivaldi et al., LAM (2024)

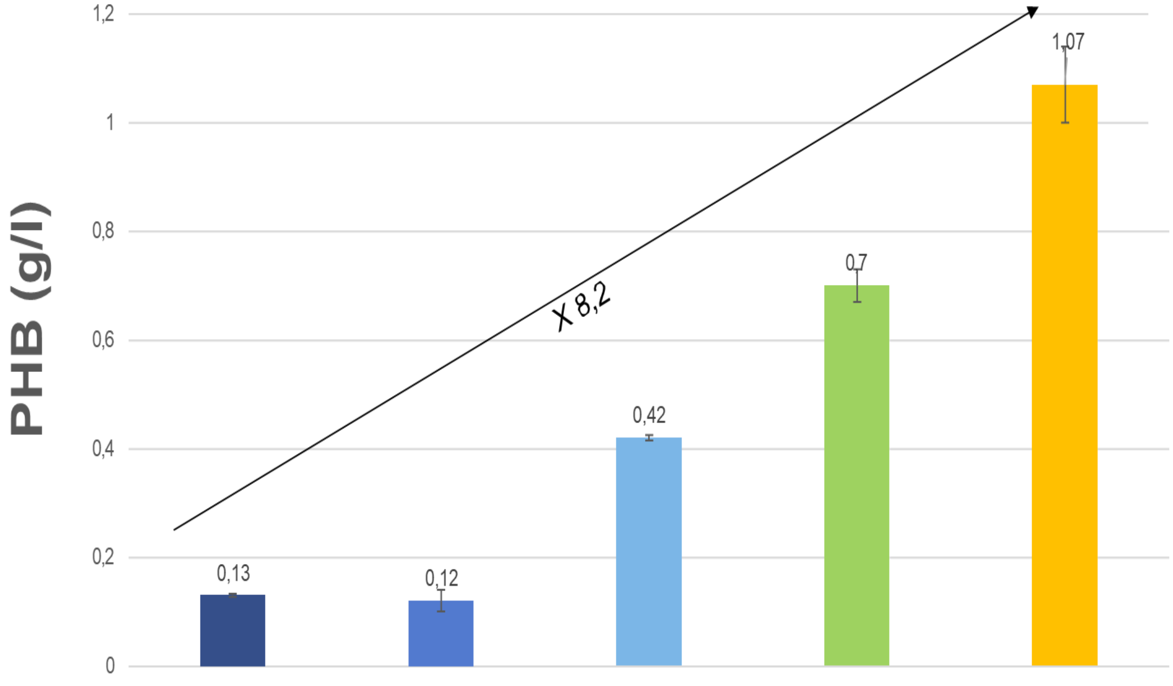
Genomic comparison of PHA metabolism by *Paraburkholderia* strains

Copy numbers of *phaA*, *phaB* and *phaC* genes in the strains studied

Souche	*phaA*	*phaB*	*phaC*
P. sacchari LMG 19450T	1	4	3
P. madseniana SEWS6	3	7	4

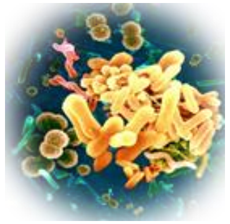


phaA : β-cétothiolase
phaB : AcétoacétylCoA réductase
phaC : PHA synthase



Production of up to 1.07 g/l PHA without pretreatment

Innovative results to be optimized



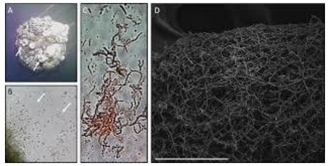
Next : Production using microbial communities dominated by this genus

Ivaldi et al., LAM (2024)

Thank you for your attention

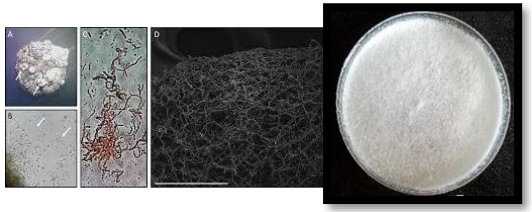
Dr Ludovic Besaury
University Reims Champagne Ardennes
UMR FARE Fractionation Agroresources Environment / AFERE Chair
ludovic.besaury@univ-reims.fr

Biomass degradation by FTIR ?

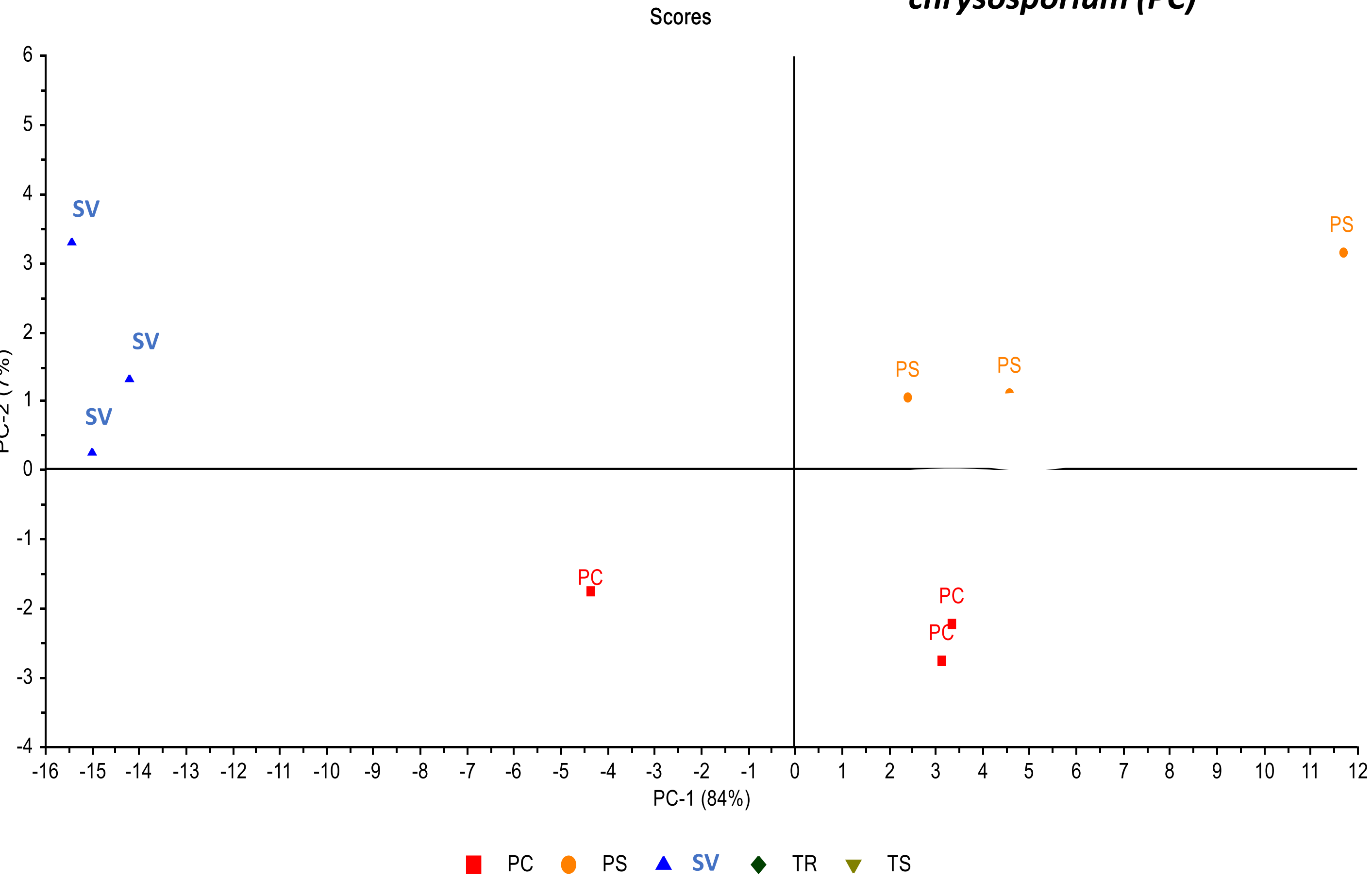


Streptomyces violaceoruber (SV)

Phanerochaete chrysosporium (PC)

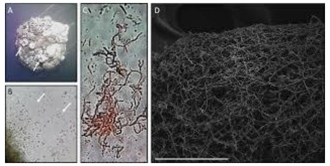


Co-culture = *PS*



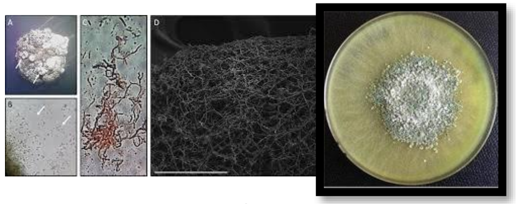
Amrani et al., in preparation

Biomass degradation by FTIR ?

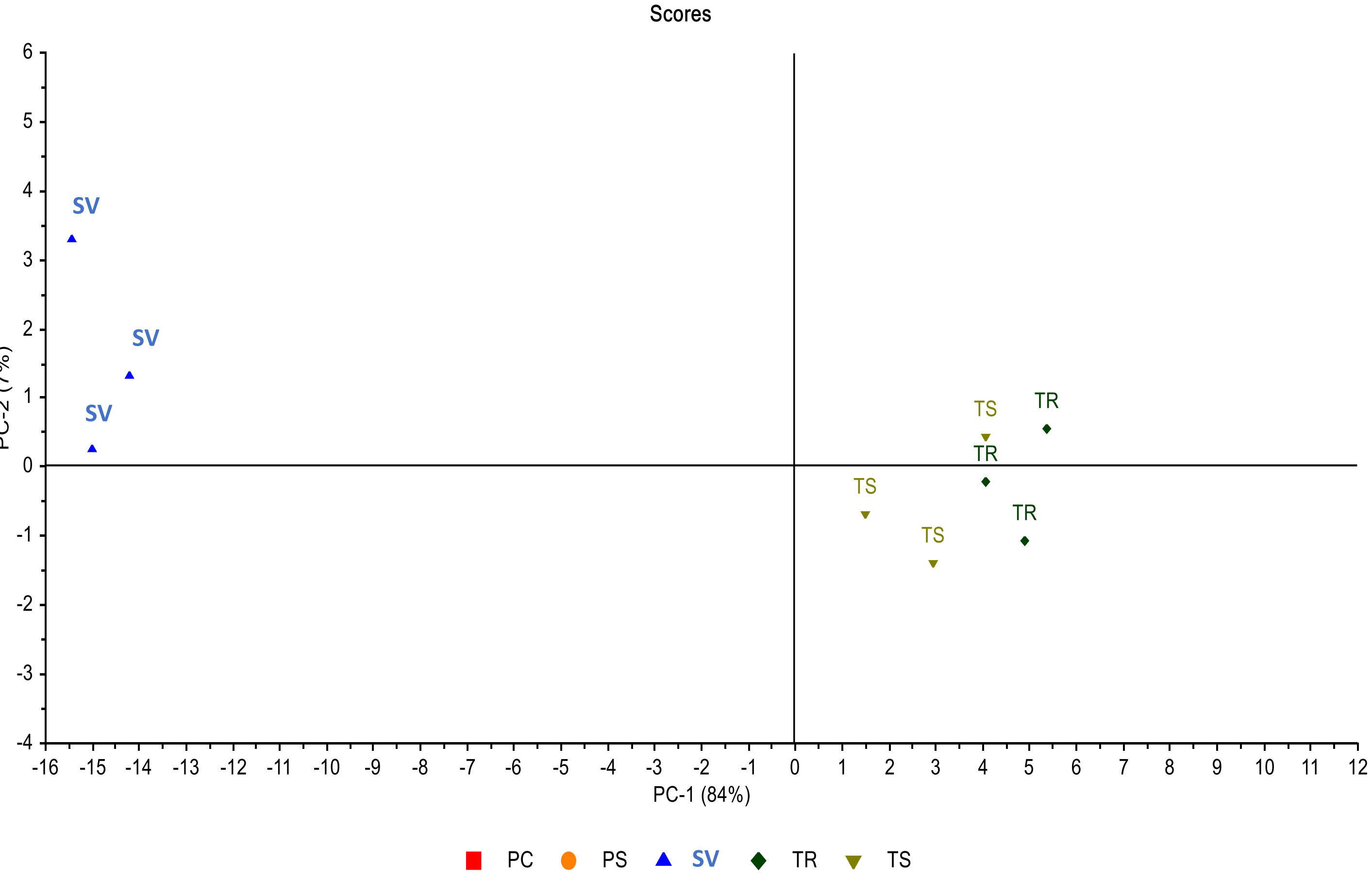


Streptomyces violaceoruber (SV)

Trichoderma reesei (TR)

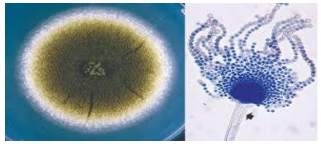


Co-culture = *TS*



Amrani et al., in preparation

Lignocellulolytic enzymatic mechanisms present in *Aspergillus*



Nova seq,
2*150bp

Aspergillus niger

Genome size = 37,5 Mbp

Carbohydrate Active Enzymes (CAZymes)

CAZypedia
carbohydrate-active ENZYMES

Glycoside Hydrolases (GHs) : hydrolysis and/or rearrangement of glycosidic bonds

GlycosylTransferases (GTs) : formation of glycosidic bonds

Polysaccharide Lyases (PLs) : non-hydrolytic cleavage of glycosidic bonds

Carbohydrate Esterases (CEs) : hydrolysis of carbohydrate esters

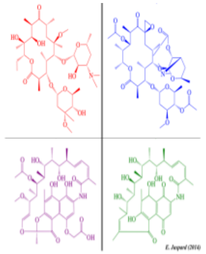
Auxiliary Activities (AAs) : redox enzymes that act in conjunction with CAZymes

Carbohydrate-Binding Modules (CBMs) : adhesion to carbohydrates

Strain	Length (MBp)	Single-coding proteins	CAZymes number	CAZymes (%)	AA	CBM	CE	GH	GT	PL
DSM 1957	35.6	10798	581	5.38	106	17	88	262	99	10
An76	34.6	10373	551	5.31	97	15	86	246	97	10
ATCC 1015	34.9	10950	565	5.16	104	17	81	256	97	10
ATCC 13496	35.7	12194	576	4.72	103	16	86	261	101	9
CBS 513.88	34	10609	576	5.43	104	15	83	249	107	18
CBS 101883	35.9	13359	581	4.35	106	15	88	262	100	10
ATCC 64974	35.5	11236	581	5.17	105	17	87	259	99	14

- **Highest content of CAZymes, AA, CE and GH compared to other Aspergillus niger strains**
- **Very high diversity of families within the AA, GH groups**

	Aspergillus niger DSM 1957
T1PKS	19
NRPS-like	14
NRPS	14
terpene	5
T1PKS,NRPS-like	3
T1PKS,NRPS	3
NRPS,T1PKS	3
betalactone	1
indole	1
NRPS,terpene	1
T3PKS	1
NRPS-like,T1PKS	1



These secondary metabolites include clavaric acid, fumonisin, phyllostictin and pyranonigrin

- **High diversity of secondary metabolite clusters**
- **Higher content of T1PKS and NRPS compared to other Aspergillus strains**

Detain et al., 2022

Parameters of the coculture

Nature of the microbial partners :
-Fungi
-Bacteria
-Archaea

Physico-chemical parameters :
-pH
-temperature
-medium culture

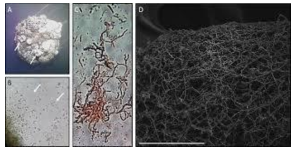
Biological interactions :
-Competition
-Mutualism
-Symbiosis

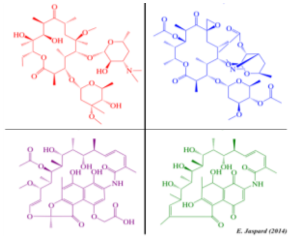
Co-culture dynamics :
-volume of inoculation
-inoculation type (spore/pellets)
-Simultaneous or delayed inoculation

- Difficulties to set up and inter-kingdom coculture

Coculture: Microbial partners

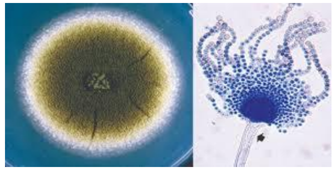
- **Bacterial partner:**
  ***Streptomyces* genus**
- Ability to degrade lignocellulose; large panel of enzymes involved in LC degradation (cellulase, laccase ...).
- One of the largest bacterial genomes = large spectrum of secondary metabolites





- <u>**Fungal partner:**</u>

***Aspergillus niger* DSM 1957**



- Ascomycete
- High content of CAZymes
- Ability to degrade lignocellulose;
- large panel of enzymes involved in LC degradation (xylanase, xylosidase...)

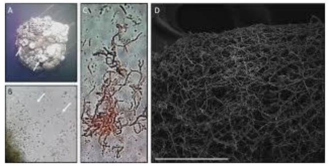
Trichoderma reesei RUT C-30 (TR)

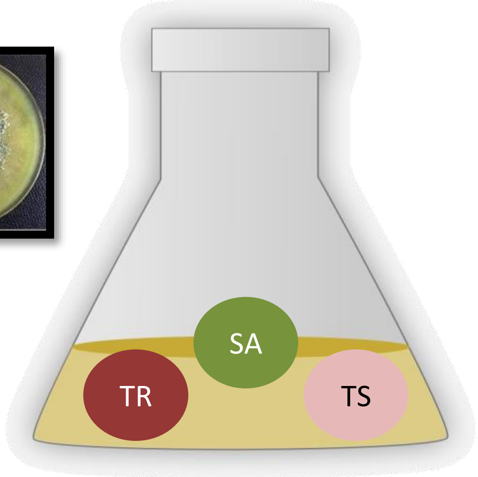
- Ascomycete
- Filamentous model fungus ; ability to degrade cellulose
- High content in CAZymes

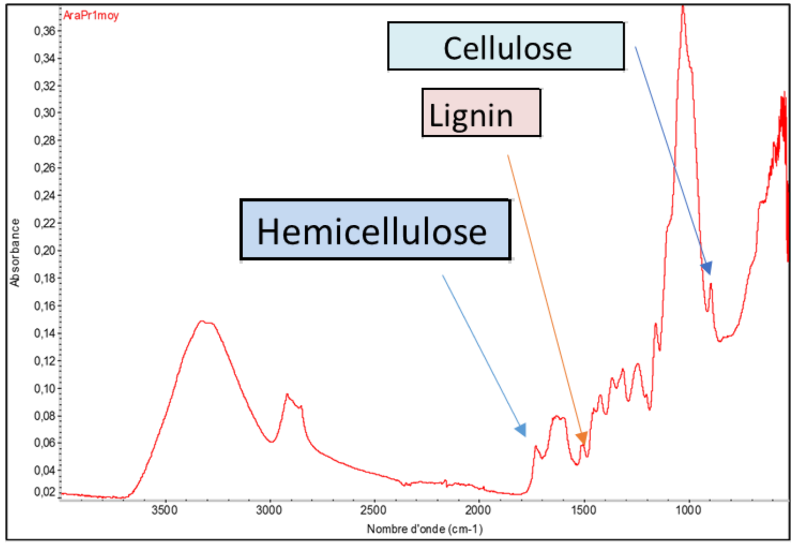
Phanerochaete chrysosporium RP-78 (PC)

- Basidiomycetes
- Model fungi known for its ability to degrade lignin
- High percentage of CAZymes in the genome

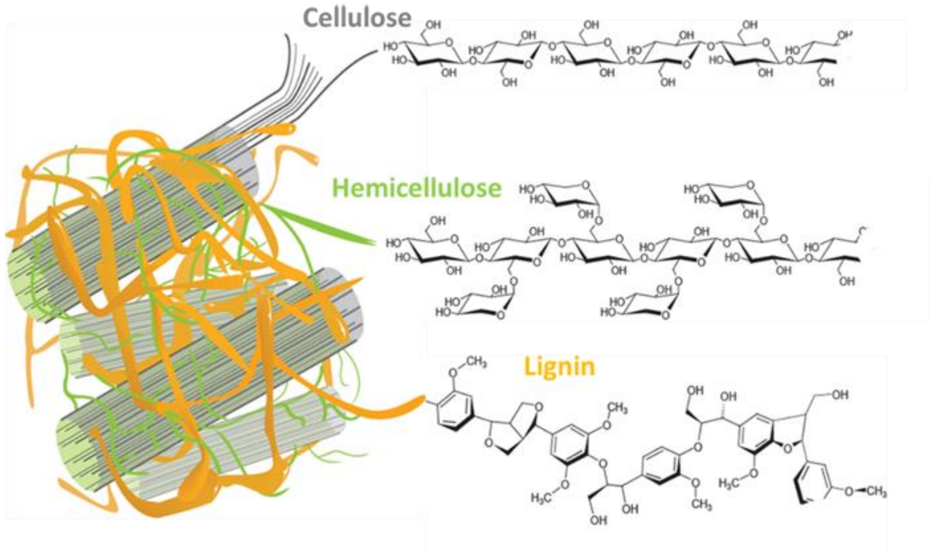
FTIR (Fourier Transform InfraRed spectroscopy)





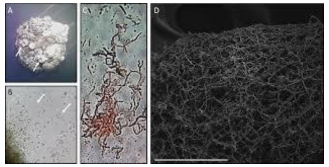


IRTF spectra



Structure modification?

Biomass degradation by FTIR and correlation with Lclytic enzymes?



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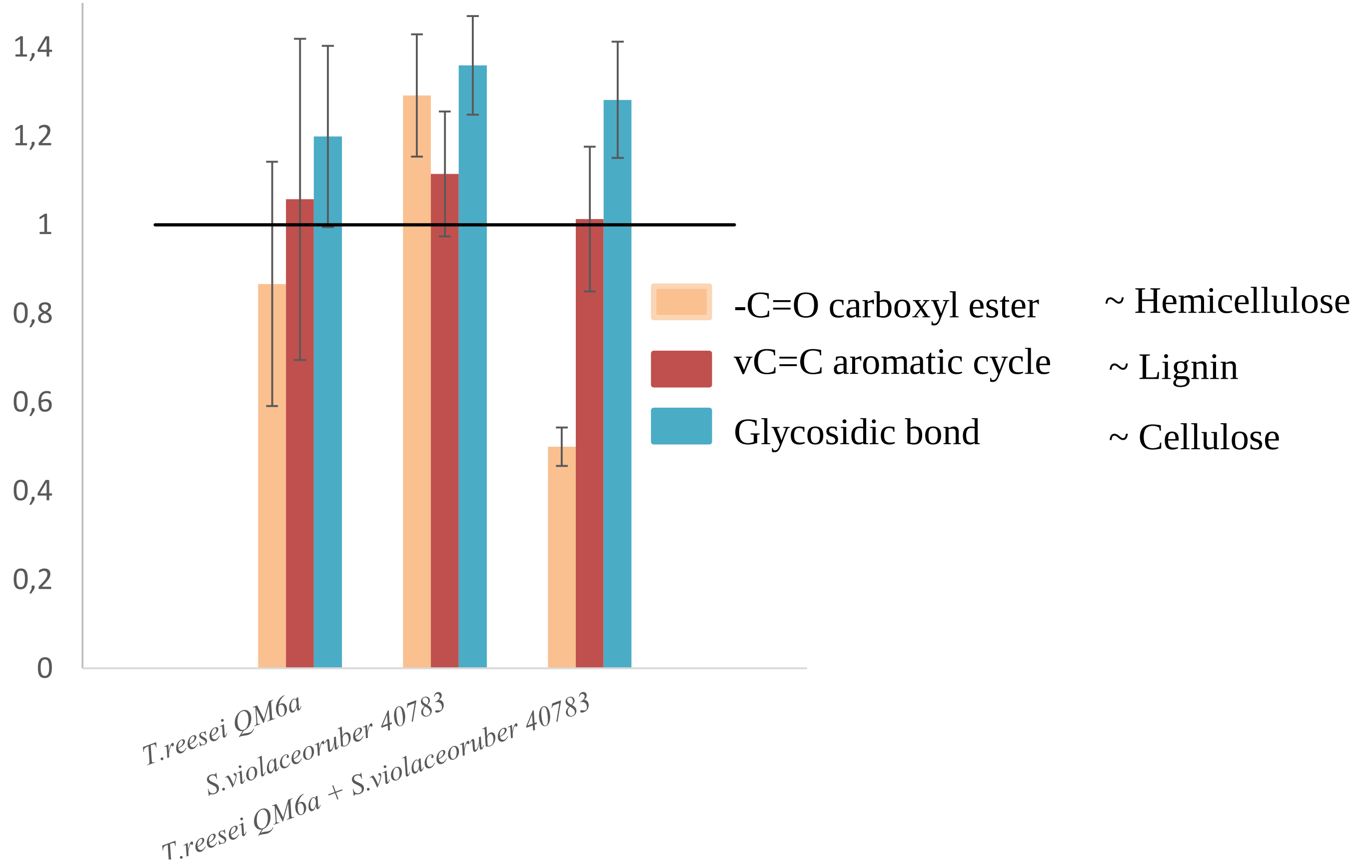
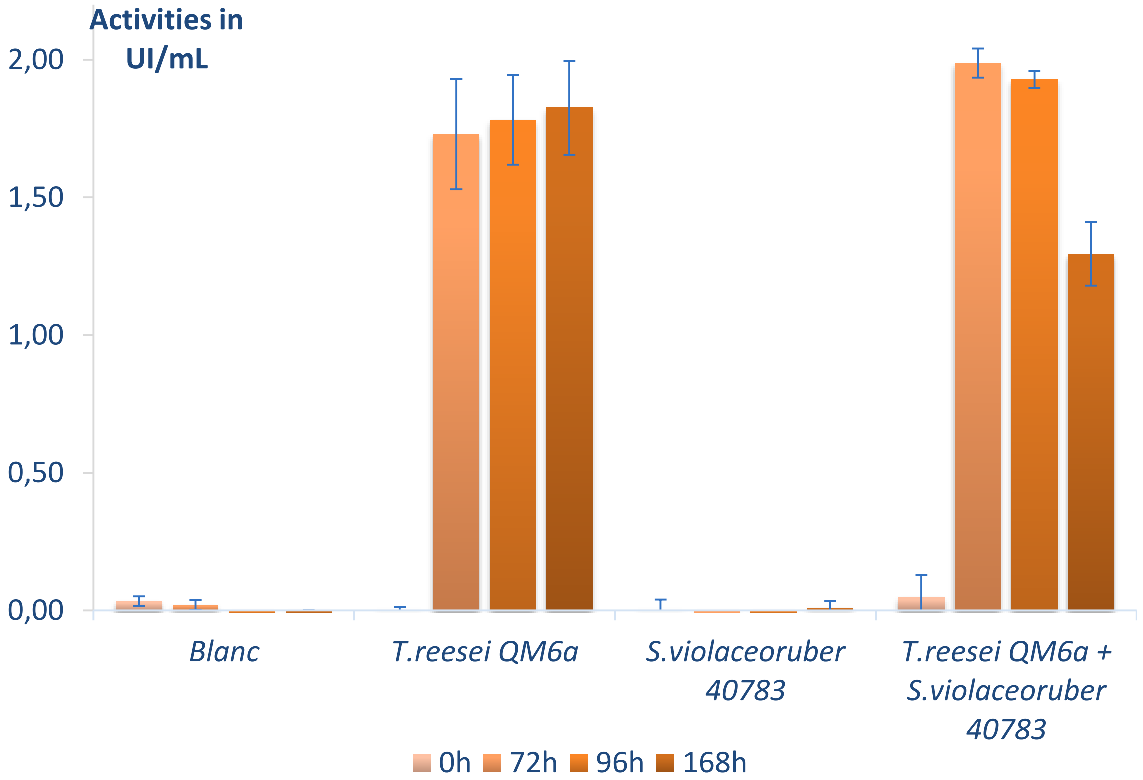


Hemicellulose degradation (++ coculture)

Xylanase activity (hemicellulose)



Correlation with enzymatic actvities

Amrani et al., in preparation