Metatranscriptomics and metabolic modeling to identify bacterial metabolic interactions during the manufacture of a model pressed cheese.

Wenfan Cao, Maxime Lecomte, Solène Le Fur, Julie J. Aubert, Marie-Bernadette Maillard, Aurélie Nicolas, Stéphanie-Marie Deutsch, Sandrine Parayre, Françoise Boissel, Arlette Leduc, et al.

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Metatranscriptomics and metabolic modeling to identify bacterial metabolic interactions during the manufacture of a model pressed cheese.

Hélène Falentin – URM STLO – RENNES

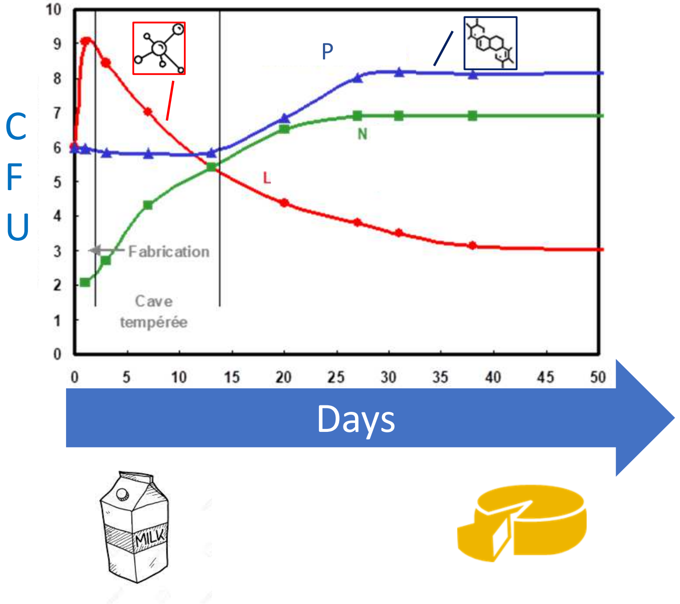
Founded by

Context et questions

Context

Activity of cheese microbiota responsible for organoleptic quality

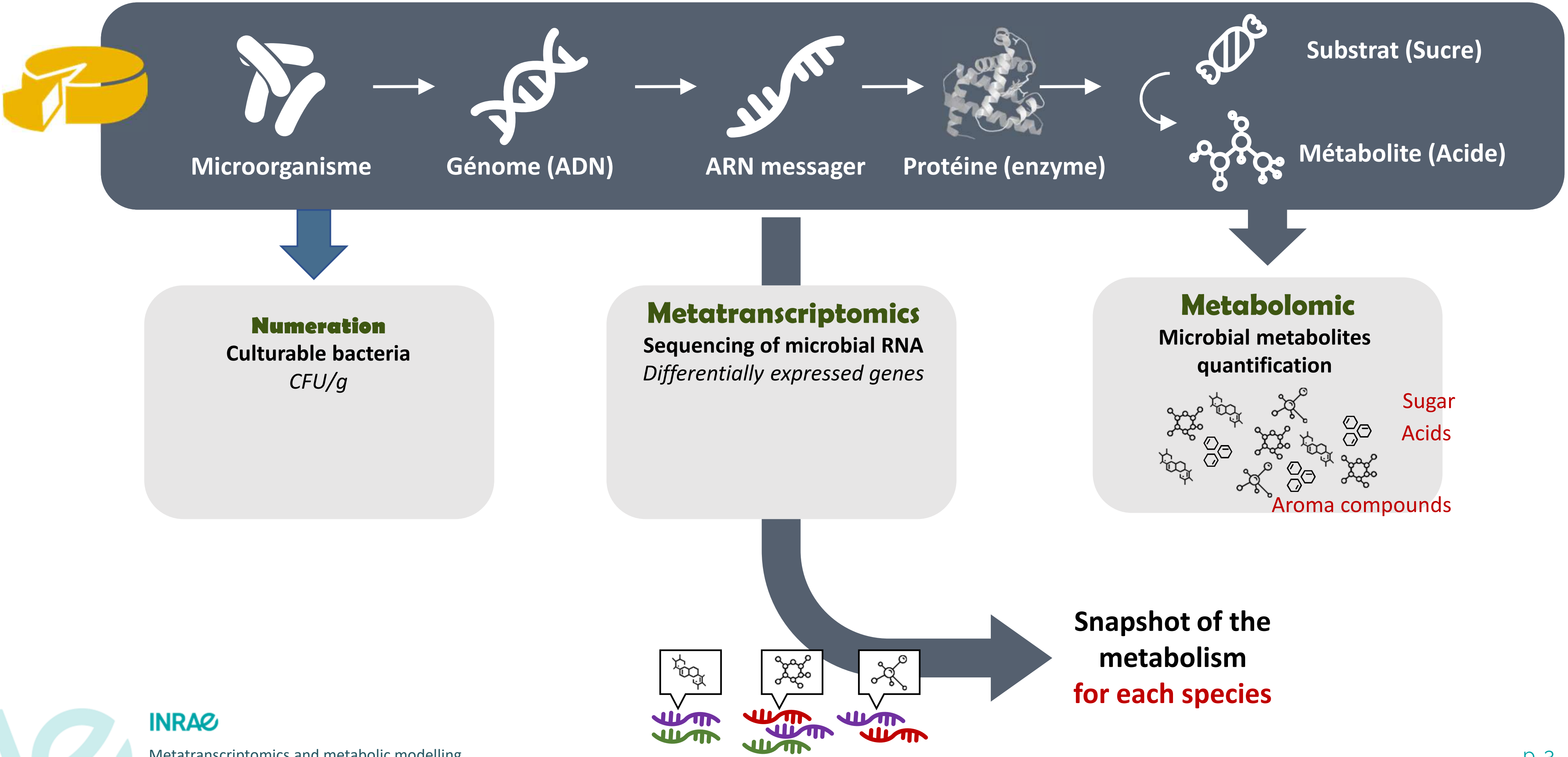
- Successive growths of microbes
- Specific and complementary microbial metabolisms
- Metabolic interactions between microbes



Scientific questions

- Which species contributes to acidification ?
- Which species contributes to aroma compound production?
- Which metabolic pathways are activated ?
- When are they activated ?
- Which bacterial metabolic interactions contributes to the functioning of microbial ecosystem ?

Strategy using multi-omics



Microorganisme → Génome (ADN) → ARN messager → Protéine (enzyme) →

Substrat (Sucre)

Métabolite (Acide)

Numeration
Culturable bacteria
CFU/g

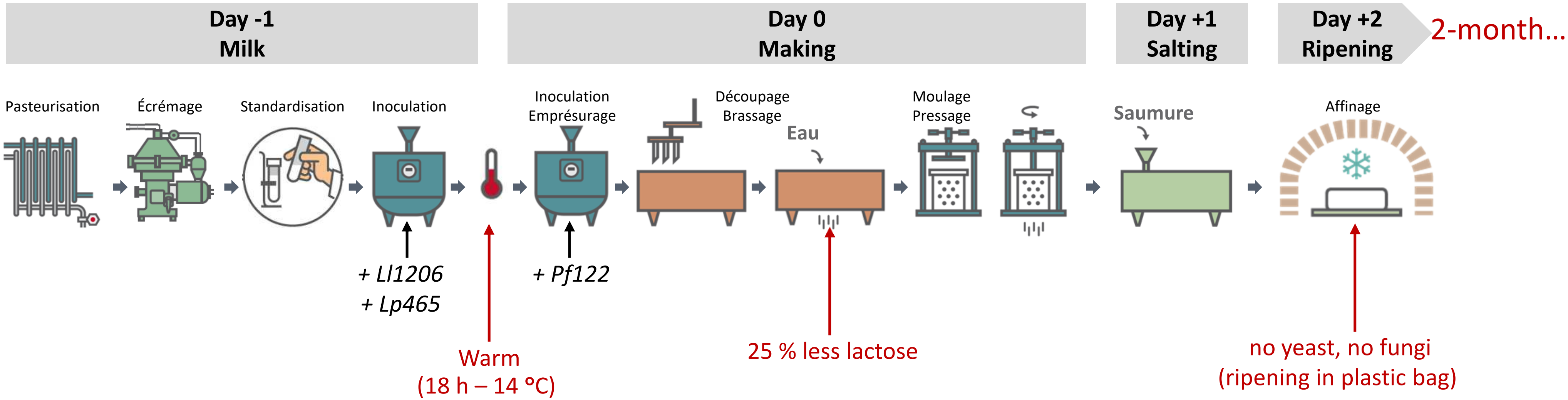
Metatranscriptomics
Sequencing of microbial RNA
Differentially expressed genes

Metabolomic
Microbial metabolites quantification

Sugar
Acids
Aroma compounds

Snapshot of the metabolism
for each species

Cheese making of semi hard cheeses



Day -1
Milk

Day 0
Making

Day +1
Salting

Day +2
Ripening

2-month…

+ *Ll1206*
+ *Lp465*

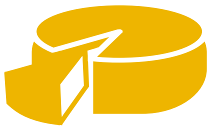
+ *Pf122*

Warm
(18 h – 14 °C)

25 % less lactose

no yeast, no fungi
(ripening in plastic bag)

Semi hard cheeses
(without yeast)



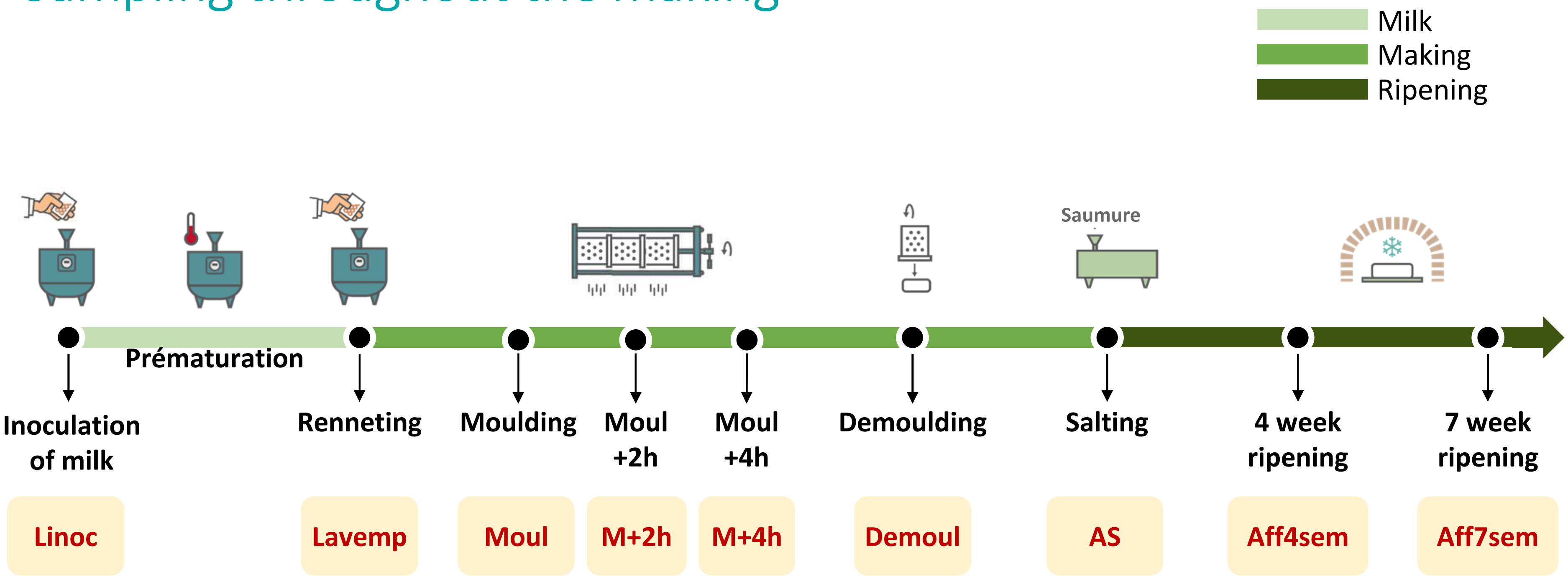
Lactococcus lactis CIRM-BIA1206

Lactiplantibacillus plantarum CIRM-BIA465

Propionibacterium freudenreichii CIRM-BIA122

Cao et al. 2021, J. Agric. Food Chem.

Sampling throughout the making



🟩 Milk
🟩 Making
🟩 Ripening

Saumure

Prématuration

Inoculation of milk – **Linoc**

Renneting – **Lavemp**

Moulding – **Moul**

Moul +2h – **M+2h**

Moul +4h – **M+4h**

Demoulding – **Demoul**

Salting – **AS**

4 week ripening – **Aff4sem**

7 week ripening – **Aff7sem**

Microbiology

Chemistry

Biochemistry

Molecular biology

Computational science

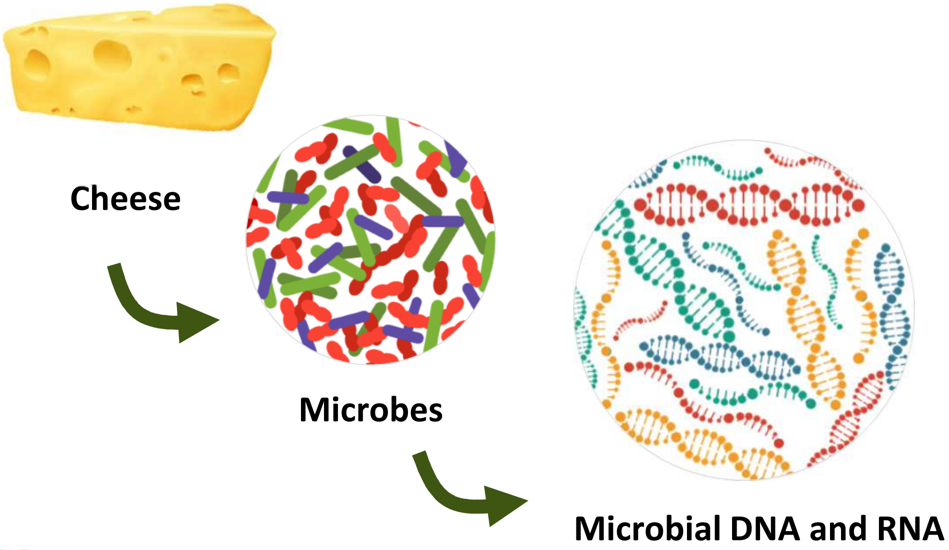
Microbiological methods

Microbiology

- ***L. lactis*** : M17 + lactose
- ***L. plantarum*** : MRS pH5.4
- ***P. freudenreichii*** : Yela

Biochemistry

- **Nitrogen**: Kjeldhal
- **Sugar and acids**: HPLC
- **Aroma compound**: head space + GCMS



Cheese

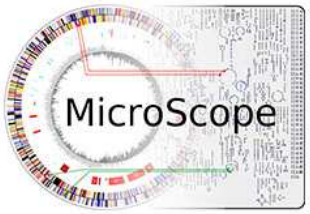
Microbes

Microbial DNA and RNA

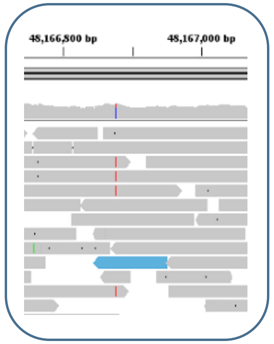
Molecular biology

- **DNA :** Dneasy Tissue kit
- **RNA :** QIAzol + Rneasy mini kit
- **Quality control :** Nanodrop, Qubit, Bioanalyzer
- **Sequencing :** Illumina pairé, PacBio

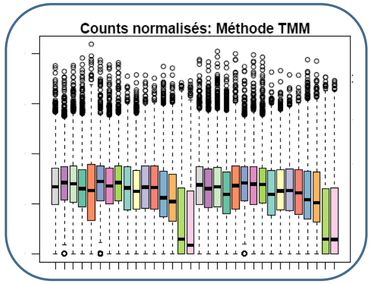
Computational methods



Automatic annotation of genomes
Reconstruction of metabolic pathways using Pathway Tools



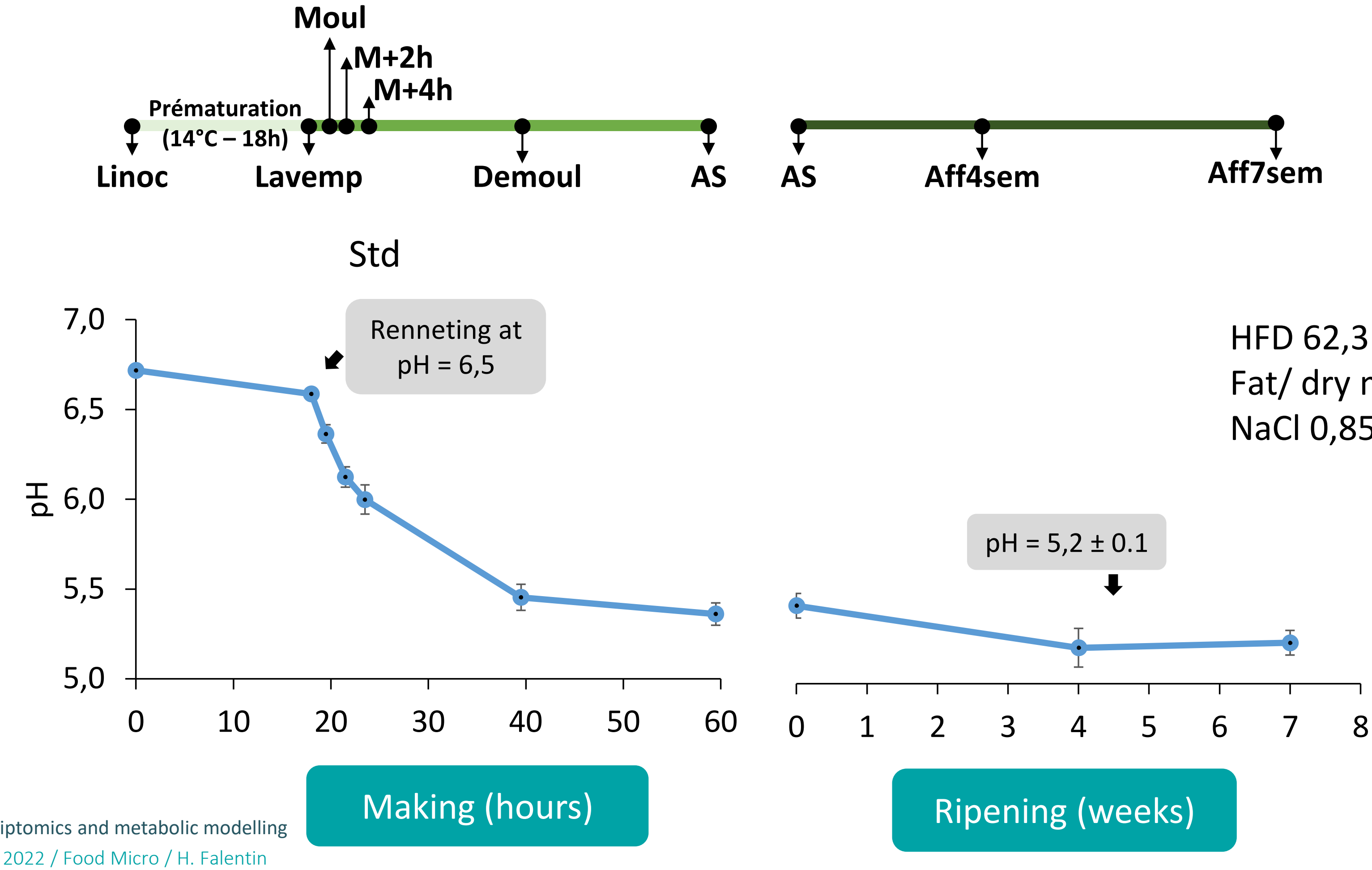
Metatranscriptomics
Reads mapping using Bowtie
Reads counting using htseq count

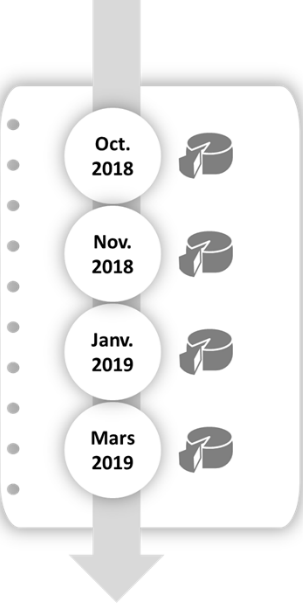


Normalisation using TMM multi species
Identification of DE genes using EdgeR
Significativity padj< 0.05

Langmead et al., 2009
Robinson et al., 2010
Karp et al., 2021

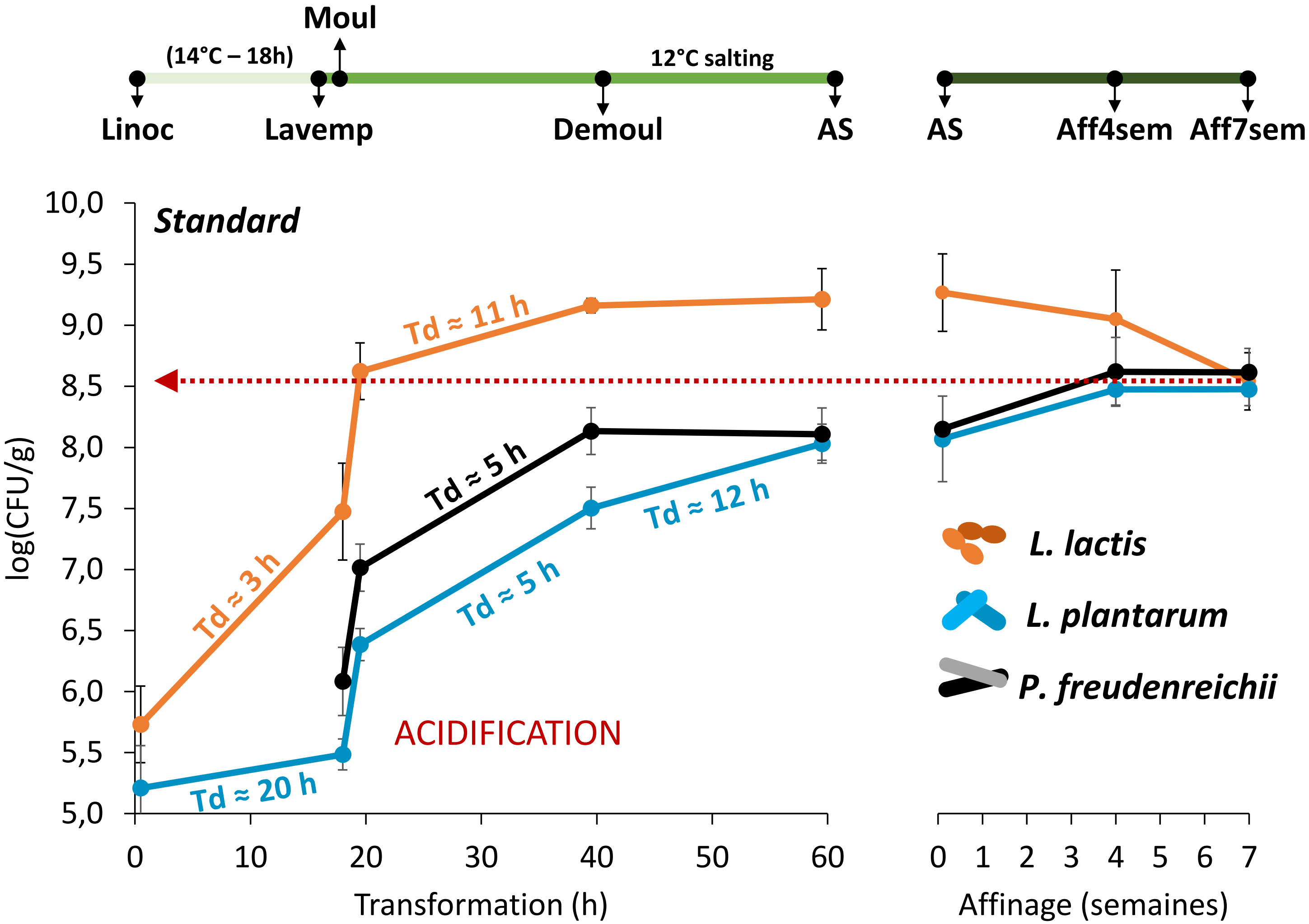
Repeatability of semi hard cheeses (X4)





HFD 62,3 %
Fat/ dry matter 47,5 %
NaCl 0,85 g / 100 g

Lactic and propionic bacterial growth



Td : time of doubling

Cao et al. 2021, J. Agric. Food Chem.

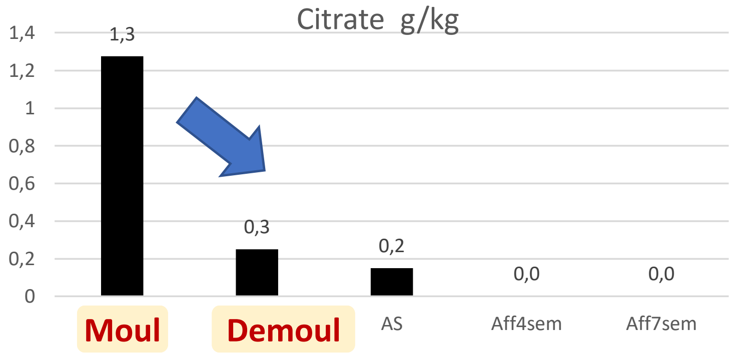
Assembly and genome annotations

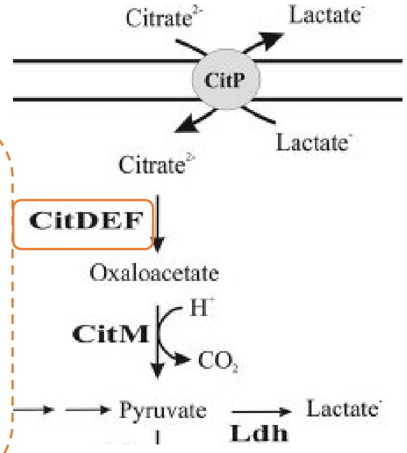
	Replicon	Seq length	% GC	CDS	tRNA	rRNA
***L. lactis* 1206**	**Chromosome**	**2 365 039**	35,34	2417	62	19
	plasmid 1	59 643	34,85	67	0	0
	plasmid 2	36 823	34,41	42	0	0
	plasmid 3	8 277	34,81	12	0	0
	plasmid 4	8 243	30,60	8	0	0
	plasmid 5	4 632	36,25	5	0	0
	plasmid 6	3 597	33,28	2	0	0
	plasmid 7	2 119	34,21	3	0	0
***L. plantarum* 465**	**Chromosome**	**3 121 980**	44,72	3016	67	16
	plasmid 1	40 748	39,81	40	0	0
	plasmid 2	30 463	41,80	27	0	0
	plasmid 3	9 152	36,21	13	0	0
	plasmid 4	2 012	37,87	2	0	0
***P. freudenreichii* 122**	**Chromosome**	**2 688 484**	67,21	2589	45	6

→ Metatranscriptomics : mapping of RNA reads / genomes

Citrate utilization by *Lc. lactis*

Moulding > Demoulding





LLACBIA1206_v1_pc0002
LLACBIA1206_v1_1007

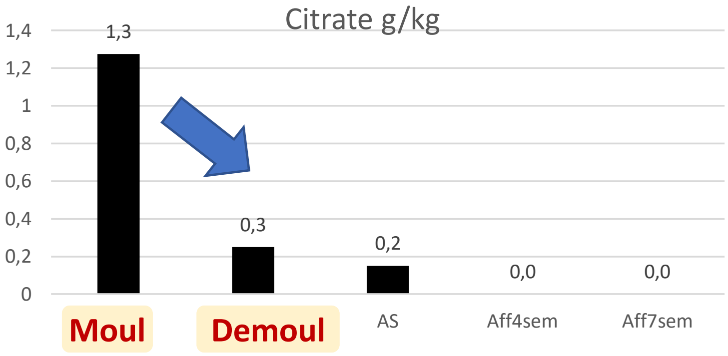
*cit*CDEFG
LLACBIA1206_v1_1311-1316

Induction logFC X 2-3 by *L. lactis*

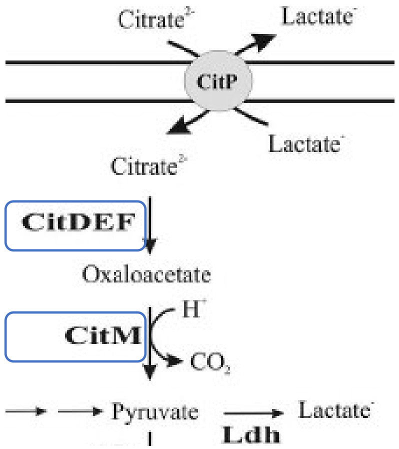
Modified from Garcia-Quintas et al. 2008, AEM

Citrate utilization by *L. plantarum*

Moulding > Demoulding



LPLABIA465_v1_0482



Induction logFC X15 chez *L. plantarum*

*cit*MCDEF
LPLABIA465_v1_0962-0966

Induction logFC X13-16 chez *L. plantarum*

Metabolic interaction = competition for citrate

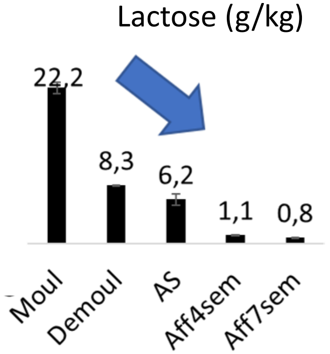
Fonctional redondancy using the same pathway induced at the same time in *L. lactis* et *L. plantarum*

Lactose utilization by *L. plantarum*

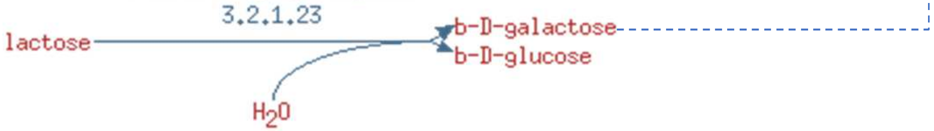
Leloir pathway

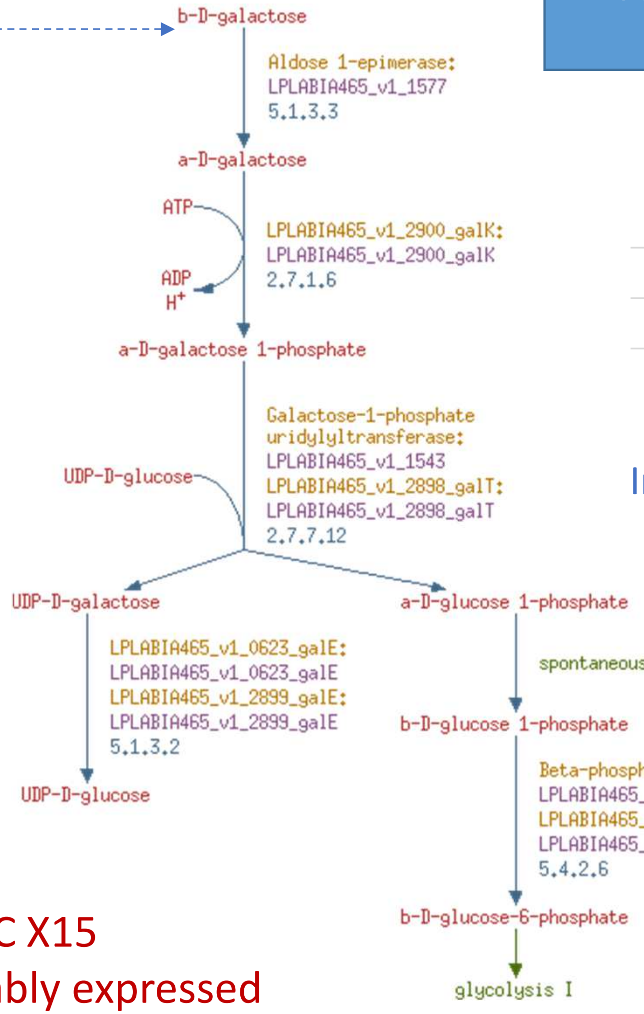
Moulding > Demoulding

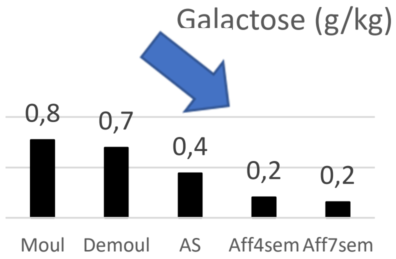
*lac*LM
Induction logFC X 6-7



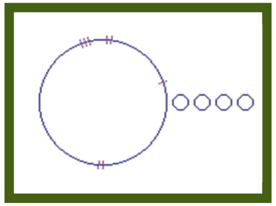
LPLABIA465_v1_2888_ganA:
LPLABIA465_v1_2888_ganA
Beta-galactosidase:
LPLABIA465_v1_2901_lacL
Beta-galactosidase:
LPLABIA465_v1_2902_lacM
3.2.1.23







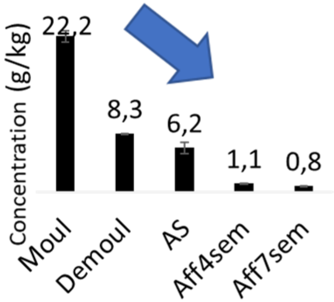
*gal*RTEK
Induction logFC X 5-6



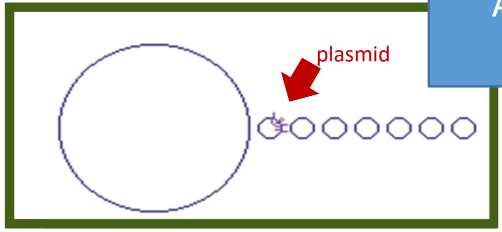
In P. freudenreichii induced logFC X15
In L. lactis same pathway but stably expressed

Lactose utilization by *Lc. lactis*

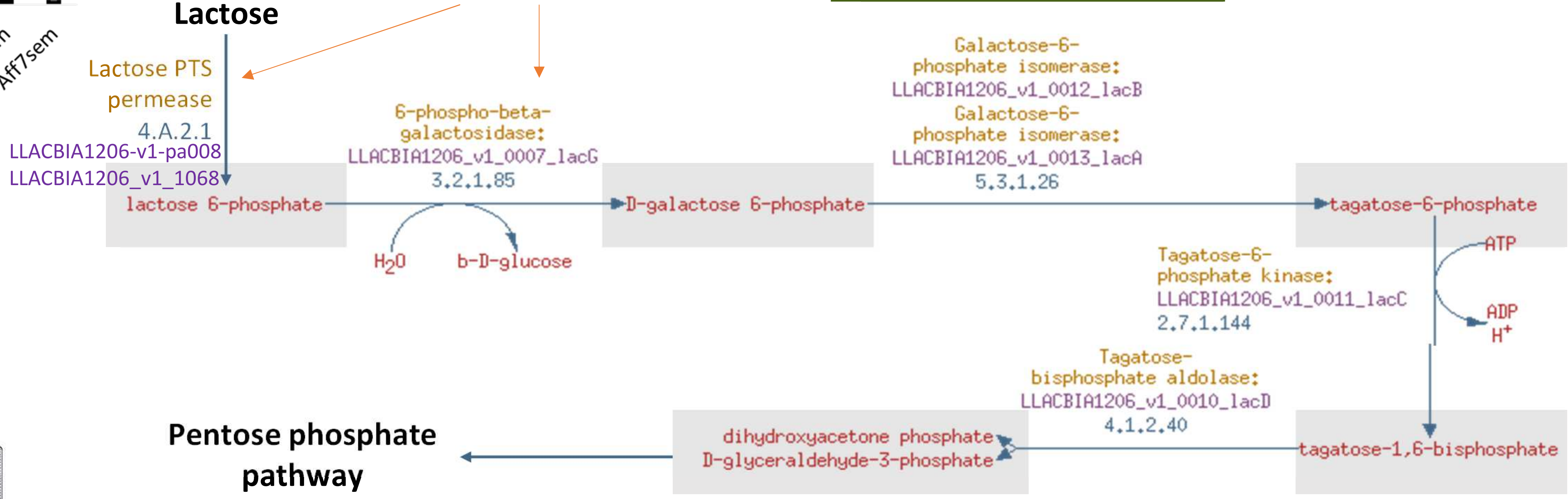
Lactose (g/kg)



AFF4sem > AFF7sem



Plasmidic induction logFC X2



Lactose

Lactose PTS permease
4.A.2.1
LLACBIA1206-v1-pa008
LLACBIA1206_v1_1068

lactose 6-phosphate

6-phospho-beta-galactosidase:
LLACBIA1206_v1_0007_lacG
3.2.1.85

H_2O

b-D-glucose

D-galactose 6-phosphate

Galactose-6-phosphate isomerase:
LLACBIA1206_v1_0012_lacB
Galactose-6-phosphate isomerase:
LLACBIA1206_v1_0013_lacA
5.3.1.26

tagatose-6-phosphate

ATP

Tagatose-6-phosphate kinase:
LLACBIA1206_v1_0011_lacC
2.7.1.144

ADP
H^+

Tagatose-bisphosphate aldolase:
LLACBIA1206_v1_0010_lacD
4.1.2.40

tagatose-1,6-bisphosphate

dihydroxyacetone phosphate
D-glyceraldehyde-3-phosphate

Pentose phosphate pathway

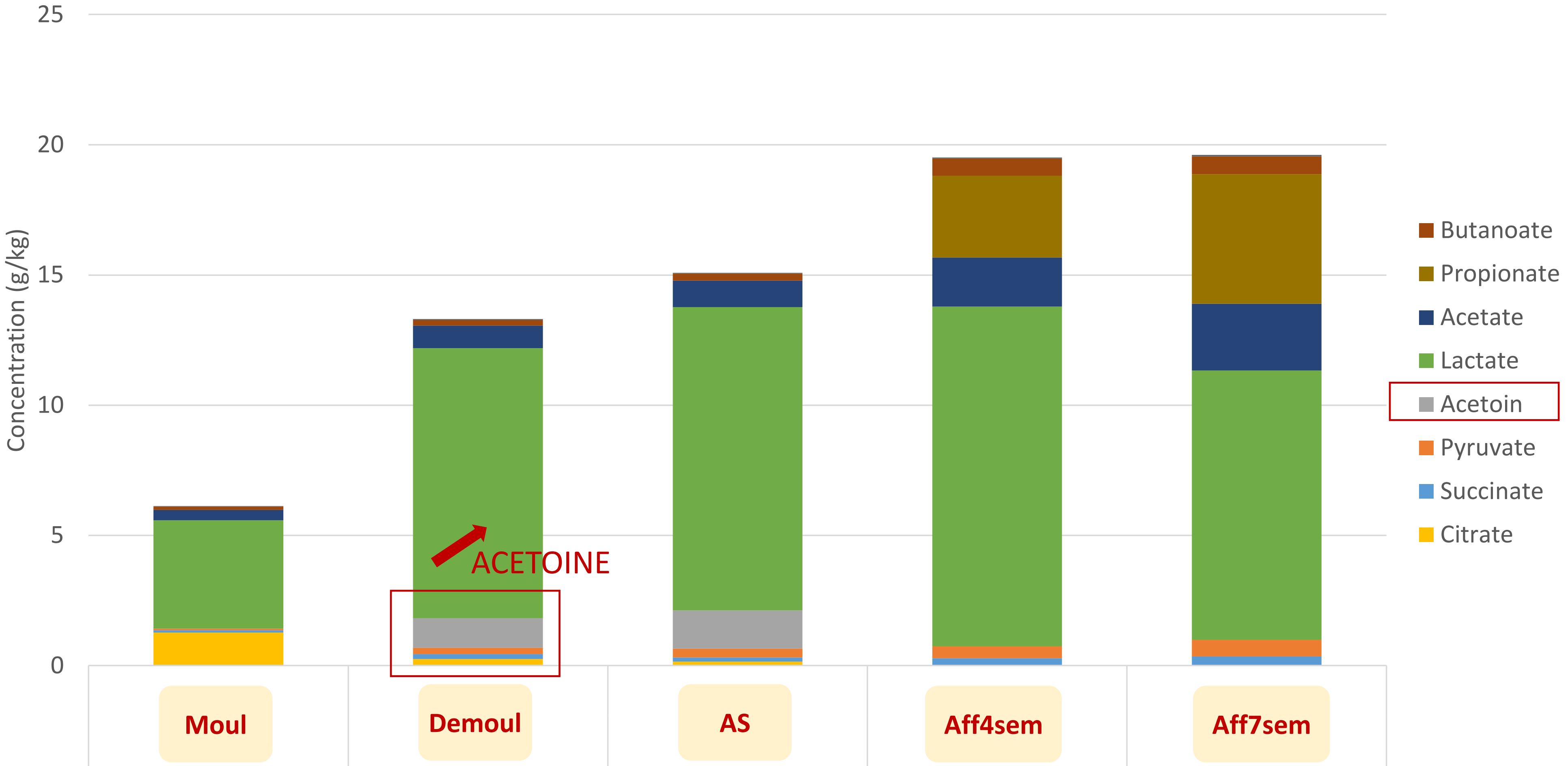
MicroScope

Pathway in agreement with Raynaud et al. 2005

Metabolic interaction = competition for lactose

Functional redondancy with <u>different pathways</u> induced at <u>different times</u>

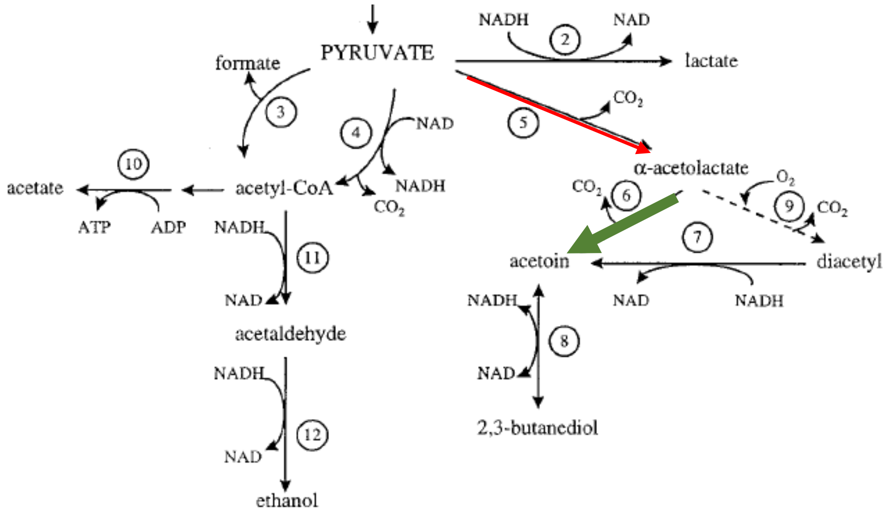
Production of acetoin (cheesy aroma coumpound)



Cao et al. 2021, J. Agric. Food Chem.

Production of acetoin

Moulage > démoulage



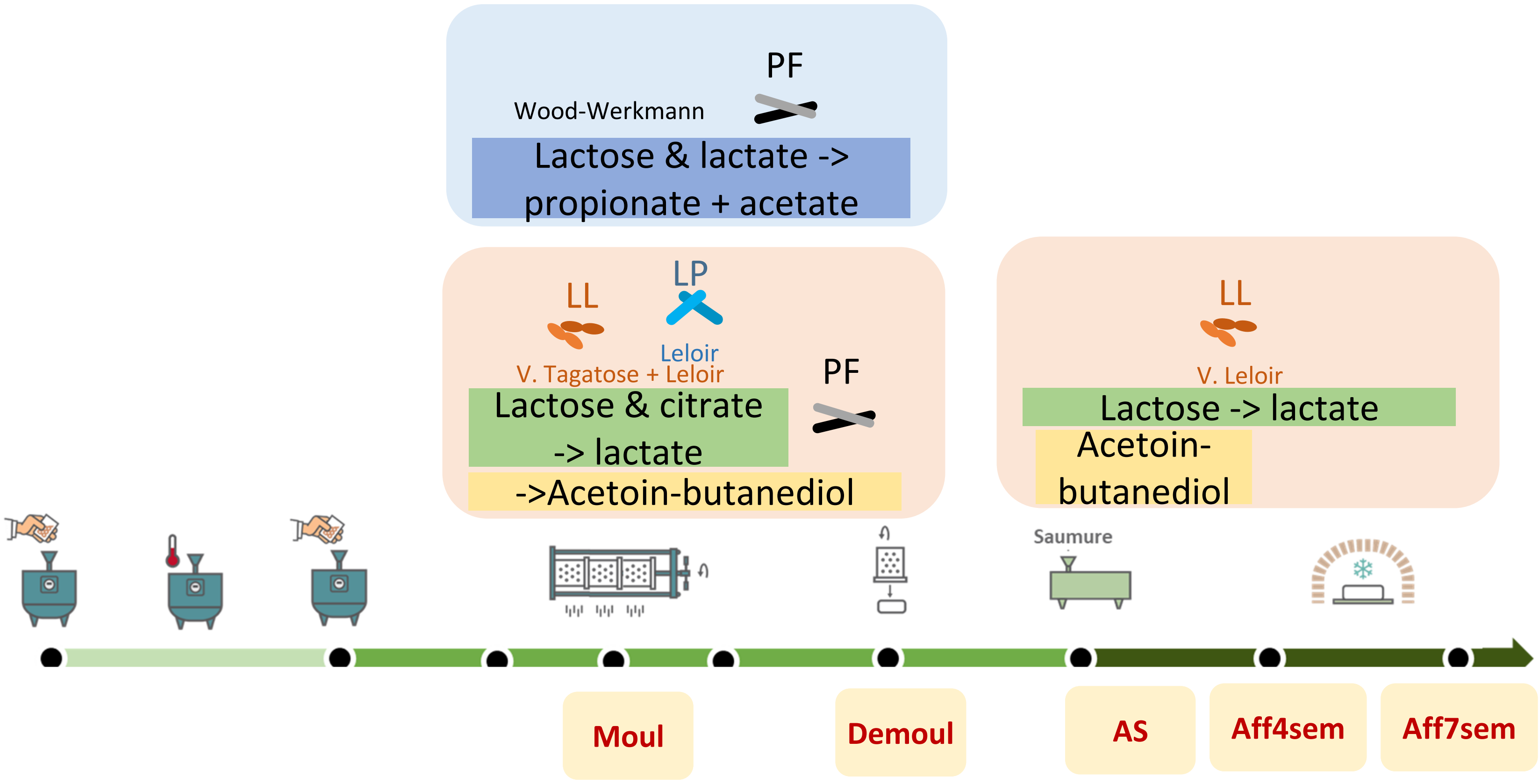
Acetolactate synthase 2.2.1.6 (*als*)
→ Induction x2-4 logFC in *Lc. lactis* and *L. plantarum*

Acetolactate decarboxylase 4.1.1.5 (*ald*)
→ Induction *14 logFC in *P. freudenreichii*

Modified from Boumerdassi et al. 1997 AEM

→ Interaction: division of labor
Functional redondancy with specific inductions of genes

Successive carbon metabolisms by each species



PF

Wood-Werkmann

Lactose & lactate -> propionate + acetate

LL

V. Tagatose + Leloir

LP

Leloir

PF

Lactose & citrate -> lactate

->Acetoin-butanediol

LL

V. Leloir

Lactose -> lactate

Acetoin-butanediol

Moul

Demoul

AS

Aff4sem

Aff7sem

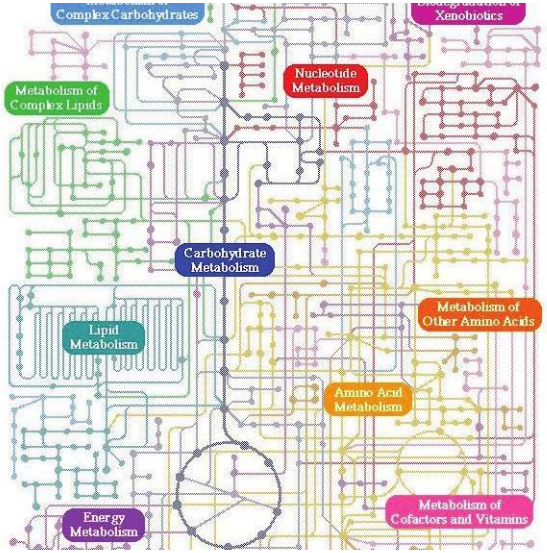
Team spirit of bacteria for degradation and production…
but do they exchange metabolites ?

Metabolic modeling to identify metabolite exchanges

INRIA-INRAE PhD student Maxime Lecomte

Annotated genome

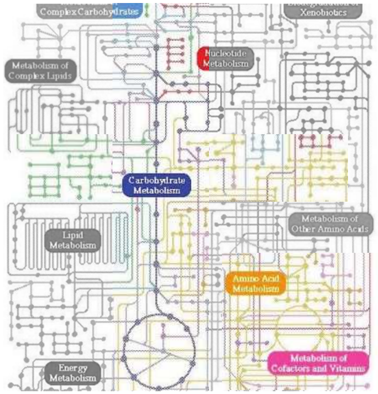
automatic
+ manual curation



Metabolic network

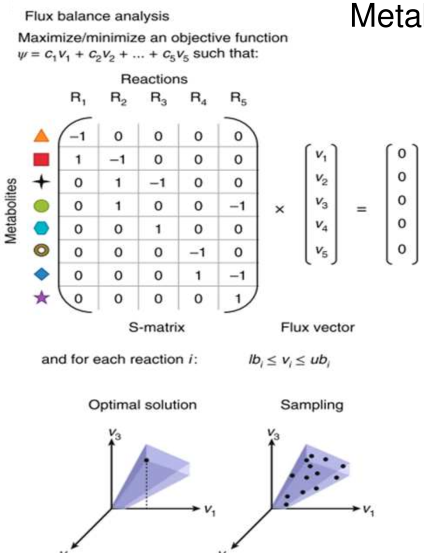
Modelisation

INPUT
Molecules present in milk



Metabolic network

OUTPUT
Microbial metabolites



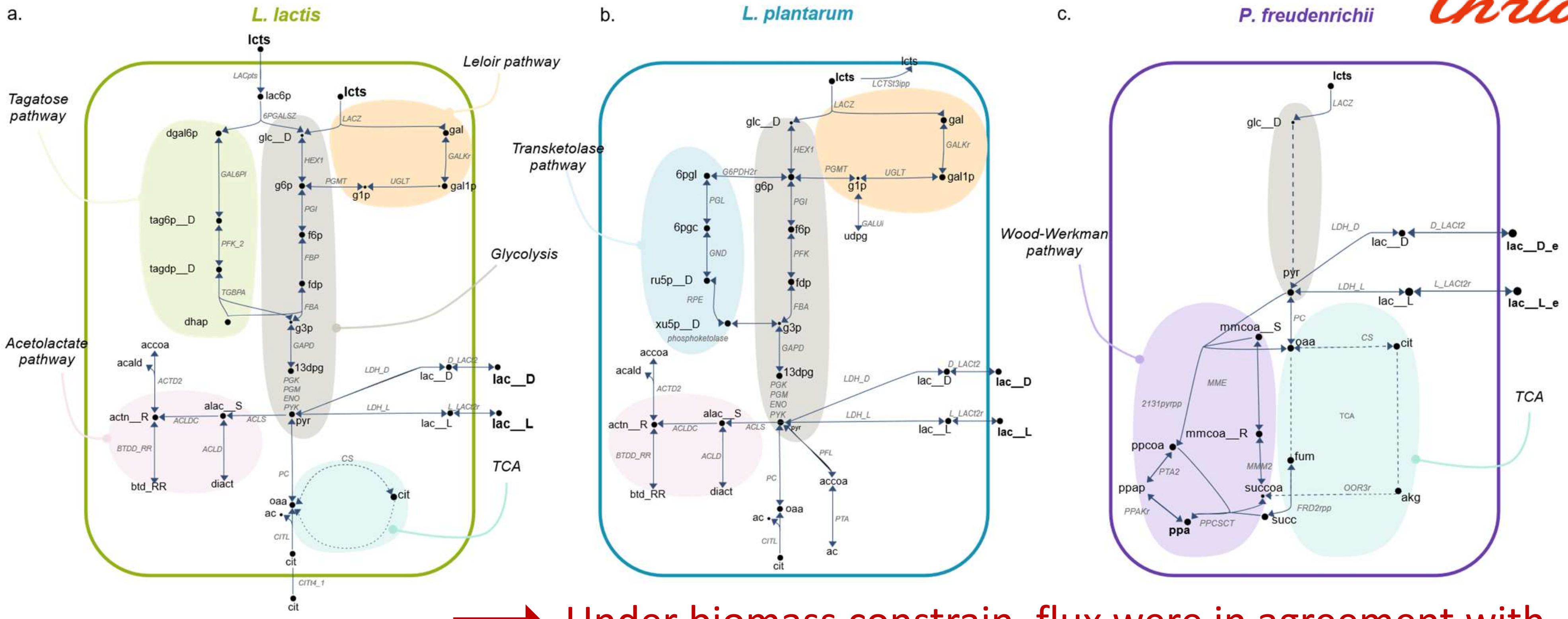
Méthodologies :

- Qualitative using CarveMe
- Quantitative modelling (FBA): parameters were adjusted using data from single strain culture grown in milk

Machado et al. 2018.
Orth et al. 2010

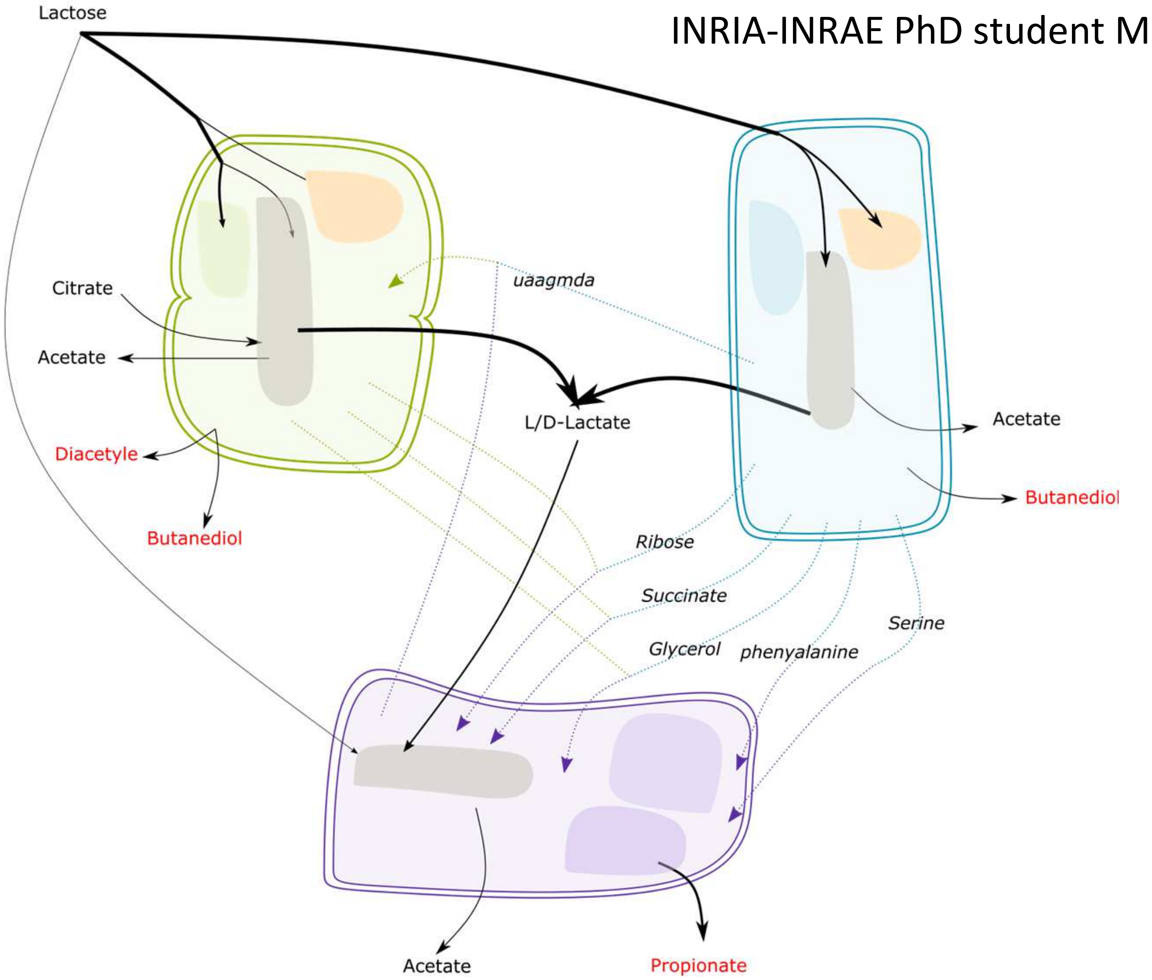
Overview of carbon metabolism of the 3 strains

INRIA-INRAE PhD student Maxime Lecomte



Modèle métabolique à l'échelle de l'écosystème : identification d'interactions

INRIA-INRAE PhD student Maxime Lecomte

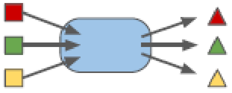


Logiciel Smetana
Zelezniak et al. 2015 PNAS

➡ Identification of potential interaction to be validated

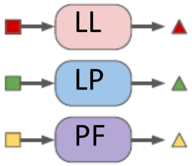
Take home messages

✓ Identification of induced pathways in each species



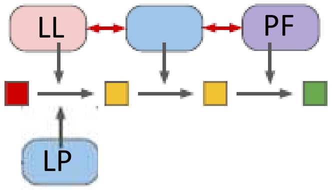
✓ Giving snapshots of successive metabolisms during cheese making

- Microbial competition for citrate and lactose utilization



Citrate
Lactose

- Division of labor for the production of acetoin



acetoin

✓ Metabolic modeling gives clues of metabolite exchanges between species

Perspectives

This strategy can be applied to any fermented food to decipher metabolisms and interactions.

Since, most identified pathways are species and strain-dependant …

This kind of knowledge opens new avenues to select and combine strains to develop new fermented food products.

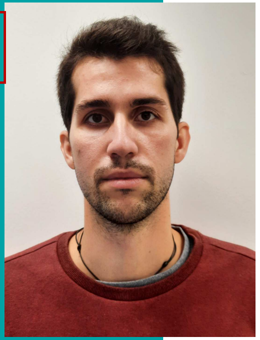
Thank you for your attention

Hélène Falentin
Stéphanie Deutsch
Sandrine Parayre
Anne Thierry
Wenfan Cao

Marie-Bernadette Maillard
Gilles Garric
Marielle Harel-Oger
Arlette Leduc

Françoise Boissel
Jean-Luc Thomas
Bénédicte Camier
Clémence Frioux

Simon Labarthe
Maxime Lecomte
Julie Aubert
Solène Le Fur
David Sherman
Clémence Frioux



Bactéries d'Intérêt Alimentaire