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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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Wenfan Cao, Maxime Lecomte, Solène Le Fur, Julie J. Aubert, Marie-Bernadette Maillard, et al.. Metatranscriptomics and metabolic modeling to identify bacterial metabolic interactions during the manufacture of a model pressed cheese.. FOODMICRO 2022, Aug 2022, Athènes, Greece. hal-03781287

HAL Id: hal-03781287

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Submitted on 20 Sep 2022

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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



INRAE



STLO

Inria

Founded by



➤ Context et questions

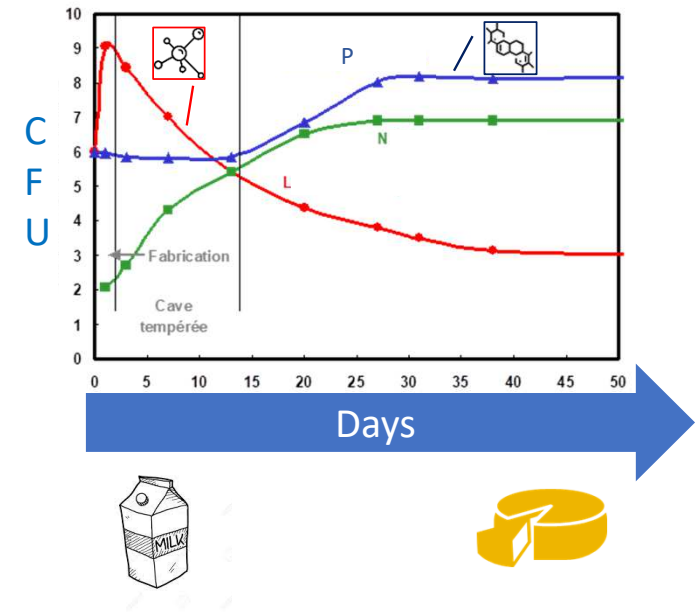
Context

Activity of cheese microbiota responsible for organoleptic quality

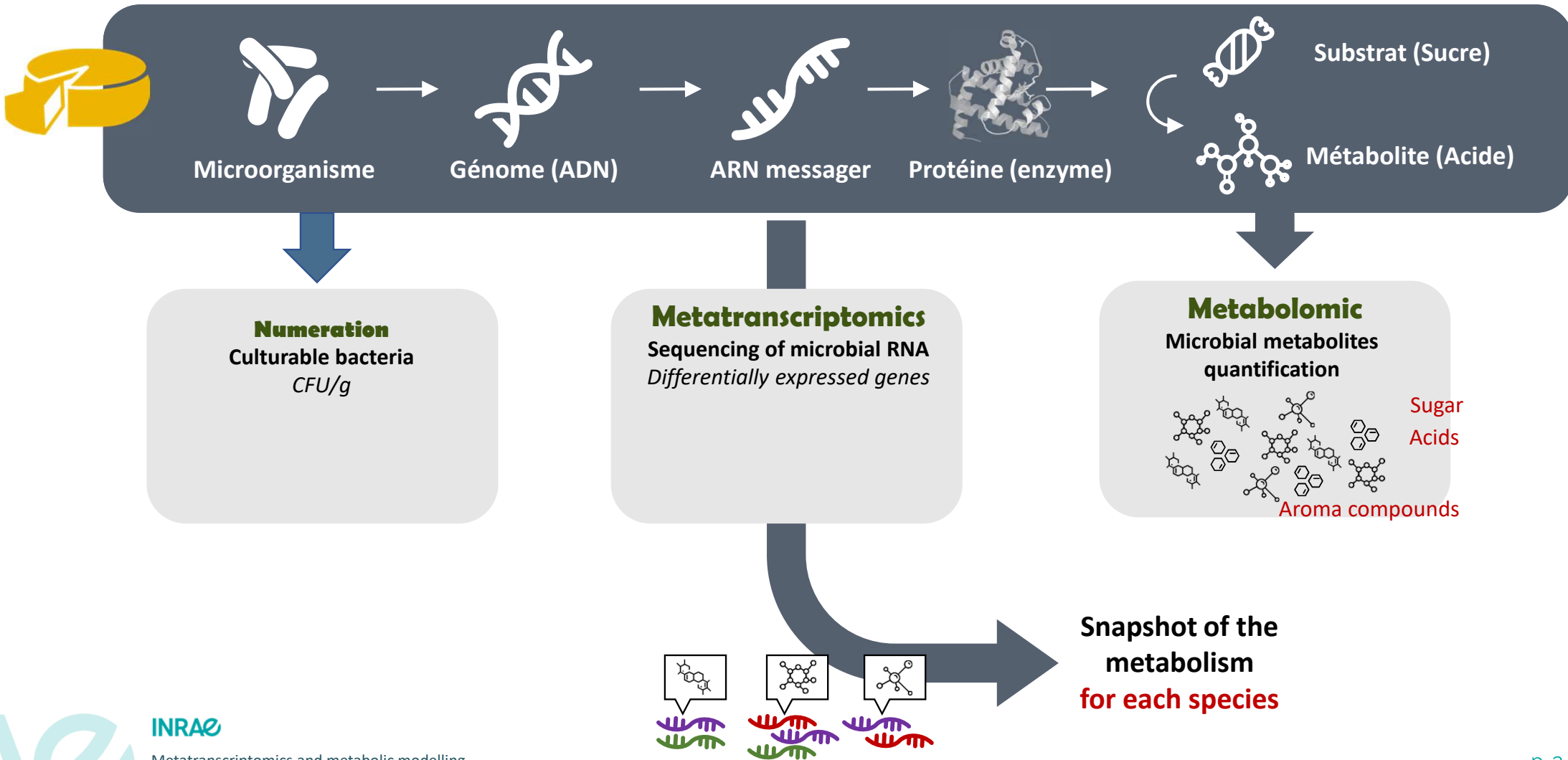
- Successive growths of microbes
- Specific and complementary microbial metabolisms
- Metabolic interactions between microbes

Scientific questions

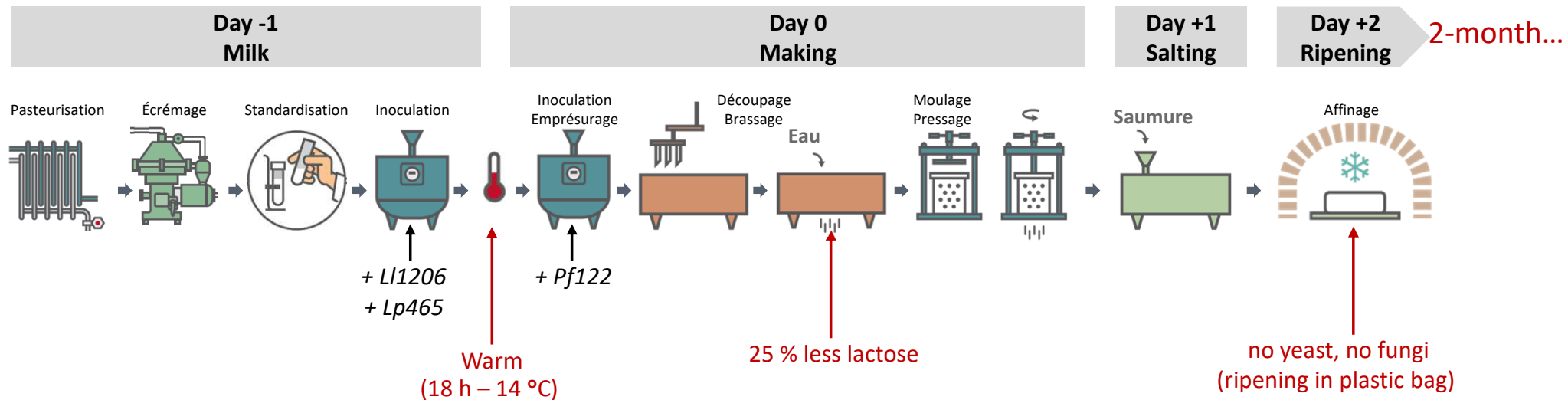
- Which species contributes to acidification ?
- Which species contributes to aroma compound production?
- Which metabolic pathways are activated ?
- When are they activated ?
- **Which bacterial metabolic interactions contributes to the functioning of microbial ecosystem ?**



➤ Strategy using multi-omics



➤ Cheese making of semi hard cheeses



Semi hard cheeses
(without yeast)



Lactococcus lactis CIRM-BIA1206



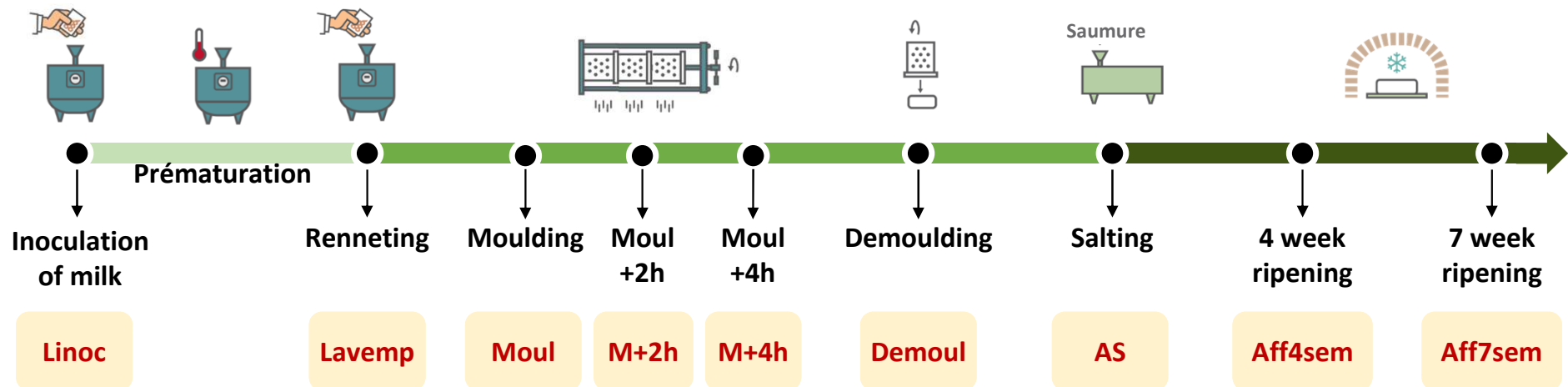
Lactiplantibacillus plantarum CIRM-BIA465



Propionibacterium freudenreichii CIRM-BIA122

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

➤ Sampling throughout the making



Microbiology

Chemistry

Biochemistry

Molecular biology

Computational science



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➤ Microbiological methods

Microbiology

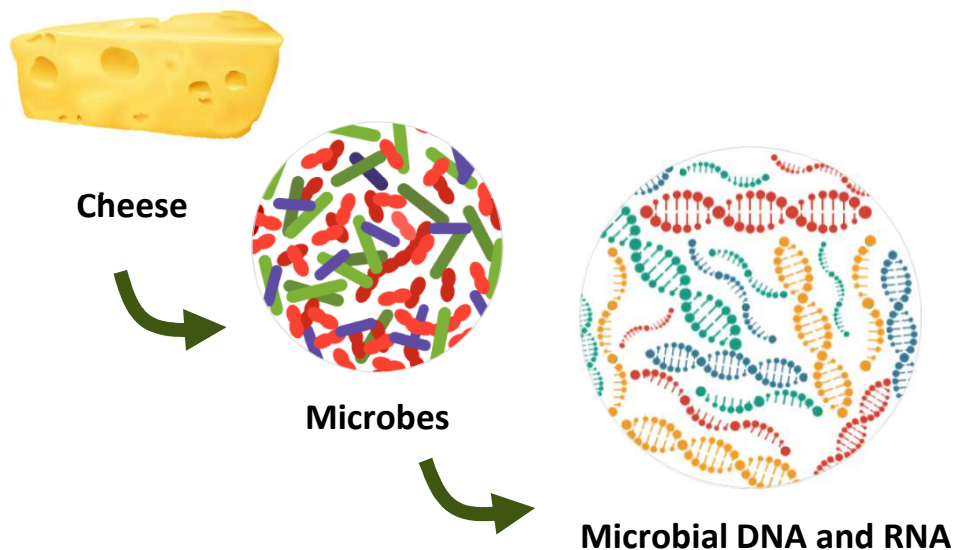
- *L. lactis* : M17 + lactose
- *L. plantarum* : MRS pH5.4
- *P. freudenreichii* : Yela

Biochemistry

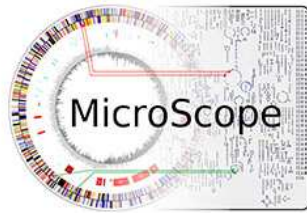
- **Nitrogen**: Kjeldhal
- **Sugar and acids**: HPLC
- **Aroma compound**: head space + GCMS

Molecular biology

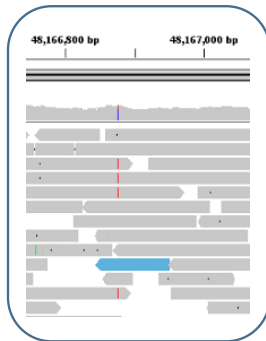
- **DNA** : Dneasy Tissue kit
- **RNA** : QIAzol + Rneasy mini kit
- **Quality control** : Nanodrop, Qubit, Bioanalyzer
- **Sequencing** : Illumina pairé, PacBio



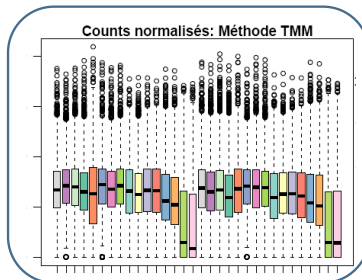
➤ Computational methods



Automatic annotation of genomes
Reconstruction of metabolic pathways using Pathway Tools



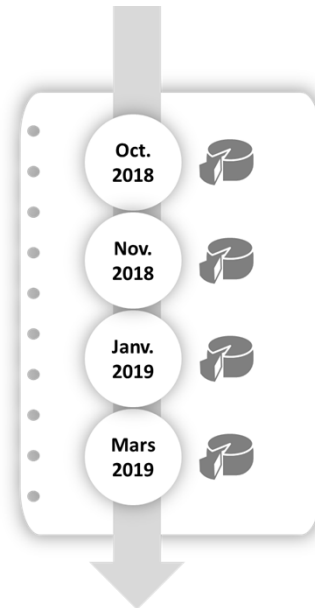
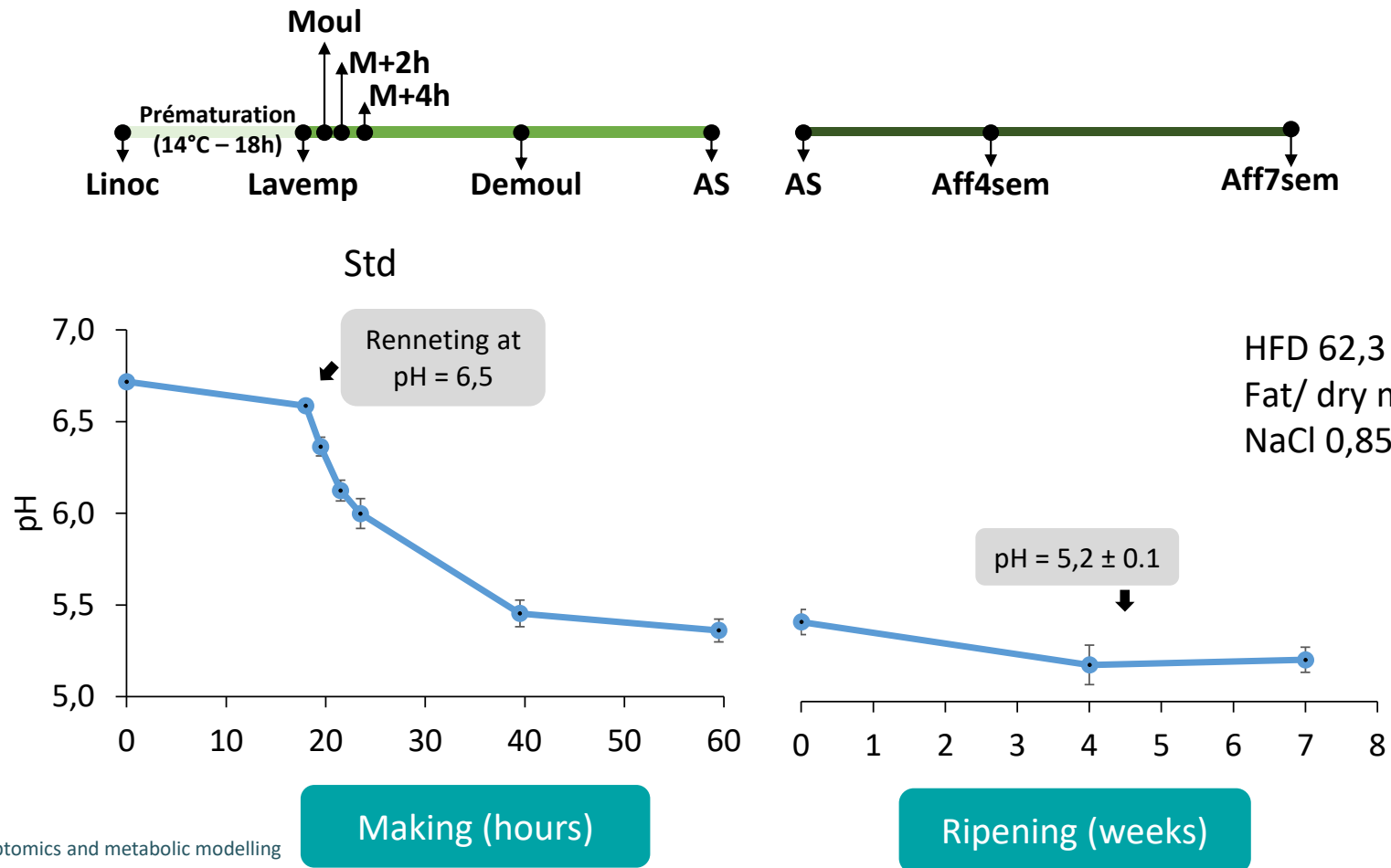
Metatranscriptomics
Reads mapping using Bowtie
Reads counting using htseq count



Normalisation using TMM multi species
Identification of DE genes using EdgeR
Significativity $p_{adj} < 0.05$

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

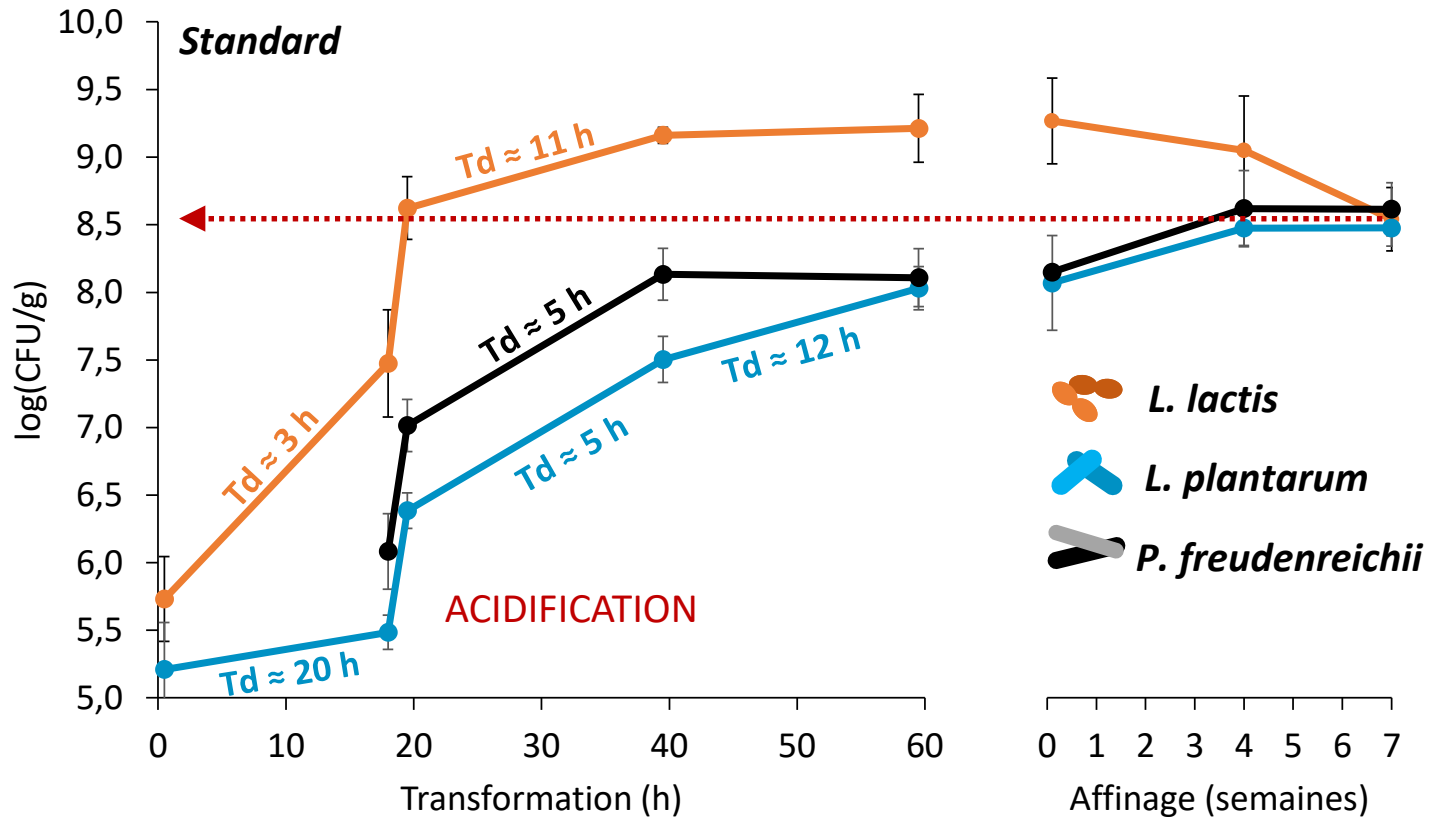
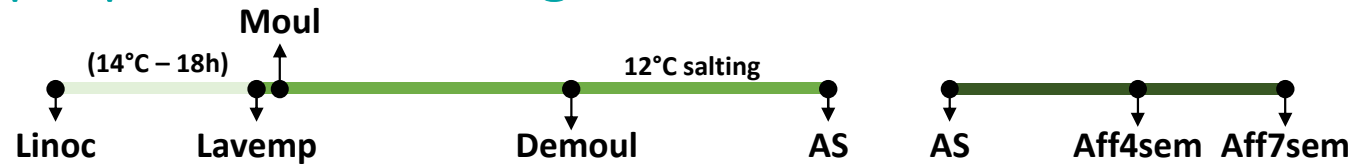
➤ Repeatability of semi hard cheeses (X4)



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➤ Lactic and propionic bacterial growth



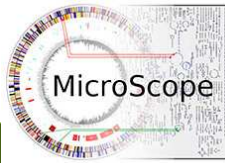
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Cao et al. 2021, J. Agric. Food Chem.

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➤ Assembly and genome annotations



L. lactis 1206

Replicon	Seq length	% GC	CDS	tRNA	rRNA
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
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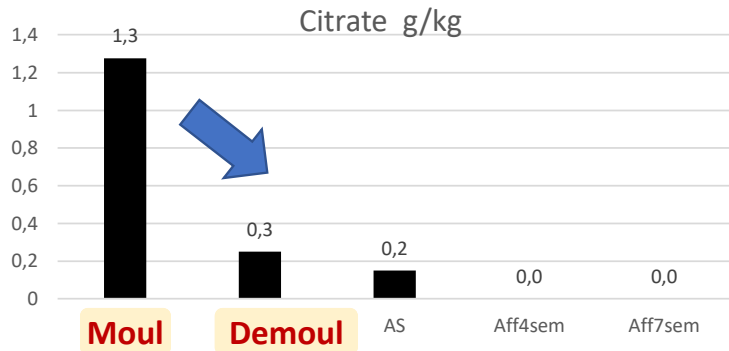
➔ **Metatranscriptomics : mapping of RNA reads / genomes**



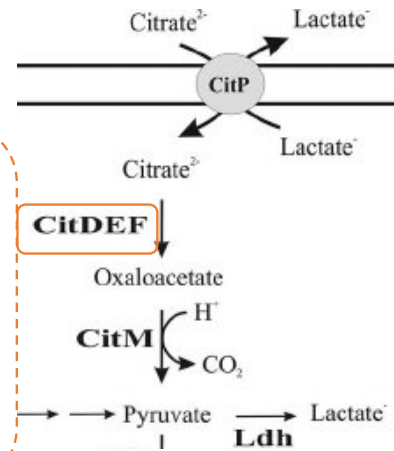
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➤ Citrate utilization by *Lc. lactis*



citCDEFG
 LLACBIA1206_v1_1311-1316
 Induction logFC X 2-3 by
L. lactis

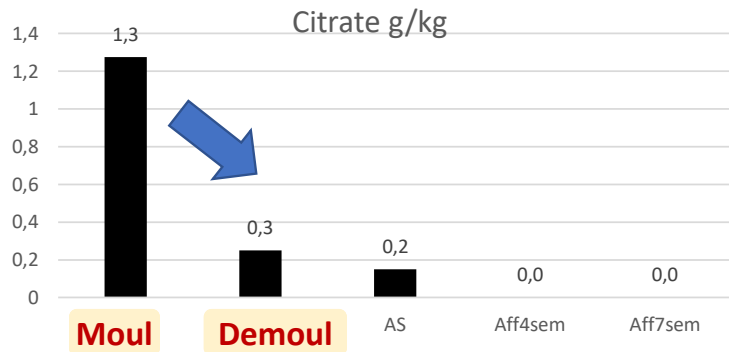


LLACBIA1206_v1_pc0002
 LLACBIA1206_v1_1007

Modified from Garcia-Quintas et al. 2008, AEM

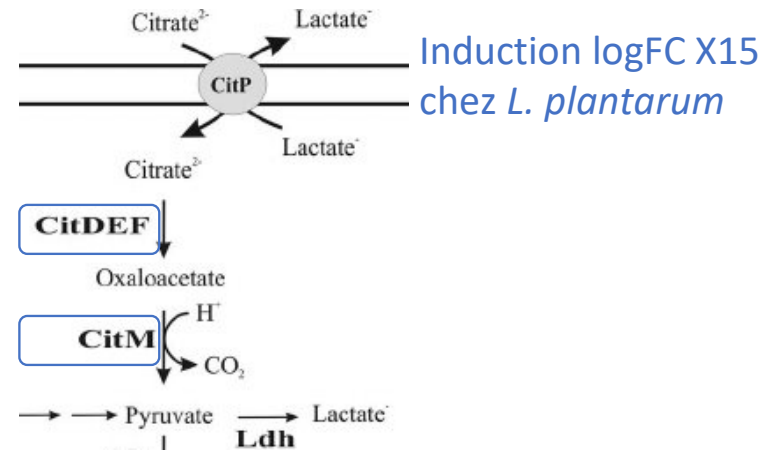


➤ Citrate utilization by *L. plantarum*



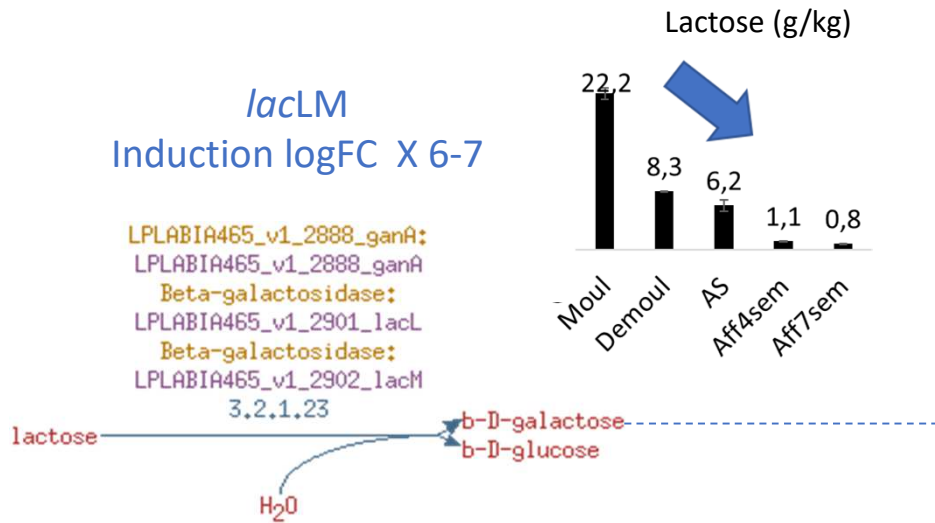
LPLABIA465_v1_0482

citMCDEF
LPLABIA465_v1_0962-0966
Induction logFC X13-16
chez *L. plantarum*

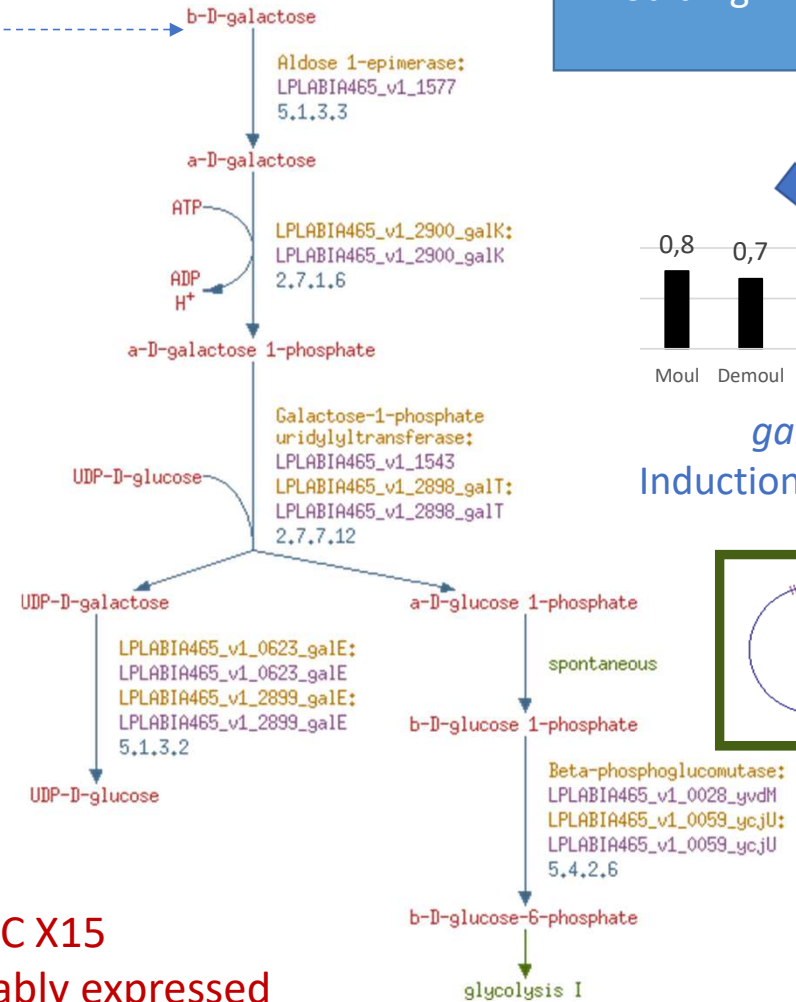


Metabolic interaction = competition for citrate
Functional redundancy using the same pathway induced at the same time in *L. lactis* et *L. plantarum*

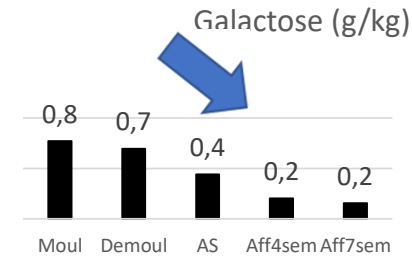
Lactose utilization by *L. plantarum*



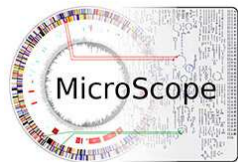
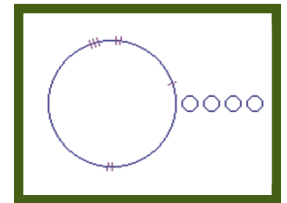
Leloir pathway



Moulding > Demoulding



gal/RTEK
Induction logFC X 5-6



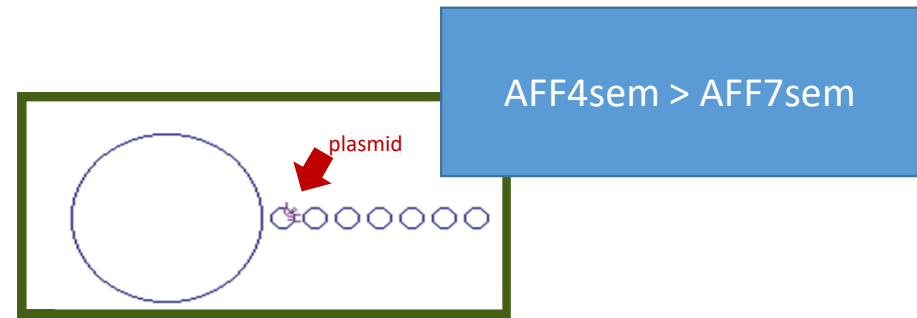
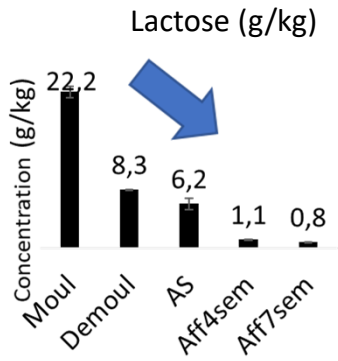
In *P. freudenreichii* induced logFC X15
In *L. lactis* same pathway but stably expressed



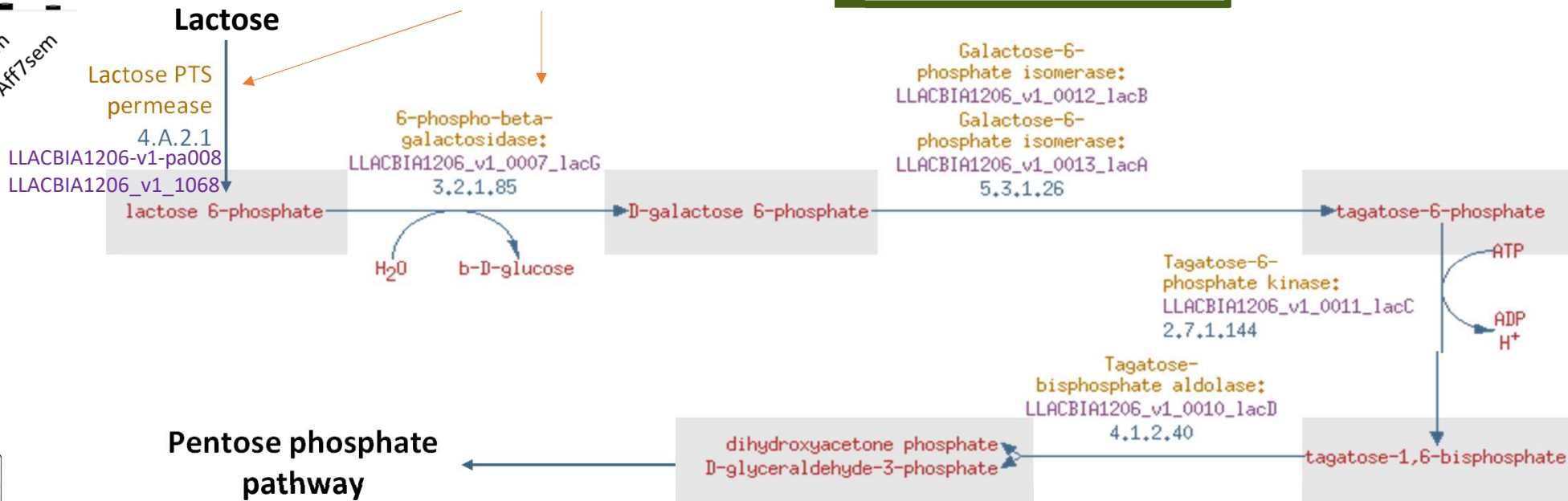
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Lactose utilization by *Lc. lactis*

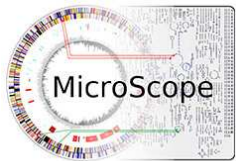


Plasmidic induction logFC X2



Pathway in agreement with Raynaud et al. 2005

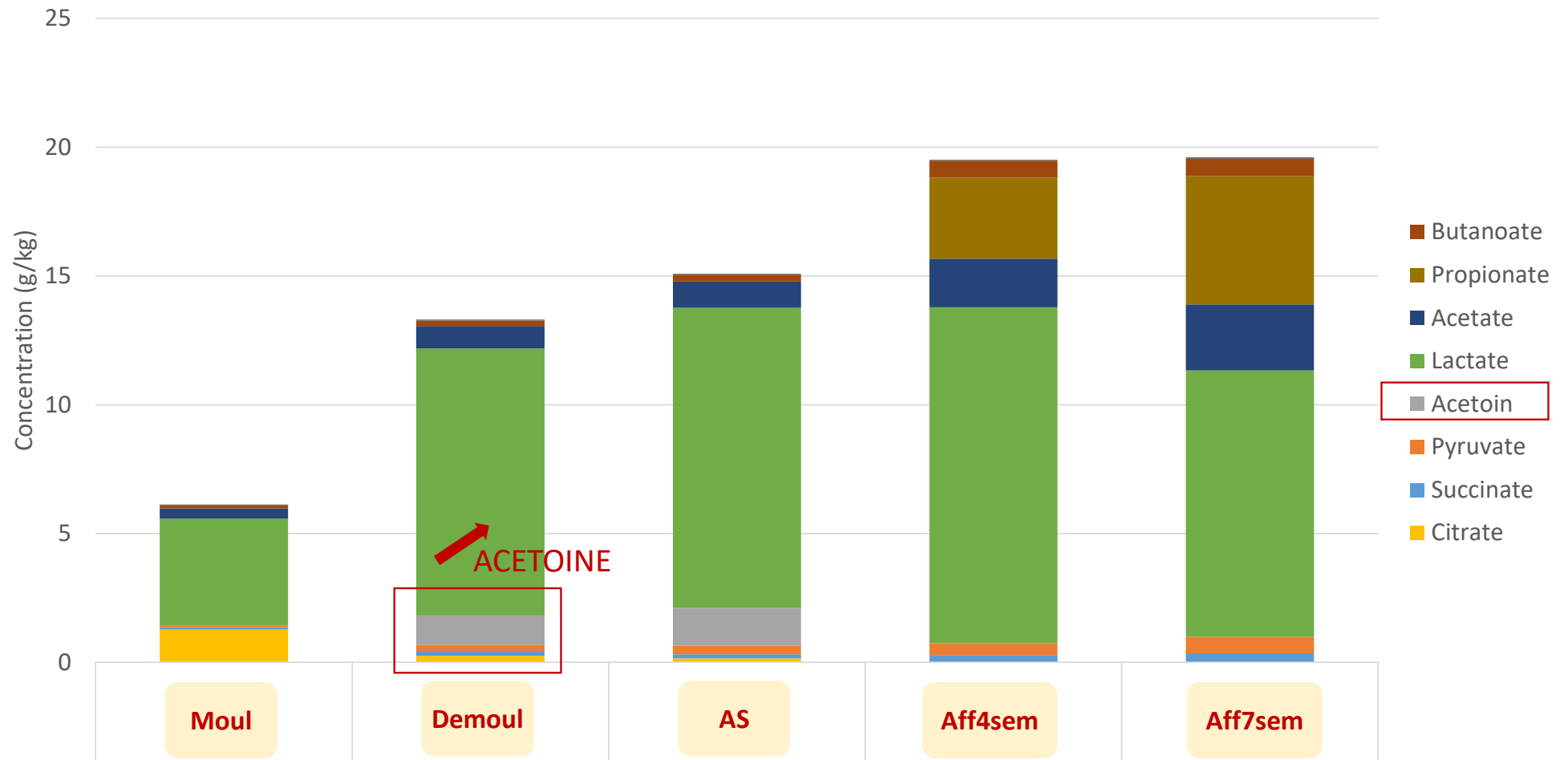
➔ **Metabolic interaction = competition for lactose**
Functional redundancy with different pathways induced at different times



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➤ Production of acetoin (cheesy aroma compound)



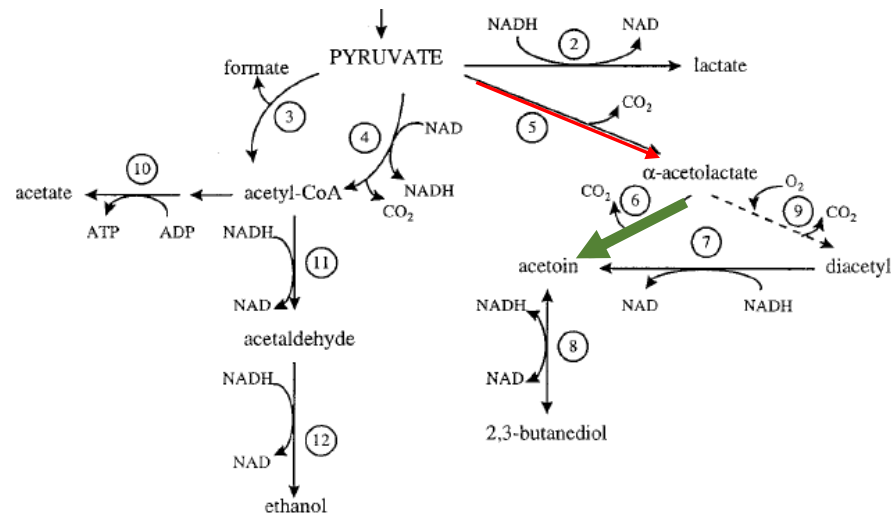
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Cao et al. 2021, J. Agric. Food Chem.

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➤ Production of acetoin



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*

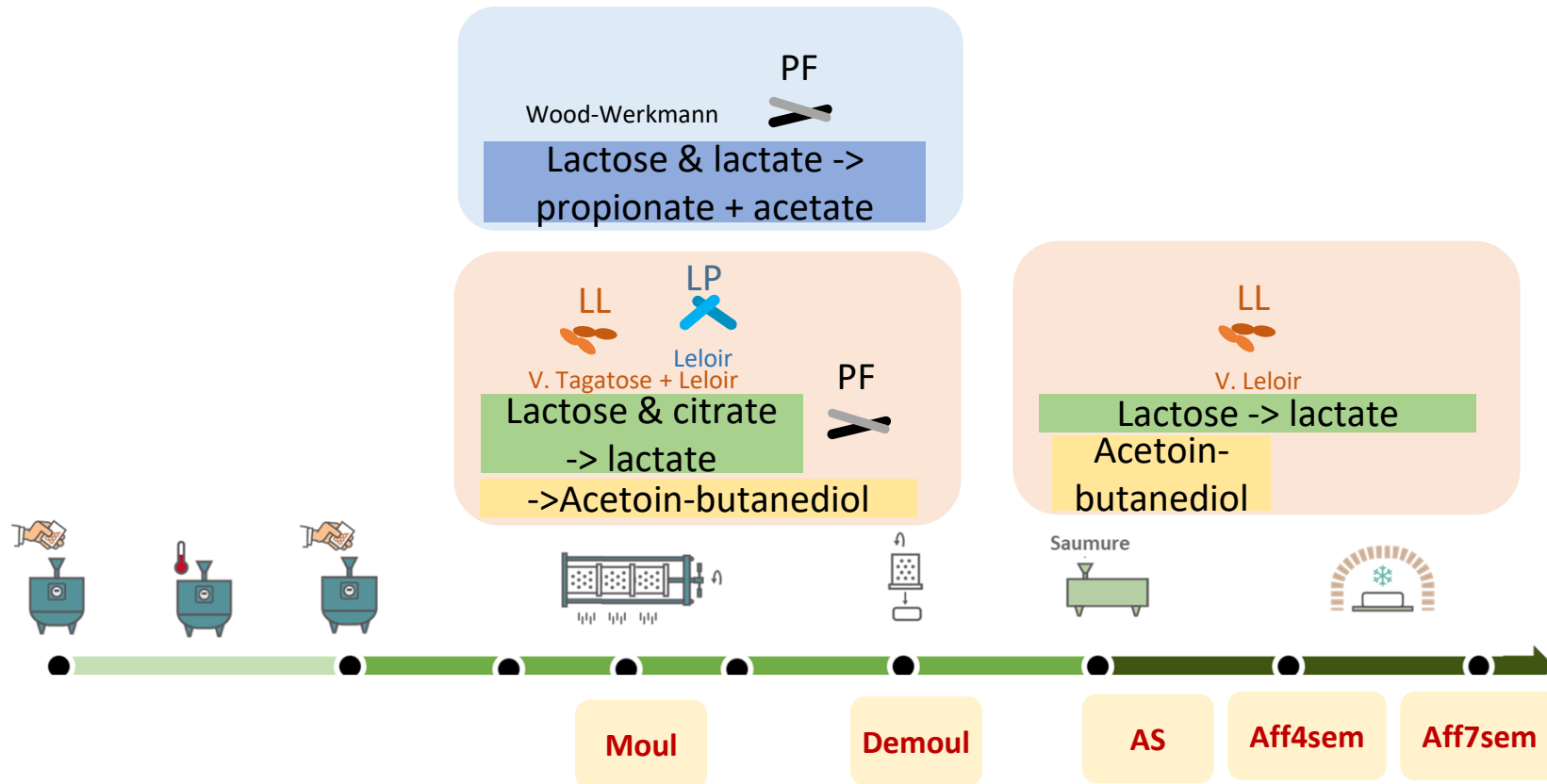
Modified from Boumerdassi et al. 1997 AEM



Interaction: division of labor

Functional redundancy with specific inductions of genes

➤ Successive carbon metabolisms by each species



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

➤ Metabolic modeling to identify metabolite exchanges

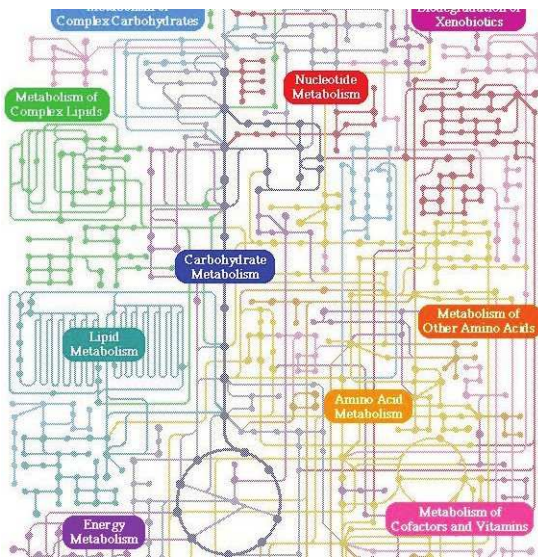


INRIA-INRAE PhD student Maxime Lecomte

Annotated genome

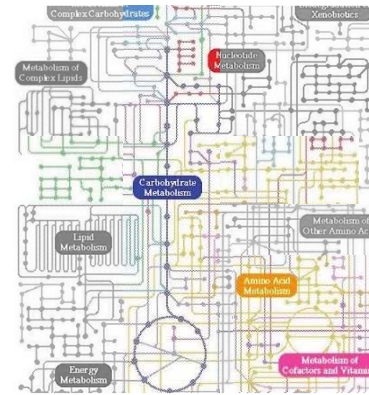
Modelisation

automatic
+ manual curation



Metabolic network

INPUT
Molecules
present
in milk



Metabolic network

OUTPUT
Microbial
metabolites



Flux balance analysis
Maximize/minimize an objective function
 $w = c_1 v_1 + c_2 v_2 + \dots + c_5 v_5$ such that:

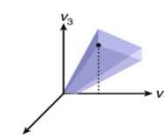
	R ₁	R ₂	R ₃	R ₄	R ₅
▲	-1	0	0	0	0
■	1	-1	0	0	0
+	0	1	-1	0	0
●	0	0	1	0	-1
○	0	0	0	1	0
◇	0	0	0	-1	0
★	0	0	0	0	1

$$\times \begin{pmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix}$$

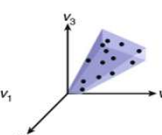
S-matrix Flux vector

and for each reaction i : $lb_i \leq v_i \leq ub_i$

Optimal solution



Sampling



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

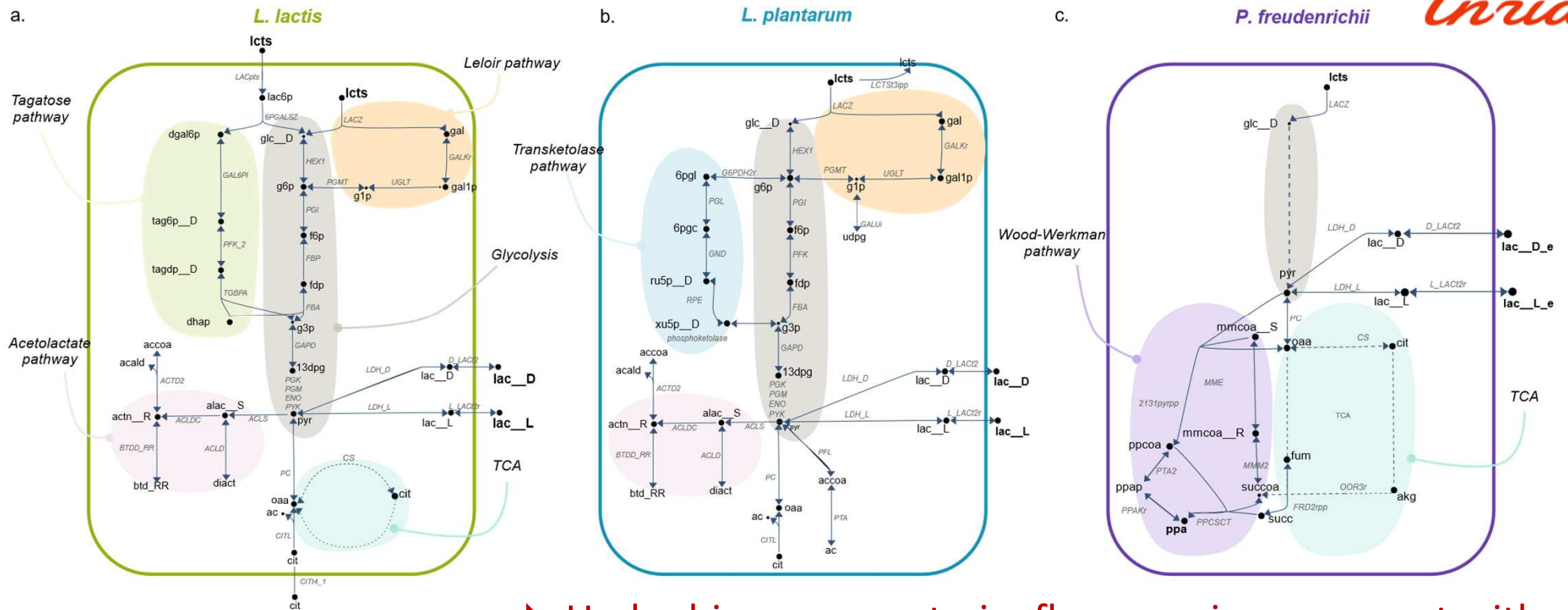
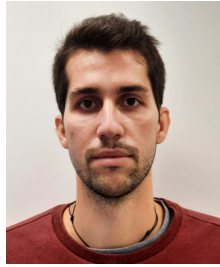


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➤ Overview of carbon metabolism of the 3 strains

INRIA-INRAE PhD student Maxime Lecomte



➔ Under biomass constrain, flux were in agreement with metatranscriptomics and targeted metabolomics

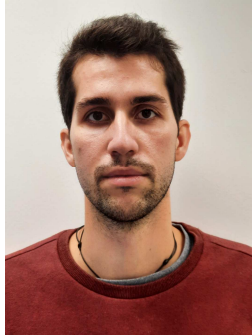


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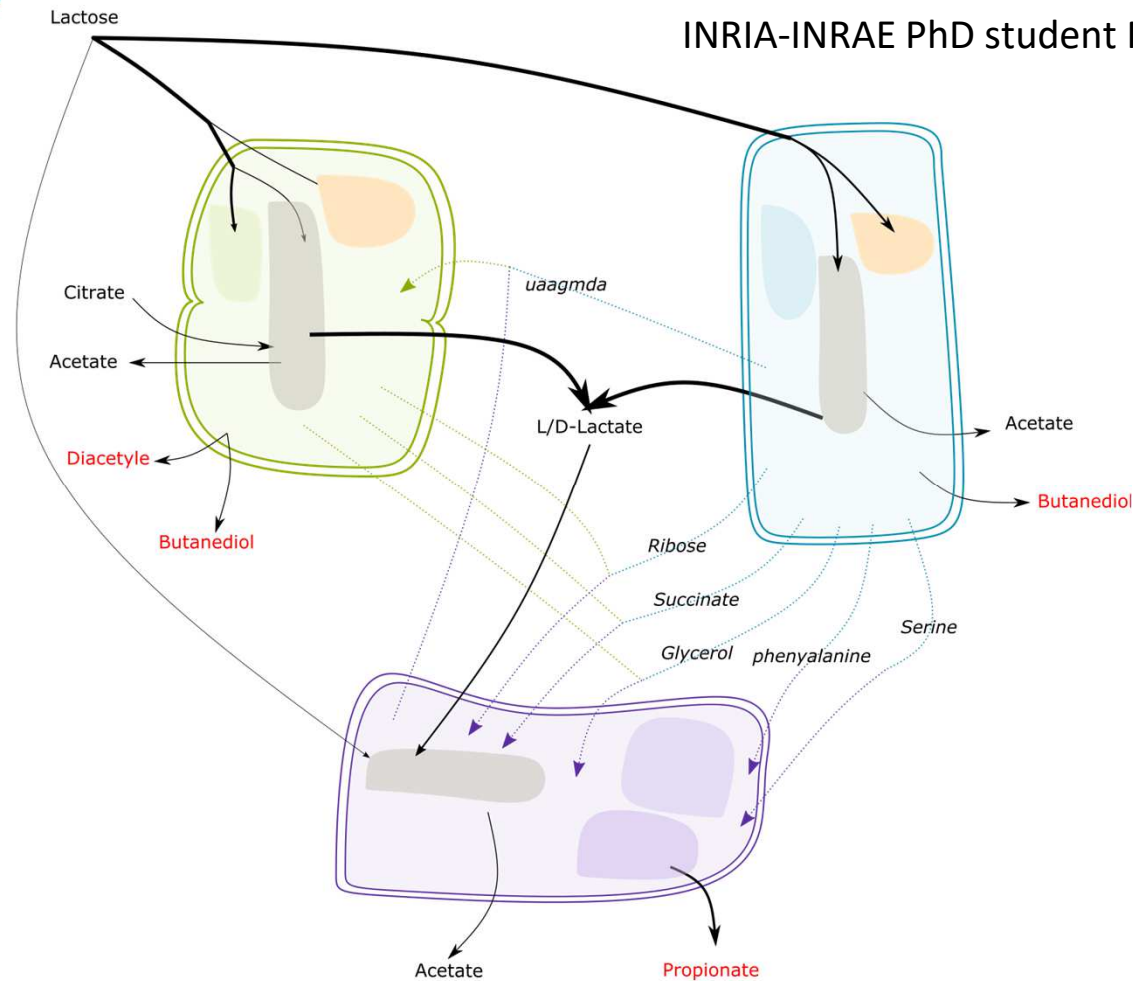
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➤ Modèle métabolique à l'échelle de l'écosystème : identification d'interactions

INRIA-INRAE PhD student Maxime Lecomte



Inria



Logiciel Smetana
Zelezniak et al. 2015 PNAS

➔ Identification of potential interaction to be validated p. 20

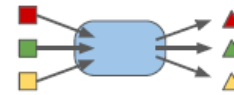


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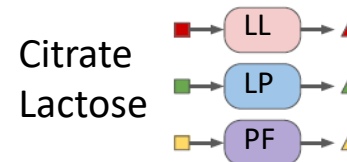
➤ Take home messages

- ✓ Identification of induced pathways in each species

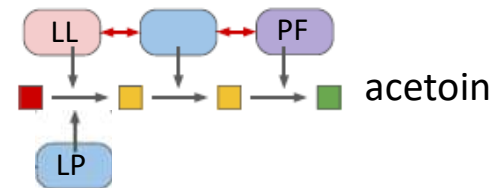


- ✓ Giving snapshots of successive metabolisms during cheese making

- Microbial **competition** for citrate and lactose utilization



- **Division of labor** for the production of 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.



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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

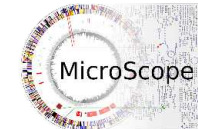
Maxime Lecomte

Julie Aubert

Solène Le Fur

David Sherman

Clémence Frioux



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Bactéries d'Intérêt Alimentaire