



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



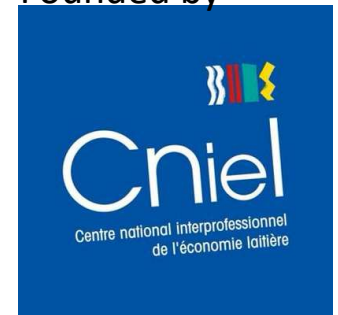
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STLO

Inria

Founded by



➤ Context et questions

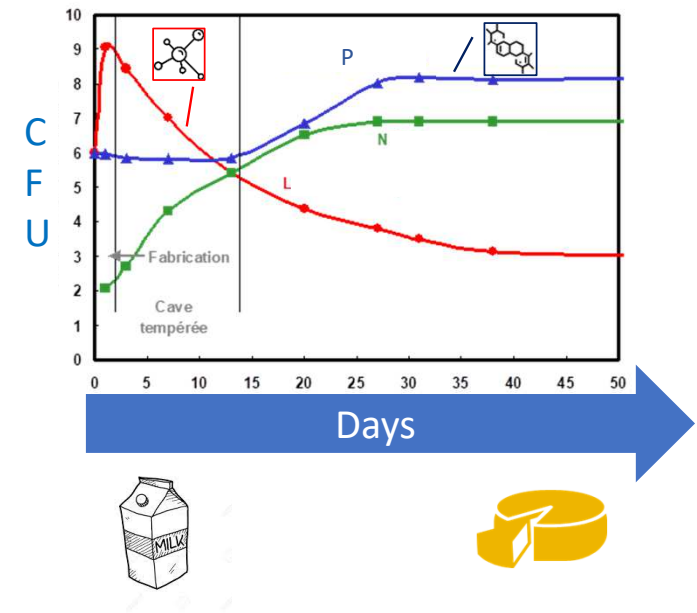
Context

Activity of cheese microbiota responsible for organoleptic quality

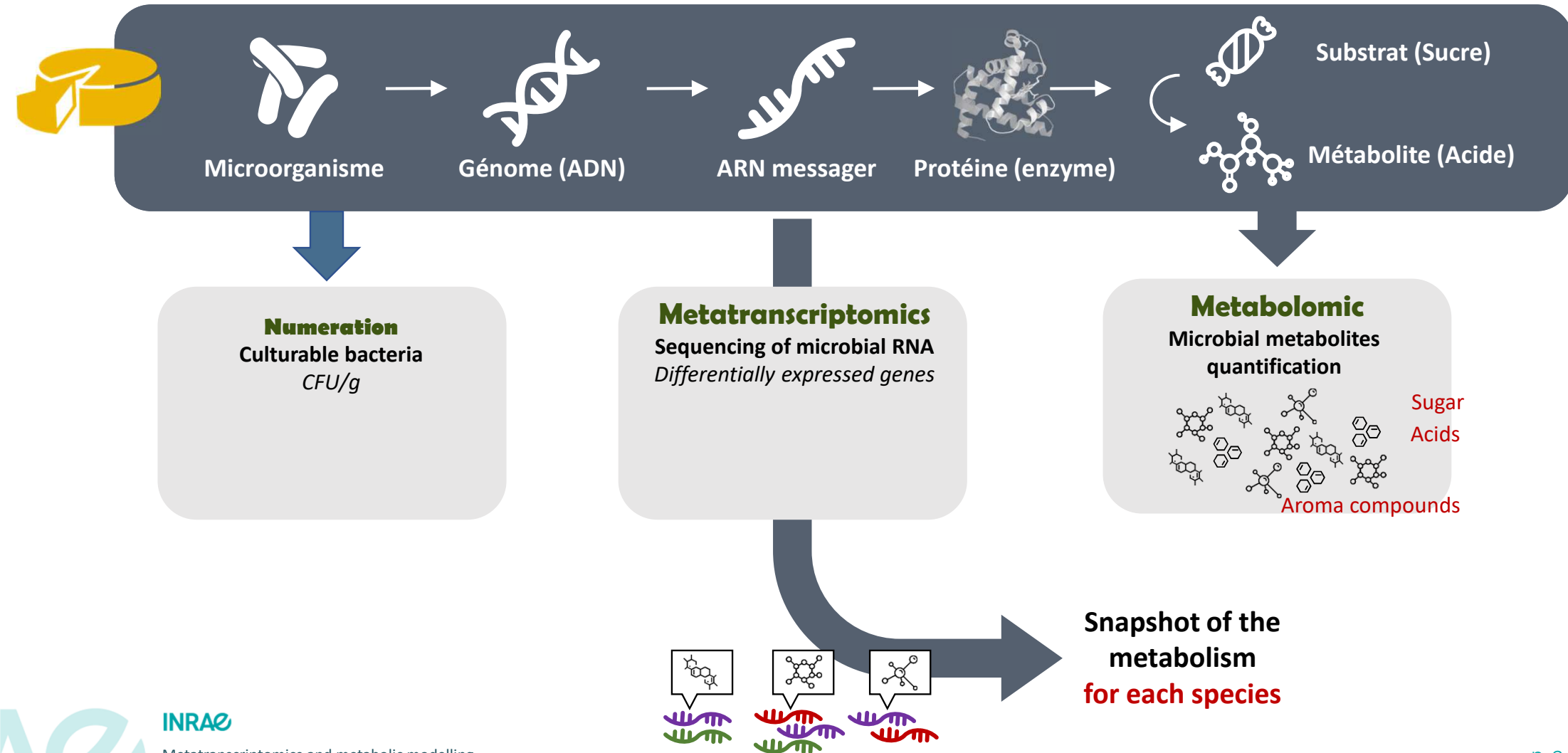
- Successive growths of microbes
- Specific and complementary microbial metabolisms
- Metabolic interactions between microbes

Scientific questions

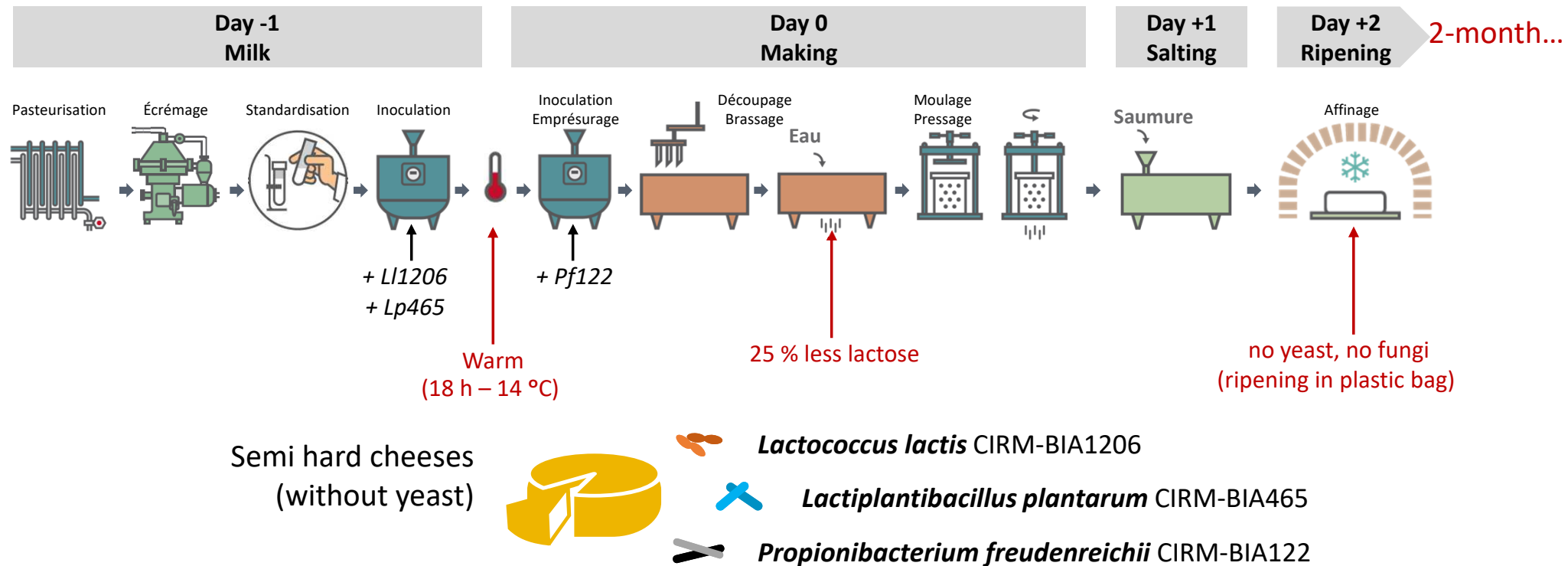
- Which species contributes to acidification ?
- Which species contributes to aroma compound production?
- Which metabolic pathways are activated ?
- When are they activated ?
- Which bacterial metabolic interactions contributes to the functioning of microbial ecosystem ?



➤ Strategy using multi-omics



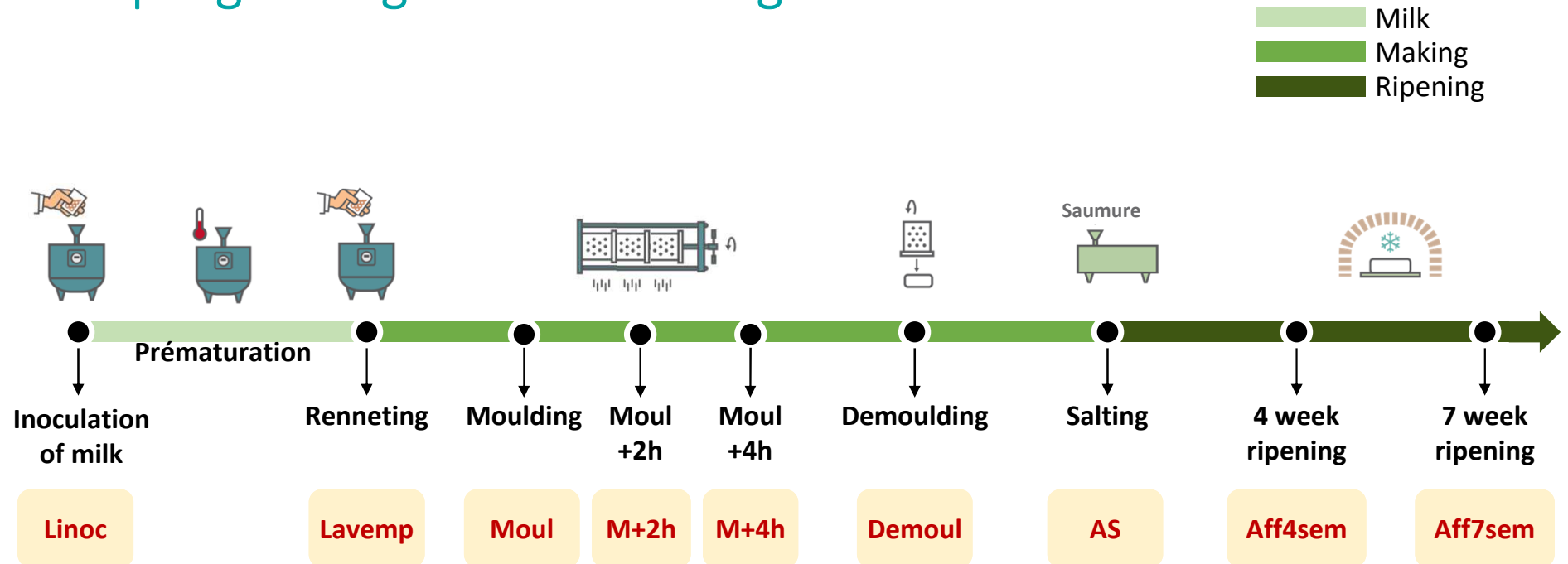
➤ Cheese making of semi hard cheeses



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

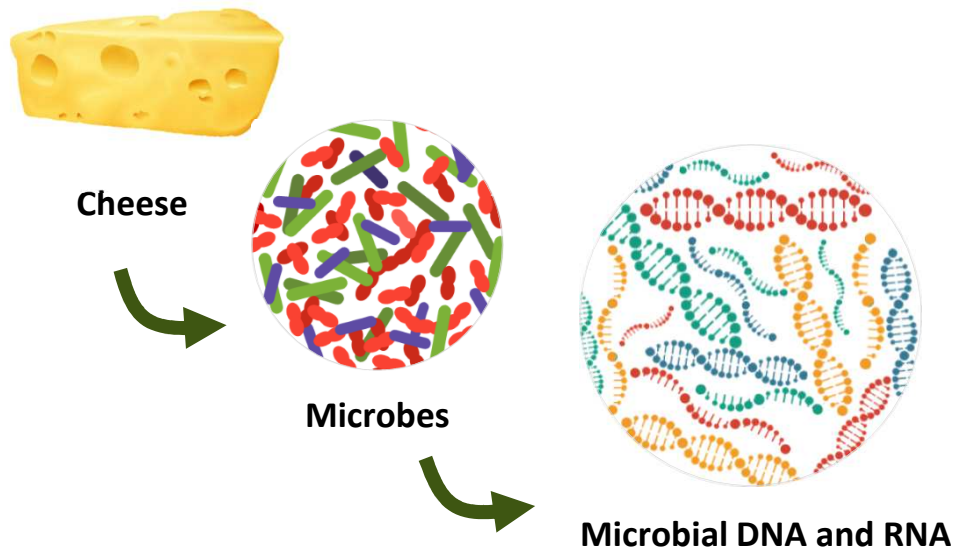
- *L. lactis* : M17 + lactose
- *L. plantarum* : MRS pH5.4
- *P. freudenreichii* : Yela

Biochemistry

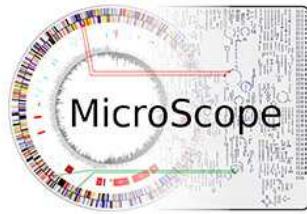
- **Nitrogen**: Kjeldhal
- **Sugar and acids**: HPLC
- **Aroma compound**: head space + GCMS

Molecular biology

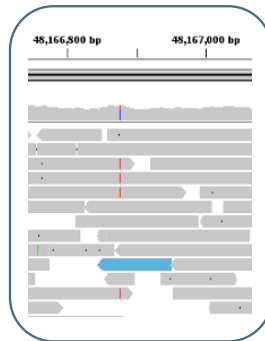
- **DNA** : Dneasy Tissue kit
- **RNA** : QIAzol + Rneasy mini kit
- **Quality control** : Nanodrop, Qubit, Bioanalyzer
- **Sequencing** : Illumina pairé, PacBio



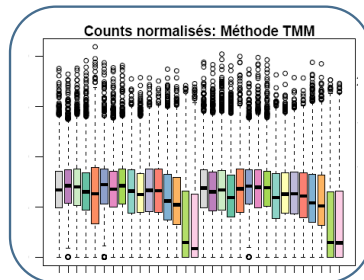
➤ Computational methods



Automatic annotation of genomes
Reconstruction of metabolic pathways using Pathway Tools



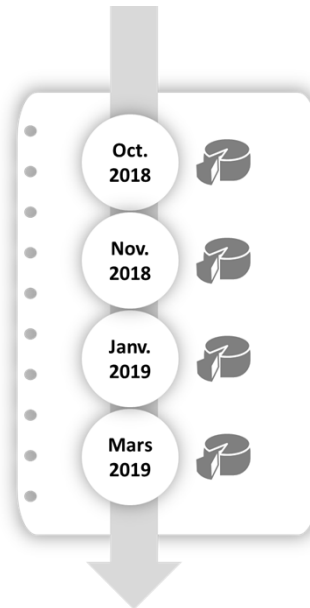
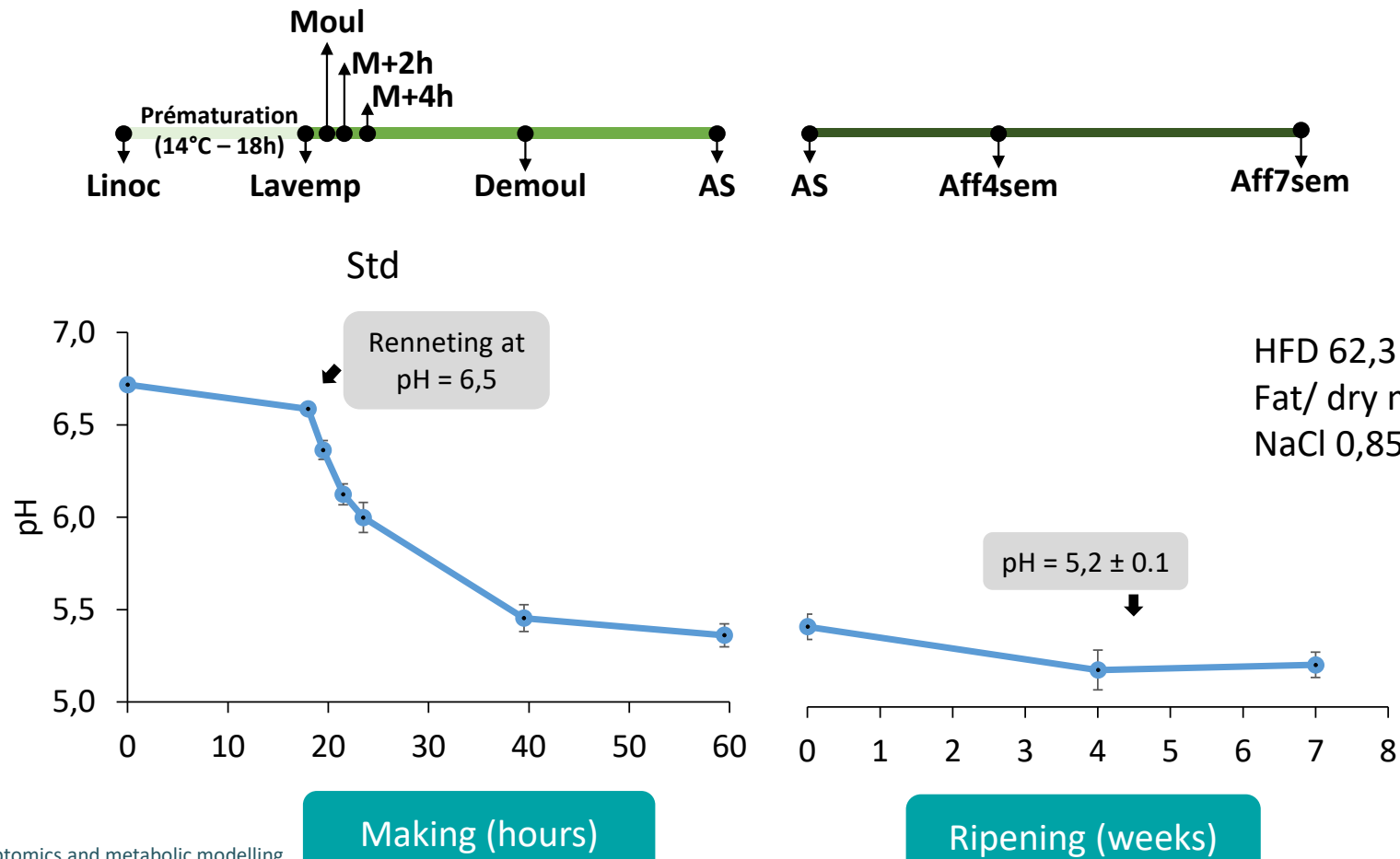
Metatranscriptomics
Reads mapping using Bowtie
Reads counting using htseq count



Normalisation using TMM multi species
Identification of DE genes using EdgeR
Significativity $\text{padj} < 0.05$

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

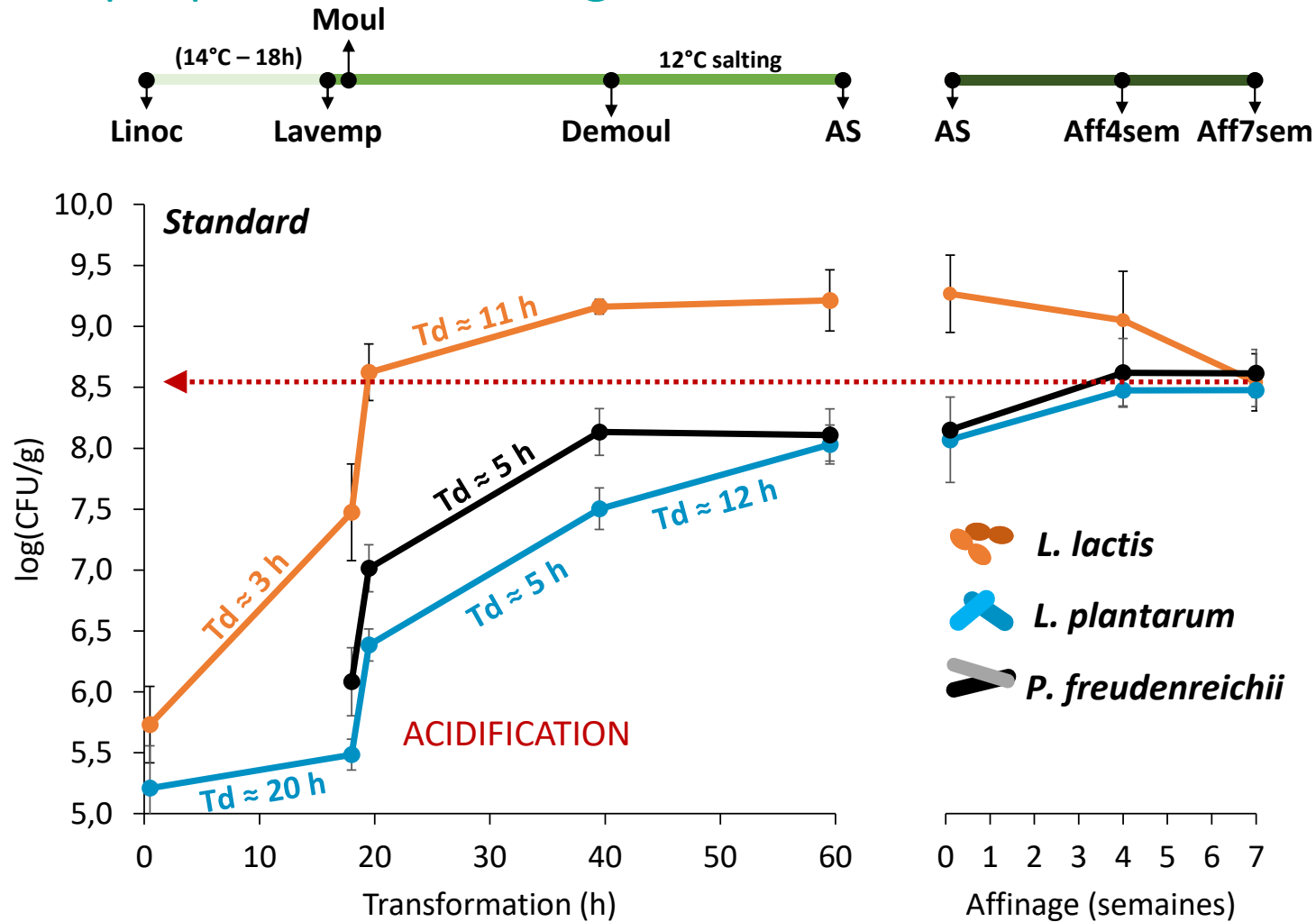
➤ Repeatability of semi hard cheeses (X4)



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➤ Lactic and propionic bacterial growth



Td : time of doubling



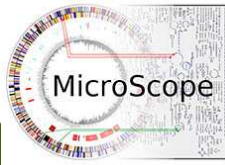
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Cao et al. 2021, J. Agric. Food Chem.

p. 9

➤ 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

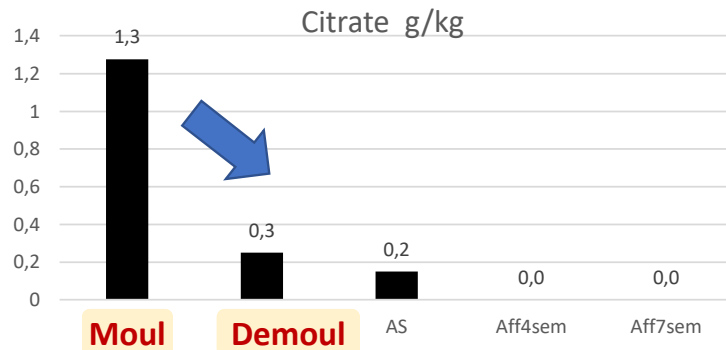
Chromosome	2 688 484	67,21	2589	45	6
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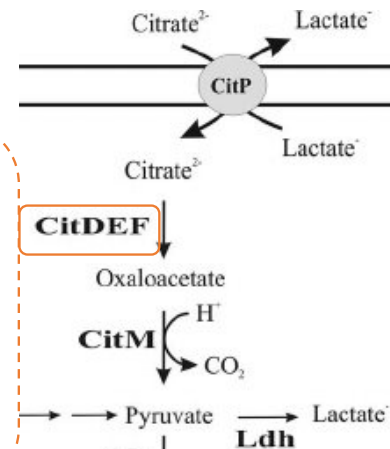
Metatranscriptomics : mapping of RNA reads / genomes

➤ Citrate utilization by *Lc. lactis*

Moulding > Demoulding



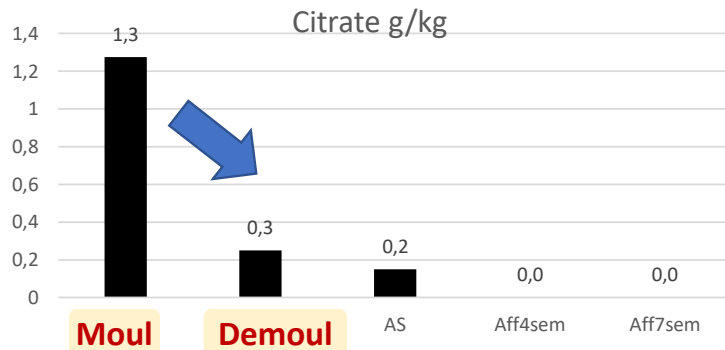
citCDEFG
 LLACBIA1206_v1_1311-1316
 Induction logFC X 2-3 by
L. lactis



LLACBIA1206_v1_pc0002
 LLACBIA1206_v1_1007

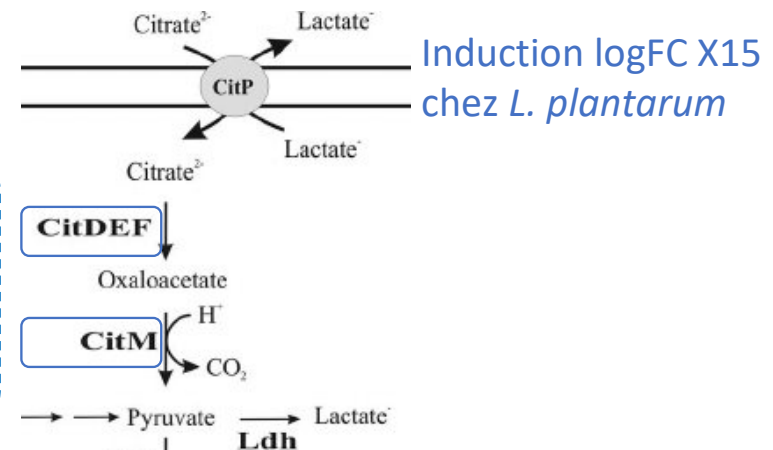
➤ Citrate utilization by *L. plantarum*

Moulding > Demoulding



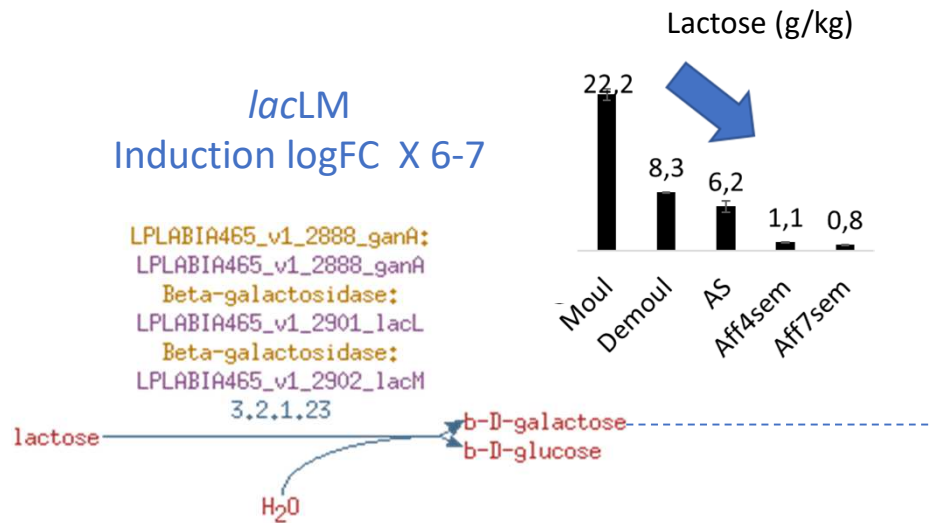
LPLABIA465_v1_0482

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

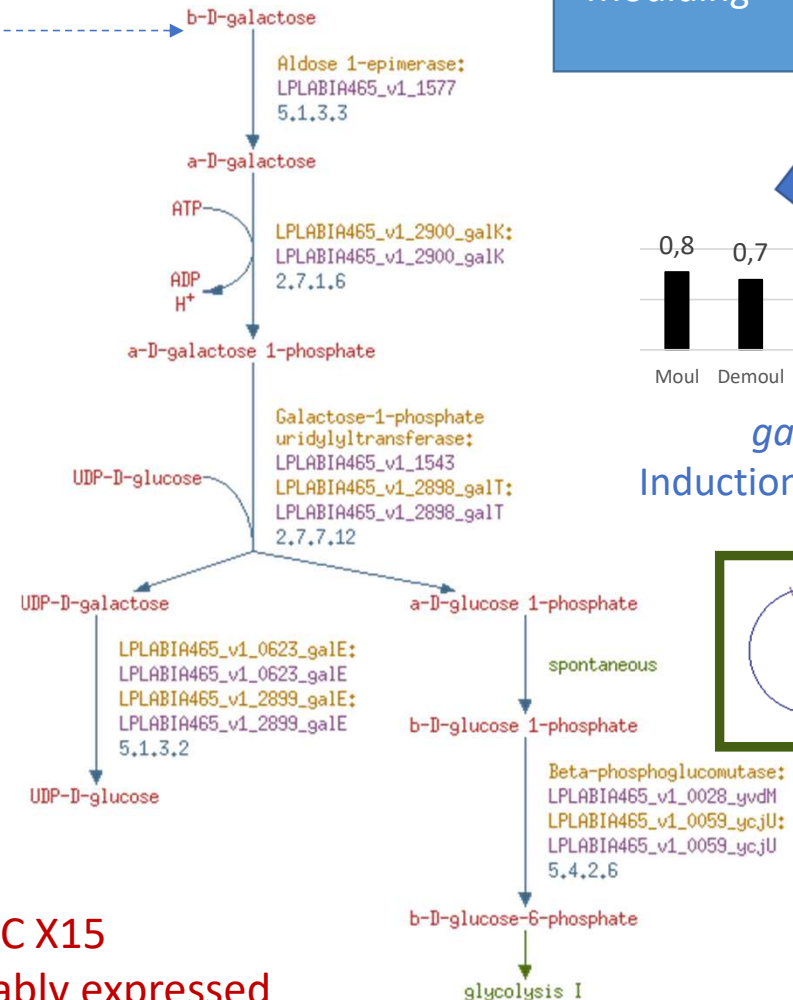


Metabolic interaction = competition for citrate
Functional redundancy using the same pathway induced at the same time in *L. lactis* et *L. plantarum*

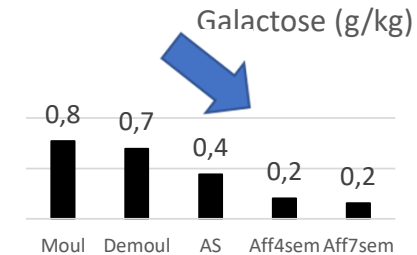
➤ Lactose utilization by *L. plantarum*



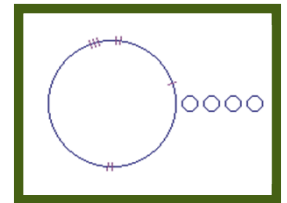
Leloir pathway



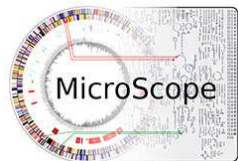
Moulding > Demoulding



gal/RTEK
Induction logFC X 5-6



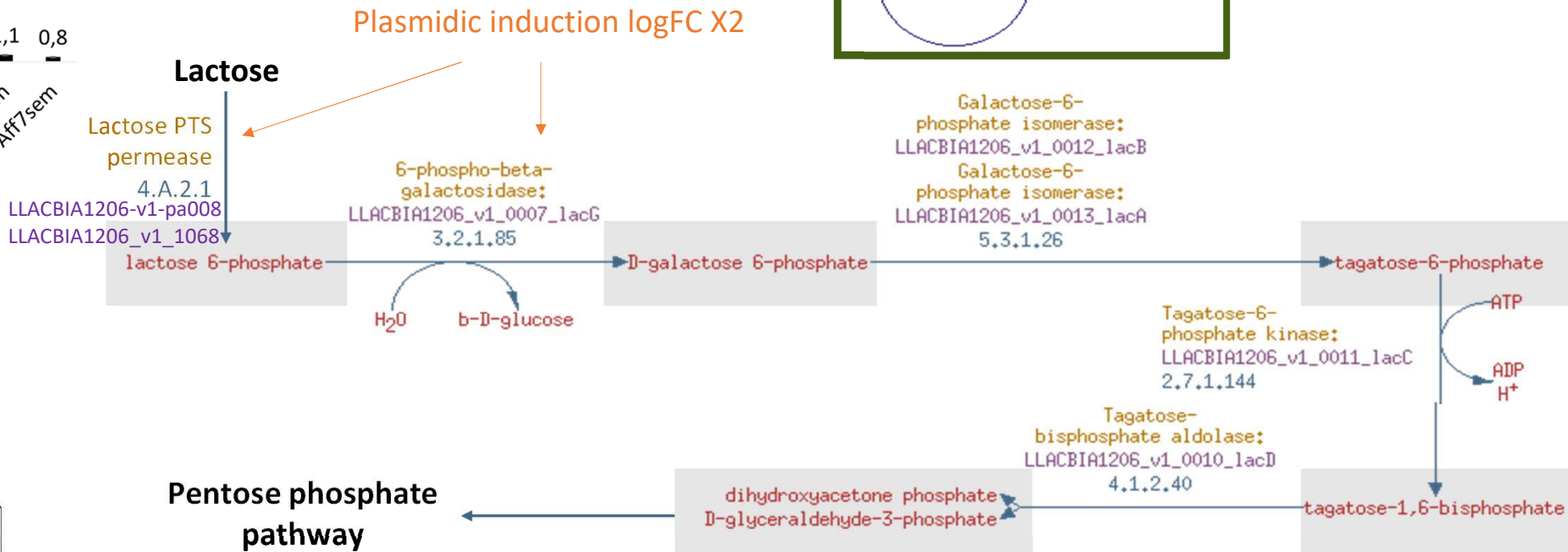
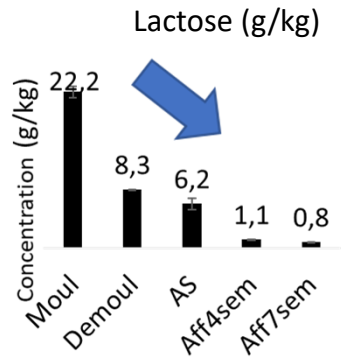
➔ *In P. freudenreichii* induced logFC X15
In L. lactis same pathway but stably expressed



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➤ Lactose utilization by *Lc. lactis*



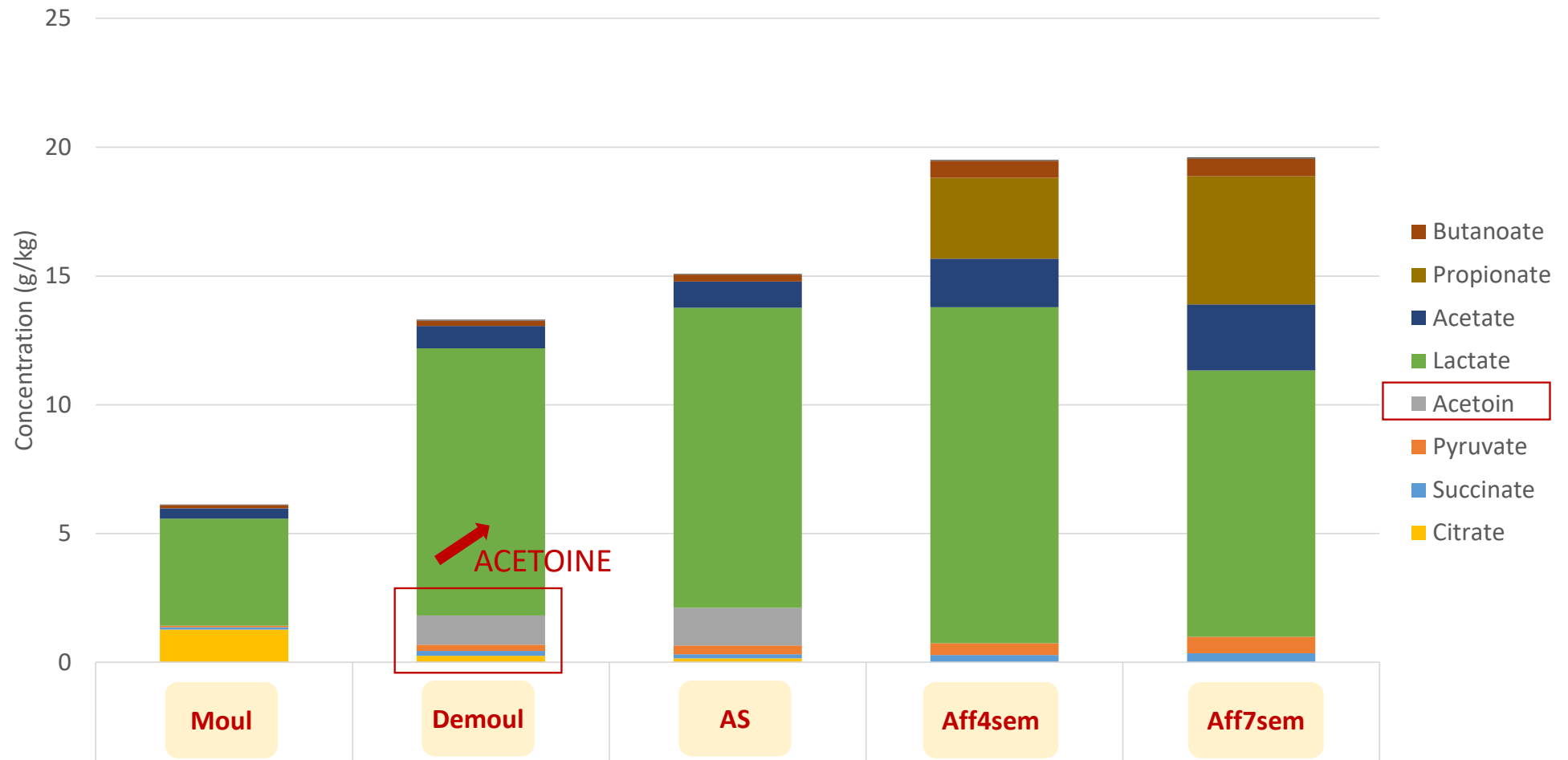
Metabolic interaction = competition for lactose
Functional redundancy with different pathways induced at different times



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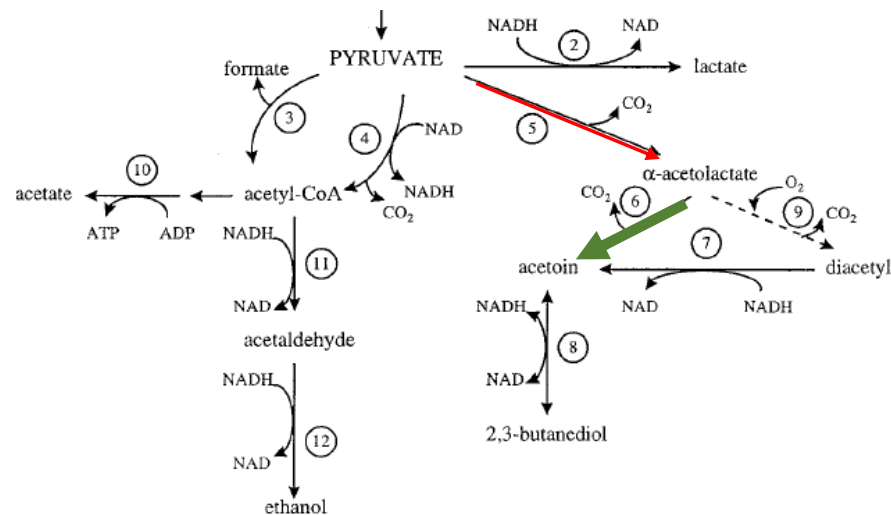
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➤ Production of acetoin (cheesy aroma compound)



➤ 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*

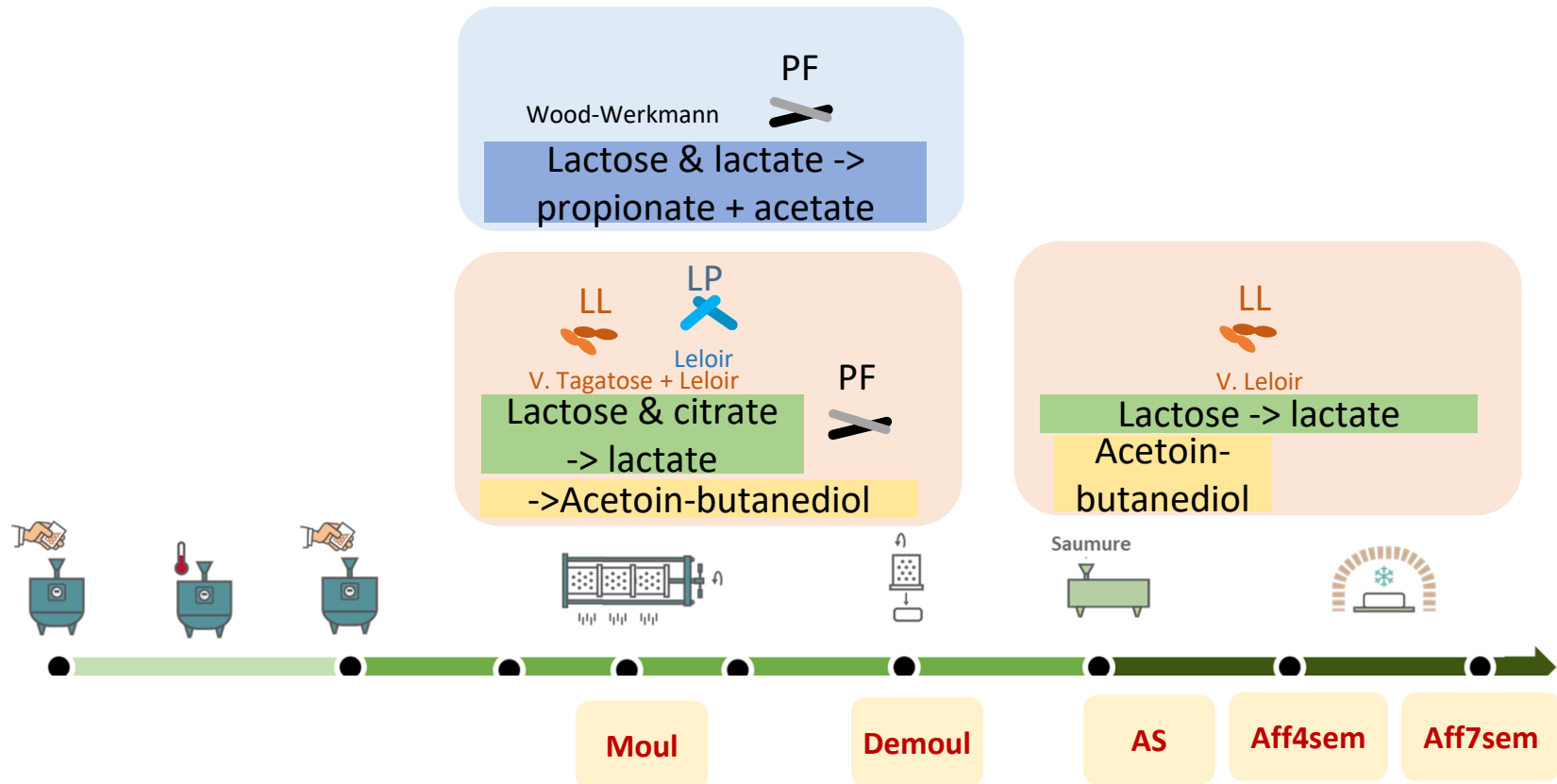
Modified from Bouverdassi et al. 1997 AEM



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 ?

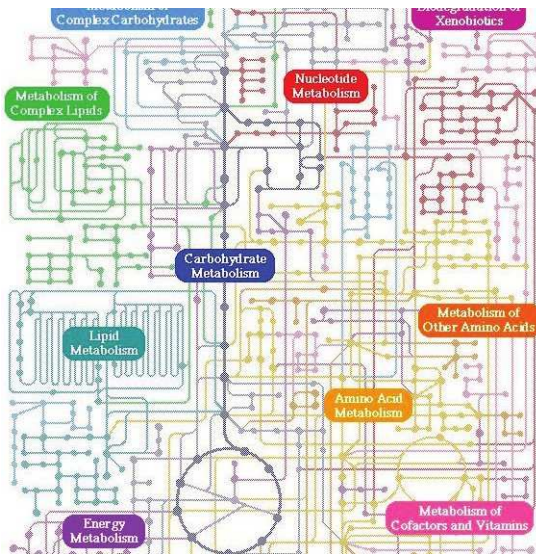
➤ Metabolic modeling to identify metabolite exchanges

INRIA-INRAE PhD student Maxime Lecomte



Annotated genome

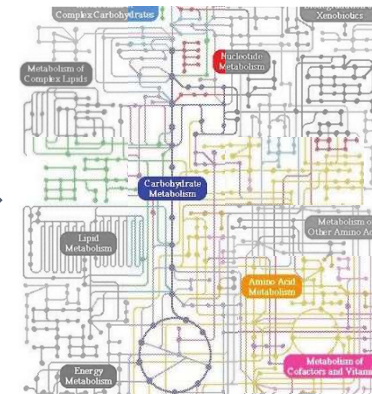
automatic
+ manual curation



Metabolic network

Modelisation

INPUT
Molecules
present
in milk



Metabolic network

OUTPUT
Microbial
metabolites

inria

Flux balance analysis
Maximize/minimize an objective function
 $\psi = c_1 V_1 + c_2 V_2 + \dots + c_5 V_5$ such that:

$$\begin{array}{c} \text{Reactions} \\ R_1 \quad R_2 \quad R_3 \quad R_4 \quad R_5 \end{array}
 \begin{array}{c} \text{Metabolites} \\ \begin{array}{c} \blacktriangle \\ \blacksquare \\ + \\ \bullet \\ \circ \\ \blacklozenge \\ \star \end{array} \end{array}
 \begin{array}{ccccc}
 -1 & 0 & 0 & 0 & 0 \\
 1 & -1 & 0 & 0 & 0 \\
 0 & 1 & -1 & 0 & 0 \\
 0 & 1 & 0 & 0 & -1 \\
 0 & 0 & 1 & 0 & 0 \\
 0 & 0 & 0 & -1 & 0 \\
 0 & 0 & 0 & 1 & -1 \\
 0 & 0 & 0 & 0 & 1
 \end{array}
 \times
 \begin{array}{c} \begin{pmatrix} V_1 \\ V_2 \\ V_3 \\ V_4 \\ V_5 \end{pmatrix} \end{array}
 =
 \begin{array}{c} \begin{pmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{pmatrix} \end{array}$$

S-matrix

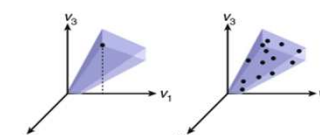
Flux vector

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

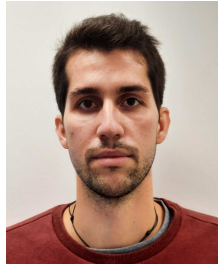
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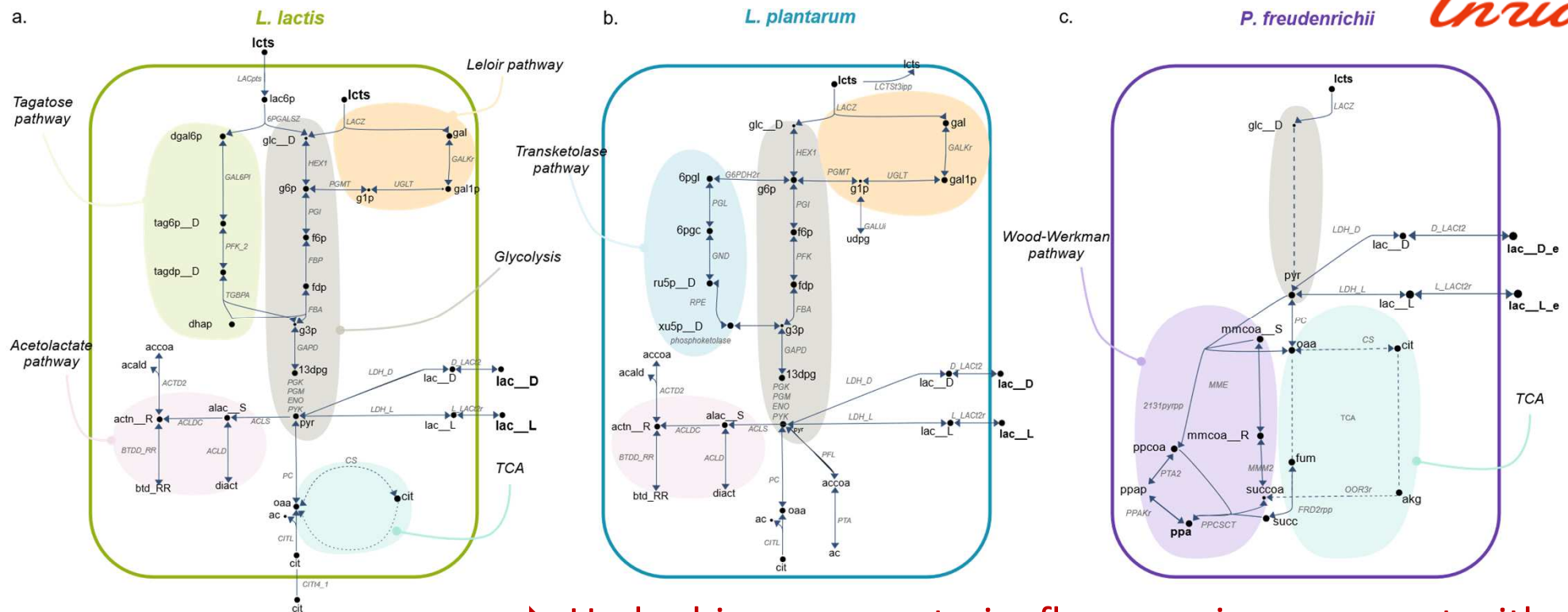
Machado et al. 2018.
Orth et al. 2010

➤ Overview of carbon metabolism of the 3 strains

INRIA-INRAE PhD student Maxime Lecomte



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

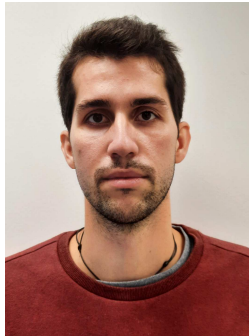


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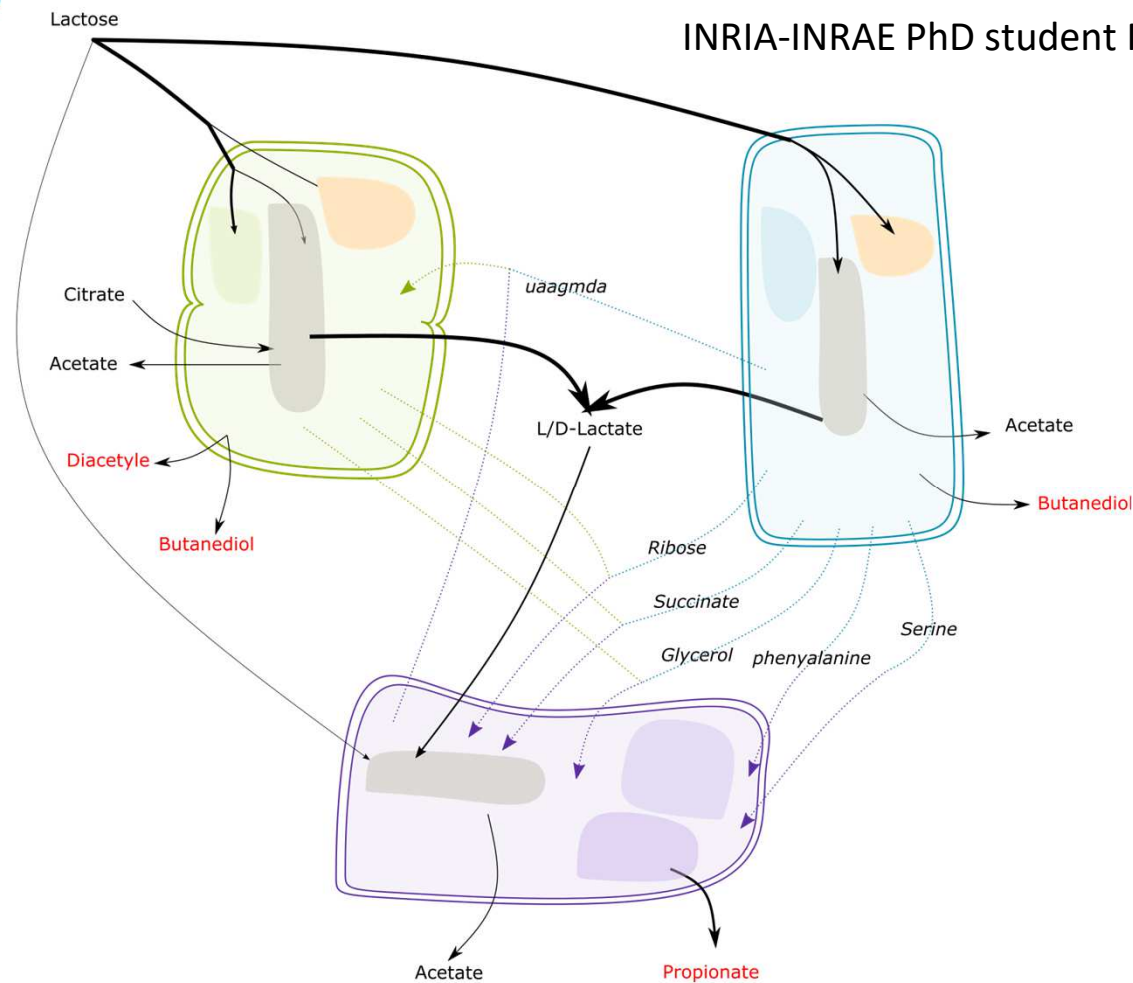
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➤ Modèle métabolique à l'échelle de l'écosystème : identification d'interactions

INRIA-INRAE PhD student Maxime Lecomte



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Logiciel Smetana
Zelezniak et al. 2015 PNAS

➔ Identification of potential interaction to be validated p. 20

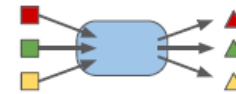


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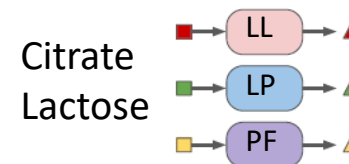
➤ Take home messages

- ✓ Identification of induced pathways in each species

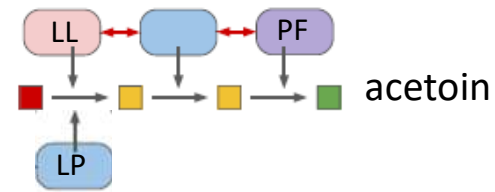


- ✓ Giving snapshots of successive metabolisms during cheese making

- 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

H  l  ne Falentin

St  phanie Deutsch

Sandrine Parayre

Anne Thierry

Wenfan Cao

Marie-Bernadette Maillard

Gilles Garric

Marielle Harel-Oger

Arlette Leduc

Fran  oise Boissel

Jean-Luc Thomas

B  n  dicte Camier

Cl  mence Frioux

Simon Labarthe

Maxime Lecomte

Julie Aubert

Sol  ne Le Fur

David Sherman

Cl  mence Frioux



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Bact  ries d'Int  r  t Alimentaire

