Valorisation de la biodiversité microbienne pour les aliments fermentés : *analyse multi-échelle du génotype au phénotype pour un choix rationnel des ferments lactiques*

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ML Daveran-Mingot, M. Cocaign-Bousquet

Toulouse, France

www.lisbp.fr

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LISBP
Ingénierie des Systèmes Biologiques et des Procédés

LISBP: a cascade of competences in biotechnology

5 scientific poles, 4 platforms, 350 people

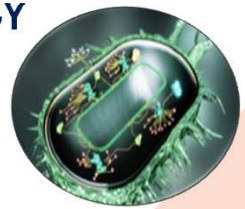
ENVIRONMENTAL ASSESSMENT, ECOCONCEPTION

PILOTS, SCALE UP

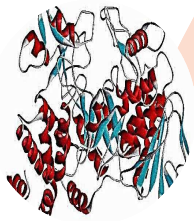
APPLICATION
chemical synthons, Bioenergy, Food supplements, Pharmaceutical compounds, Waste and water treatment

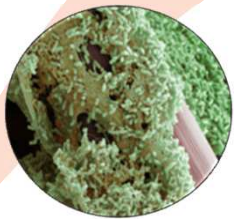
MICRO-ORGANISMS, PHYSIOLOGY

GENOMES SYNTHETIC BIOLOGY



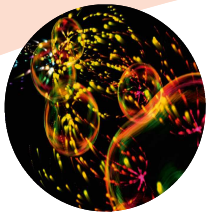
BIOCATALYSIS, ENZYMES





BIOFILMS, GRANULES

BIOREACTOR



INTERFACE MASS TRANSFER

SEPARATION PROCESSES

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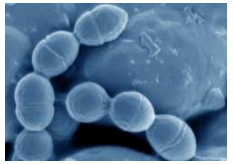
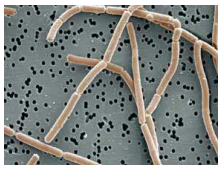
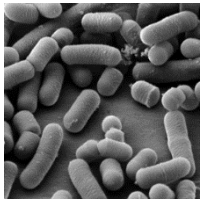
Lactic Acid Bacteria

Gram-positive, non-spore forming cocci and rods
Anaerobic aerotolerant, catalase negative
GRAS (Generally Recognized As Safe) organisms







Large number of species for a variety of fermented foods

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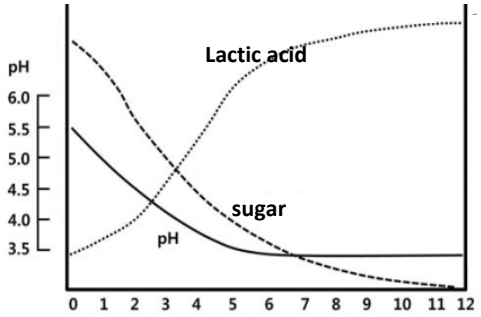
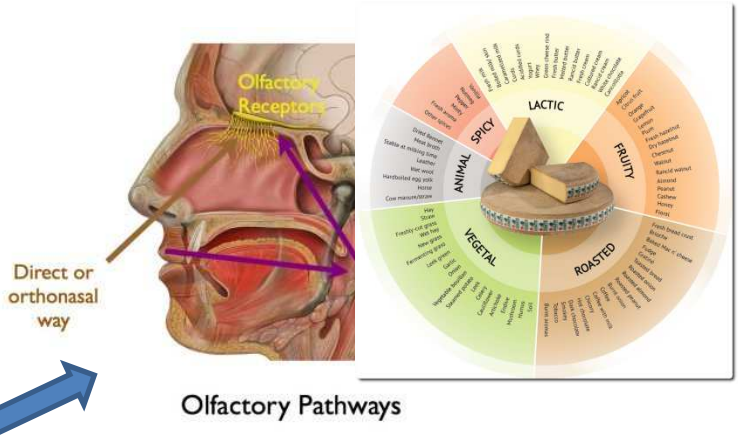
Lactic acid bacteria and food fermentation

Acidification (Lactic acid production)

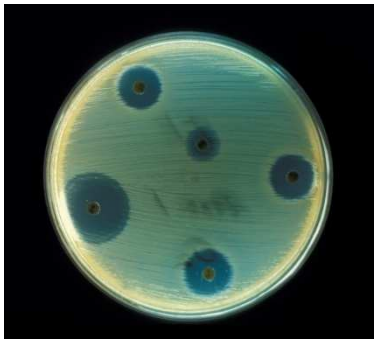


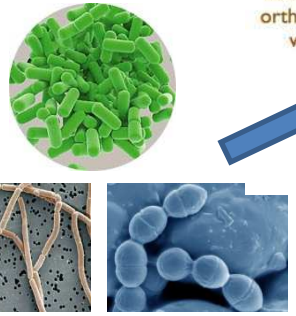
Flavor



Olfactory Pathways

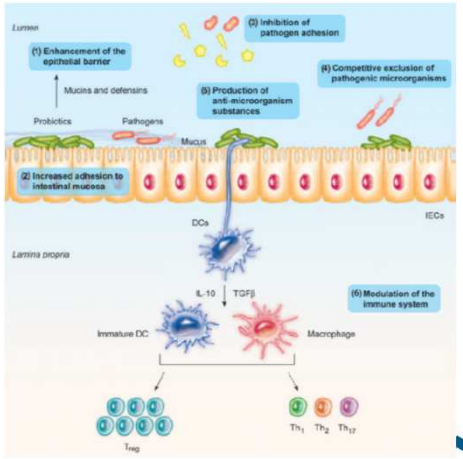
Antimicrobial compounds





Texture

Nutraceutic production, Probiotic bacteria



LISBP

Diversity and functional characterization of starters: an integrated approach

GENOTYPE

PHENOTYPE

Predict

Understand

DIVERSITY
subsp. *lactis*
?

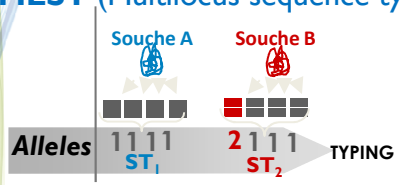
Genetics

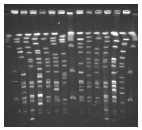
Genes content and

genes variation

CGH (Comparative Genomic hybridation)

MLST (Multilocus sequence typing)





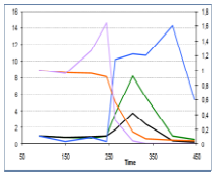
Genomics

Genome sequencing **NGS**

Genome pattern analysis

PFGE (Pulsed Field Gel Electrophoresis)

Physiology

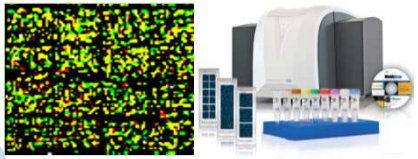


Growth, metabolites and acidification kinetics

Enzymatic analysis

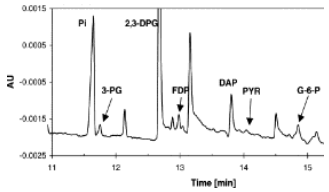
Microbial energetics

Transcriptomics



Proteomics

Metabolomics

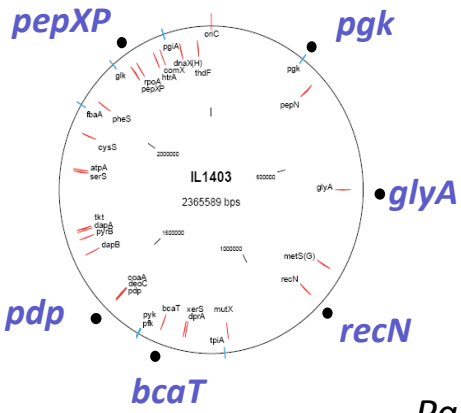


LISBP

Genetic diversity of *Lactococcus lactis* subsp *lactis* strains

MLST (Multi Locus Sequence Typing)

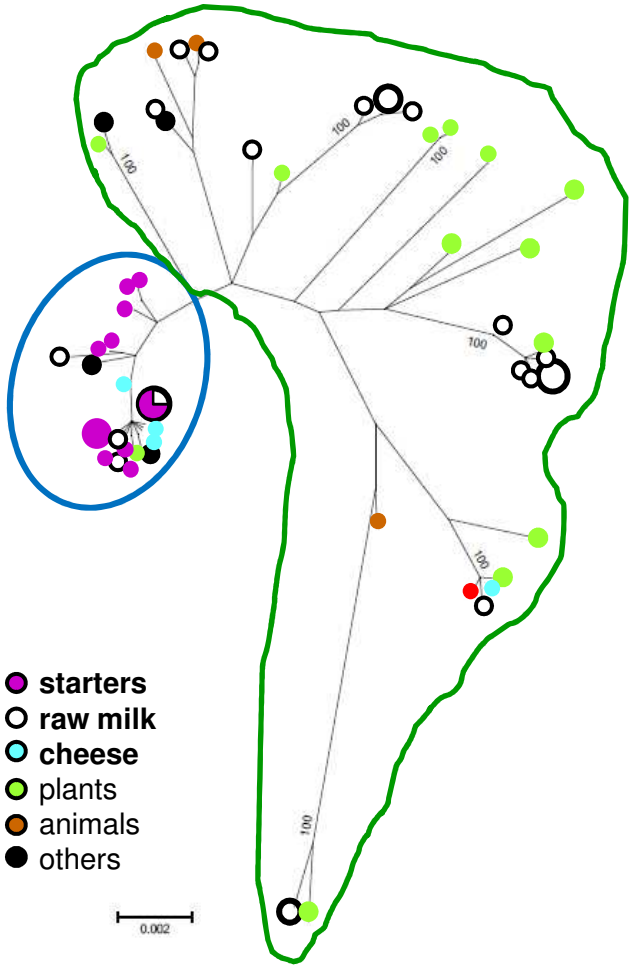
6 loci



154 strains -> 70 ST

L. lactis* subsp. *lactis

Passerini et al., Plos One 2010



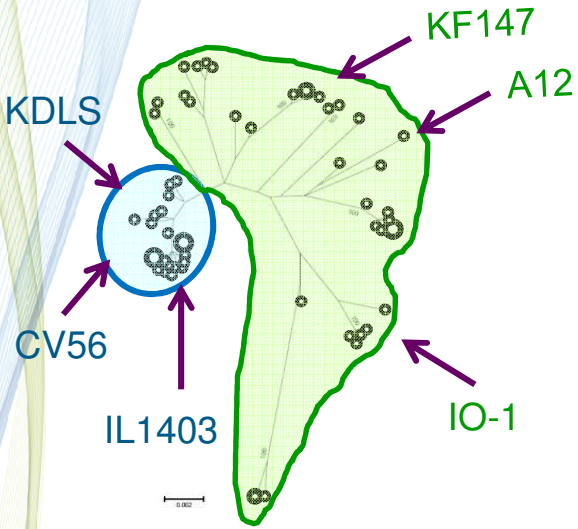
ML (T92+G+I) – boostrap 500

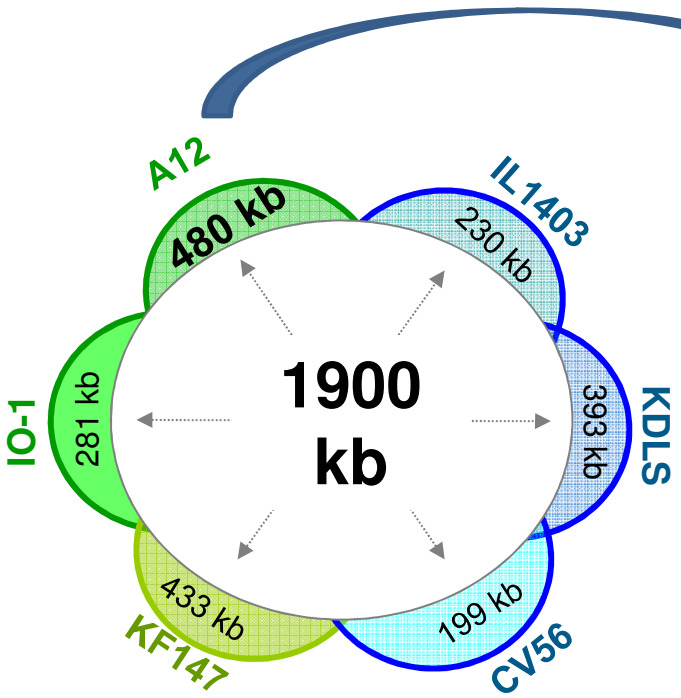
- **Environmental strains**
  - high genetic diversity
  - plants, animals, raw milk
- **Domesticated strains**
  - low genetic diversity
  - industrial processes (starters, cheeses)

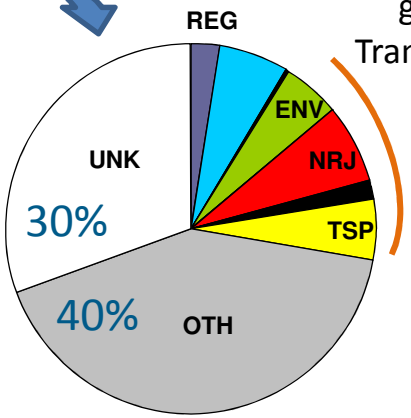
LISBP

Genomic diversity of *L. lactis* subsp *lactis* strains

40 chromosome sequences comparison







Enveloppe, Catabolisme glucides, Transporteurs, **20%**

A12 strain (sourdough)

- **« Strain-specific » genomic content (200 to 480 kb)**
- **A12 strain = 20% of genes related to sugar transport /catabolism**

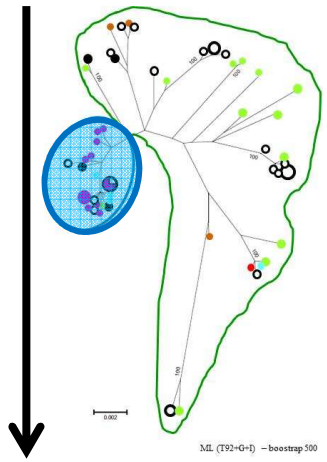
Efficient metabolism of plant sugars (*i.e.*, raffinose) → adaptation to sourdough

(Passerini et al., AEM 2013)

LISBP

Functional diversity of *L. lactis* subsp *lactis* strains

Six "domesticated" strains

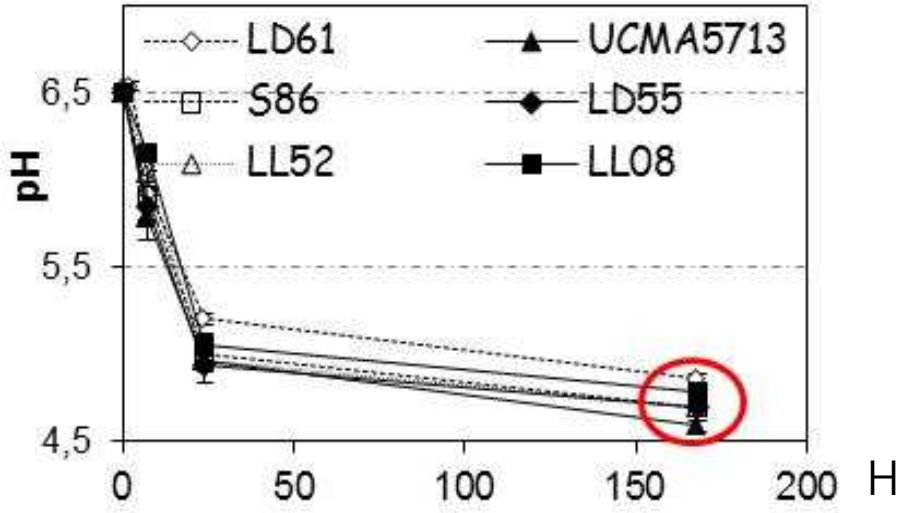


Cultivated as a model Cheese

Ultra-filtered cheese

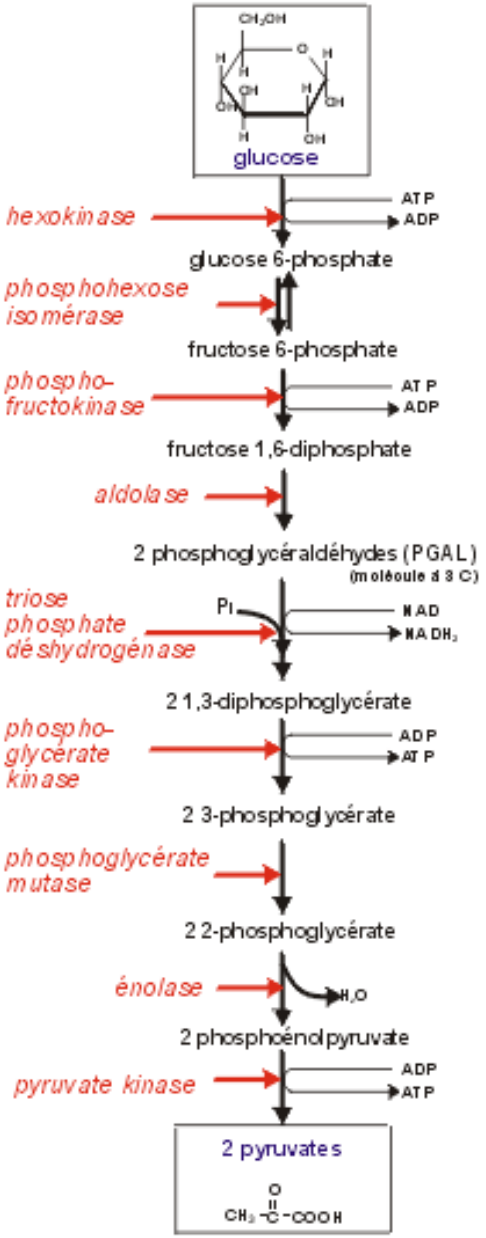
Six different phenotypes:
pH of the cheese at 7 days varies from 4.1 to 4.6

Significant differences in properties (texture, flavor) and bacterial viability



Tan-a-Ram et al., AEM 2011

Metabolic and energetic analysis of *L. lactis* subsp *lactis* strains



Metabolic analysis:

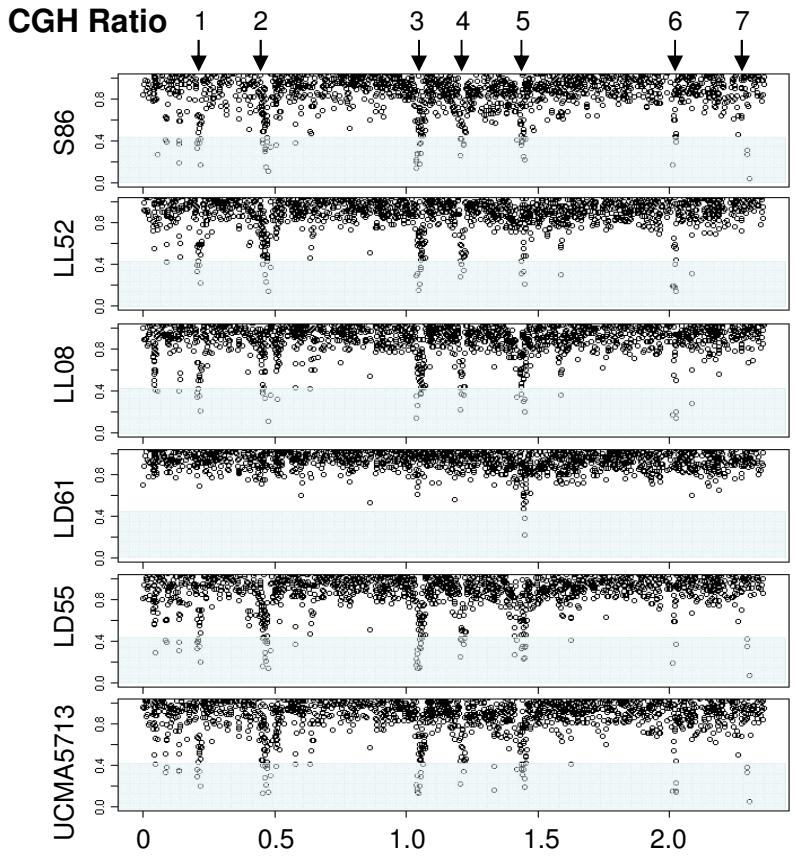
- **enzyme activities** all along the culture
- In vivo enzyme characterization : effect of external factors (pH, T°C, cofactors…)
- Kinetic analysis of these factors
- In vivo **carbon flux** measurement

Modelisation of glycolysis in the culture, during growth, at growth arrest, and at post-acidification arrest

= **identification of limiting enzymes**, inhibited by low pH (PFK, PK)

Functional diversity of *L. lactis* subsp *lactis* strains

Core genome of 1915 genes between the 6 strains. Few genetic differences by CGH



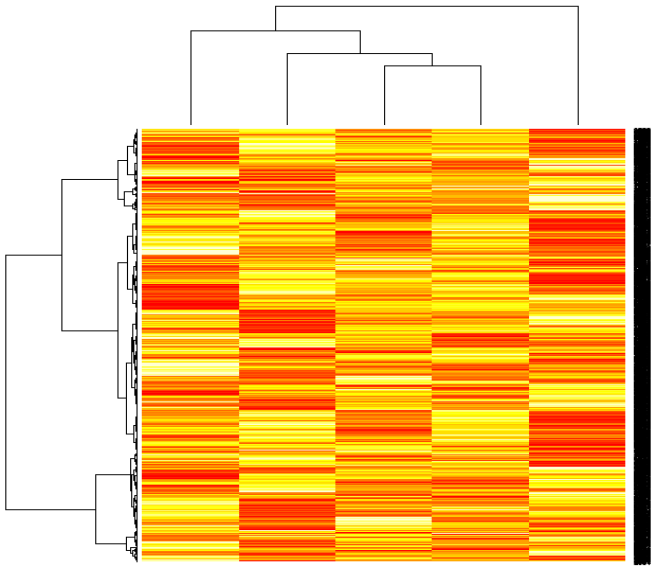
Gene position on the chromosome (Mbp)

Comparison to the sequence strain *IL1403*

Tan-a-Ram et al., AEM 2011

Dynamic transcriptome of *L. lactis*

Wide expression changes in the core genome, between 250 and 600 genes differentially expressed *vs* LD61



Differential expression in:

Response to acidic or oxidative stress,
Carbon limitation,
Carriers,
Some metabolic features (cit, arg, glu, ATPase…)

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Functional analysis of *L. lactis* subsp *lactis* strain

Kinetic transcriptome of *L. lactis* LD61 in UF-cheese

Genes involved	Positive	Negative
Growth rate response *rpl B,M,Q,U,V rpmGB,J rpsA,B,C,J,K,L,N,N2*		
General stress response *clpE, clpP, groES, hrcA-grpE-dnaK regulon, ytgH*		
Amino acid starvation response *oppA,B,C,D,F optC,D,S pep O,N,C,XP, pepXP,O,N,C*		
Acidic response *atp G,D, atpA,B,E,F,H arcC1 arcD1,D2*		
Carbon starvation response *lacC,Z galM,K,T msmK rbsB celB ptbA ptcA,B,C,N,D scrK xylA bglA..*		

Growth arrest and stress

Moderate acidic stress

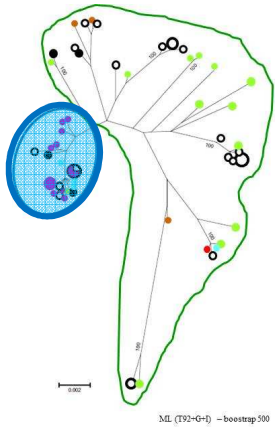
Carbon limitation

Low diffusion of lactose inside the cheese

Laroute et al, AEM, 2011

Functional diversity of *L. lactis* ssp *lactis* strains

Nine "domesticated" strains

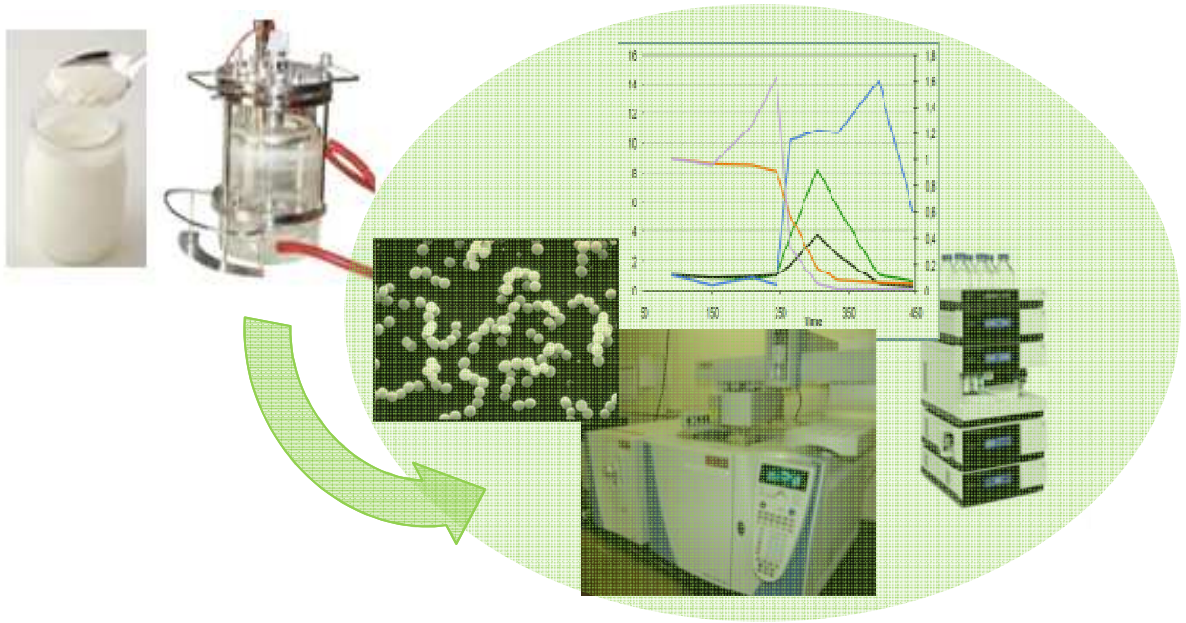


In a model dairy product (fermented milk)

Dhaisne et al. , AEM, 2013

Deep phenotype investigation of *L. lactis*

Fermented milks



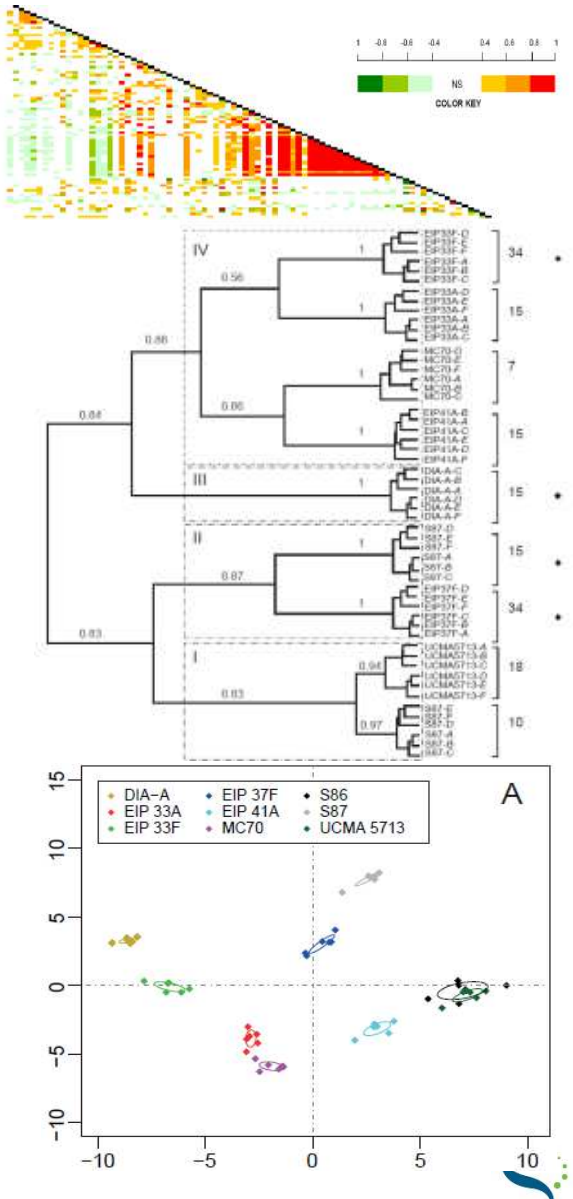
Physiological descriptors and metabolite concentrations in dynamic and at 14 Days

Functional diversity of *L. lactis* ssp *lactis* strains

82 variables selected as important features in dynamic during 24h and at 14 Days

- Sugars,
- Organic acids (lactate, acetate, formate, acetaldehyde),
- Amino Acids ,
- 36 VOCs: Volatile Organic Compounds,
- Growth rate, acidity & redox activity

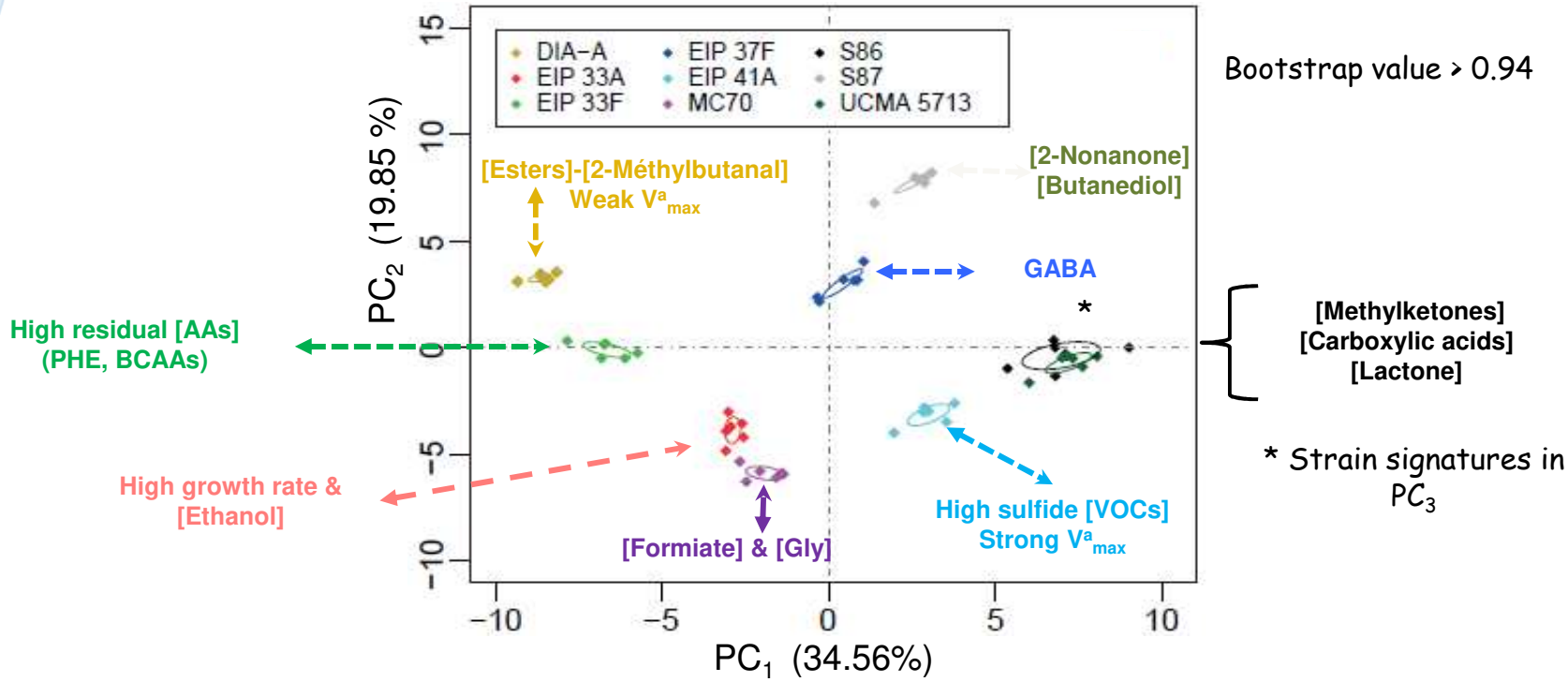
Statistical analysis
Unsupervised: PCA, HAC
Supervised: PLS-DA, Random Forest



Functional diversity of *L. lactis* ssp *lactis* strains

Nine strains = nine signatures

Accurate strain identification



Bootstrap value > 0.94

* Strain signatures in PC_3

Principal Component Analysis of the 82 phenotypic variables.
Ellipses show strain category at a 95% confidence level.

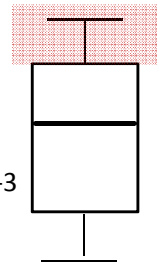
Functional diversity of *L. lactis* ssp *lactis* strains

82 variables

Earn time & money: variable selection

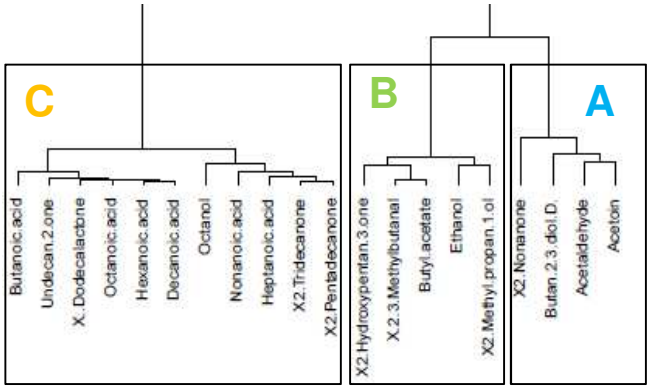
20 variables

Variable selection : 25% of the most contributive variables on PC_{1-3}



Variable clustering

20 variables = 20 VOCs

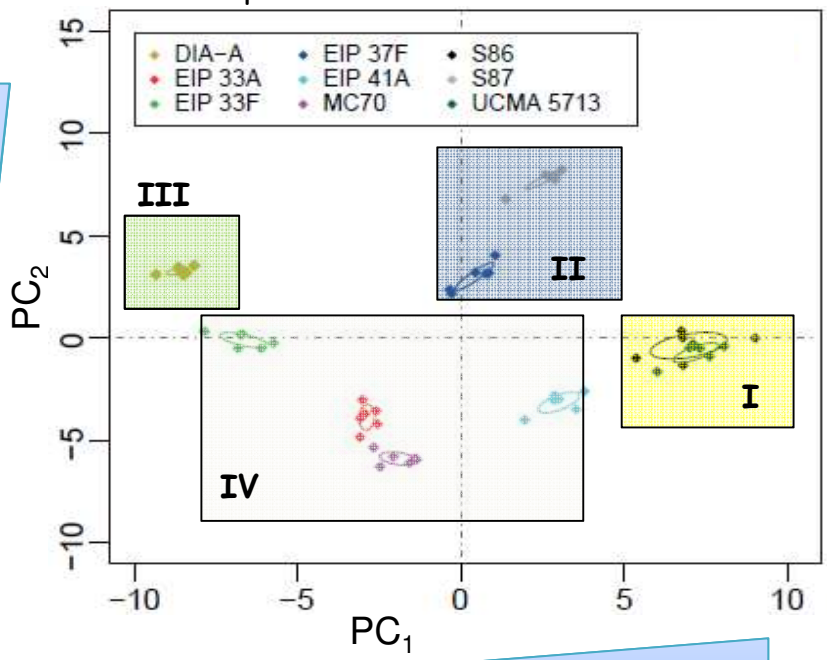


Lypolysis

Glycolysis

Proteolysis

Bootstrap value > 0.86



Glycolysis

Lypolysis

Proteolysis

Four robust strain clusters

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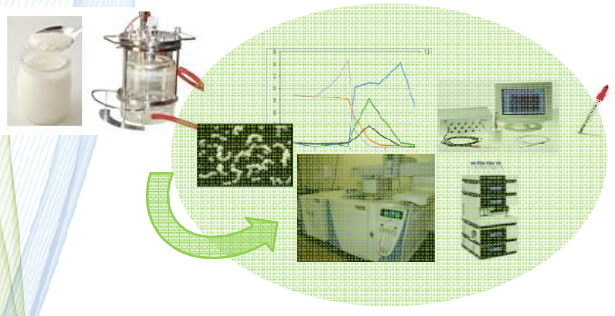
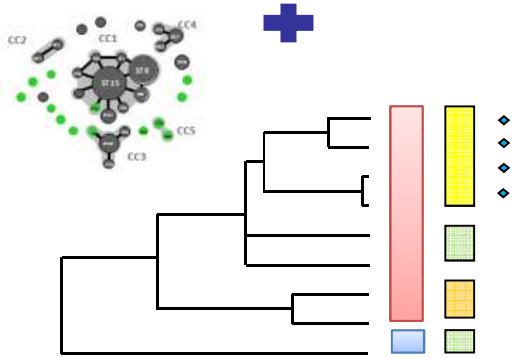
Functional diversity of *L. lactis* ssp *lactis* strains

Integration….. towards prediction

Dhaenne et al. AEM 2013

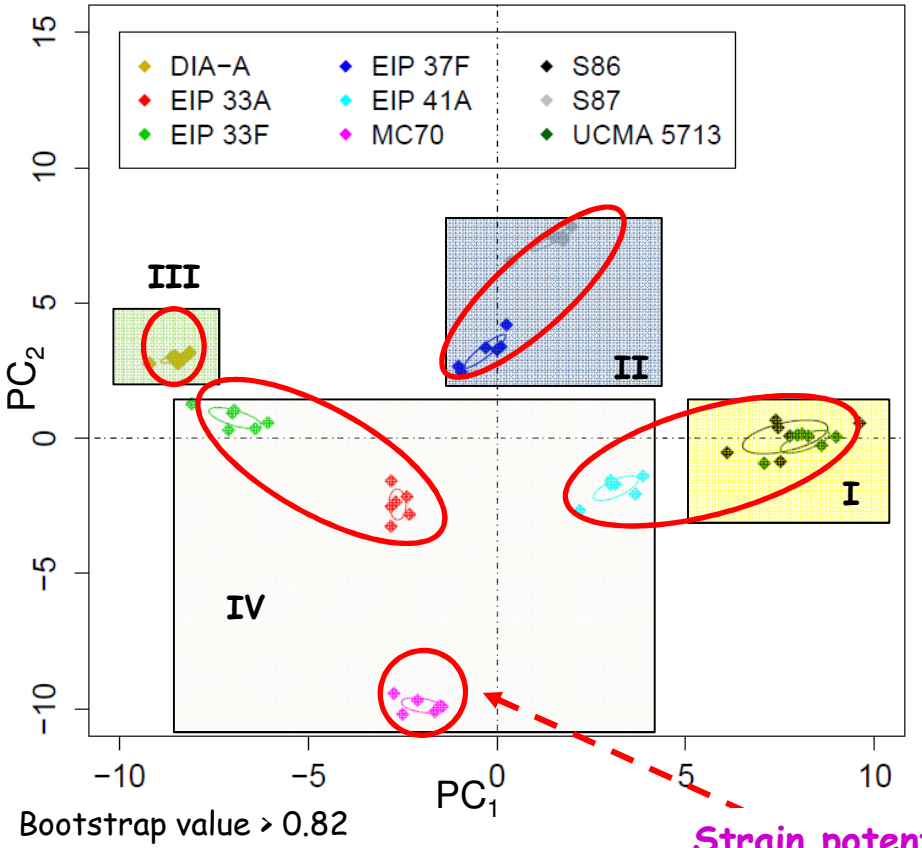
Phenotype





Genotype

Inclusion of genotypic diversity led to 5 rather than 4 clusters



Bootstrap value > 0.82

Strain potentalities encoded in the genotype

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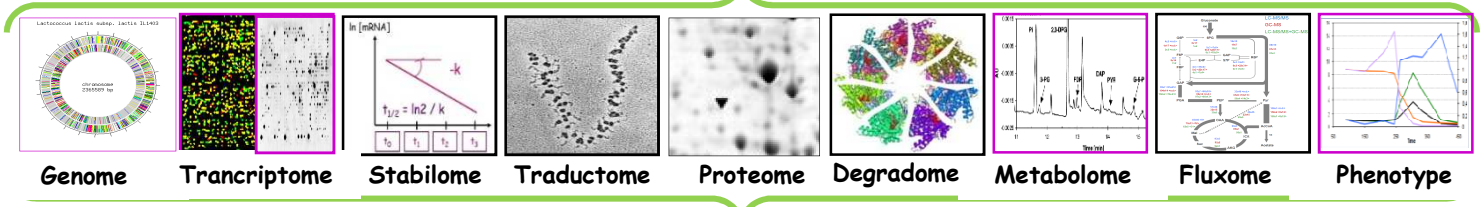
Fermented Food product

Natural strain diversity and/or Starter addition → **functional responses**

Data



Tools

INTEGRATION

Real understanding of bacterial potentialities to strain/starter selection & phenotype prediction

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