



Innovative pulse and cereal-based food fermentations for human health and sustainable diets

HOW GENOMICS CAN SUPPORT IMPROVED STARTER CULTURE SELECTION

Prof. Dr. Stefan Weckx

Vrije Universiteit Brussel, Brussels, Belgium

HealthFerm Conference, August 25-26, 2026



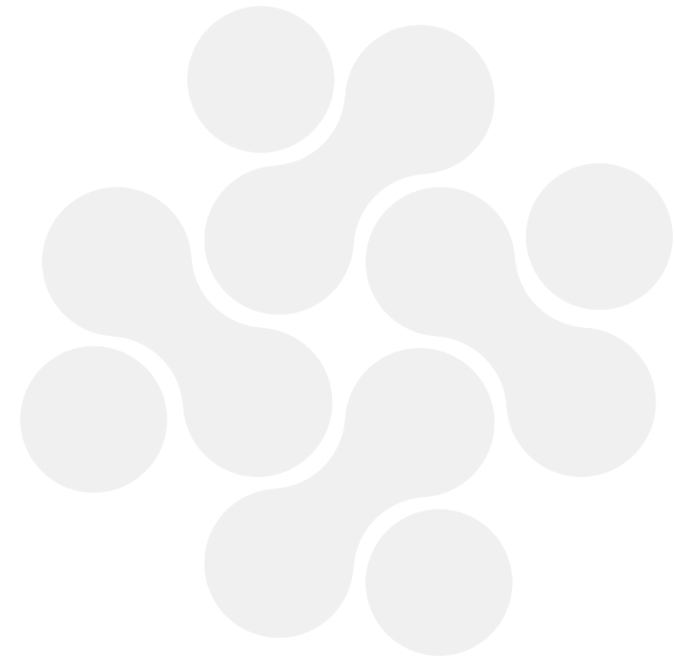
**Co-funded by
the European Union**



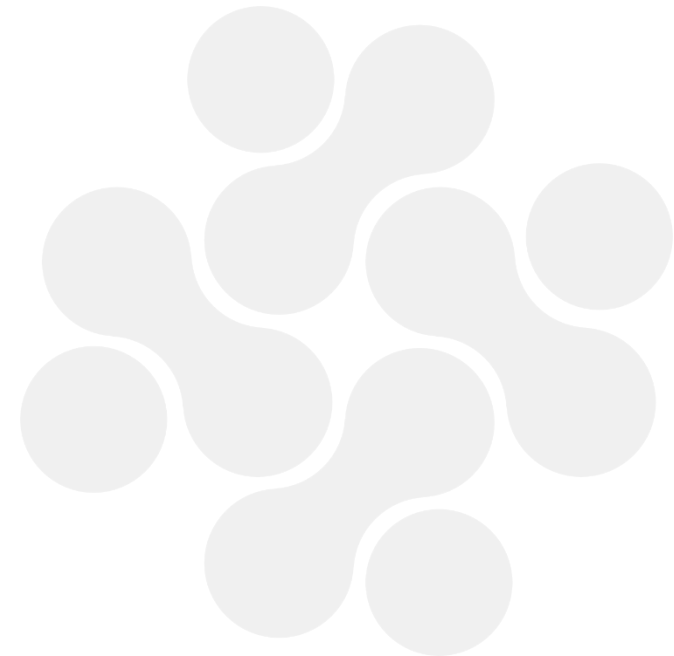
Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra



- Starter cultures and high-quality genomes
- Utilising genomic information
- Conclusions

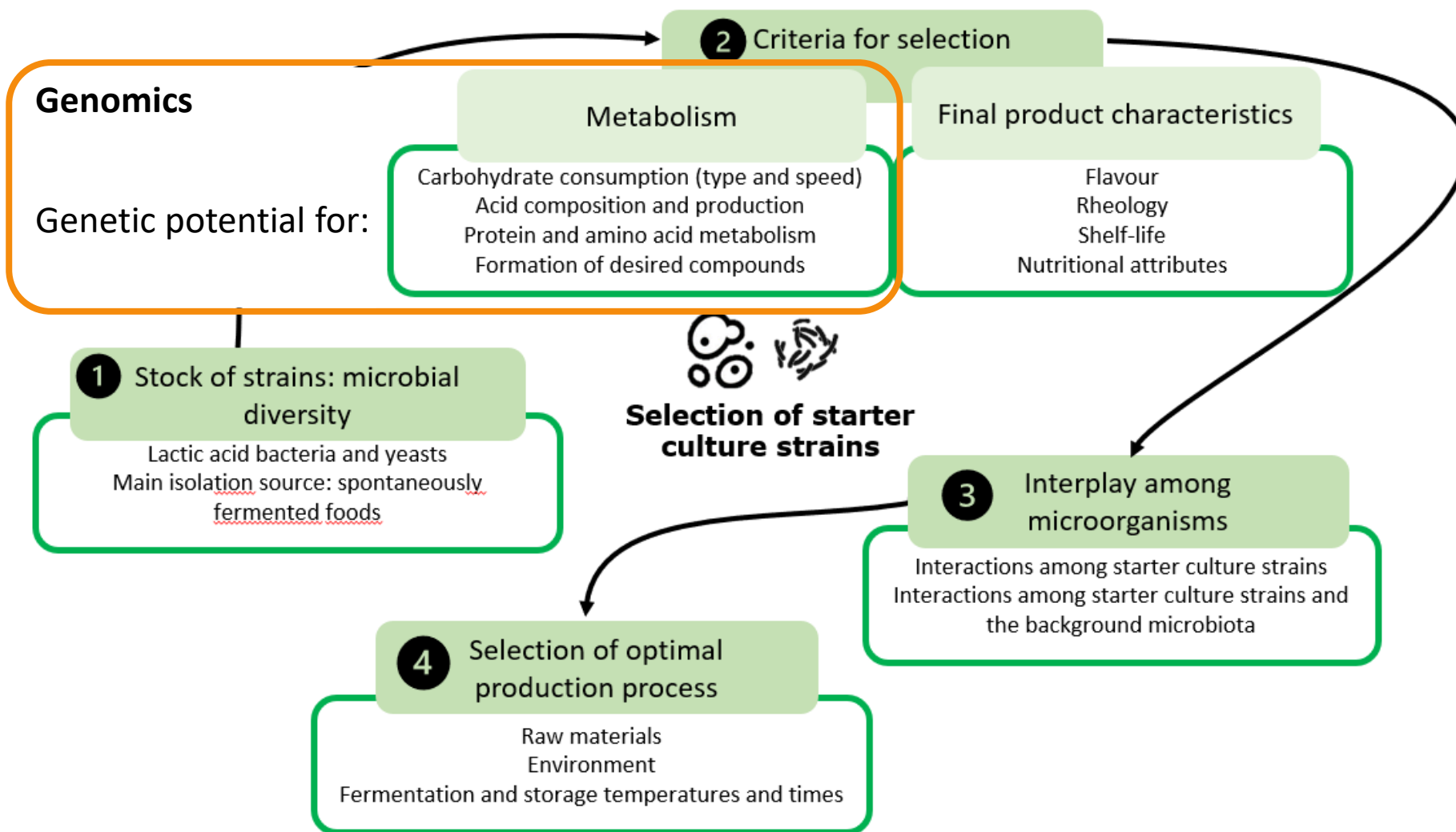


- Starter cultures and high-quality genomes
- Utilising genomic information
- Conclusions



HealthFerm

Starter culture selection



Target property	Matrix
Proteolysis: solubility	
Proteolysis: digestibility	
Proteolysis: foaming	
Proteolysis: emulsification	
Dietary fiber breakdown	
Exopolysaccharides production	
Short chain fatty acids production	
Vitamin B12 production	
Starch digestibility decrease	
Phytate degradation	
β-glucosidase production	
RFOs (raffinose family oligosaccharides)	
FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols)	

101 target genes



Need for high-quality genomes

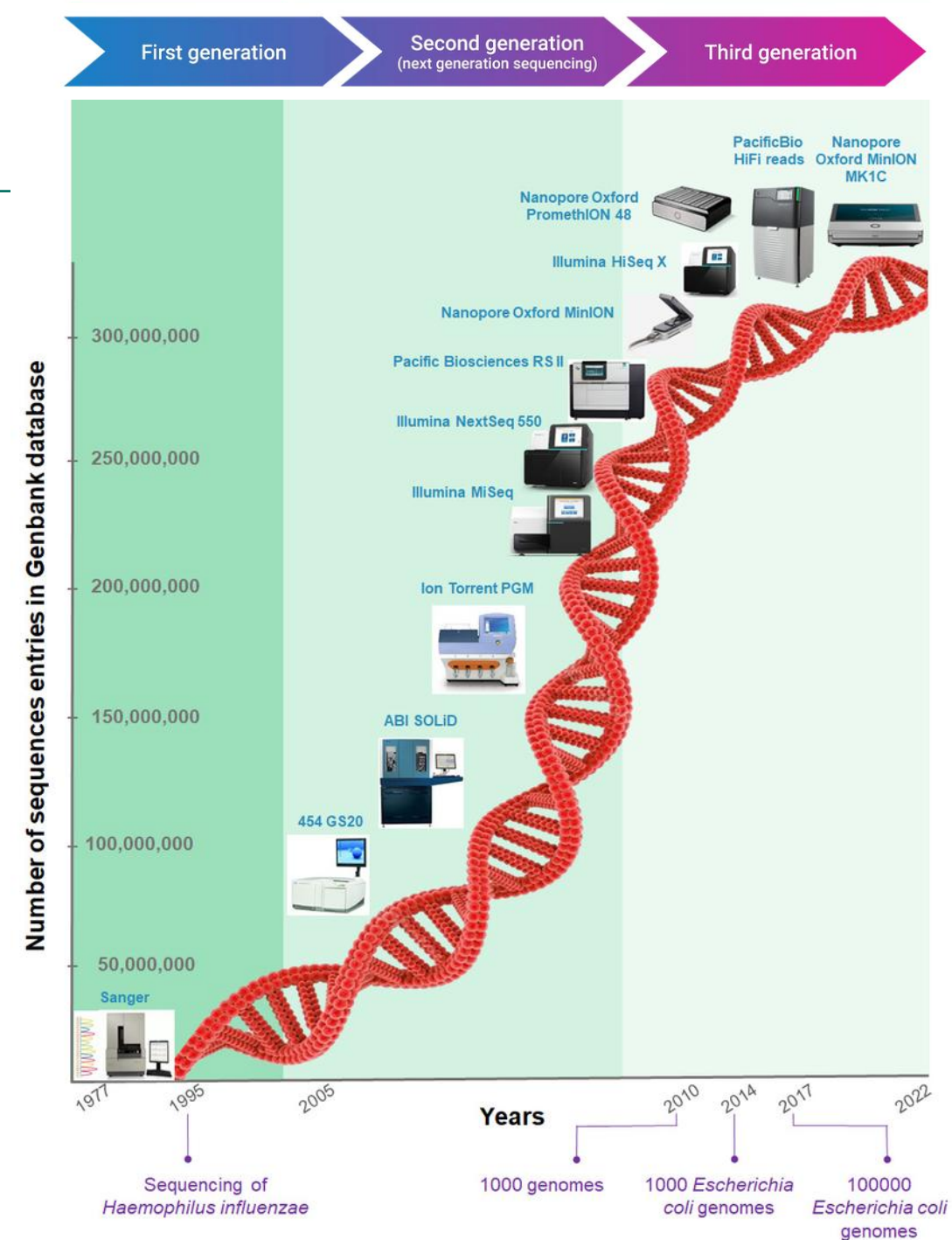
Oxford Nanopore Technologies – PromethION

100-200 Gb native gDNA reads
Up to 96 bacterial genomes



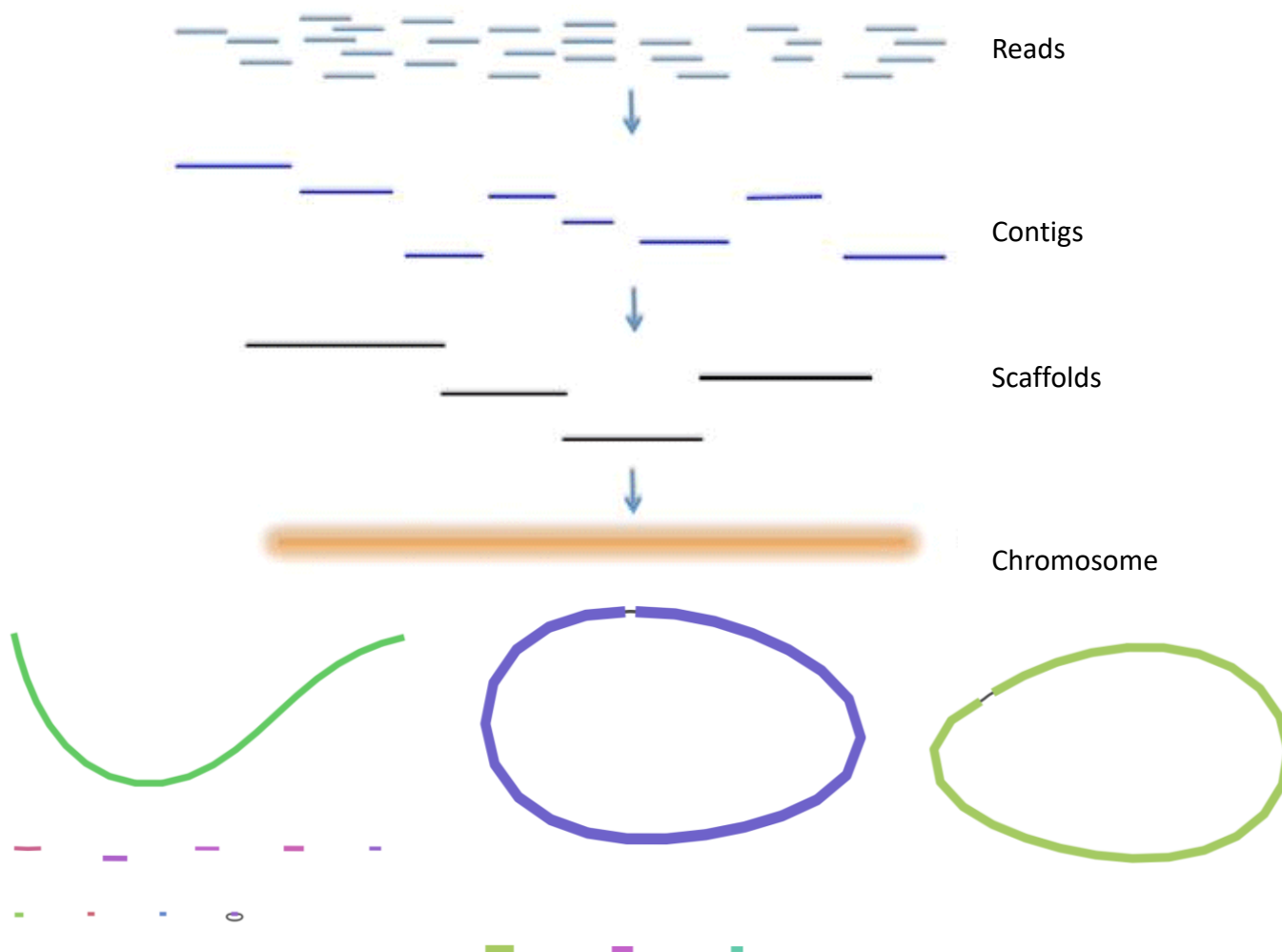
PacBio Vega or Revio

Up to 90 or 120-180 Gb HiFi reads (up to 20 kb)
Up to 384 bacterial genomes



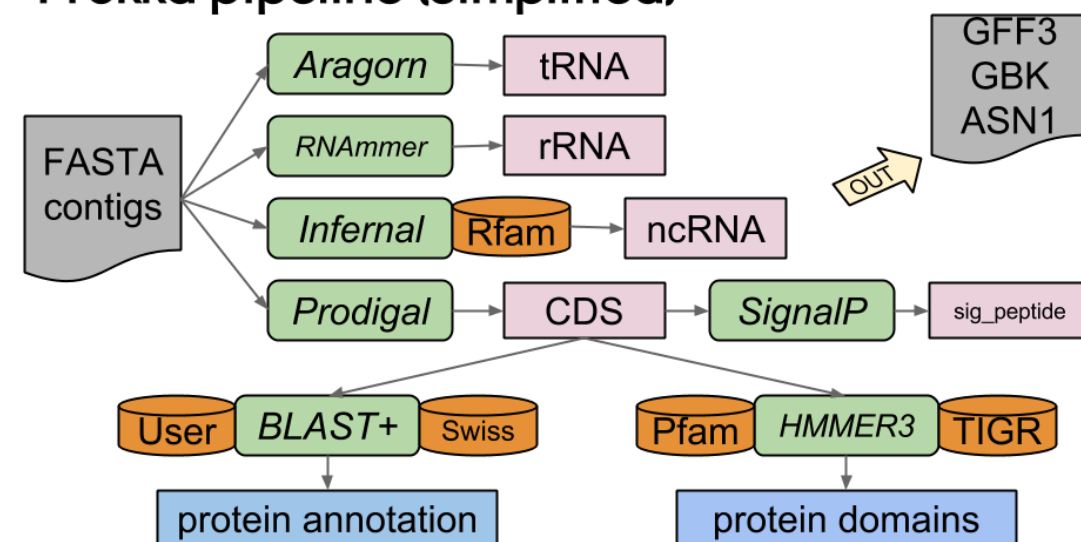
Need for high-quality genomes

Assembly – complete genomes



Genome annotation

Prokka pipeline (simplified)



High-quality genomes in HealthFerm

Type	Genus	Species	Strains		
AAB	<i>Acetobacter</i>	7	10		11%
	<i>Gluconobacter</i>	2	2		
LAB	<i>Lactiplantibacillus</i>	2	13		66%
	<i>Levilactobacillus</i>	4	10		
	<i>Furfurilactobacillus</i>	3	10		
	<i>Fructilactobacillus</i>	1	8		
	<i>Leuconostoc</i>	2	7		
	<i>Companilactobacillus</i>	3	5		
	<i>Lactobacillus</i>	2	4		
	<i>Lacticaeibacillus</i>	2	3		
	<i>Pediococcus</i>	2	3		
	<i>Lentilactobacillus</i>	1	2		
	<i>Limosilactobacillus</i>	1	2		
	<i>Lactococcus</i>	1	1		
	<i>Latilactobacillus</i>	1	1		
	<i>Weissella</i>	1	1		
PAB	<i>Propionibacterium</i>	1	3		3%
Yeast	<i>Maudiozyma</i>	3	8		20%
	<i>Saccharomyces</i>	2	5		
	<i>Naumovozyma</i>	1	5		
	<i>Schizosaccharomyces</i>	1	1		
	<i>Torulaspora</i>	1	1		
	<i>Zygosaccharomyces</i>	1	1		
Total		45	106		

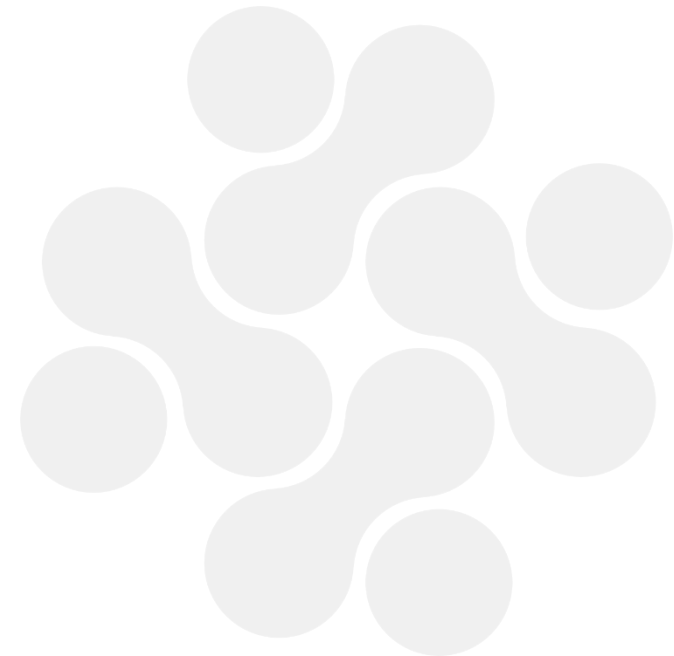


92% complete
circular bacterial
genomes

+250k genes found
and annotated

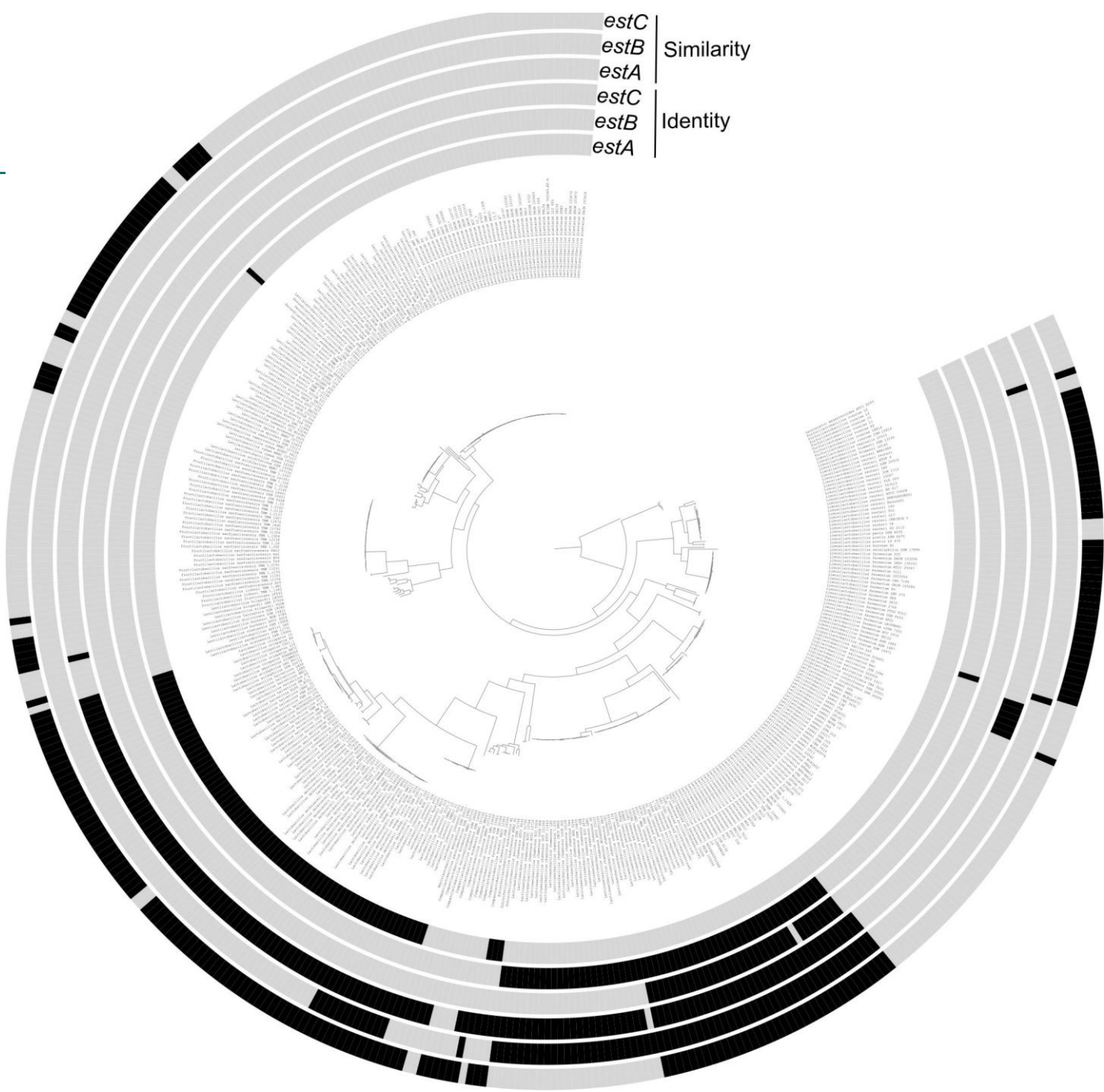


- Starter cultures and high-quality genomes
- Utilising genomic information
- Conclusions

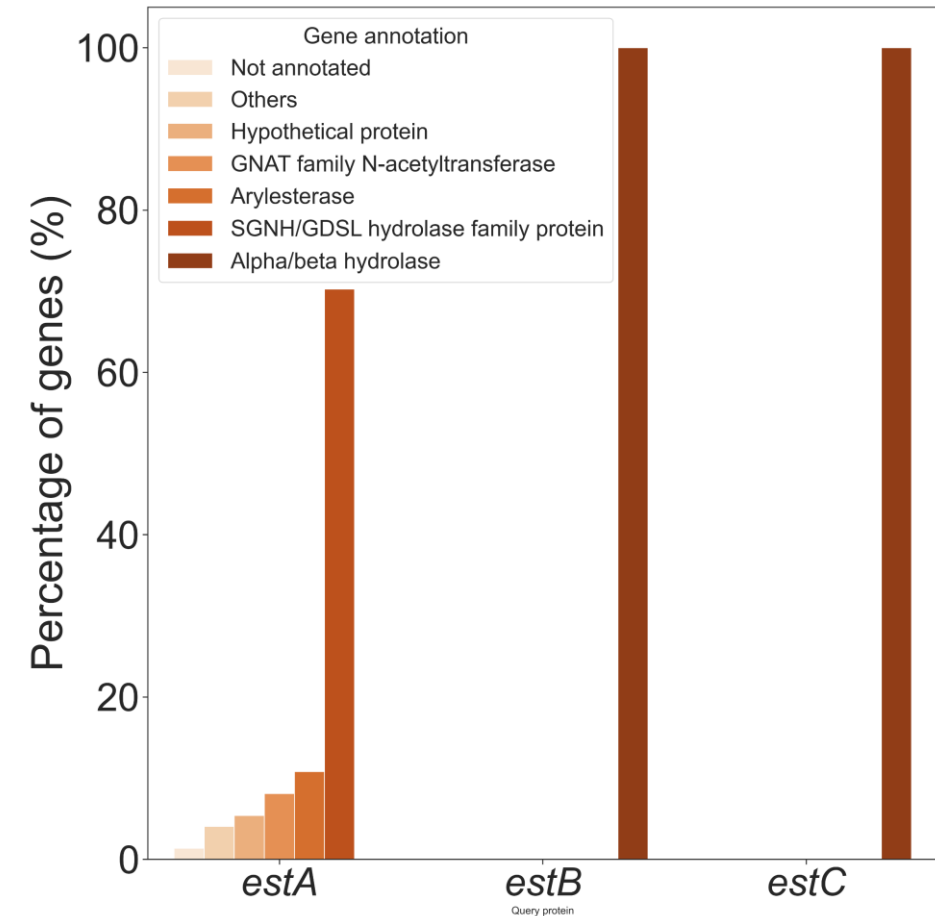


Ester synthesis by LAB

- Esters provide fruity notes (cocoa, cheese, beer, wine, and sourdough)
- **Can LAB synthesise esters from their precursors in a sourdough matrix?**
- Only the esterases EstA, EstB, and EstC have been studied under comparable conditions
 - EstA of *Lactobacillus helveticus*, EstB and EstC of *Lactocaseibacillus casei*
 - Biosynthesis of ethyl esters (ethyl butanoate, ethyl pentanoate, ethyl hexanoate) *in vitro* and in a cheese model
- Screening (tblastn alignment with AA sequences of verified enzymes) of 401 LAB genomes from strains of species described in sourdoughs

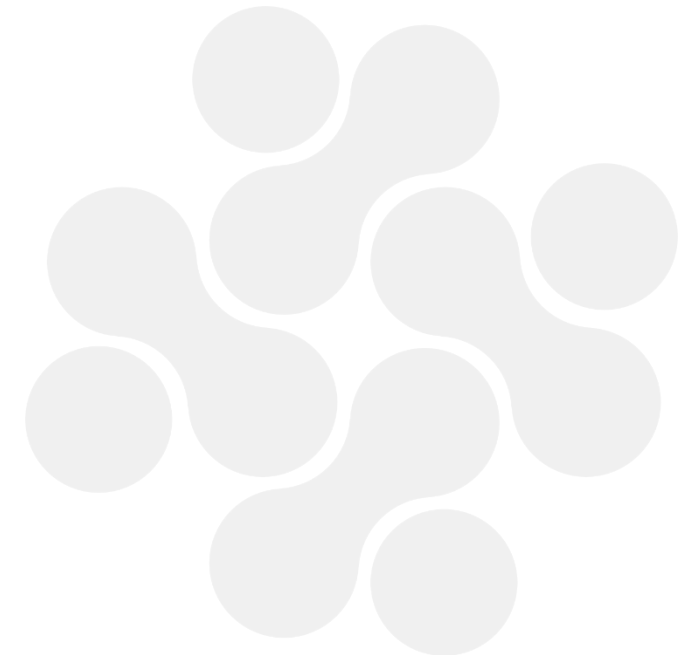
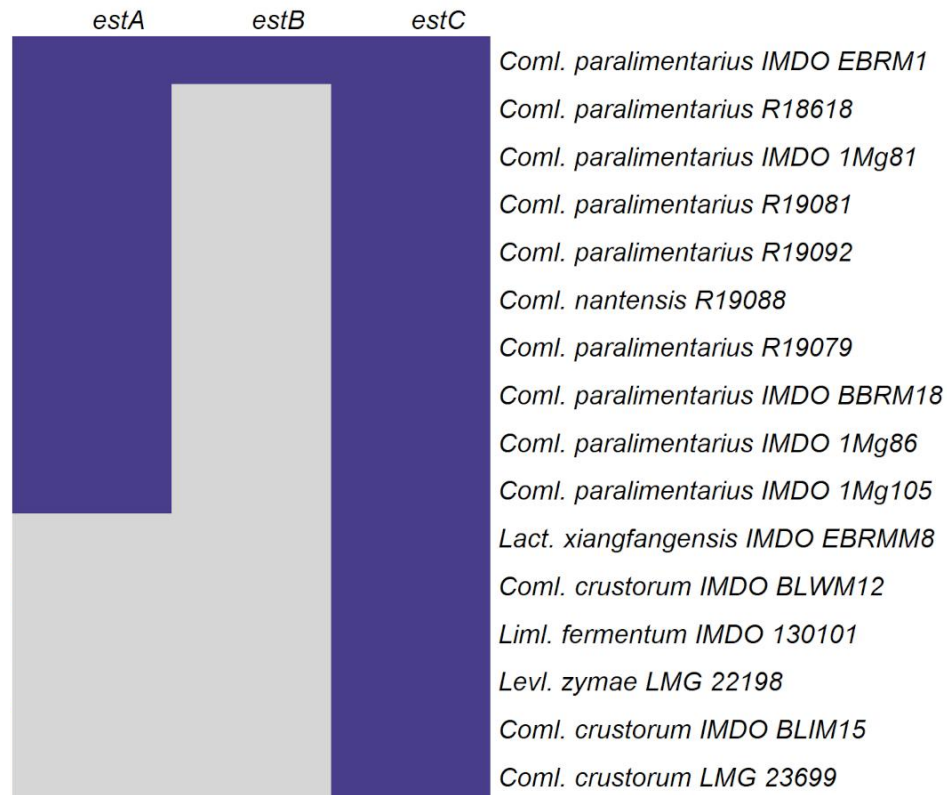


- The presence of *estA*, *estB*, and *estC* genes was species-dependent
- Sourdough-relevant species containing at least one of the genes:
 - *Companilactobacillus paralimentarius*
 - *Companilactobacillus crustorum*
 - *Limosilactobacillus fermentum*
- *Fructilactobacillus sanfranciscensis* did not harbour any of the genes
- Genes were mostly annotated as α/β hydrolases



Genome mining

- Analysed the genomes of 16 culture collection strains from species that contained the *estA*, *estB*, and/or *estC* genes, as shown by screening the 401 genomes



In vitro fermentations to assess ester biosynthesis by LAB strains



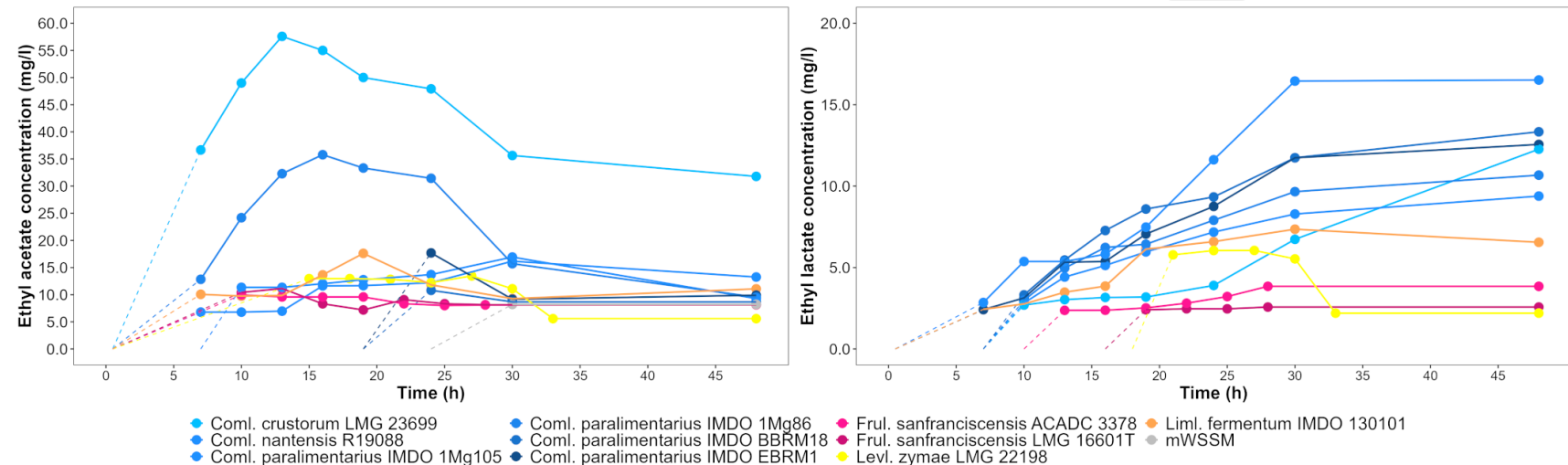
WSSM, wheat sourdough simulation medium

mWSSM: WSSM + ester precursors

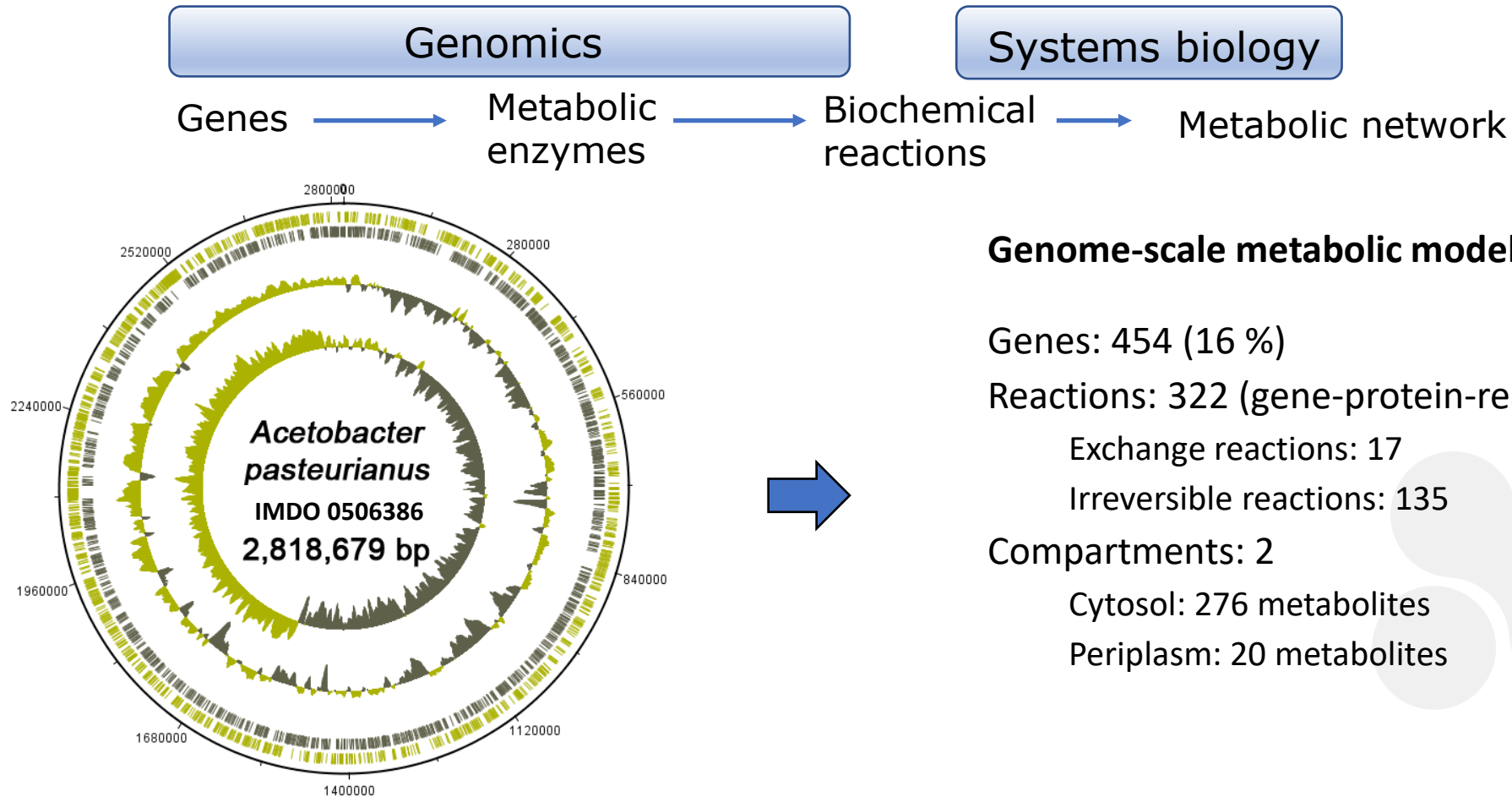
- Ethanol: 1 %
- Acetic acid: 0.5 %
- Organic acids (propionic acid, butyric acid, pentanoic acid, hexanoic acid, decanoic acid, lactic acid): 5 mg/l each
- Higher alcohols (1-propanol, 1-butanol, 1-pentanol, 1-hexanol, isoamyl alcohol): 5 mg/l each

Some strains, mainly of *Companilactobacillus* species, could produce esters in the medium and under the conditions used

Likely, *estC* was involved in ester biosynthesis

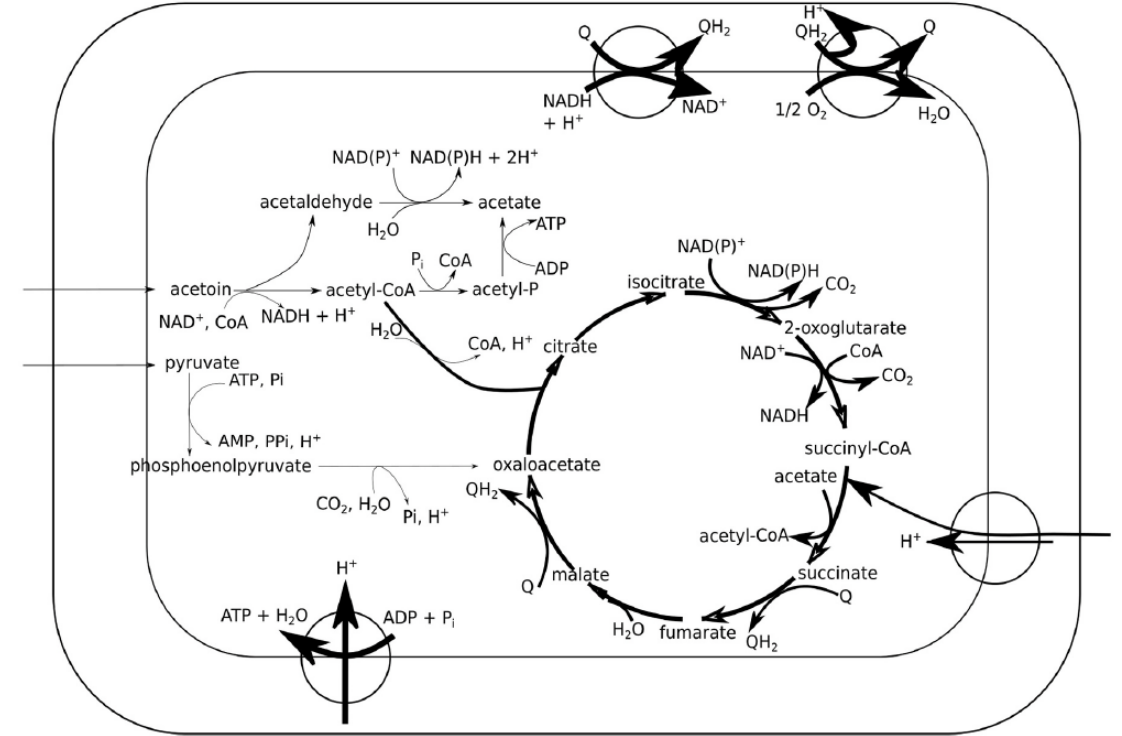
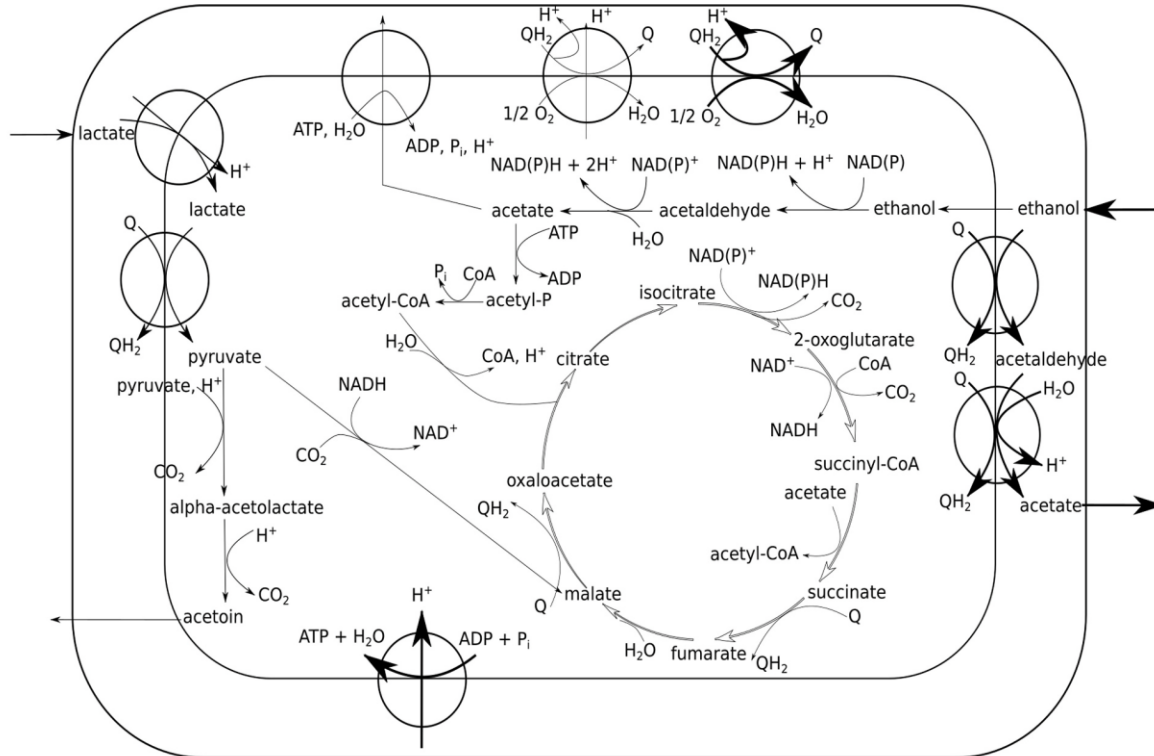


Specific LAB strains can synthesise esters; their ester biosynthesis capabilities is dependent on precursor concentrations



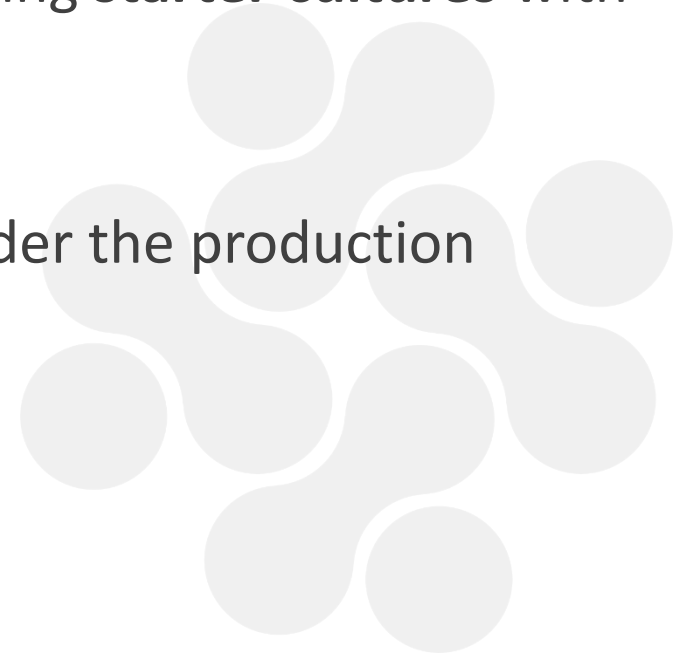
Gene prediction: **2595** -> **2854** protein-encoding genes + functional reannotation

Flux Balance Analysis



- **Evidence for two growth phases** (diauxic growth) of *Acetobacter pasteurianus* IMDO 0506386 under cocoa fermentation conditions, as previously found
 - Different sets of ethanol-oxidising enzymes in **cytoplasm** and **periplasm**
 - Potential role of **ethanol** and **lactate** as a source of **carbon, energy, and NADPH** in the first growth phase
- ⇒ **Is it possible to aim for acetic acid depletion by extensive oxygenation during the AAB stage of the fermentation process?**

- Obtaining **high-quality microbial genomes** is easier and more affordable than ever before
- **Genome mining and comparative genomics**, driven by good knowledge of genes and pathways related to desired traits, allow for **rational choices** regarding **starter cultures** with desired properties to produce plant-based fermented foods
- A **multi-omics approach** is needed to prove the strains' abilities under the production process parameters applied



Acknowledgements

Prof. Dr. ir. Luc De Vuyst

Dr. Inés Pradal

Dr. Rudy Pelicaen

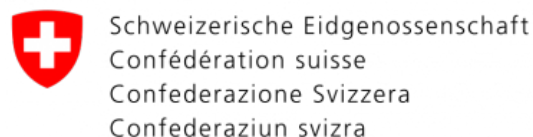
MSc. Gabriela Samaniego

IMDO team

HealthFerm consortium members



VLAAMS
SUPERCOMPUTER
CENTRUM



Co-funded by
the European Union



Innovative pulse and cereal-based food fermentations for human health and sustainable diets

HOW GENOMICS CAN SUPPORT IMPROVED STARTER CULTURE SELECTION

Prof. Dr. Stefan Weckx

Vrije Universiteit Brussel, Brussels, Belgium

HealthFerm Conference, August 25-26, 2026



**Co-funded by
the European Union**



Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

