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# Substrate-driven pre-adaptation during propagation shapes the metabolic behavior of *Leuconostoc mesenteroides* GSL1

Chiara Viretto, Gabriela Samaniego, Ali Zein Alabiden Tlais, Petra Wojnar, Kashika Arora, Stefan Weckx, Raffaella Di Cagno, & Marco Gobbetti

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# Background

↑ High protein content
↑ Source of dietary fibers
↓ Low glycemic index
↓ Gluten-free

Faba beans
Yellow peas

↑ High anti-nutrient contents
↑ Off-flavors
↓ Low proteins bioaccessibility
↓ Digestive discomfort

Fermentation

↑ Nutritional value
↓ Anti-nutrient contents
↑ Protein digestibility
↑ Techno-functional properties
Conditions for spoilage

Toward prediction and customization

Functional gap in conventional fermentation strains

Predicting microbial functionality under pulse-based conditions

| Genomics | Transcriptomic | Proteomics | Metabolomics | Phenomics |
|---|---|---|---|---|

Integrating multi-omics approaches for comprehensive strain characterization

Genotype-phenotype

Tailored starter cultures

This study **aims** to investigate **how the mode of propagation** in pulse-based substrates, compared with optimal growth in modified de Man, Rogosa and Sharpe (mMRS) medium, **reshapes** the **metabolic behavior** and subsequent **fermentation performance** of *Leuc. mesenteroides* GSL1.

# Propagation history modulates metabolic activity and fermentation kinetics: Experimental design

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## Pulse-based model media preparation

Faba bean (FB) flour

Yellow pea (YP) flour

20 g of flour suspended in 180 mL water

Incubation 30 °C, 200 rpm, 16 h

Centrifugation 11,000 x g, 20 min, 4 °C

Supernatant adjusted to pH 5.6 ±0.2

Autoclaving

Re-centrifugation 11,000 x g, 20 min, 4 °C

Filtration 0.22 µm

### Sterile pulse-based media

FB medium

YP medium

### Control medium

Modified MRS (mMRS) supplemented with 0.5% (w/v) maltose and 0.5% (w/v) yeast extract, adjusted to pH 5.6 ± 0.2

## Strain propagation and adaptation

*Leuconostoc mesenteroides* GSL1 (frozen stock)

Routine propagation in mMRS broth 30 °C, 24h

3 propagations (4% inoculum)

-Control (mMRS)

-Faba bean

-Yellow pea

P-C

P-F

P-Y

## Cross-inoculation design (fermentation media)

P-C → P-C-M, P-C-Y, P-C-F

P-F → P-F-M, P-F-Y, P-F-F

P-Y → P-Y-M, P-Y-Y, P-Y-F

30 °C for 48 h

## Monitoring growth and acidification

**Growth kinetics ($OD_{620}$)**

- Lag phase ($\lambda_2$)
- Maximum growth rate ($\mu_2$)
- Growth capacity ($A_2$)
- Area under the curve ($AUC_2$)

## Genomics

DNA extraction

Whole-genome sequencing
Assembly and annotation

## Phenotyping

OmniLog™ Phenotype MicroArray PM1 plates – 95 carbon sources

Carbon utilization profiles of **P-C**, **P-F**, and **P-Y**

## Gene expression (RT-qPCR)

Total RNA extracted from cells cultivated under the three conditions

Expression analysis of 7 genes involved in carbohydrate metabolism and adaptation (disaccharide breakdown, sugar transport, and sugar phosphorylation/modification)

## Quantification of sugars, organic acids, and ethanol

High-Performance Liquid Chromatography (HPLC) system Ultimate 3000

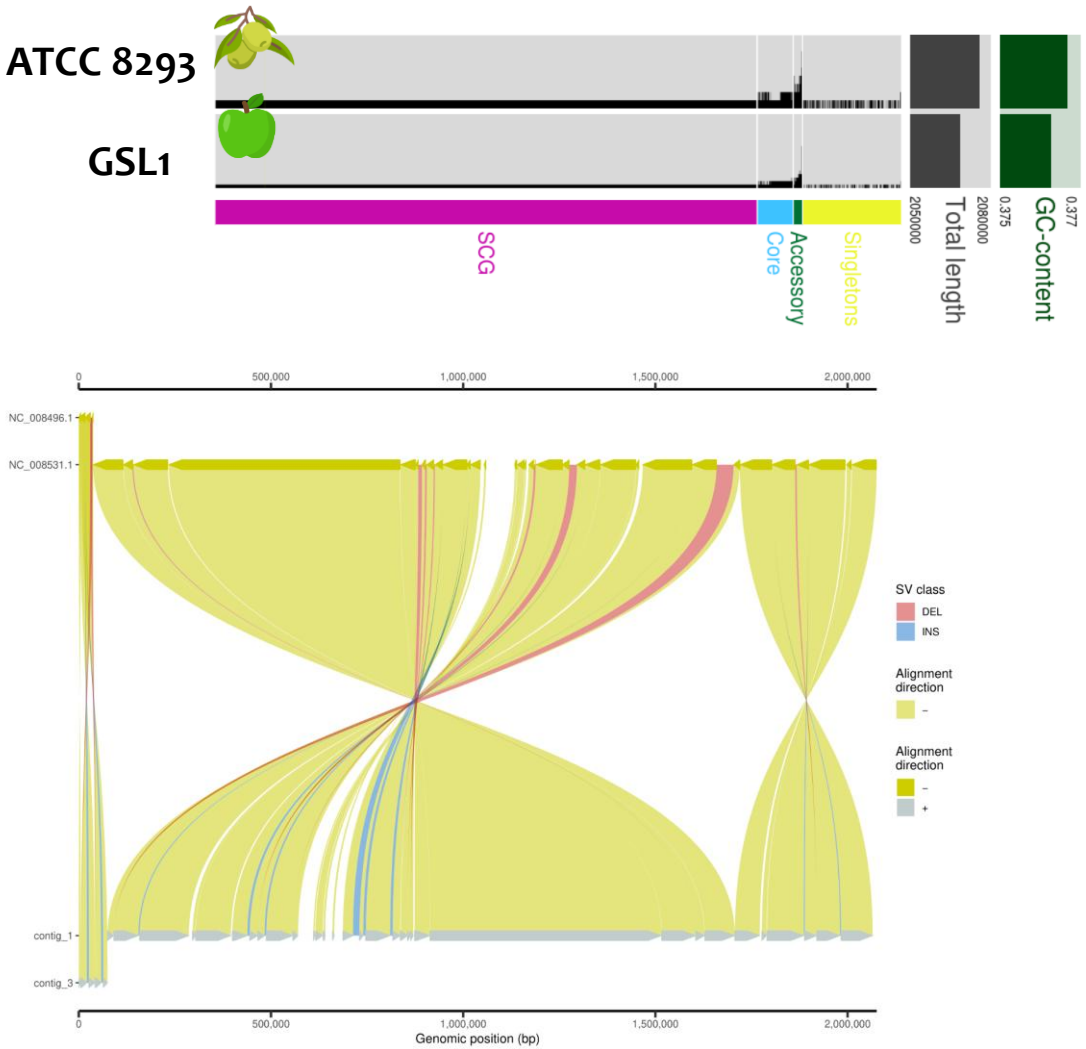
- Glucose
- Fructose
- Mannitol
- Lactic acid
- Acetic acid
- Ethanol

## Quantification of free amino acids and phenolics

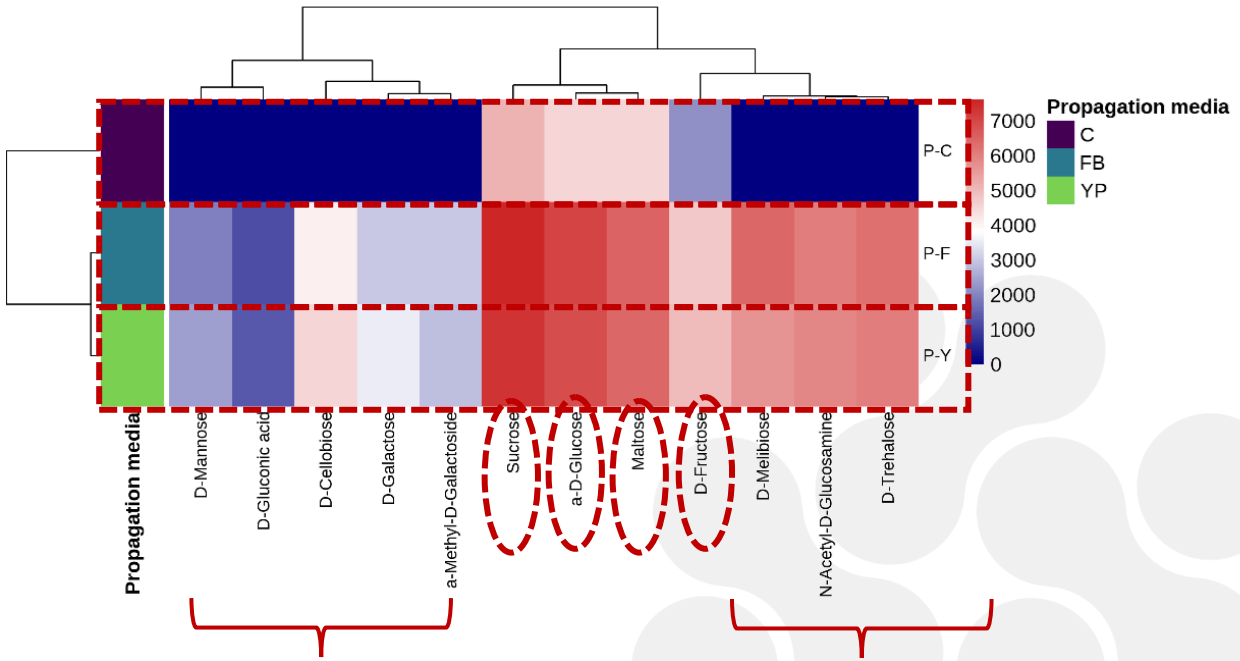
Liquid Chromatography-Mass Spectrometry (Q-Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer)

# Strain propagation and adaptation: Integrating phenotypic and genomic data for *Leuc. mesenteroides* GSL1

## Genome characteristics of GSL1




## Propagating conditions define the metabolic phenotype of GSL1



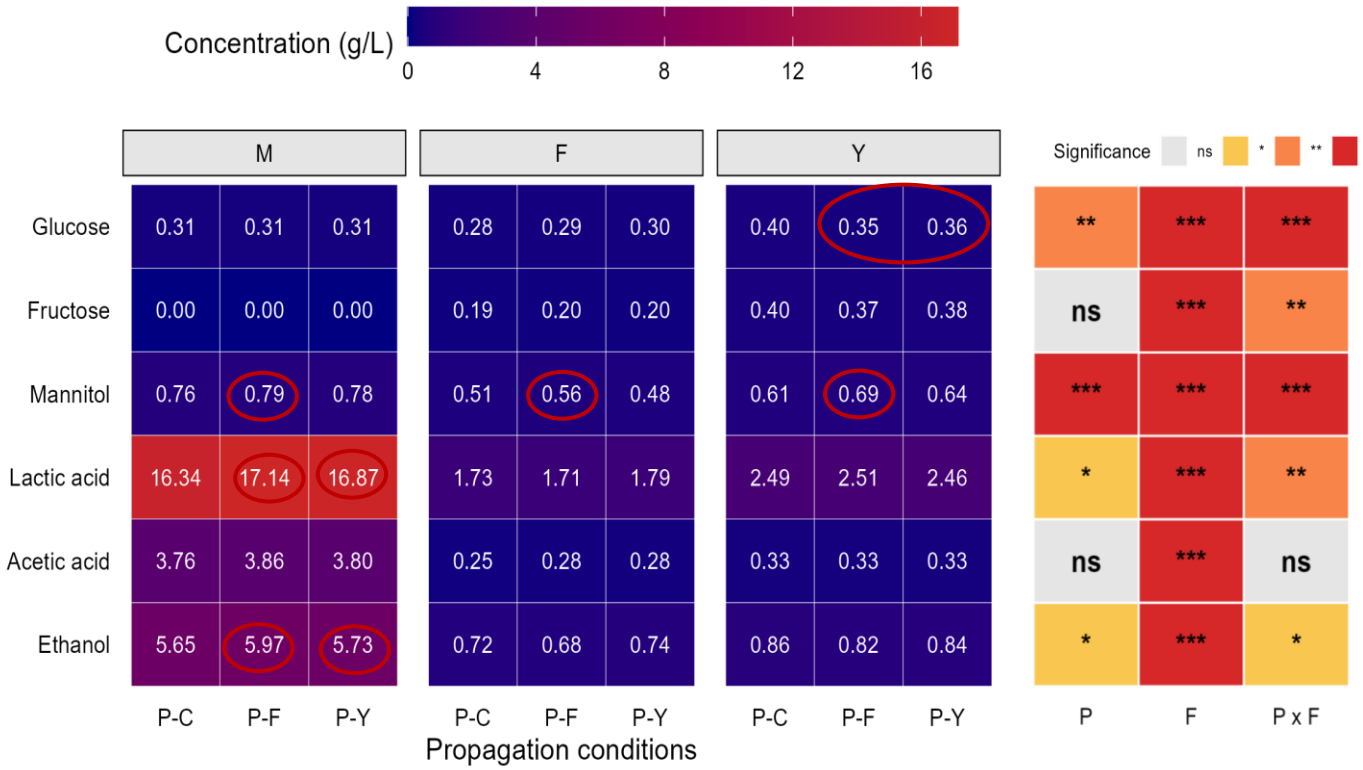

**Relative expression of genes involved in the uptake/metabolism of carbon sources:**

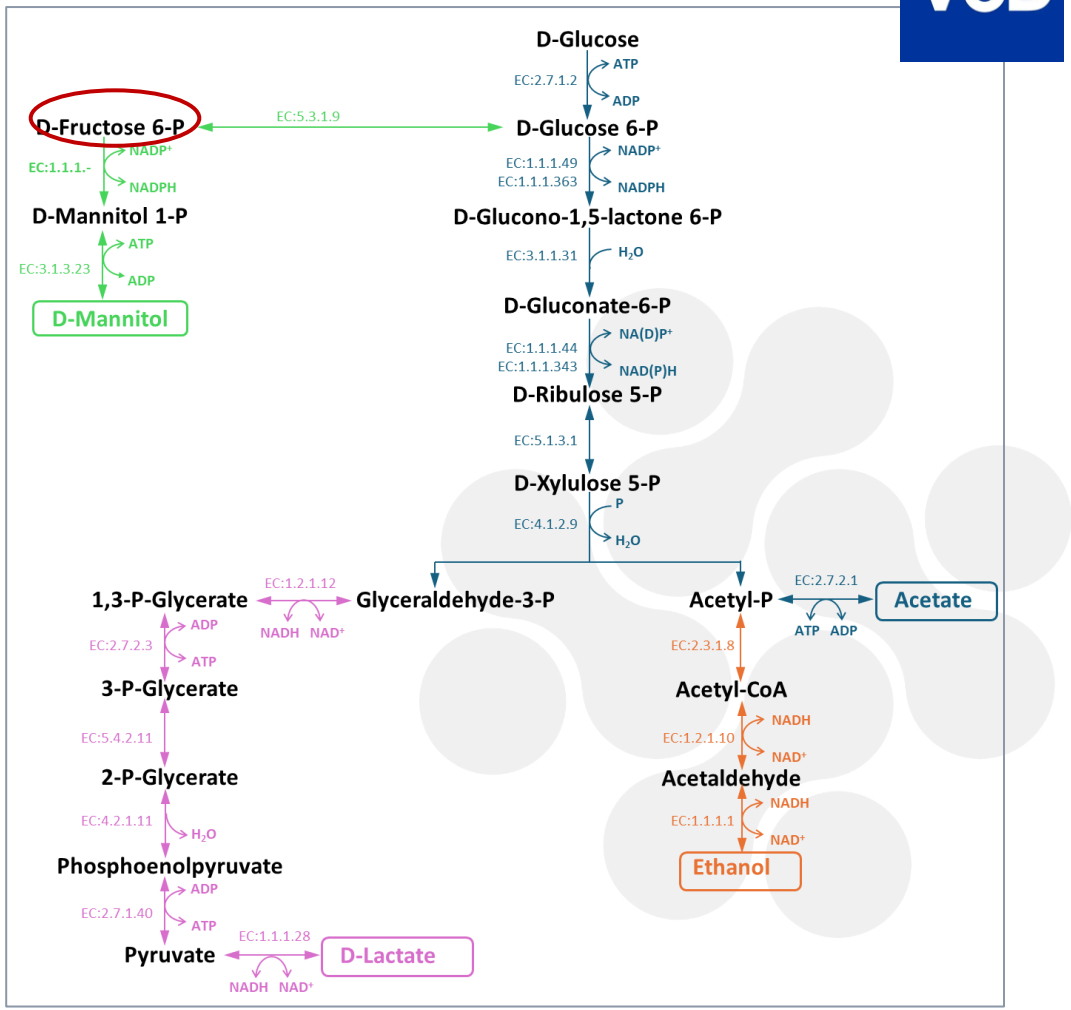
FB

YP

# Cross-inoculation design: Metabolic shifts in *Leuc. mesenteroides* GSL1 during fermentation (according to propagation)

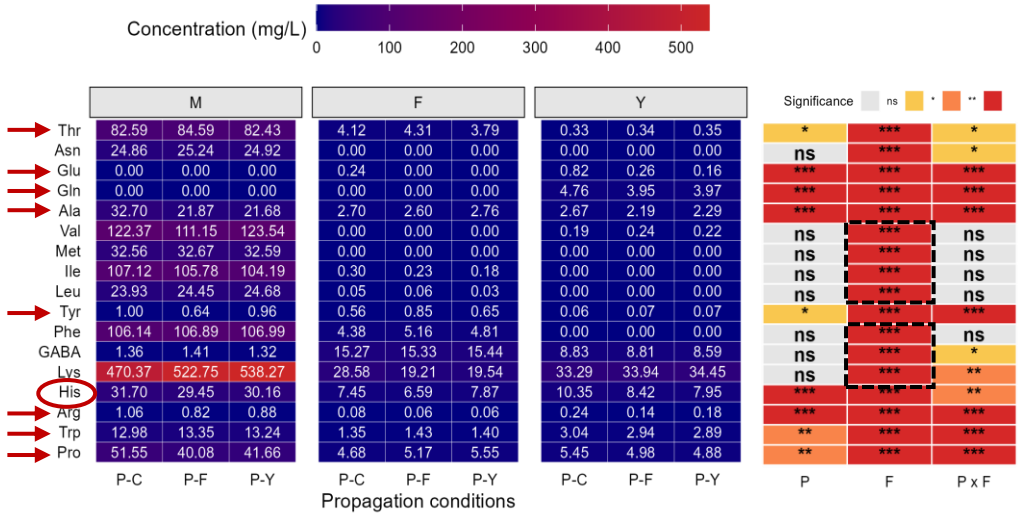
## Sugars consumption, organic acids, and ethanol production



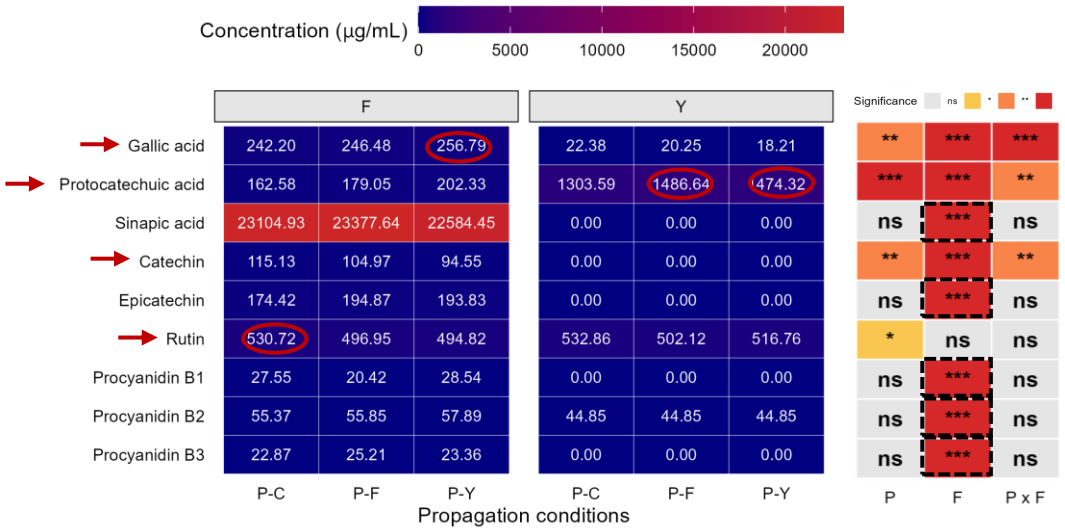



# Cross-inoculation design: Metabolic shifts in *Leuc. mesenteroides* GSL1 during fermentation (according to propagation)

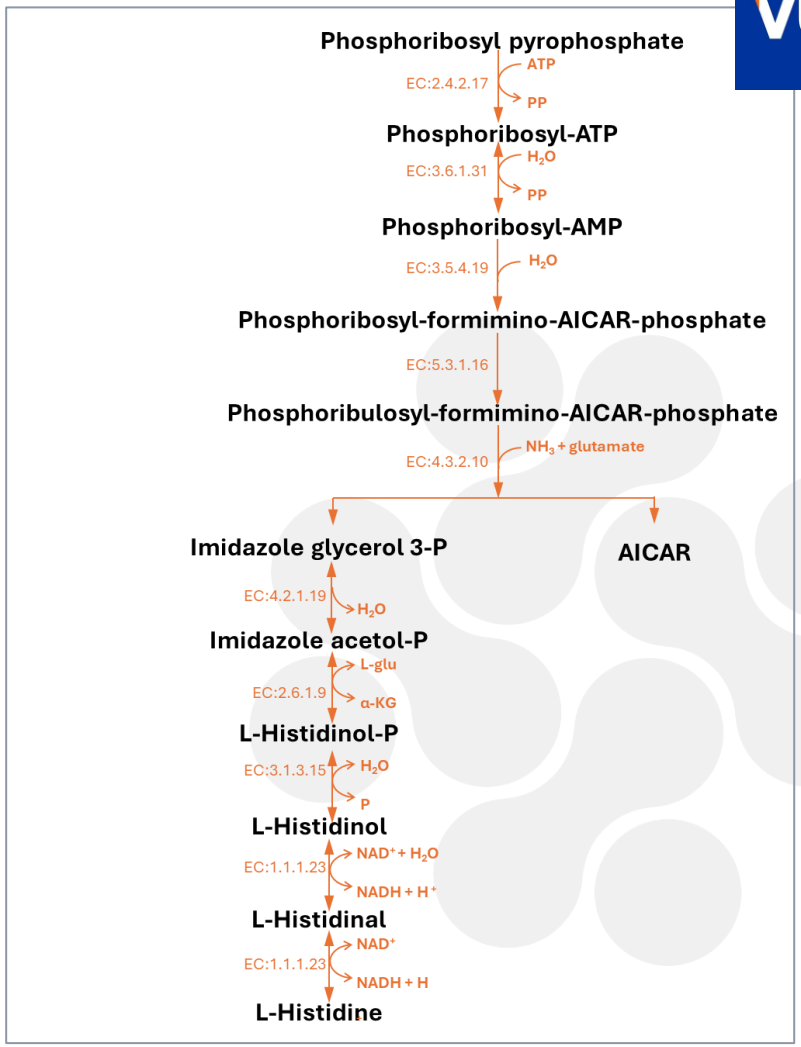
## Free amino acids metabolism




## Phenolic compounds metabolism




caffeic acid
*p*-coumaric acid
ferulic acid



# Take home messages

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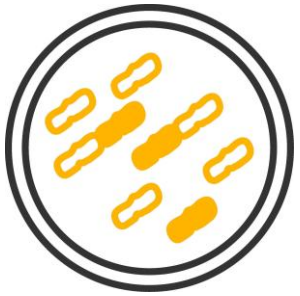
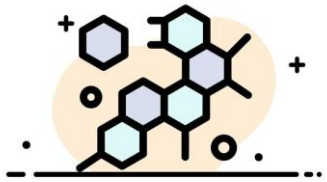


Propagation in pulse-based matrices critically shapes phenotype and fermentation behavior.

Pulse-based propagation enhances substrate utilization and activates sugar metabolism pathways, supported by gene upregulation.



Propagation history significantly influenced fermentation performance and metabolite production (organic acids, ethanol, free amino acids, and phenolics).

Strategic use of pulse-based propagation can pre-adapt starter cultures, enabling tailored pulse-based food fermentations.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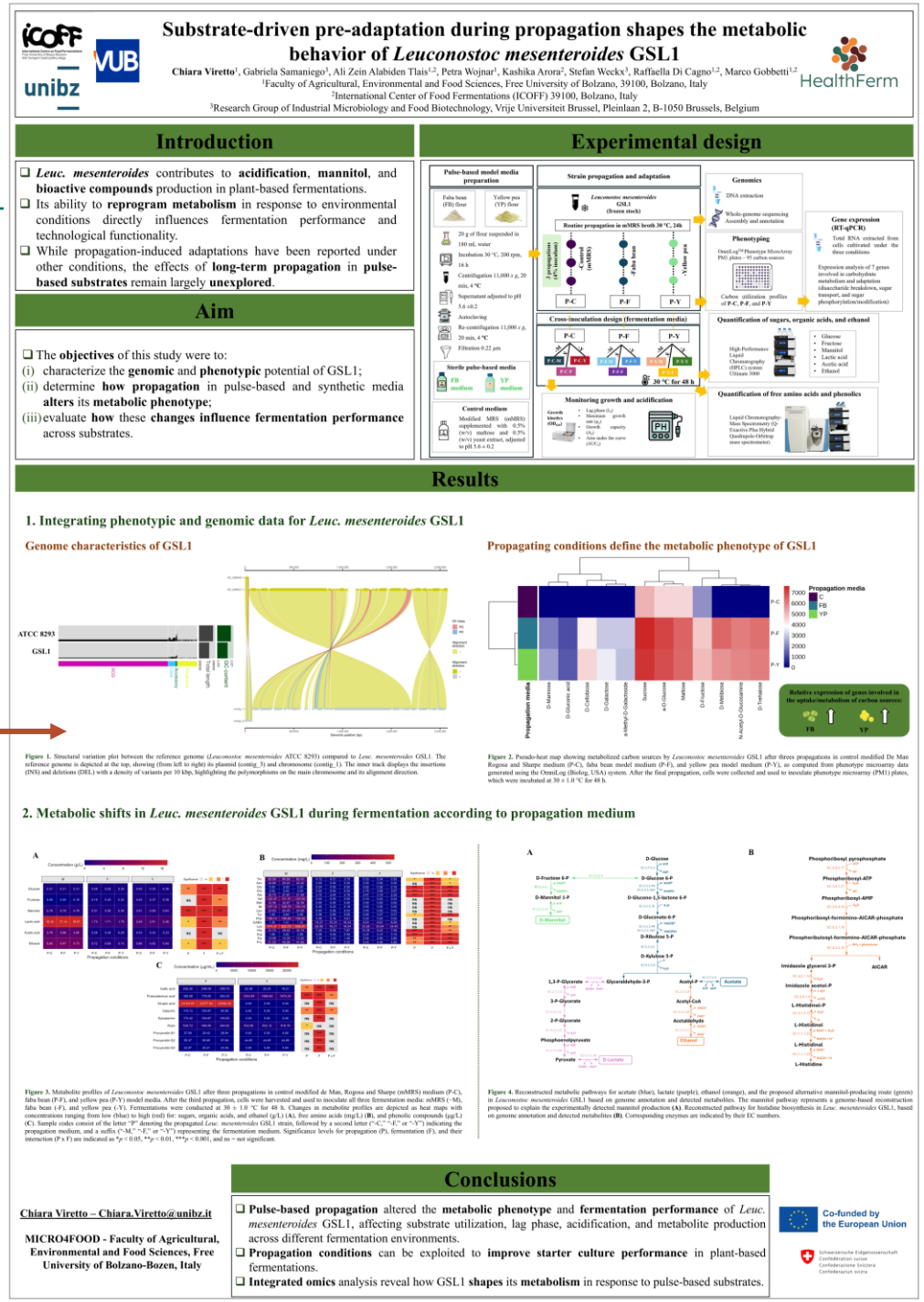

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## Introduction

- ***Leuc. mesenteroides*** contributes to **acidification**, **mannitol**, and **bioactive compounds** production in plant-based fermentations.
- Its ability to **reprogram metabolism** in response to environmental conditions directly influences fermentation performance and technological functionality.
- While propagation-induced adaptations have been reported under other conditions, the effects of **long-term propagation** in **pulse-based substrates** remain largely **unexplored**.

## Aim

The **objectives** of this study were to:
(i) characterize the **genomic** and **phenotypic** potential of GSL1;
(ii) determine **how propagation** in pulse-based and synthetic media **alters** its **metabolic phenotype**;
(iii) evaluate **how** these **changes influence fermentation performance** across substrates.

## Experimental design




## Results

### 1. Integrating phenotypic and genomic data for *Leuc. mesenteroides* GSL1

**Genome characteristics of GSL1**




**Propagating conditions define the metabolic phenotype of GSL1**




Figure 1. Structural variation plot between the reference genome (*Leuconostoc mesenteroides* ATCC 8293) compared to *Leuc. mesenteroides* GSL1. The reference genome is depicted at the top, showing (from left to right) its plasmid (contig_3) and chromosome (contig_1). The inner track displays the insertions (INS) and deletions (DEL) with a density of variants per 10 kbp, highlighting the polymorphisms on the main chromosome and its alignment direction.

Figure 2. Pseudo-heat map showing metabolized carbon sources by *Leuconostoc mesenteroides* GSL1 after threes propagations in control modified De Man Rogosa and Sharpe medium (P-C), faba bean model medium (P-F), and yellow pea model medium (P-Y), as computed from phenotype microarray data generated using the OmniLog (Biolog, USA) system. After the final propagation, cells were collected and used to inoculate phenotype microarray (PM) plates, which were incubated at 30 ± 1.0 °C for 48 h.

### 2. Metabolic shifts in *Leuc. mesenteroides* GSL1 during fermentation according to propagation medium







Figure 3. Metabolite profiles of *Leuconostoc mesenteroides* GSL1 after three propagations in control modified de Man, Rogosa and Sharpe (mMRS) medium (P-C), faba bean (P-F), and yellow pea (P-Y) model media. After the third propagation, cells were harvested and used to inoculate all three fermentation media: mMRS (−M), faba bean (-F), and yellow pea (-Y). Fermentations were conducted at 30 ± 1.0 °C for 48 h. Changes in metabolite profiles are depicted as heat maps with concentrations ranging from low (blue) to high (red) for: sugars, organic acids, and ethanol (g/L) (**A**), free amino acids (mg/L) (**B**), and phenolic compounds (µg/L) (**C**). Sample codes consist of the letter "P" denoting the propagated *Leuc. mesenteroides* GSL1 strain, followed by a second letter ("-C","-F," or "-Y") indicating the propagation medium, and a suffix ("-M," "-F," or "-Y") representing the fermentation medium. Significance levels for propagation (P), fermentation (F), and their interaction (P x F) are indicated as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and ns = not significant.

Figure 4. Reconstructed metabolic pathways for acetate (blue), lactate (purple), ethanol (orange), and the proposed alternative mannitol-producing route (green) in *Leuconostoc mesenteroides* GSL1 based on genome annotation and detected metabolites. The mannitol pathway represents a genome-based reconstruction proposed to explain the experimentally detected mannitol production (**A**). Reconstructed pathway for histidine biosynthesis in *Leuc. mesenteroides* GSL1, based on genome annotation and detected metabolites (**B**). Corresponding enzymes are indicated by their EC numbers.

## Conclusions

- **Pulse-based propagation** altered the **metabolic phenotype** and **fermentation performance** of *Leuc. mesenteroides* GSL1, affecting substrate utilization, lag phase, acidification, and metabolite production across different fermentation environments.
- **Propagation conditions** can be exploited to **improve starter culture performance** in plant-based fermentations.
- **Integrated omics** analysis reveal how GSL1 **shapes** its **metabolism** in response to pulse-based substrates.


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