

ADVANCED FERMENTATION TECHNOLOGIES – LILLE 2026

Advancing Lignocellulosic Biomass Conversion into Lipopeptides through Knowledge-Driven *B. subtilis* Engineering

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Presentation Overview

1

Context: Lipopeptides, Process, Substrate

2

BioComputing: Modeling, Predictions

3

Strain Optimization: *B. subtilis* Mutants, Test

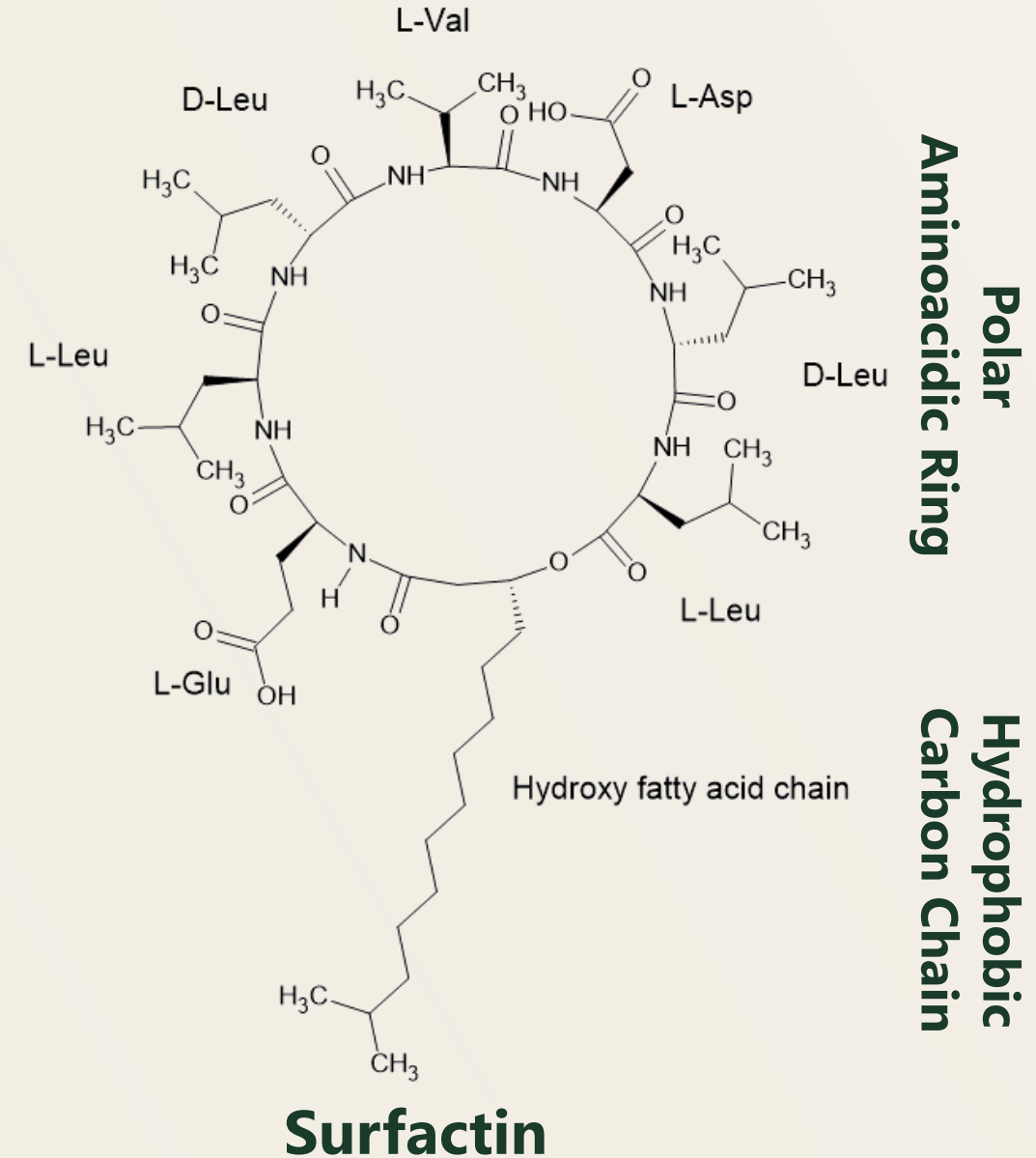
4

Conclusion and Perspectives

Context: Lipopeptides

Field of Use:

- Agronomy
 - Elicitor & Bio-stimulants [1]
- Environment
 - Bioremediation [2]
- Cosmetic Industry [3]
- Medicine [4]

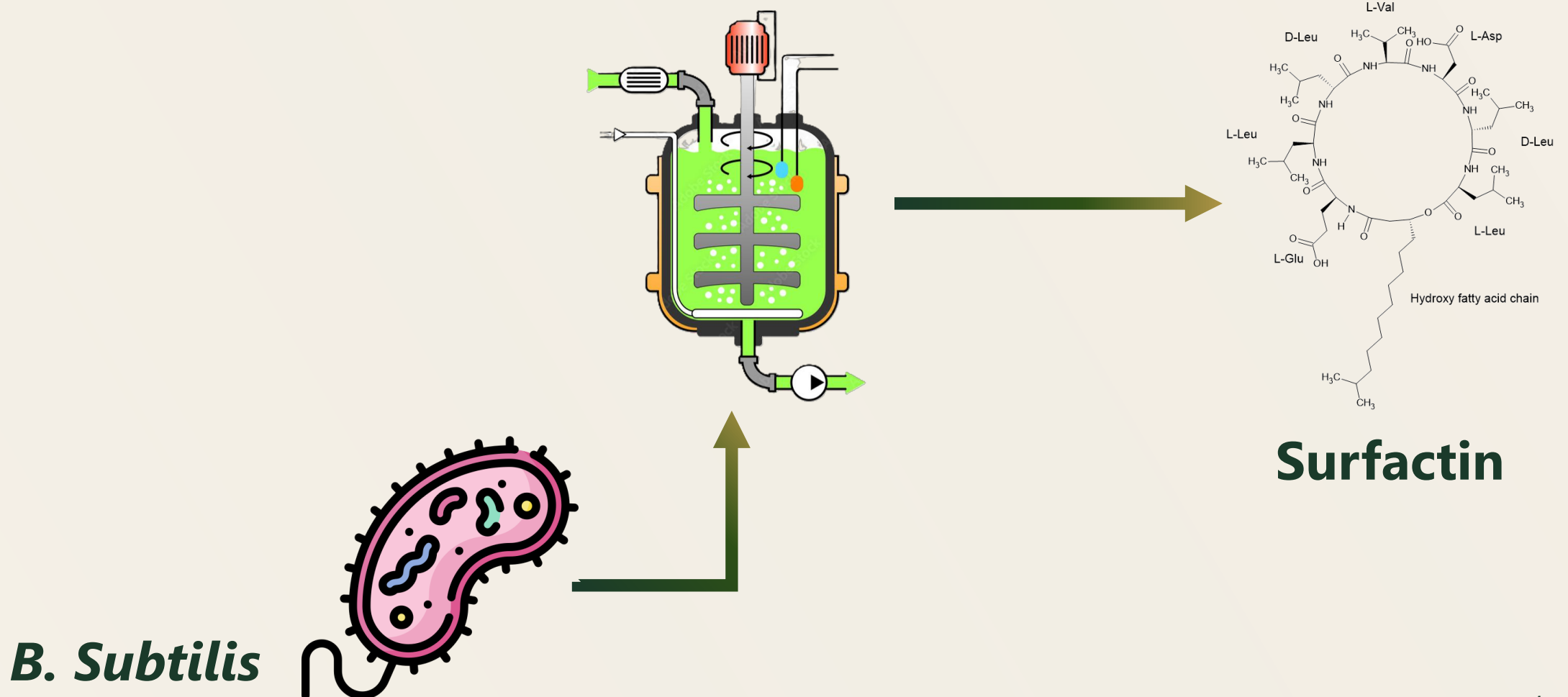


[3] M. Klimek-Szczykutowicz et al., 2024 [2] M. D. G. C. Silva et al., 2024

[4] V. Thakur et al., 2024 [1] T. M. Rocha et al., 2024

Context: Production Process

Fermentation



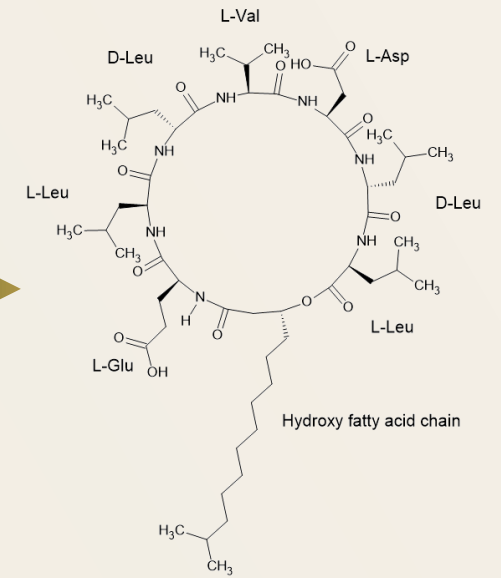
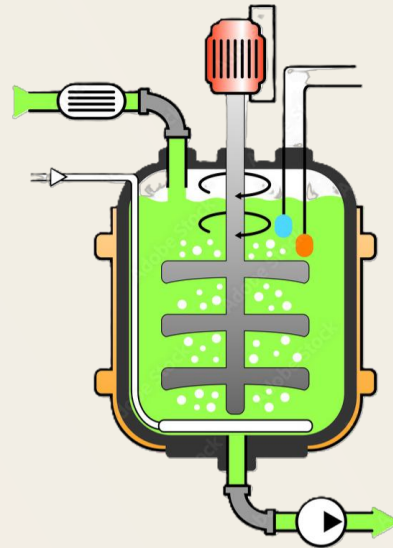
Context: Lignocellulosic Substrate

Fermentation

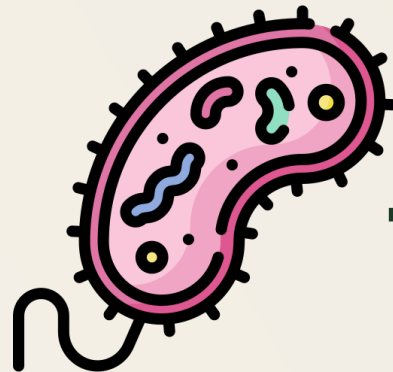


Substrate
Miscanthus

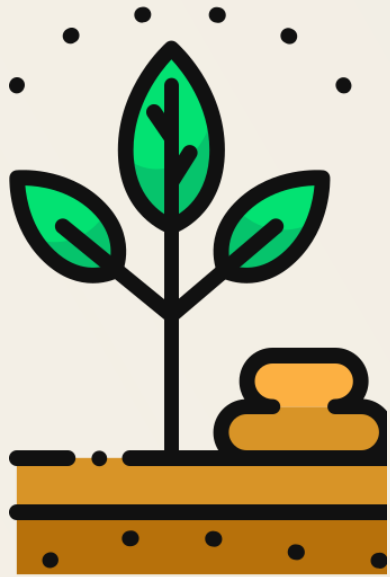
Acid
Hydrolysis



B. Subtilis



Context: Lignocellulosic Substrate



Substrate

Miscanthus

Advantage:

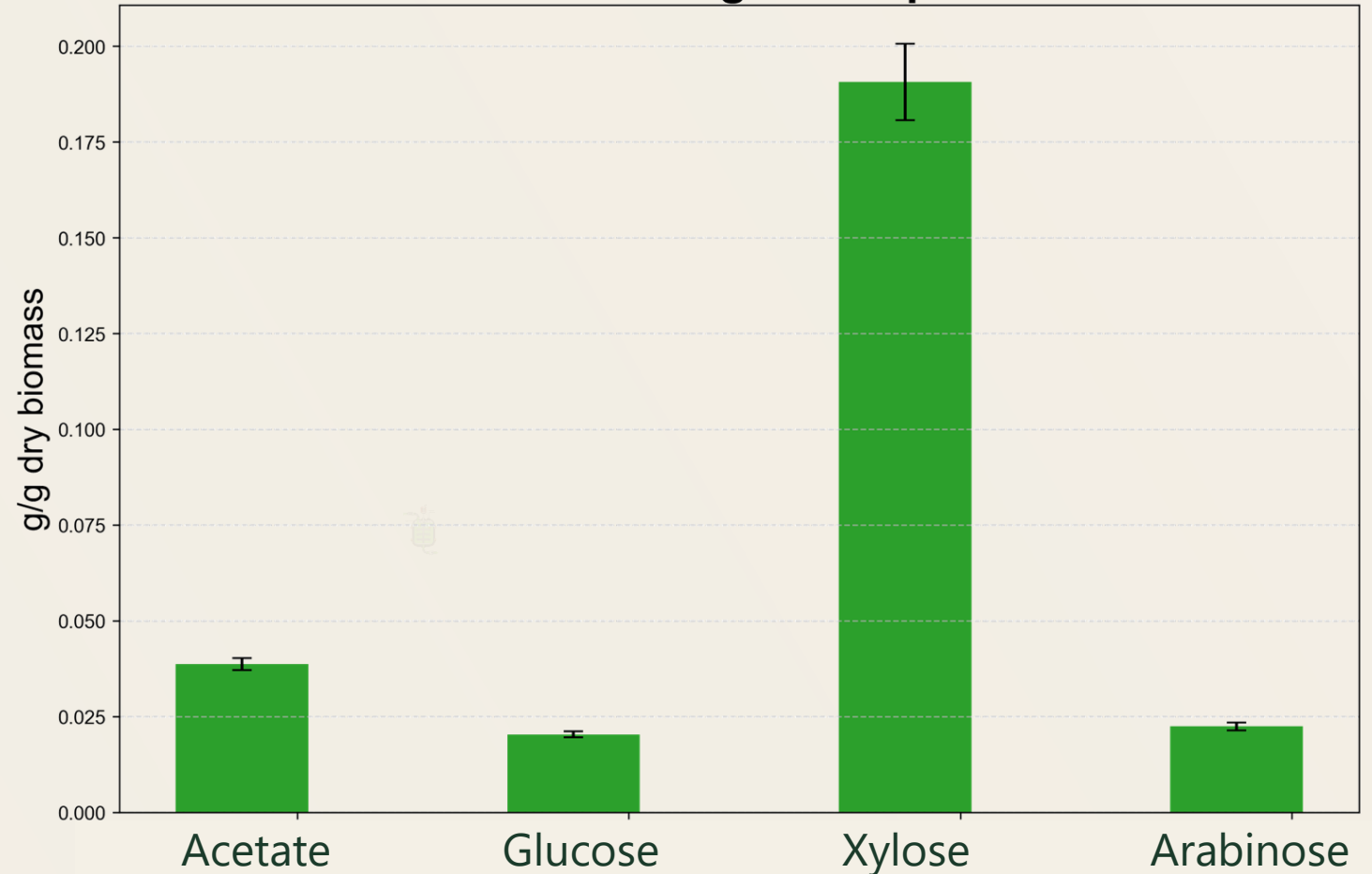
- Higher economic feasibility [7]
- Lower CO2 emissions [6]

Context: Lignocellulosic Substrate



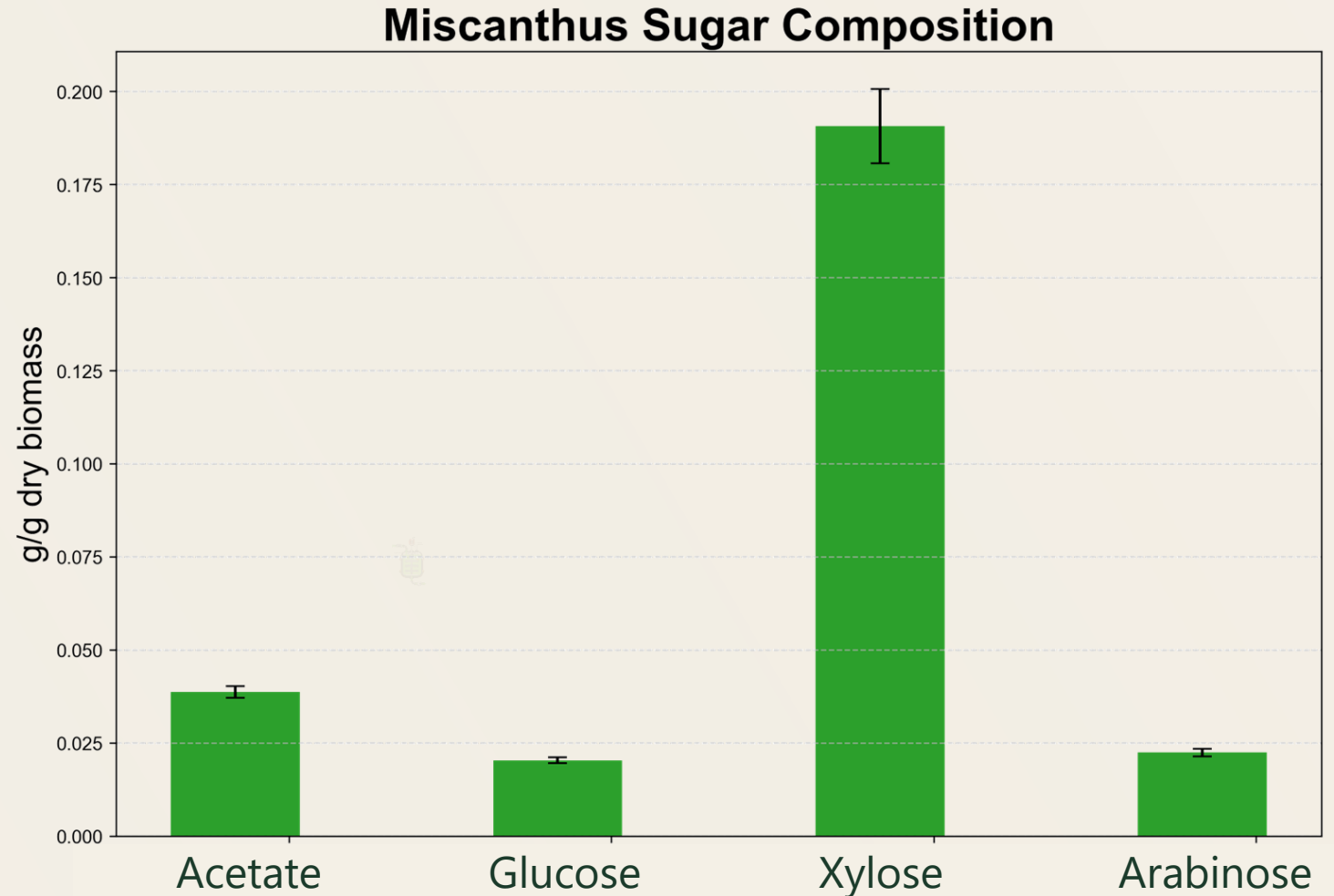
Substrate
Miscanthus

Miscanthus Sugar Composition



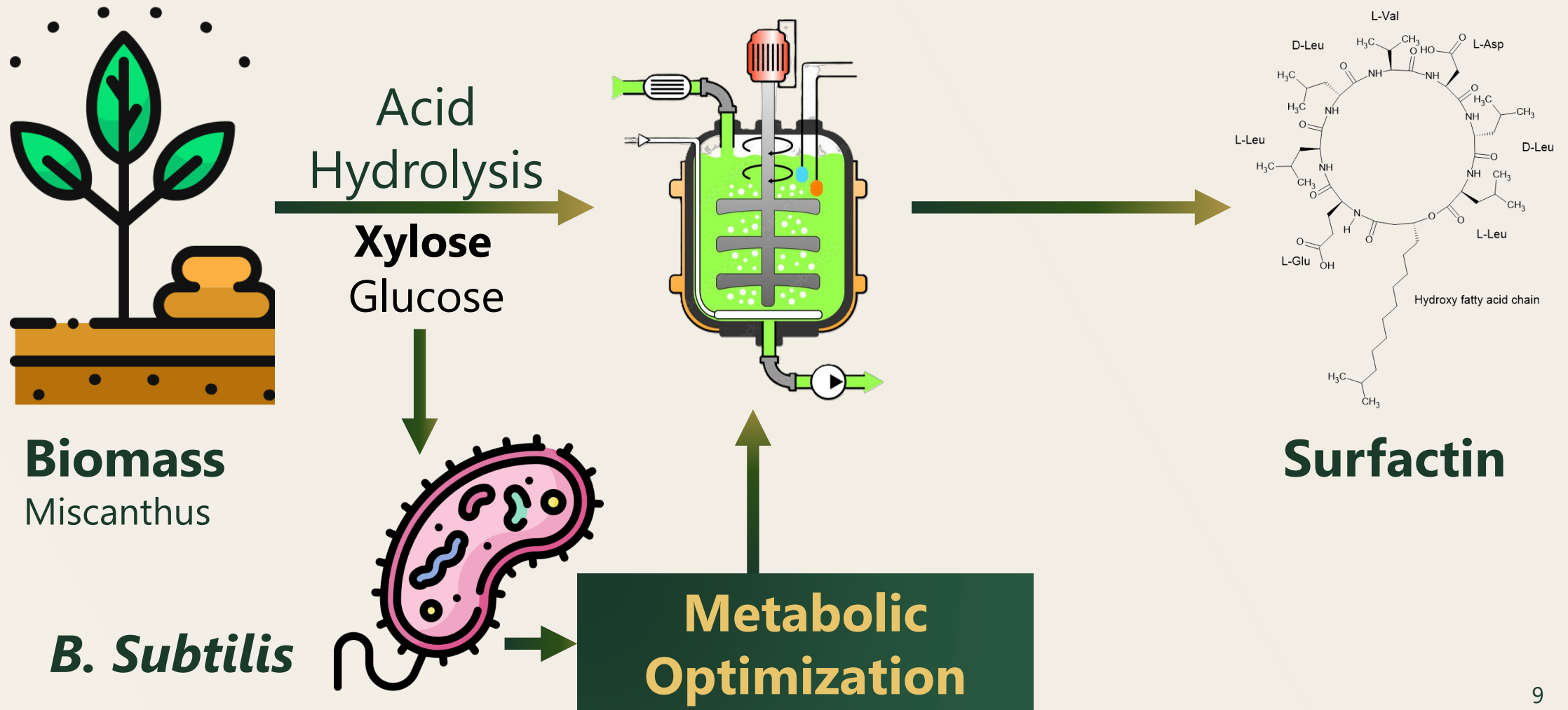
Context: Lignocellulosic Substrate

Xylose
↓
Main C5 sugar
↓
Lower efficiency
for microbial
processes

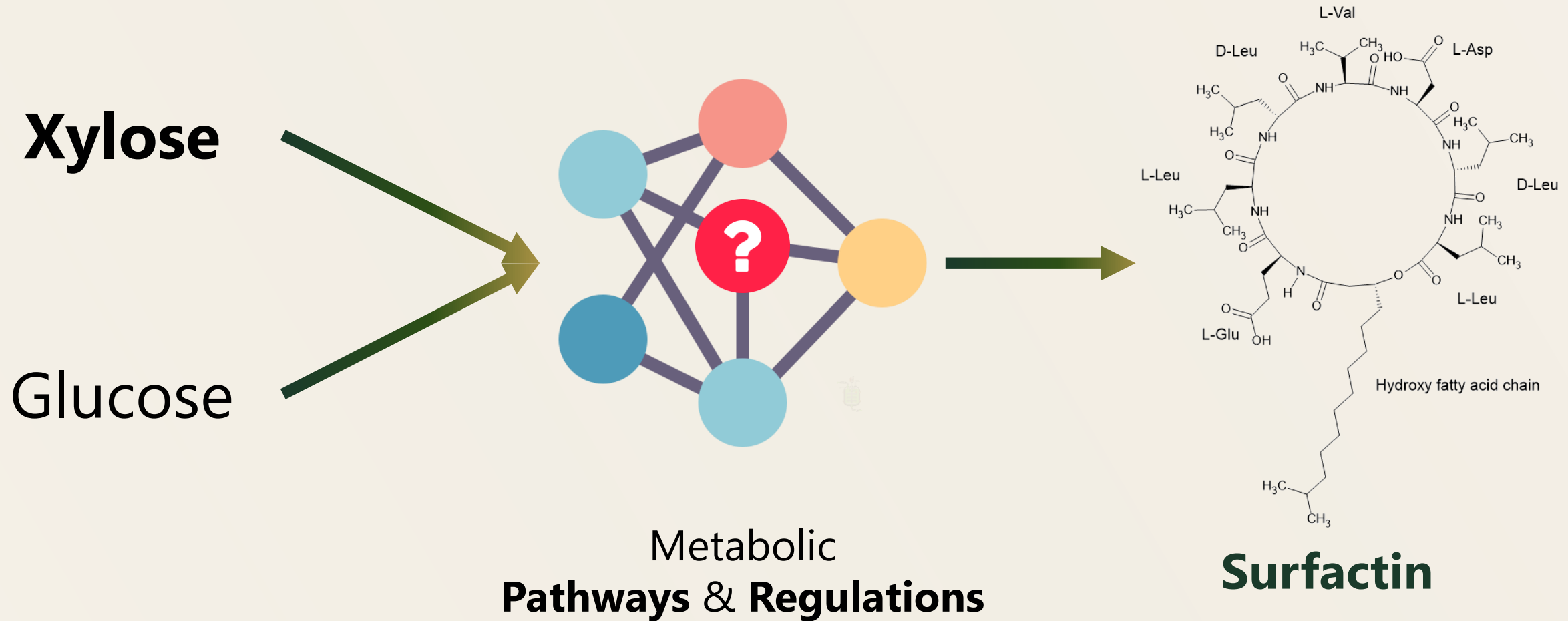


Context: Microbial Limitations

Fermentation

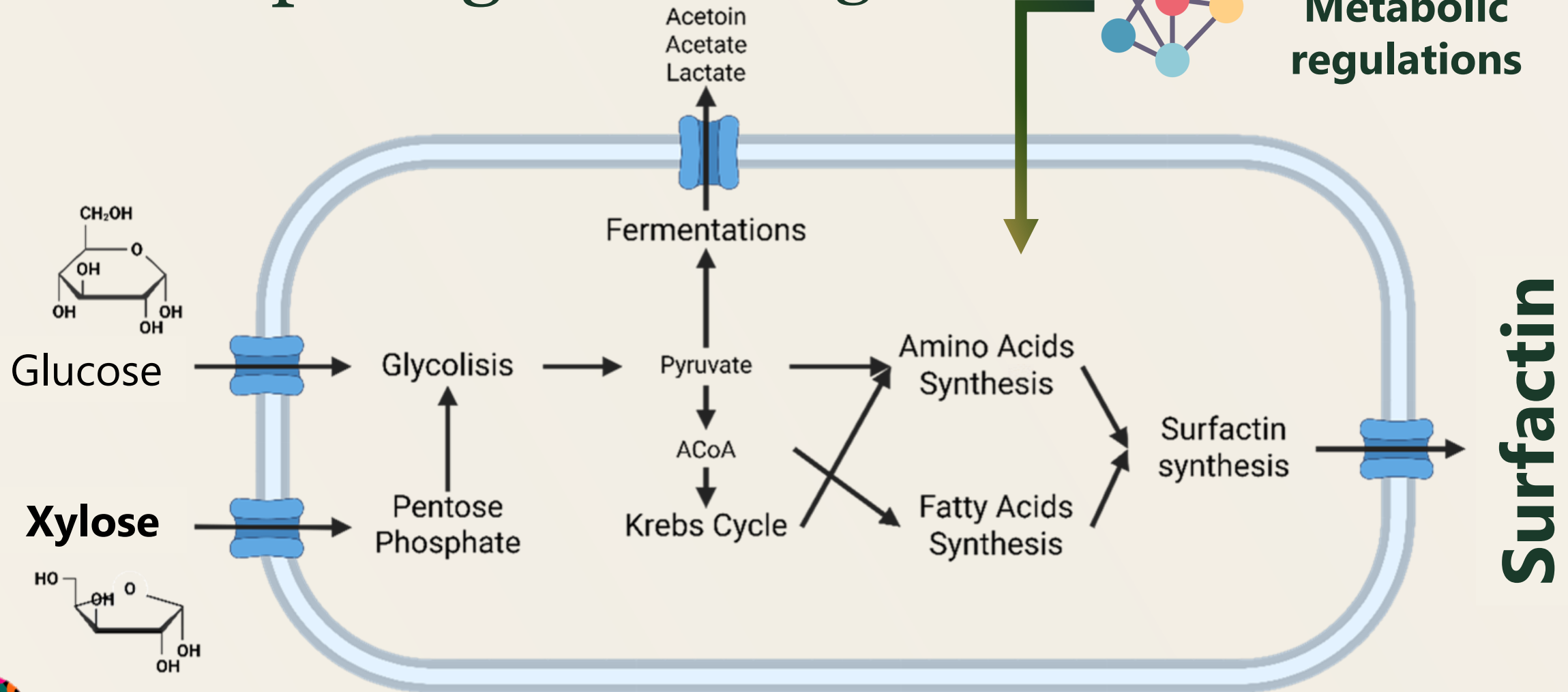


Context: Microbial Limitations



BioComputing: Modelling

**New:
Metabolic
regulations**



BioComputing: Partial Reaction Networks (PRNs) [7]

Reaction

Specie

Gene

Metabolite

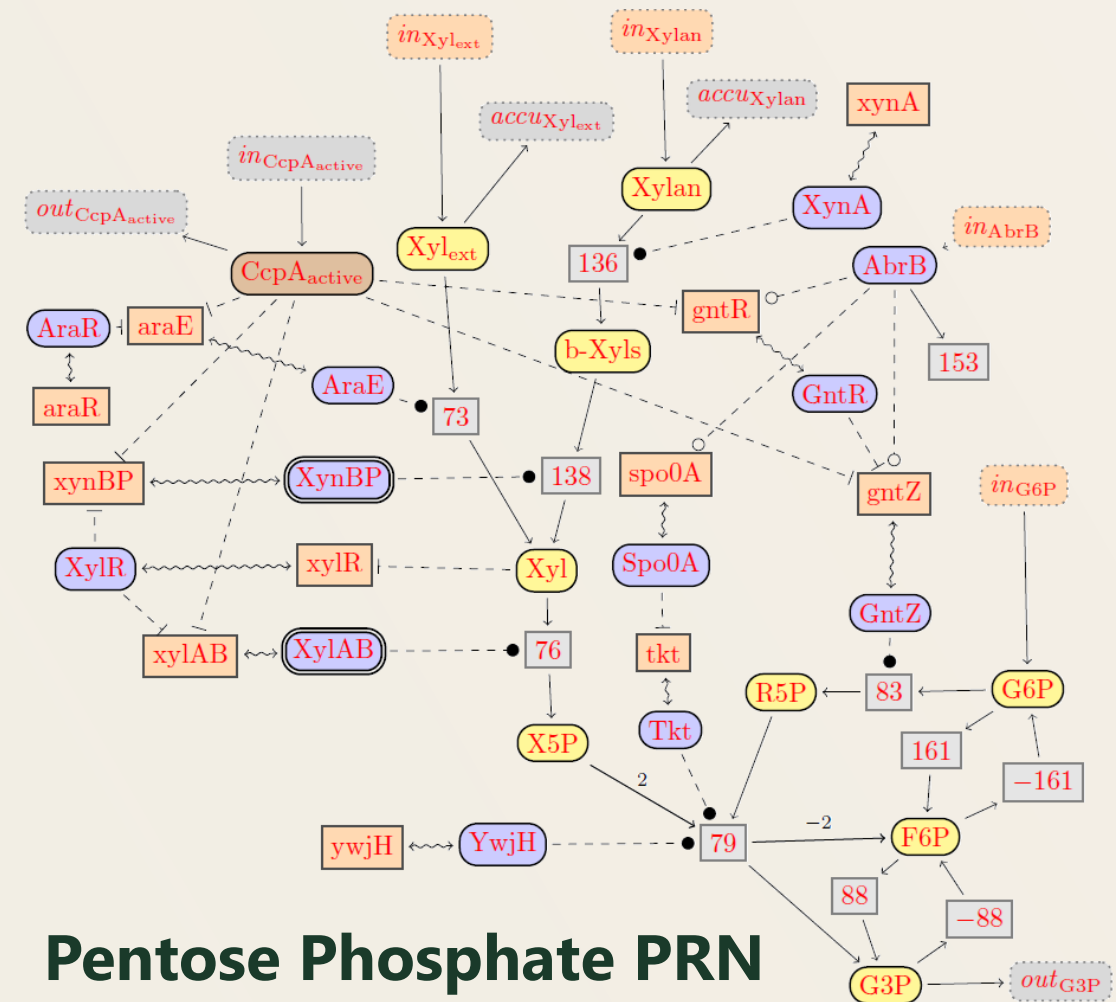
Metabolic
Reaction

Protein

Outflow

Actor
Species

Inflow

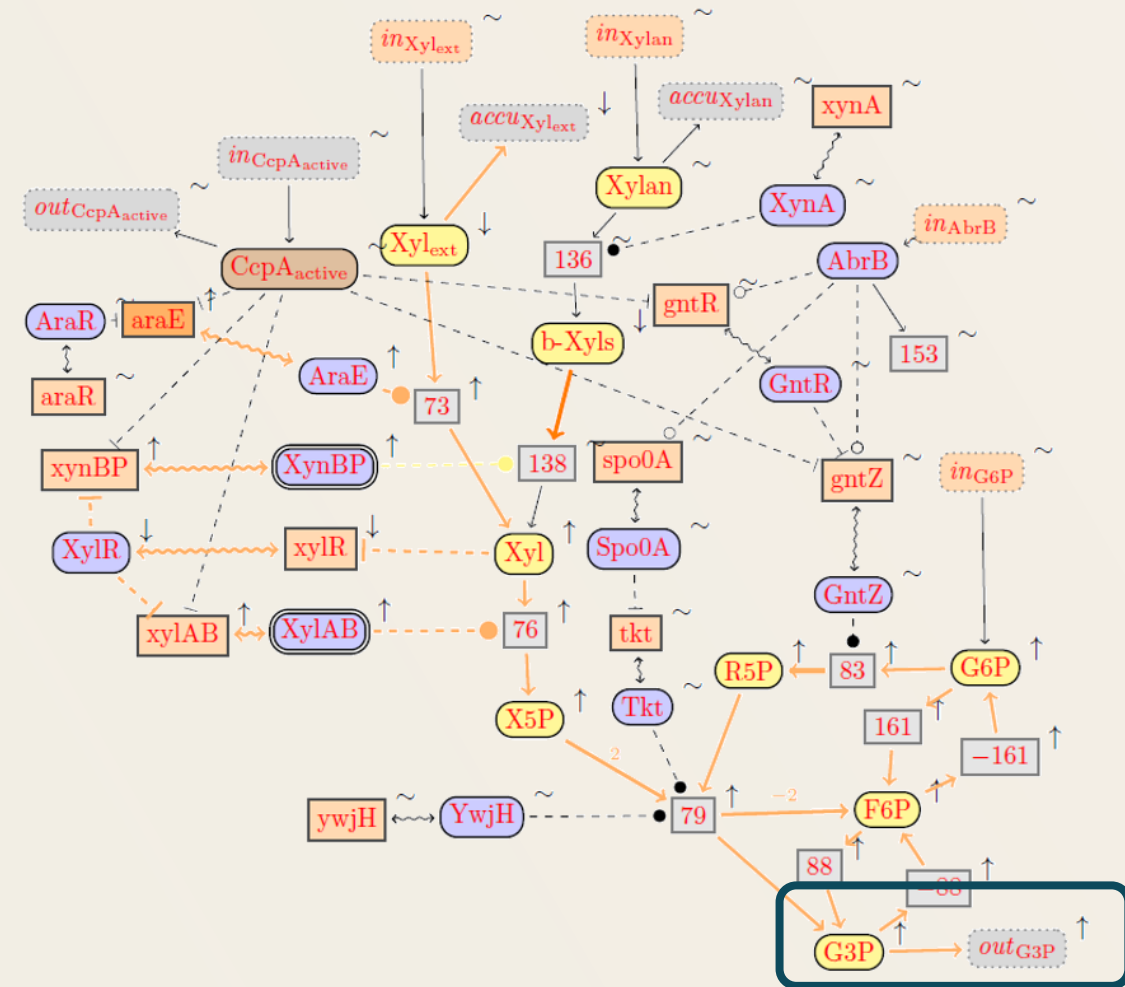


Pentose Phosphate PRN

BioComputing: Single Change Prediction [7]

Pentose Phosphate PRN

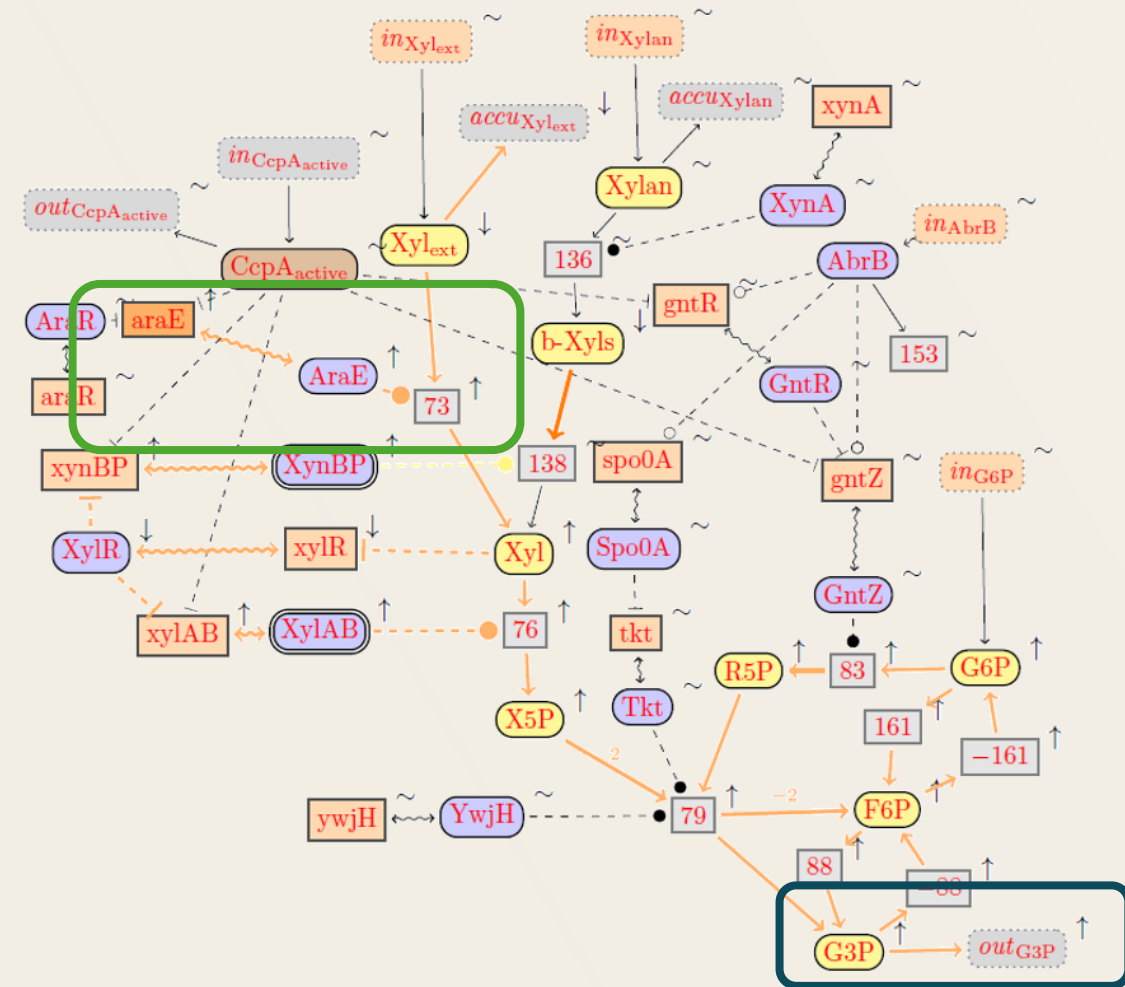
1. $Out_G3P = \uparrow$: Requested target



BioComputing: Single Change Prediction [7]

Pentose Phosphate PRN

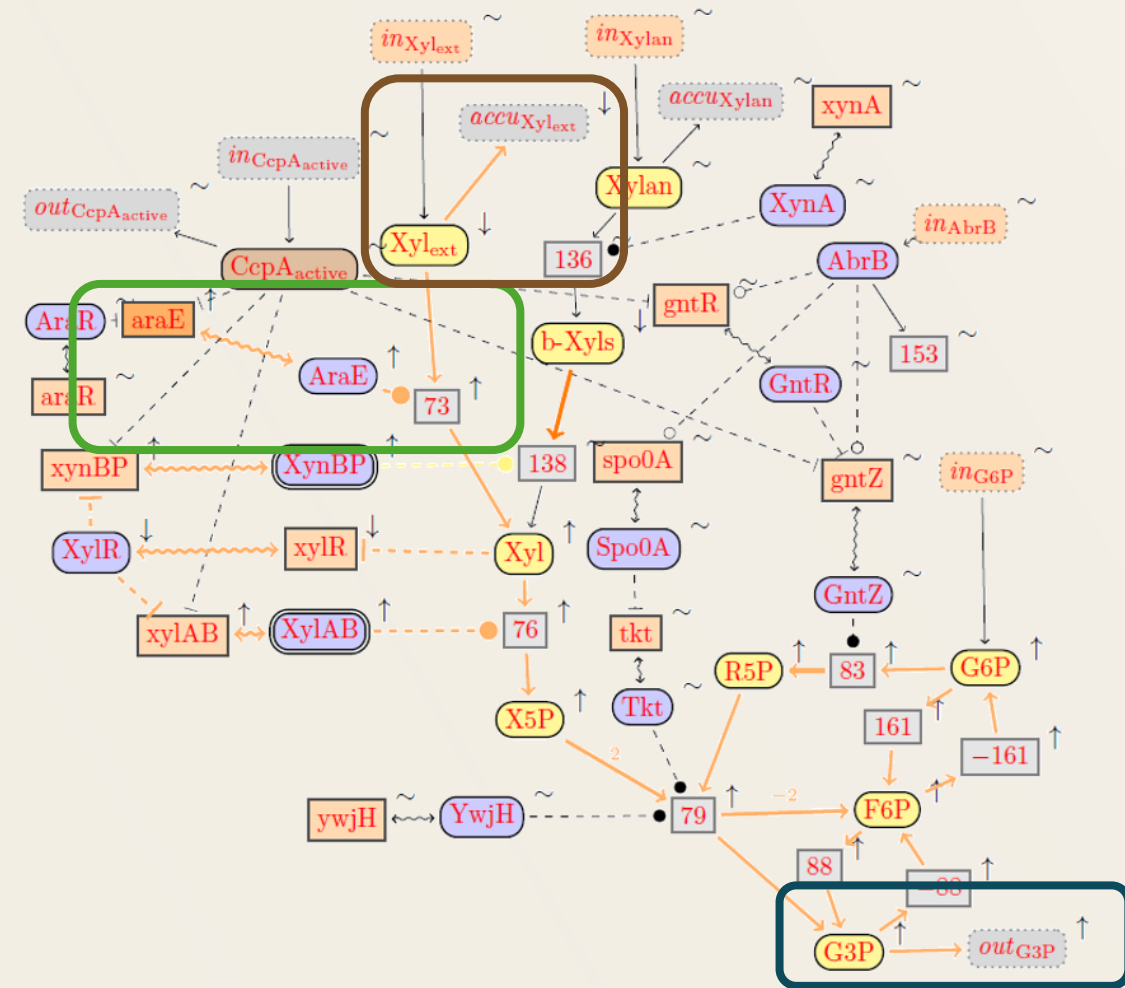
1. $Out_G3P = \uparrow$: Requested target
2. $araE = \uparrow$: Computed change



BioComputing: Single Change Prediction [7]

Pentose Phosphate PRN

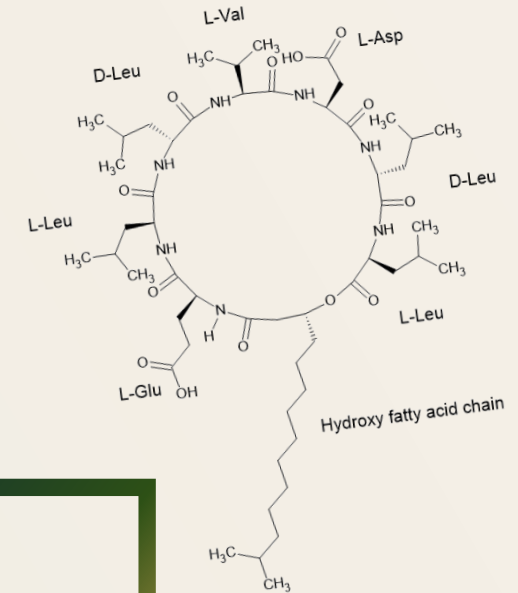
1. $Out_G3P = \uparrow$: Requested target
2. $araE = \uparrow$: Computed change
3. $Accu_xyl_ext = \downarrow$: Implication



BioComputing: Multi-level single change prediction

Xylose

PRNs	Single change	Aim
Pentose Phosphate	<i>araE</i> = ↑ [10]	Increase Xyl consumption
Fermentations	<i>alsR</i> = ↓	Reduce carbon Outflows
BCAA [8]	<i>Pilv-leu</i> = ↑	Increase Leucine synthesis



Surfactin

Strain Optimization: *B. subtilis* Mutants

Control Strain

BBG258 ^[9]

BS168, trp+, sfp+

Leucine-Overproducer strain

BBG-FR403

ΔalsR, PrepU::ilvBH

Xylose-Adapted strain

BBG-FR412

ΔalsR, PrepU::ilvBH, Pveg::araE

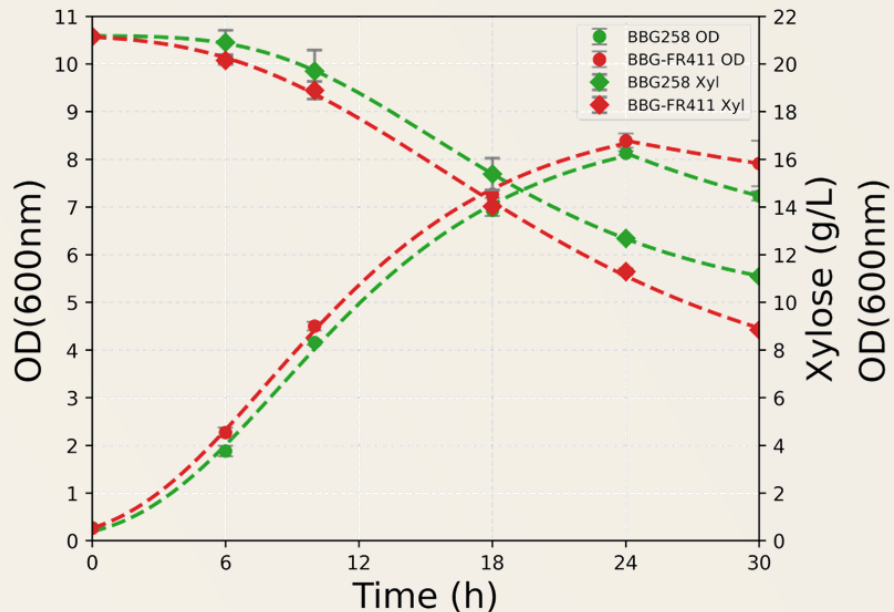
BBG-FR411

Pveg::araE

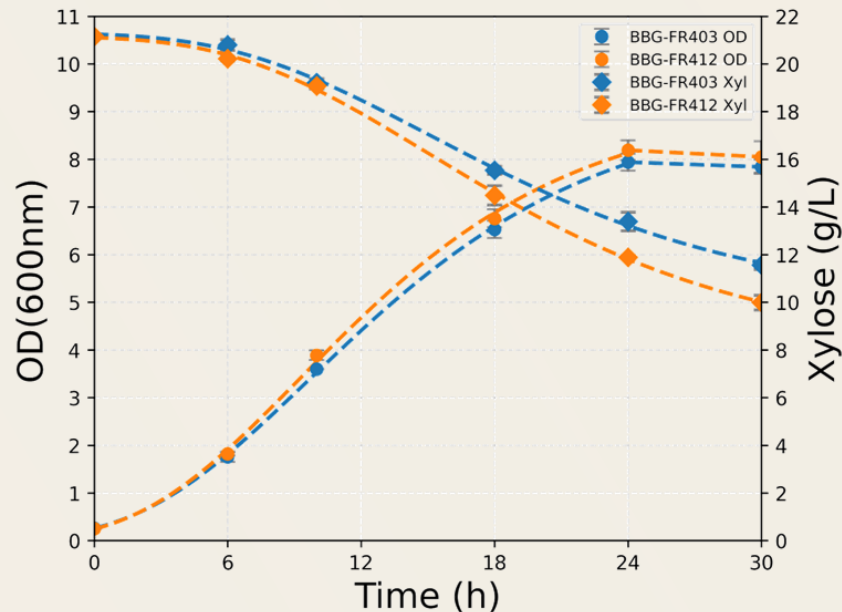
Strain Optimization: Higher xylose consumption

B. subtilis performance on Landy-Xylose

BBG258 (BS168, *trp* + , *sfp* +) Vs
BBG-FR411 (BBG258, *Pveg* : : *araE*)



BBG-FR403 (BBG258, $\Delta alsR$, *PrepU* : : *ilvB*) Vs
BBG-FR412 (BBG258, $\Delta alsR$, *PrepU* : : *ilvB*, *Pveg* : : *araE*)



Method

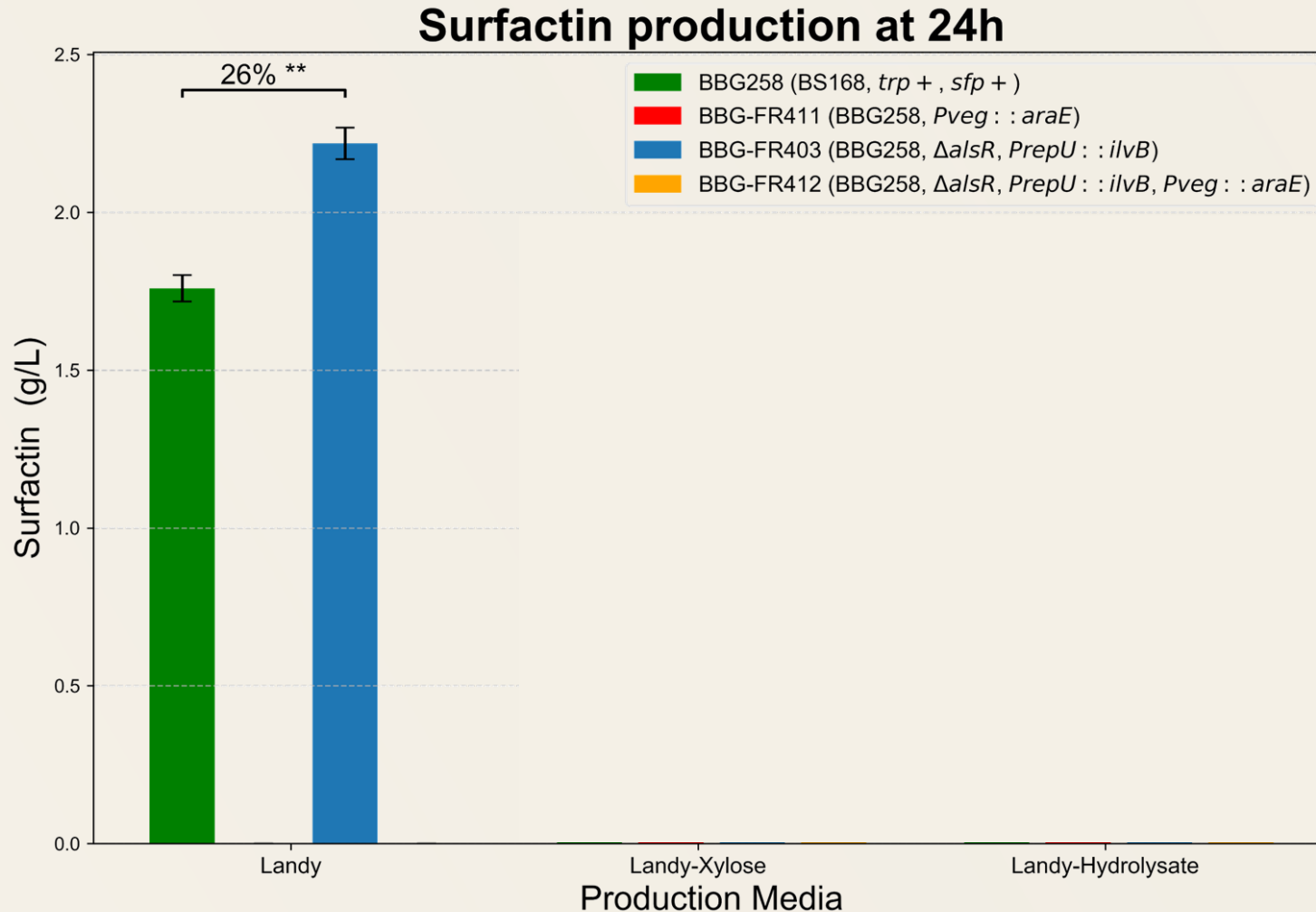
Media:

Landy-Xylose (20g/L xylose)

Results

+10% xylose consumption at 24h for
BBG-FR411 & **BBG-FR412**

Strain Optimization: Impact of carbon sources



Methods

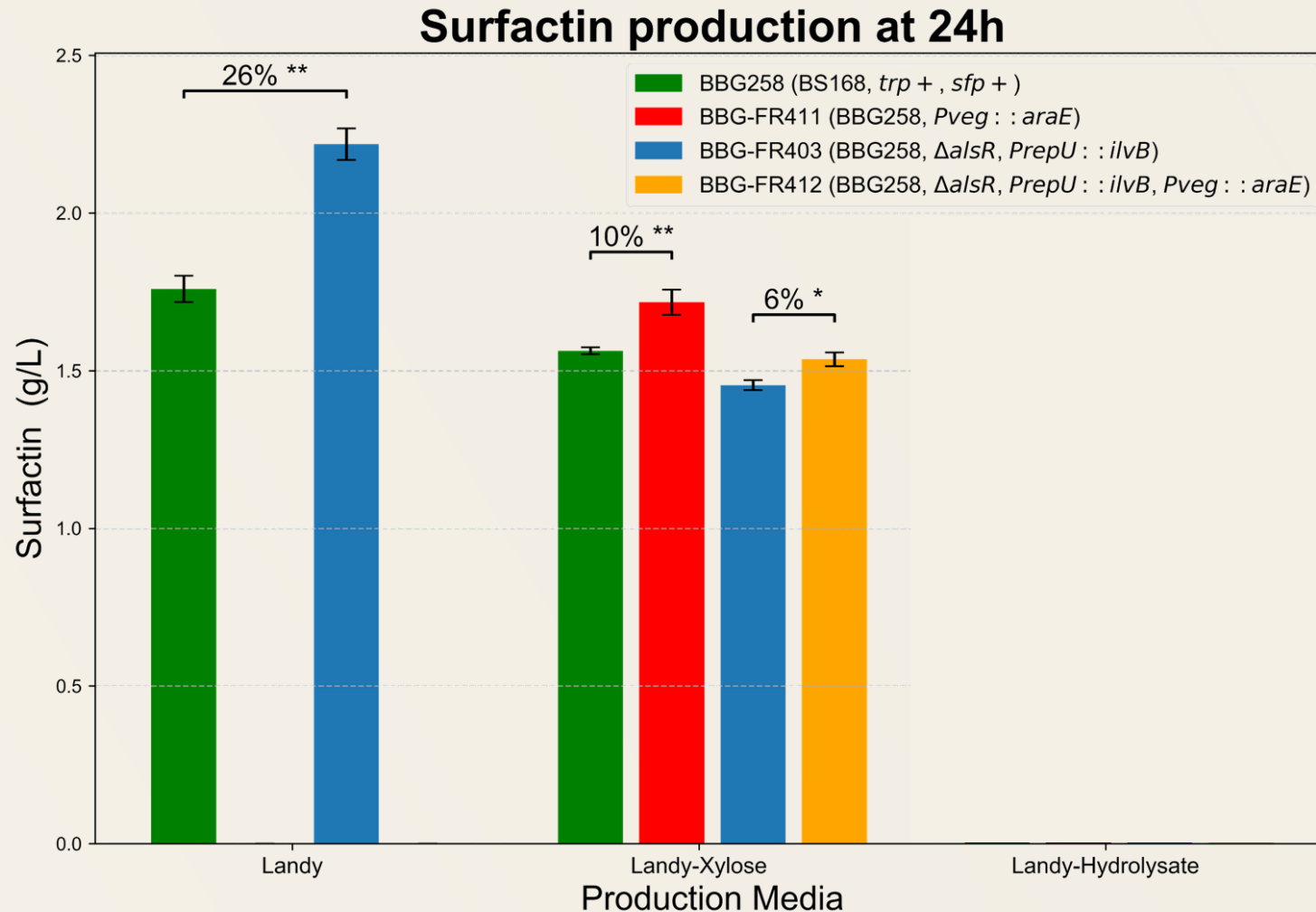
Media:

Landy (20g/L glucose)

Results

+26% production for
BBG-FR403 on Landy

Strain Optimization: Impact of carbon sources



Methods

Media:

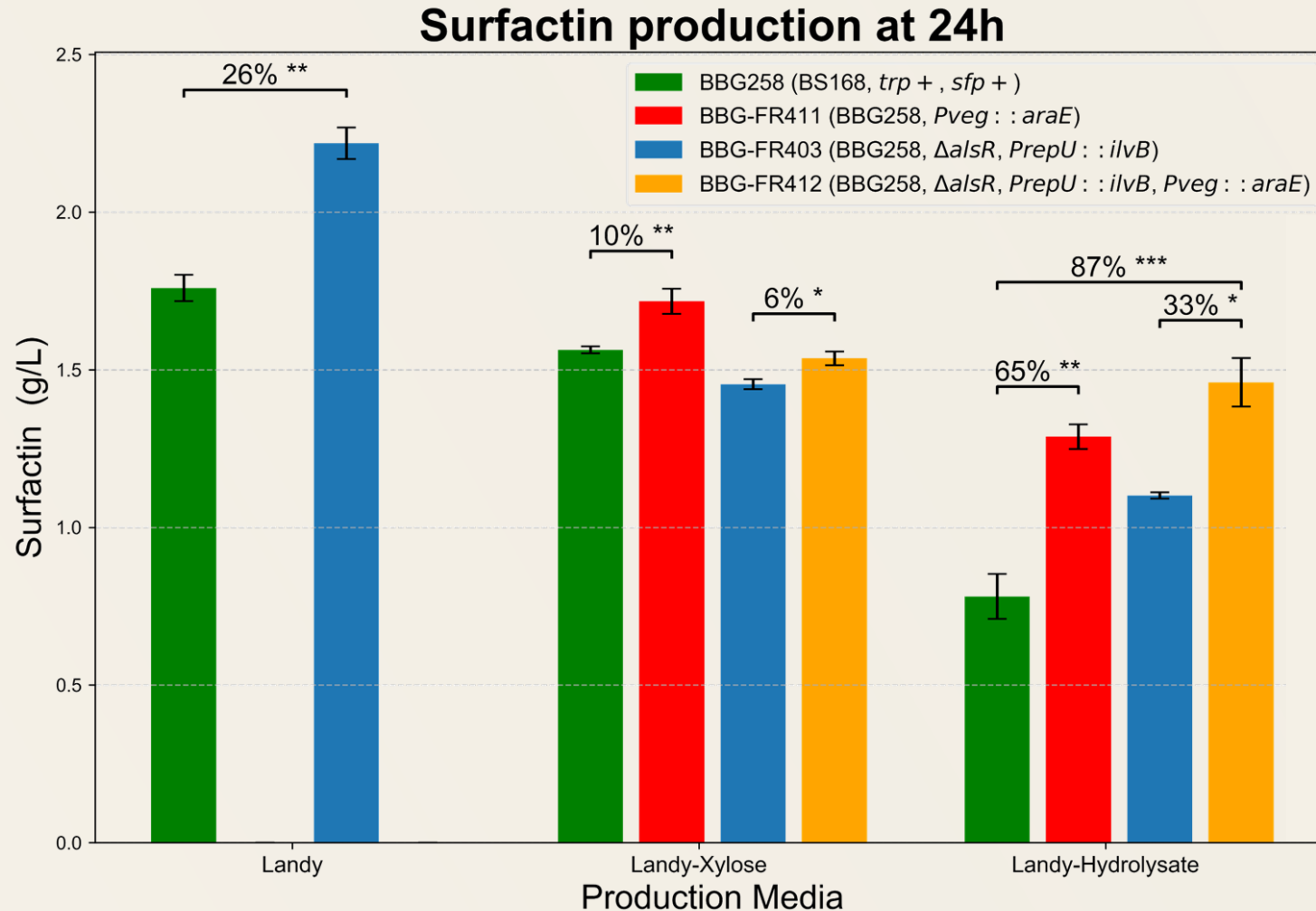
Landy (20g/L glucose)

Landy-Xylose (20g/L xylose)

Results

+10~6% production
for **BBG-FR411** and **BBG-FR412** on Landy-Xylose

Strain Optimization: Impact of carbon sources



Methods

Media:

Landy (20g/L glucose)

Landy-Xylose (20g/L xylose)

Landy-Hydrolysate (1.5g/L glc, 11g/L xyl, 1g/L ara)

Results

+87% production for **BBG-FR412** on Landy-Hydrolysate

Conclusions & Perspectives

1. Develop two new **Partial Reaction Network** to describe *B. subtilis* metabolism
 - a) **Pentose Phosphate PRN**
 - b) **Fermentation PRN**
2. Develop and proved **bioinformatic pipe** to support time-efficient strain engineering process
3. Adapted **surfactin**-producer *B. subtilis* on lignocellulosic substrate

Conclusions & Perspectives

1. Further develop *B. subtilis* metabolism **Partial Reaction Network** to describe the whole metabolism
2. Refine the bioinformatic constraints for **more accurate predictions**
 - a) **Transcriptomic** analysis
3. **Systemic editing** of *B. subtilis* for higher surfactin production
4. **Scale-up** the production process

AFT 2026

Advanced Fermentation Technology



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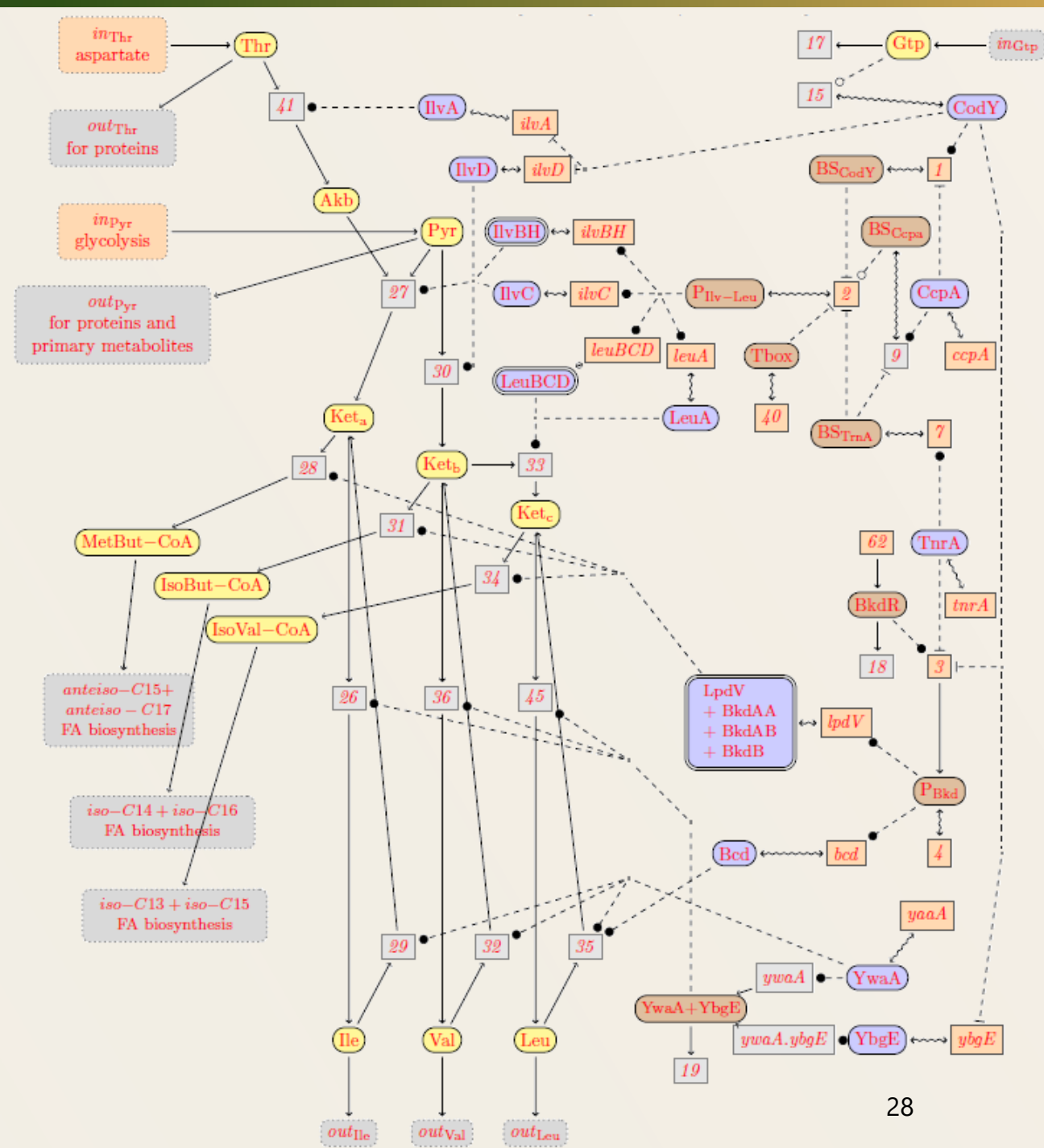


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- [2] **M. D. G. C. Silva et al.**, 'Biosurfactants: Promising Biomolecules for Agricultural Applications', *Sustainability*, vol. 16, no. 1, p. 449, Jan. **2024** [Online]. Available: 10.3390/su16010449.
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- [10] **Y. Yin et al.**, 'Construction of *Bacillus subtilis* for efficient production of fengycin from xylose through CRISPR-Cas9', *Front. Microbiol.*, vol. 14, p. 1342199, Jan. **2024** [Online]. Available: 10.3389/fmicb.2023.1342199.

BioComputing: Partial Reaction Networks (PRNs)

Branched Chain Amino Acids PRN



BioComputing: Partial Reaction Networks (PRNs)

Fermentation PRN

