Metabolic control modelling and Optimization of *B. subtilis* for Surfactin Production from Xylose-Rich substrates

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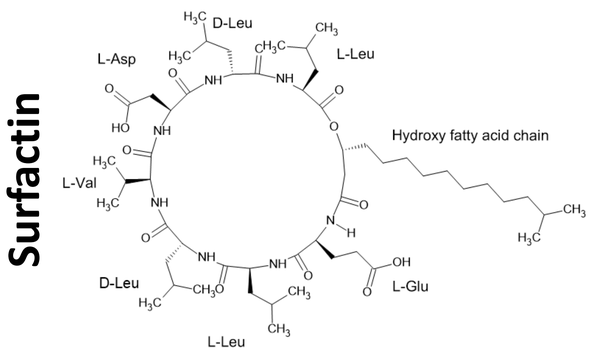
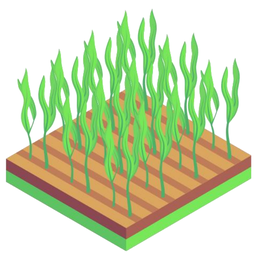
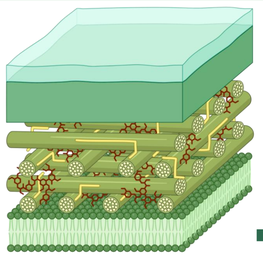
Introduction: Lipopeptide production from *B. subtilis* 168 on Lignocellulosic biomass





Lignocellulosic biomass



C sources:
- Xylose
- Glucose

Metabolic Network

Application of lipopeptides

- Cosmetic
- Pest-control and Bio-stimulant agent
- Anti-microbial
- Biosurfactant

Limitations

- High costs of production
- Feedstock account for 50% of the costs [3]
- Complex **metabolic regulation**

Aim of the study

- Unveil connection between **Carbon source** and **Lipopeptides** on **lignocellulosic biomass hydrolysate** and optimize their production

Modelling and Methods

Partial Reaction Network (PRN):

Reactions

- Gene
- Metabolic Reaction
- Outflow
- Inflow
- Prediction

Species

- Metabolite
- Protein
- Actor Species

Partial Kinetic

- Activation
- Acceleration
- Flow
- Inhibition

Modelling PRN [1]

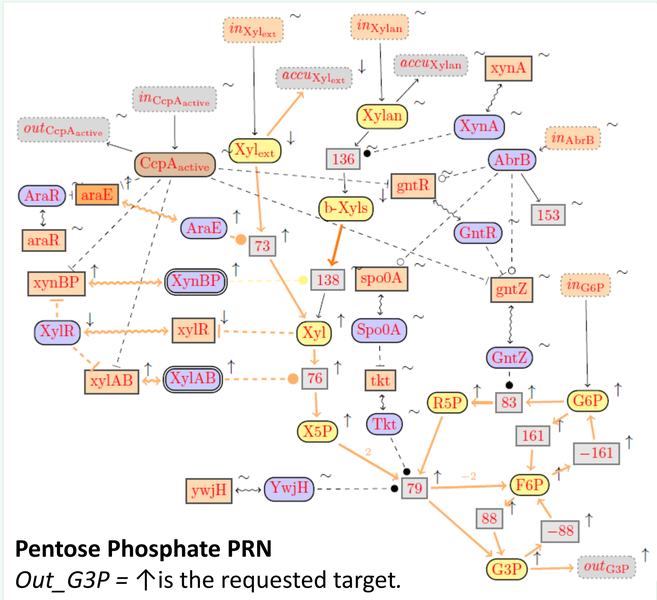
Single Change:

- ⇓ Knock-Out
- ↑ Increase
- ↓ Decrease

Predictions [1] :

Model	Prediction
BCAA[4]	*Pilv-leu* = ↑
C Outflows	*alsR* = ⇓
Pentose Phosphate	*araE* = ↑

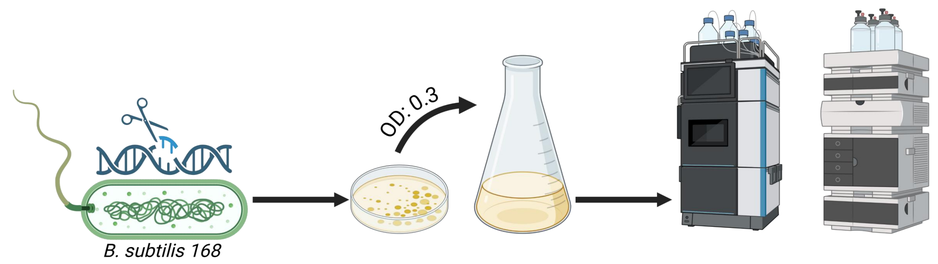
Single Change Prediction



Pentose Phosphate PRN
Out_G3P = ↑is the requested target.
araE = ↑ is the computed change.
Yellow arrows are the implications of the change on the PRN

Single Change Explanation

Methods



B. subtilis 168

Genome Editing: CRISPR-Cas9 [2]

Production: **Medium:** Landy and Landy-Xyl 37°C, pH 7, 160rpm

Analysis: **Sugars:** Ionic Chromatography **Lipopeptides:** UPLC

Production Media [6]:
Landy: 20g/L of Glucose
Landy-Xylose: 20g/L of Xylose
Landy-Hydrolysate: 1.5g/L Glucose, 11.5g/L Xylose, 1g/L arabinose, 3.5g/L acetate

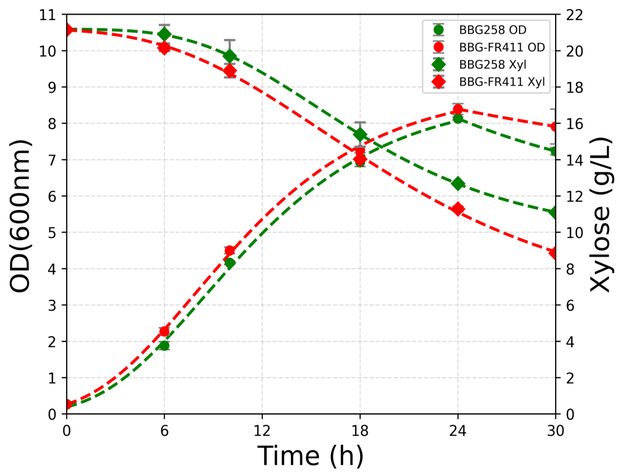
Mutant Construction — ***In-vitro* Validation**

Results & Discussion

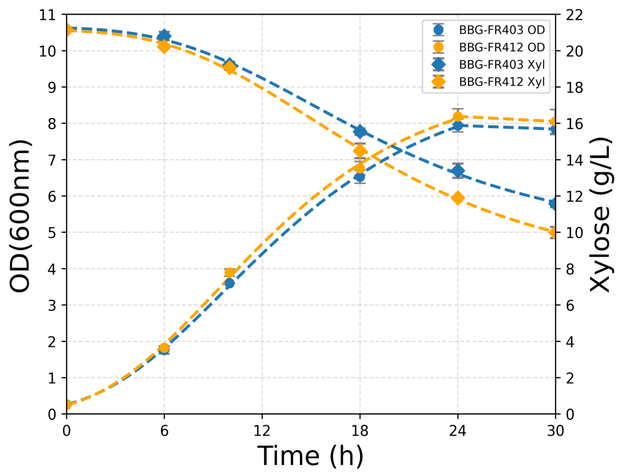
The effect of *araE* Knock-Up on xylose consumption

***B. subtilis* Performance on Landy-Xylose**

BBG258 (BS168, *trp+*, *sfp+*) vs **BBG-FR411** (BBG258, P*veg::araE*)



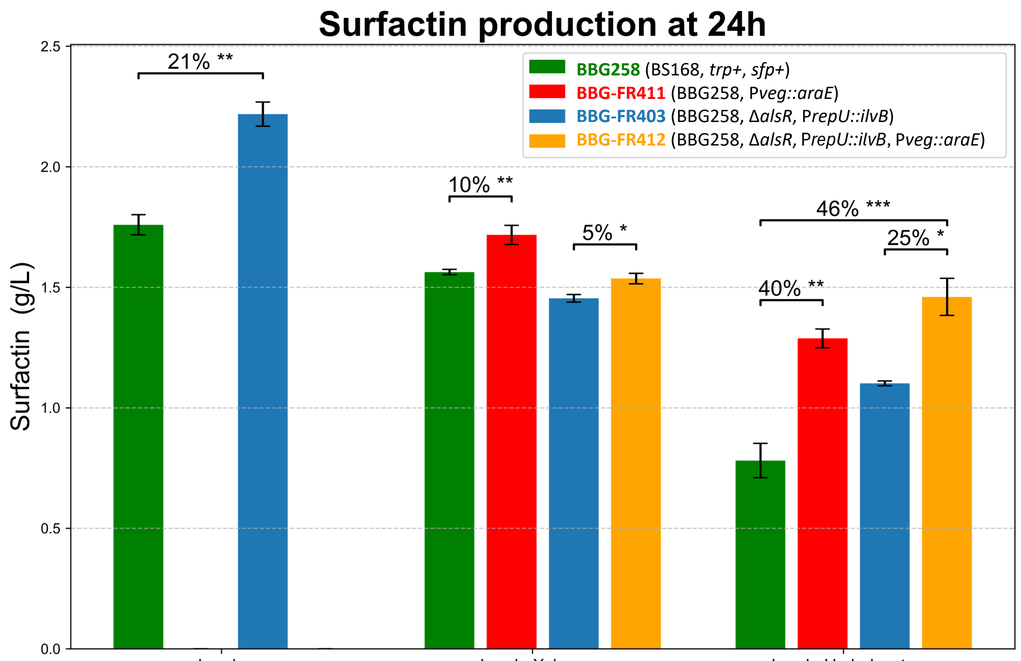
BBG-FR403 (BBG258, Δ*alsR*, P*repU::ilvB*) vs **BBG-FR412** (BBG258, Δ*alsR*, P*repU::ilvB*, P*veg::araE*)



+11% xylose consumption: *ParaE* Vs *Pveg*
Landy-Xylose at 24h

***In-silico* Prediction verified: Less xyl accumulated**
Constraint: G3P = ↑, Solution: *araE* = ↑, Implication: accu_Xyl_ext =↓

Surfactin production on different carbon sources



-56% Surfactin production for BBG258
From Landy (glucose) to Landy-Hydrolysate (glucose + xylose)

+46% Surfactin production for BBG-FR412
BBG258 Vs **BBG-FR412**: Effect of *Pveg::araE* and *PrepU::ilvBH*

Conclusions

- Two new Partial Reaction Networks proposed: **Pentose Phosphate** and **C Outflows (Fermentation)**
- Proved the **prediction explanations** of BioComputing's Reaction-network Tool using **partial kinetic information**
- Identified 3 compatible genes with more surfactin production on lignocellulosic biomass: ***araE, alsR, ilvBH***

Check all the models, Single Change Explanation and References ▶

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