



2024 | DANI DOKTORATA BIOTEHNIČKOG PODRUČJA



Sucrose as an electron source for cofactor regeneration in recombinant *E. coli* expressing invertase and a Baeyer Villiger monooxygenase

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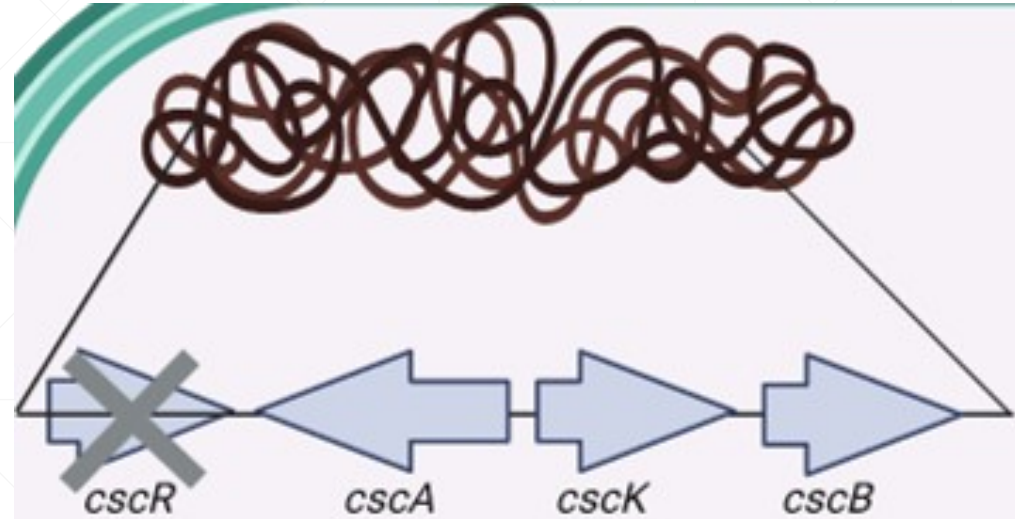
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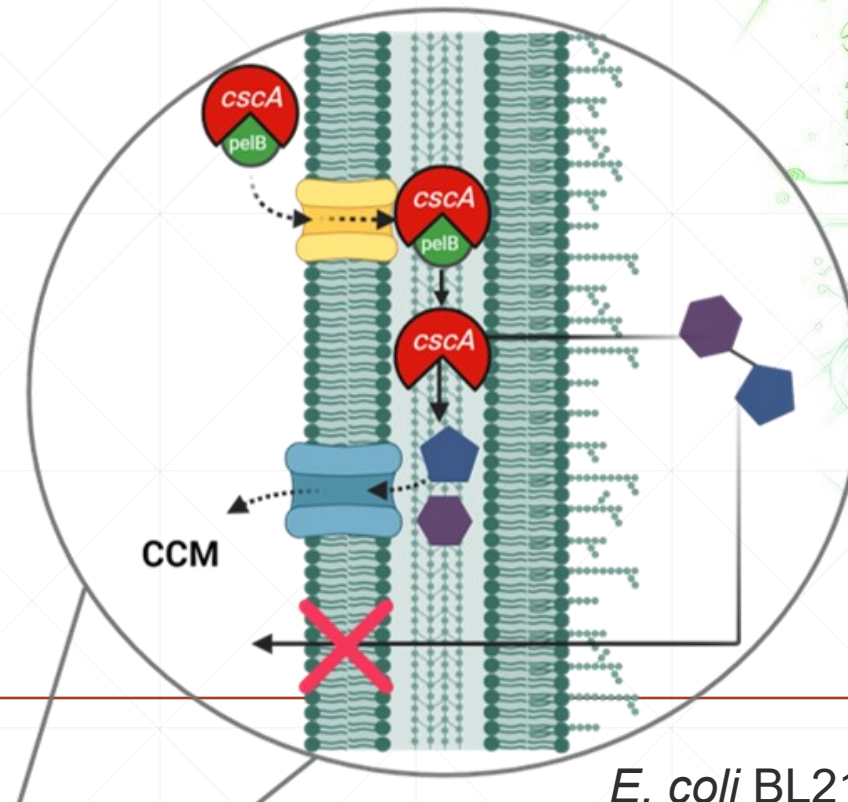
Most *E. coli* laboratory strains do not utilize sucrose

- Presence of genome-encoded *csc* regulon
- *E. coli* W $\Delta cscR$ utilizes sucrose below 6 mM (2 g L^{-1})





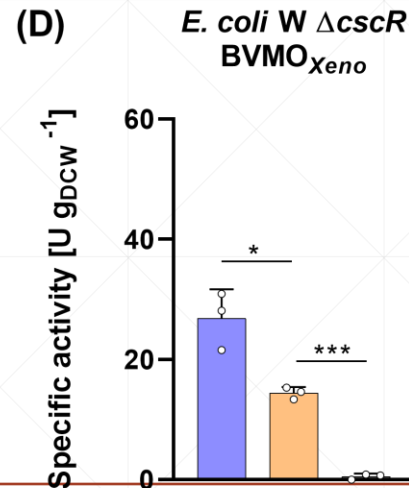
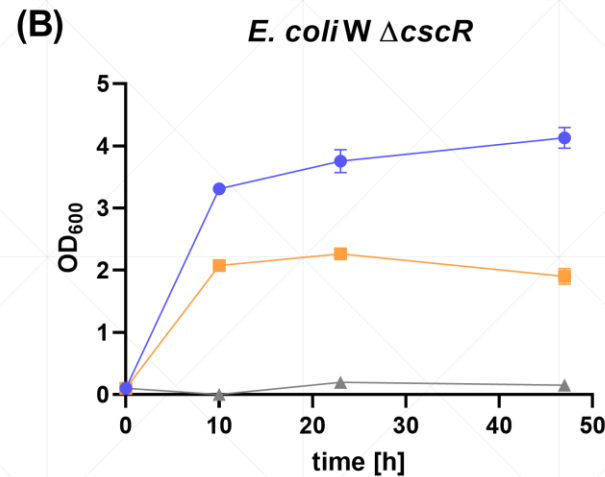
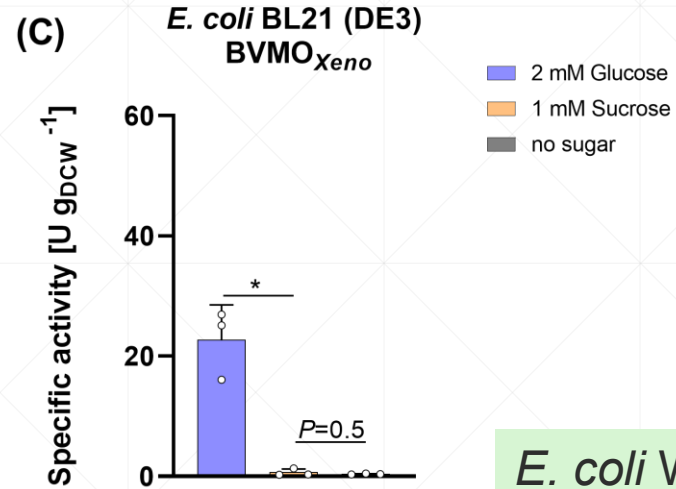
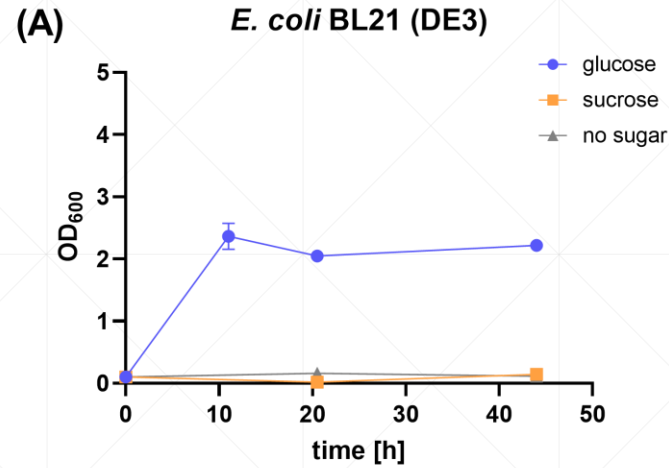
E. coli W $\Delta cscR$



E. coli BL21 (DE3)

Control strains

E. coli BL21 (DE3) is unable to grow on sucrose (A) or to utilize it as an electron donor (C)



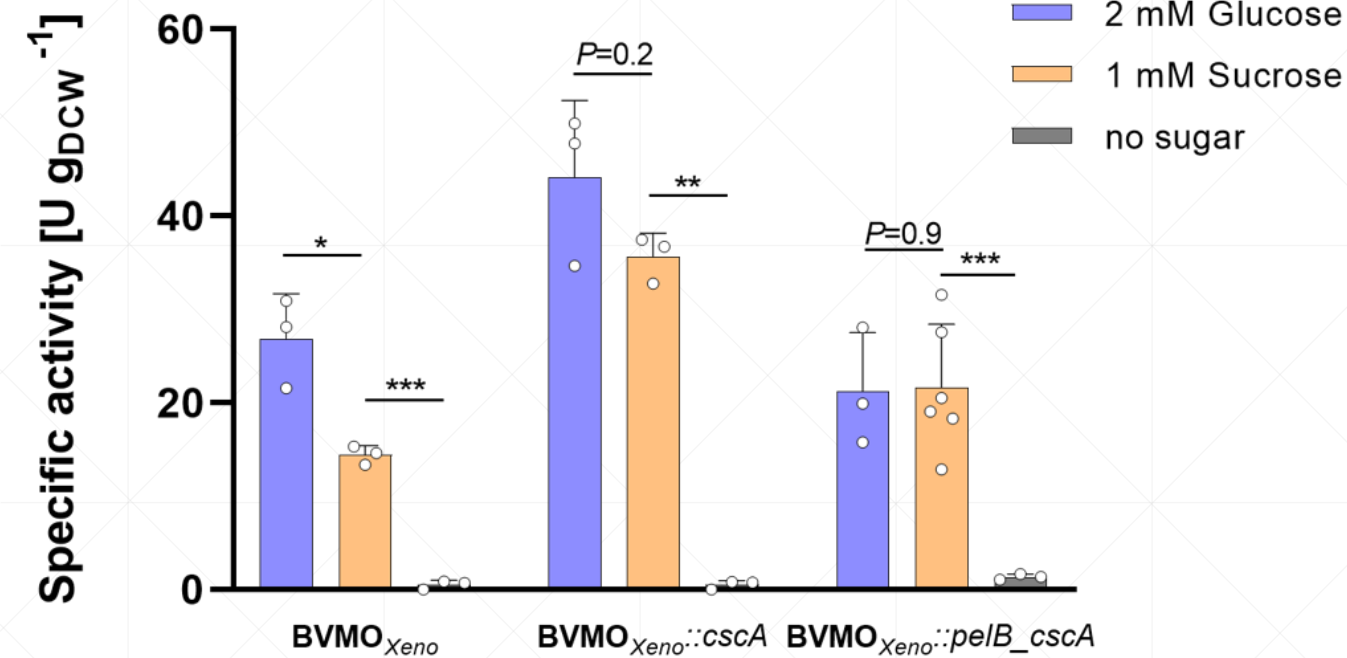
E. coli W Δ cscR formed product using sucrose due to the genomically-encoded cscA (D)



Comparing the invertase allocation: cytosol (*cscA*) or periplasmic space (*pelB_cscA*)

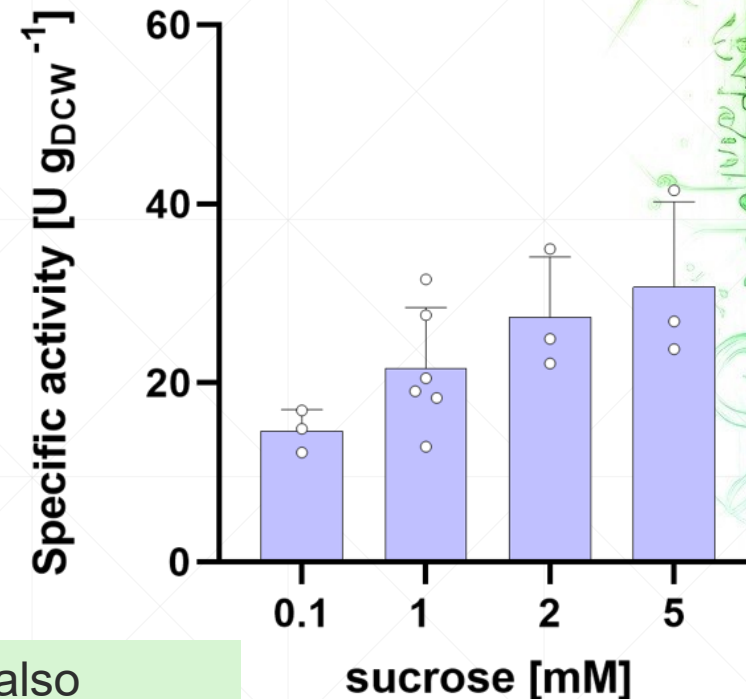
A

E. coli W $\Delta cscR$



B

E. coli W $\Delta cscR$
BVMO_{Xeno::pelB_cscA}



Overexpression of *cscA* is also beneficial for the *E. coli* W $\Delta cscR$ (A)

Whole-cell biotra

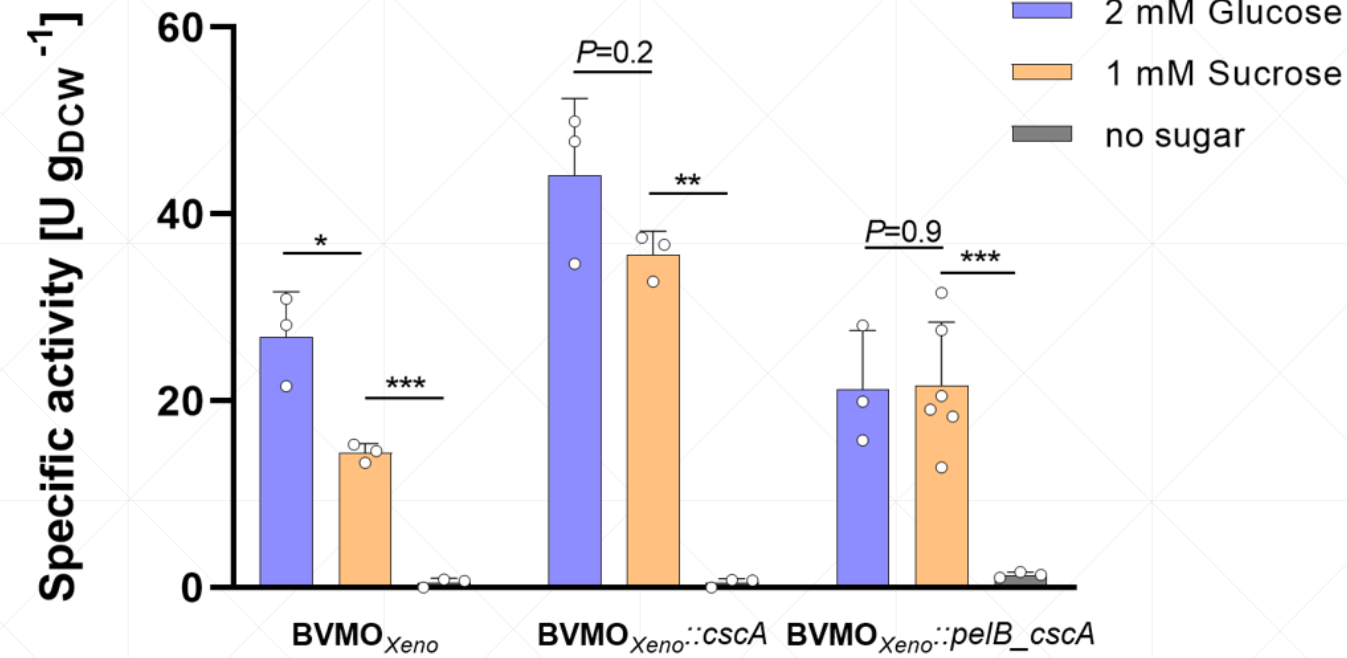
Reaction conditions: 30 mL, M9 salts as medium, 1.5 g_{DCW} L⁻¹, 5 mM 1a, 25 °C, 160 rpm, N ≥ 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations. P values were calculated using Welch's t test (*P < 0.05)

in the presence of sugars (2 mM glucose or 1 mM sucrose).

Comparing the invertase allocation: cytosol (*cscA*) or periplasmic space (*pelB_cscA*)

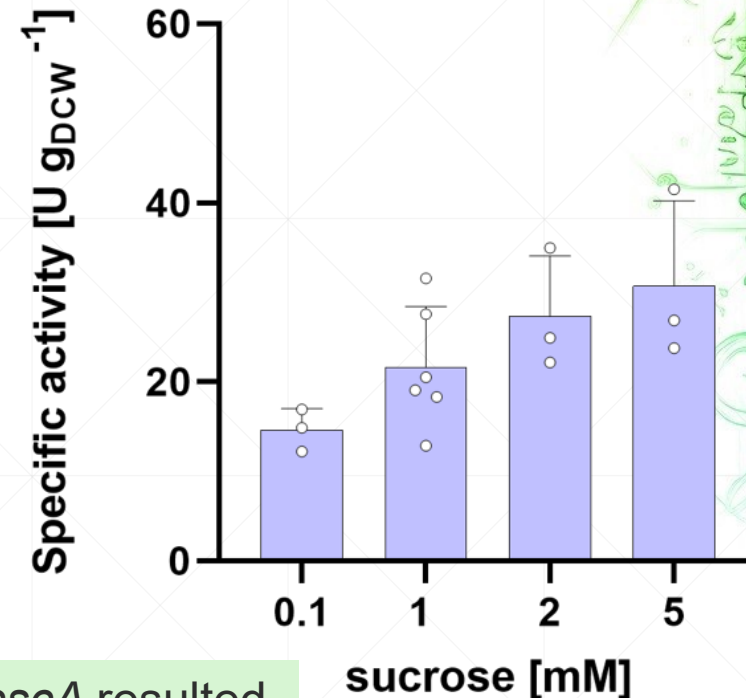
A

E. coli W $\Delta cscR$



B

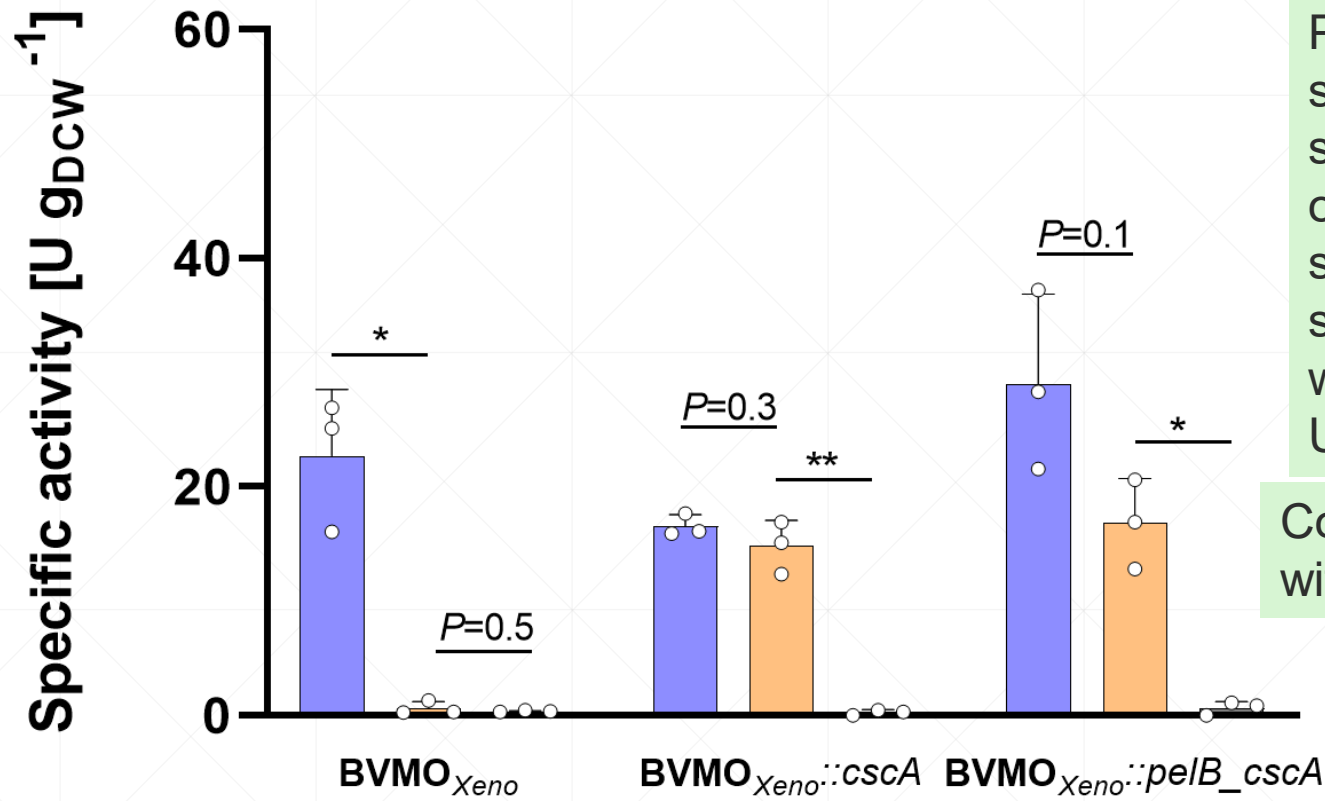
E. coli W $\Delta cscR$
BVMO_{Xeno::pelB_cscA}



Cytosolic production of *cscA* resulted with highest specific activities

Transferability of the sucrose utilization to *E. coli* BL21 (DE3)

E. coli BL21 (DE3)



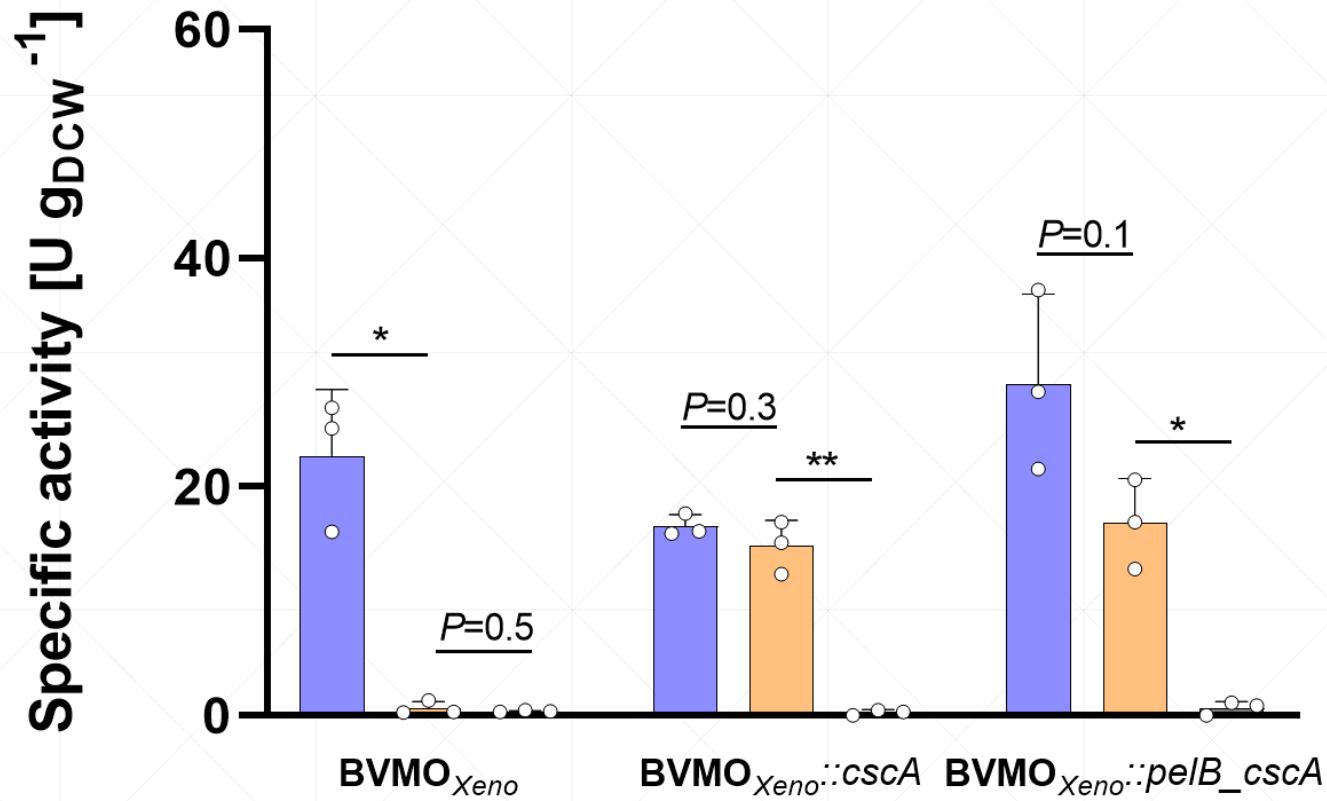
Periplasmic production strain (*peIB_cscA*) showed a specific activity of 17 U g_{DCW}⁻¹ with sucrose, similarly to specific activities obtained with *E. coli* W Δ cscR (22 U g_{DCW}⁻¹)

Comparable results obtained with both constructs

Whole-cell biotransformation of 1a mediated by different *E. coli* constructs harboring BVMO_{Xeno} in the presence of sugars (2 mM glucose or 1 mM sucrose). Reaction conditions: 30 mL, M9 salts as medium, 1.5 g_{DCW} L⁻¹, 5 mM 1a, 25 °C, 160 rpm, N ≥ 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations. P values were calculated using Welch's t test (*P < 0.05)

Transferability of the sucrose utilization to *E. coli* BL21 (DE3)

E. coli BL21 (DE3)



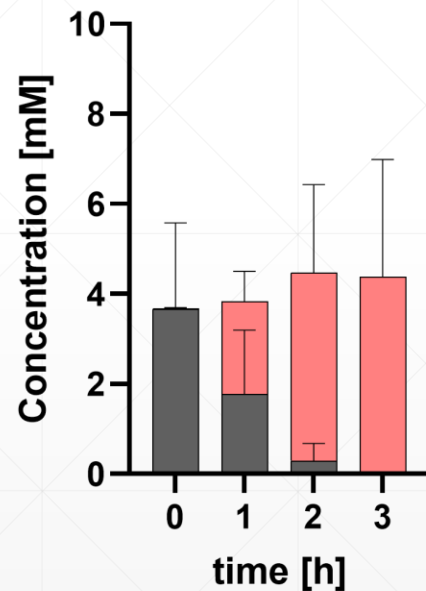
Simple co-expression of *cscA* was sufficient to confer the sucrose uptake in *E. coli* BL21 (DE3)

Whole-cell biotransformation of 1a mediated by different *E. coli* constructs harboring BVMO_{Xeno} in the presence of sugars (2 mM glucose or 1 mM sucrose). Reaction conditions: 30 mL, M9 salts as medium, 1.5 g_{DCW} L⁻¹, 5 mM 1a, 25 °C, 160 rpm, N ≥ 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations. P values were calculated using Welch's t test (*P < 0.05)

Photosynthetically-derived sucrose (9 mM) fuels oxidation reaction using recombinant *E. coli*

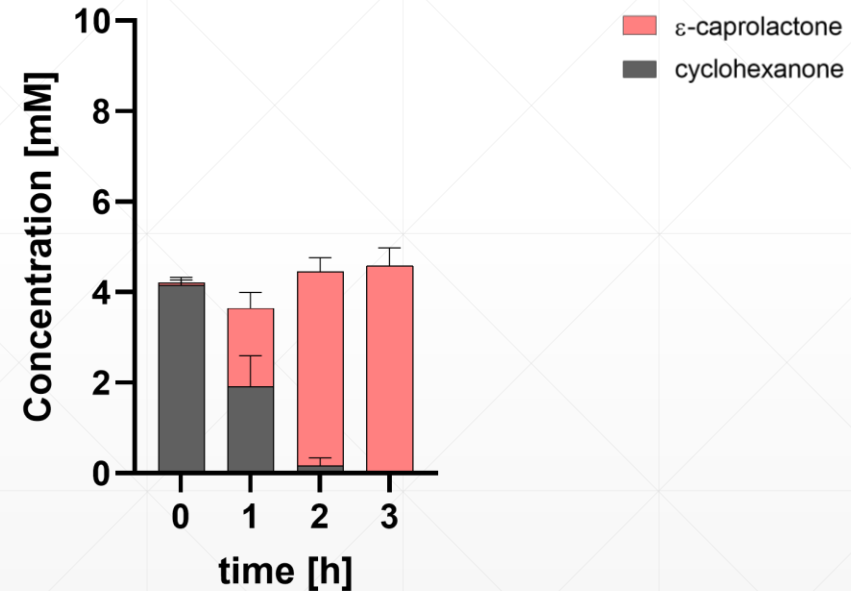
E. coli W $\Delta cscR$

a) $BVMO_{Xeno}::pelB_cscA$



E. coli BL21 (DE3)

b) $BVMO_{Xeno}::pelB_cscA$



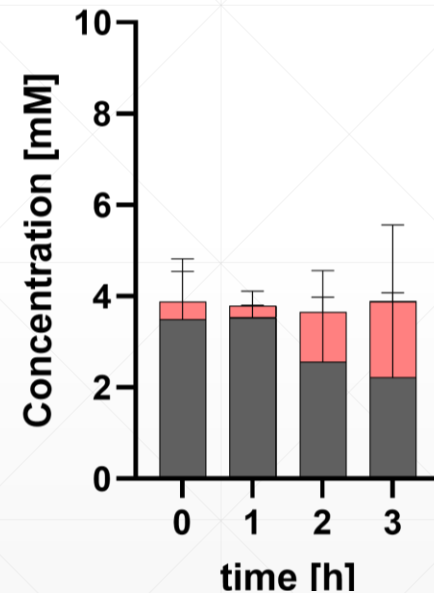
E. coli W $\Delta cscR$ $BVMO_{Xeno}::pelB_cscA$ was deemed to be the best-performing strain resulting in a volumetric productivity of $1.7 \text{ mmol L}^{-1} \text{ h}^{-1}$

Reaction conditions: sucrose-enriched BG11 medium containing 400 mM NaCl, 1% CO₂, 30 °C, 100 rpm, initial concentration 5 mM 1a, N = 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations

Photosynthetically-derived sucrose (9 mM) fuels oxidation reaction using recombinant *E. coli*

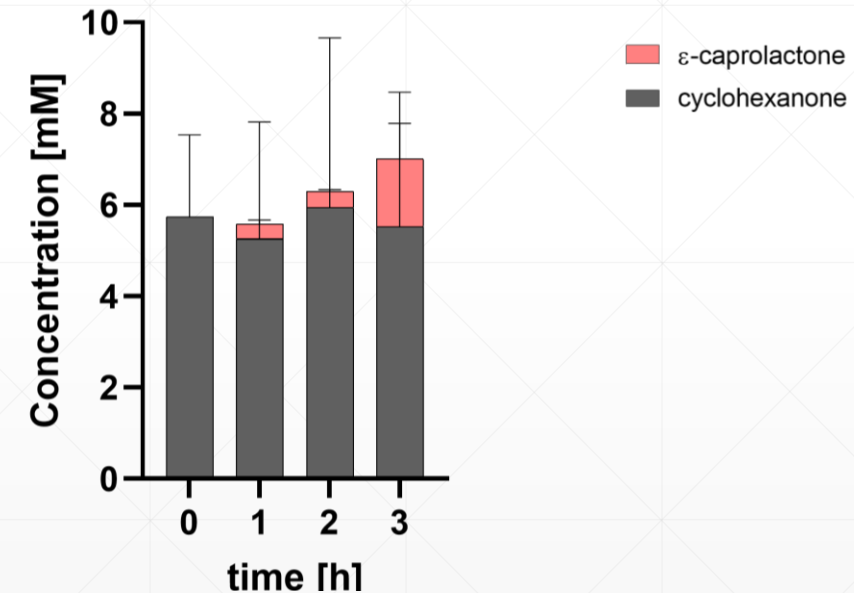
E. coli W $\Delta cscR$

c) $BVMO_{Xeno}::cscA$



E. coli BL21 (DE3)

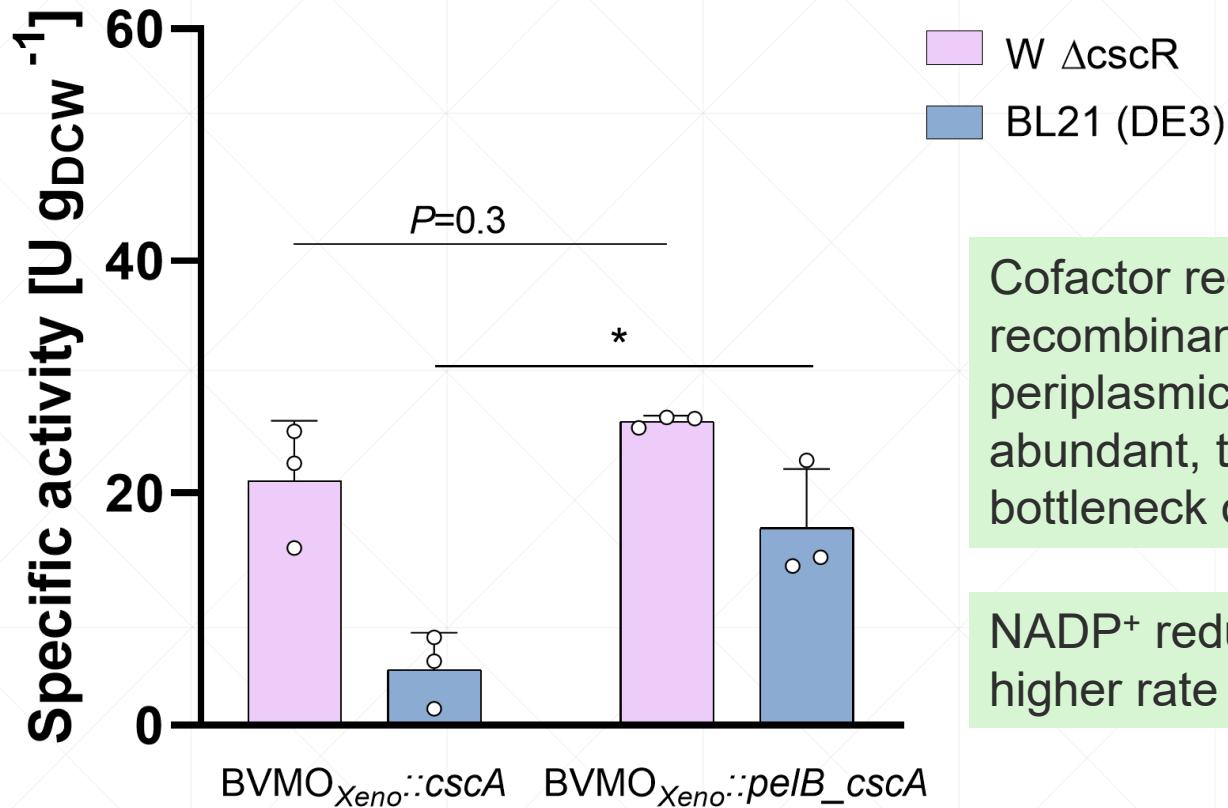
d) $BVMO_{Xeno}::cscA$



No particular difference between the *E. coli* W $\Delta cscR$ and BL21 (DE3) strains while carrying the same $BVMO_{Xeno}::cscA$ cassette, whereas the same variants resulted in different activities in the initial study

Reaction conditions: sucrose-enriched BG11 medium containing 400 mM NaCl, 1% CO₂, 30 °C, 100 rpm, initial concentration 5 mM 1a, N = 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations

1 mM sucrose derived the opposite results as opposed to 9 mM – why?



Cofactor regeneration is faster if the recombinant invertase is produced in the periplasmic space, while sucrose is highly abundant, thus overcoming a possible bottleneck of NADPH limitation

NADP⁺ reduction is taking place at a higher rate than the BVMO_{Xeno} oxidation

Whole-cell biotransformation employing 10 mM sucrose. Reaction conditions: 30 mL, M9 salts as medium, 1.5 g_{DCW} L⁻¹, 5 mM 1a, 25 °C, 160 rpm, N ≥ 3. All bars represent data generated from reactions stemming from biological replicates with individual values depicted. Error bars represent standard deviations

Thank you for your attention! Questions?

Acknowledgments

- Robert Kourist
- Lenny M.-Yap
- Gabor Tóth
- AG Kourist
- Golden Coffice

