Proteome constraints shape *Lactococcus lactis*' metabolic behavior

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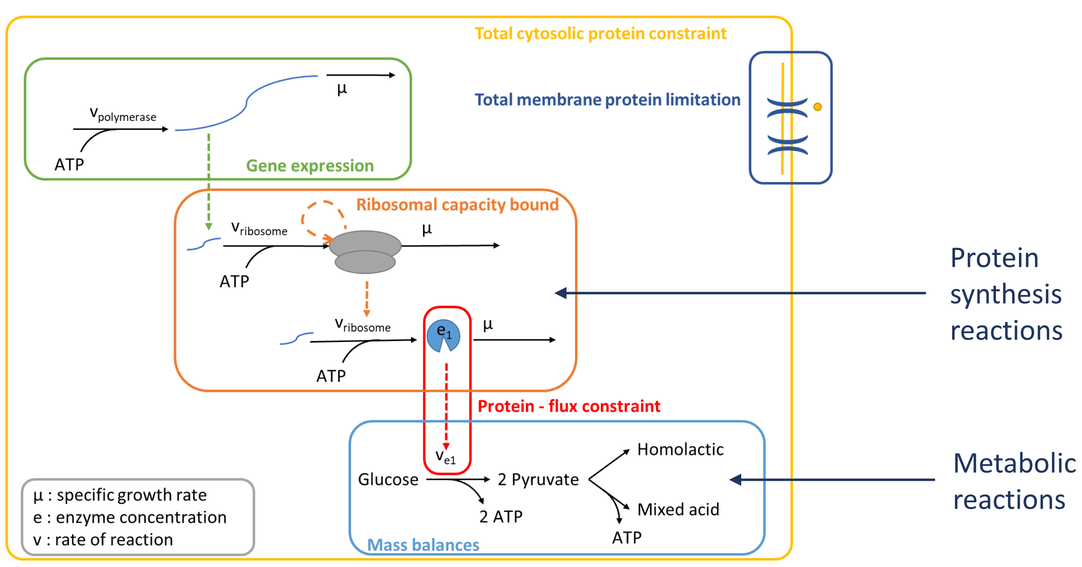
1. Can we understand LAB physiology from cost-benefit analysis of the underlying metabolic network?

- Limited nutrients and protein capacities constrain growth.
- Constraints can result in trade-offs and resource allocation choices.
- We developed a Proteome Constrained model of *L. lactis* - pcLactis[1] - to study metabolic benefits and the associated resource costs.

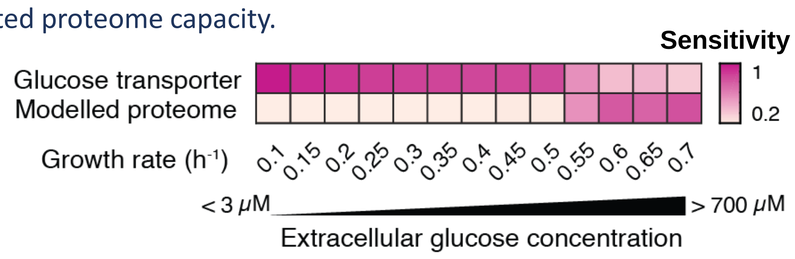
2. pcLactis couples the metabolic network to protein synthesis machinery at genome-scale

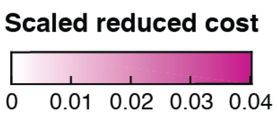


3. pcLactis predicts growth-limiting constraints

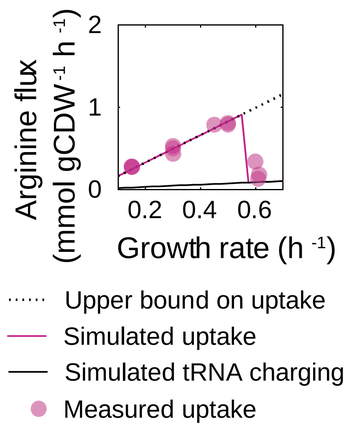
A. Growth is modeled as function of glucose availability. A high reduced cost reflects an active constraint.

- At low growth rate, only glucose transport is limiting growth rate.
- Above 0.5 h^{-1}, inactive enzyme becomes zero and an extra constraint becomes active, reflecting limited proteome capacity.





B. Arginine uptake is also growth-limiting, but only when proteome capacity is not limiting, as shown by the high reduced cost (purple) at growth rates <0.5 h^{-1}.

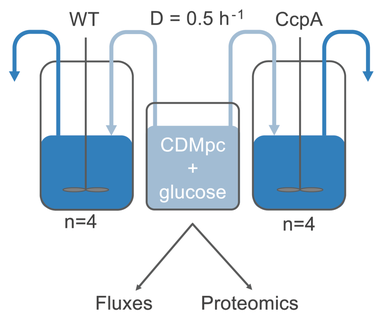


C. Arginine is taken up more than needed for biomass (solid black line), but only at growth rates <0.5 h^{-1}.

D. Explanation: Under proteome limitation, the proteome efficiency (ATP yield/protein cost) of a pathway determines the optimal strategy. Lactate production is then preferred as best investment.

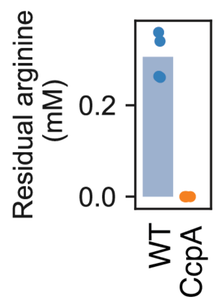
Pathway	ATP yield	Protein cost	Protein efficiency
Mixed acid fermentation	3	0.0066	456
Lactic acid fermentation	2	0.0022	897
Arginine catabolism	1.67	0.0153	109

4. pcLactis predicts active constraints as sites of adaptative evolution



A. Laboratory evolution in glucose-limited environment resulted in selection of a CcpA mutant with better adapted phenotype.[2]

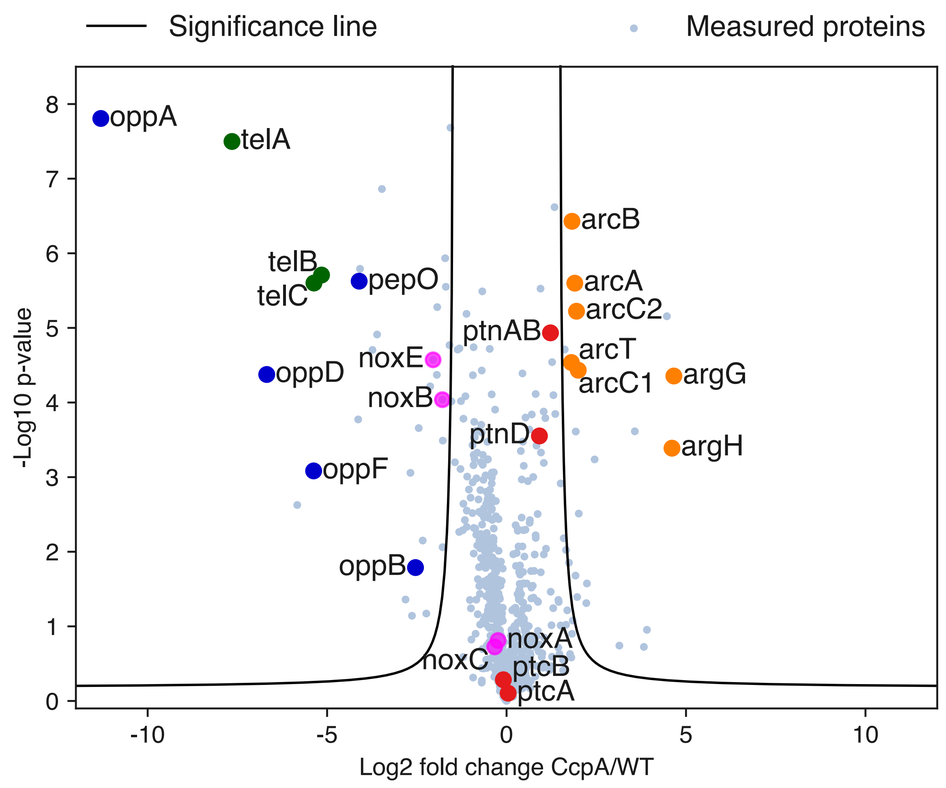
Proteome and fluxes of CcpA mutant were compared with WT *L. lactis* at D=0.5 h^{-1}, where arginine uptake is predicted to be limiting



B. Arginine uptake increased in the CcpA mutant to such an extent that the residual arginine concentration became zero.

C. Proteome of CcpA mutant is changed where the PC-model predicts active constraints:

- Glucose transporter and arginine catabolism proteins are upregulated in the CcpA mutant.
- Expression of non-used (membrane) proteins is decreased.



5. Conclusion: physiology of *L. lactis* is the optimal strategy under nutrient and proteome constraints

- pcLactis can identify the growth-limiting constraints.
- The growth-limiting constraints explain changes in *L. lactis'* metabolism.
- This provides insights in likely targets of evolutionary change.
- Arginine catabolism is used as additional ATP source only when proteome space is available.

6. References

[1] Chen, Y., van Pelt-KleinJan, E., van Olst, B., Douwenga, S., Boeren, S., Bachmann, H., Molenaar, D., Nielsen, J., Teusink, B. Proteome constraints reveal targets for improving microbial fitness in nutrient-rich environments. Mol. Syst. Biol. 2021, 17: e10093.

[2] Price, C. E., Branco Dos Santos, F., Hesseling, A., Uusitalo, J. J., Bachmann, H., Benavente, V., ... Kuipers, O. P. Adaption to glucose limitation is modulated by the pleotropic regulator CcpA, independent of selection pressure strength. BMC Evolutionary Biology, 2019, 19:15.

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