Characterizing microbial interactions in controlled and natural microbial communities

Maxime Lecomte, Simon Labarthe, David James Sherman, Hélène Falentin, Clémence Frioux

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Workshop SymBioDiversity 2021-2022

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Industrial cheese starter bacterial community as a controlled ecosystem

L. lactis → 2 lactic bacteria

L. plantarum → 2 lactic bacteria

P. freudenreichii → 1 propionic bacterium

Bacterial fermentation

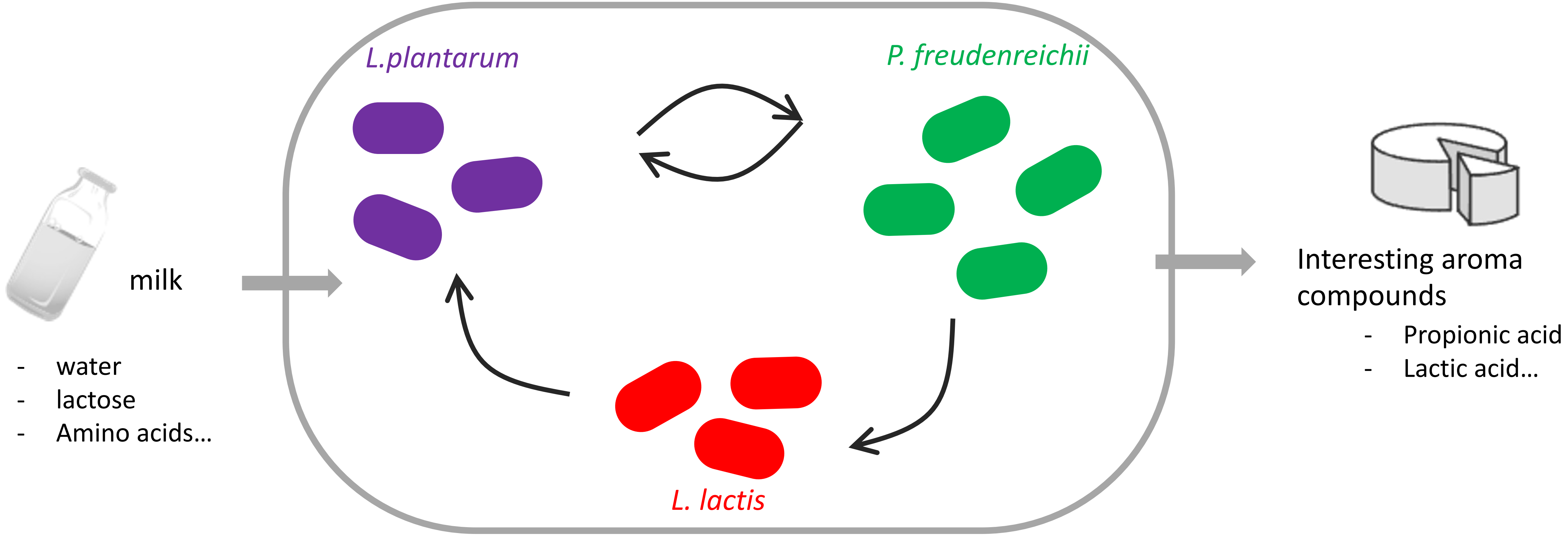
→ Aroma compounds released

→ Media acidification

Which biological phenomena rules aroma compounds production ?

Cao et al, 2021,*Journal of Agricultural and Food Chemistry*

Bacterial fermentation process in cheese



Model the metabolism enable us to monitor compounds

Cao et al, 2021,*Journal of Agricultural and Food Chemistry*

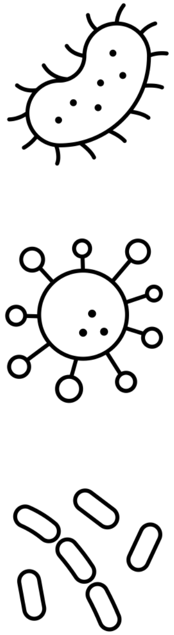
Multi-omics strategy

Annotated genomes

Genes expression (metatranscriptomics)

Acétate-HPLC-F1	Acétate-HPLC-F3
0,01	0,01
0,04	0,05
0,44	0,36
0,92	0,81
1,05	0,97
2,00	1,77
2,59	2,52

Metabolomics data

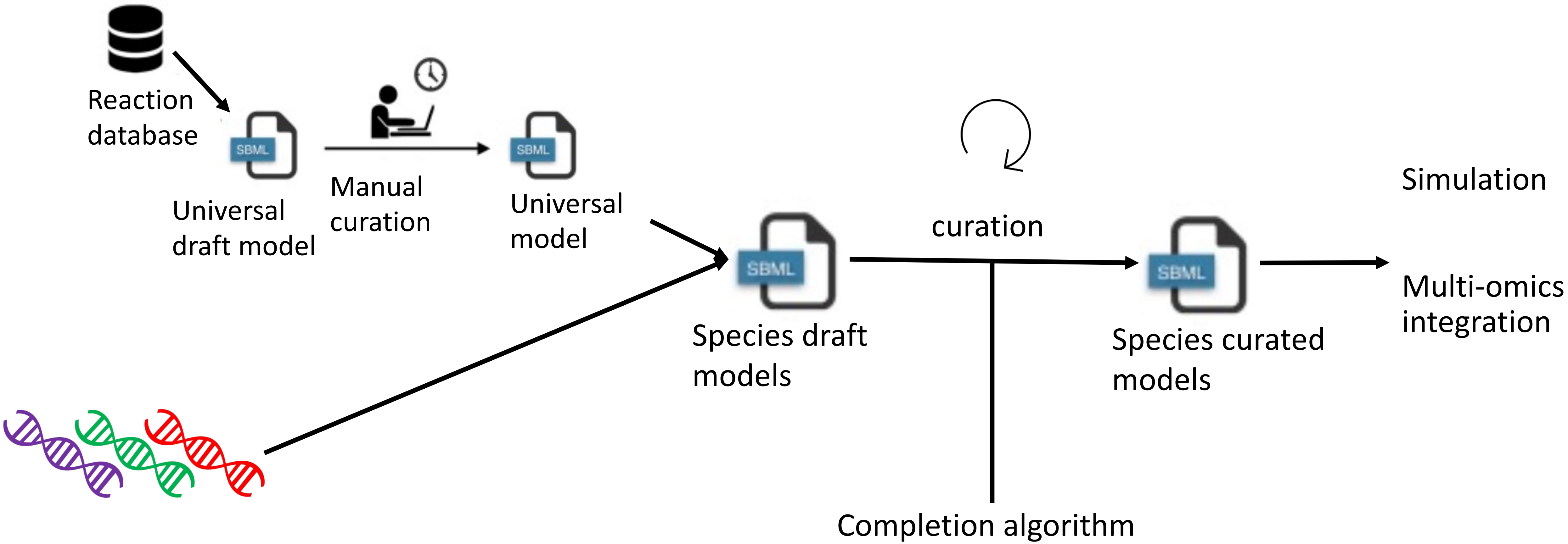


Growth and pH data in pure cultures

To integrate all this data, we first reconstruct the metabolism

Cao et al,2021,*Journal of Agricultural and Food Chemistry*

Inference of genome-scale metabolic networks



Machado, D. et al, 2018, *Nucleic Acids Research*

FBA as a numerical model of metabolism

Stoichiometric matrix =
$$\begin{array}{cc} \text{metabolites} & \begin{array}{ccc} r_1 & \dots & r_n \end{array} \\ \begin{array}{c} \text{A} \\ \text{B} \\ \text{C} \end{array} & \begin{pmatrix} -1 & \dots & 0 \\ 1 & \dots & -2 \\ 0 & \dots & 1 \end{pmatrix} \end{array}$$

$$\max v_{growth}$$
$$\text{such that } S.v = 0$$
$$\text{and } v_{min} \leq v \leq v_{max}$$

Orth, J. D. et al,2010,*Nature Biotechnology*

FBA as a numerical model of metabolism

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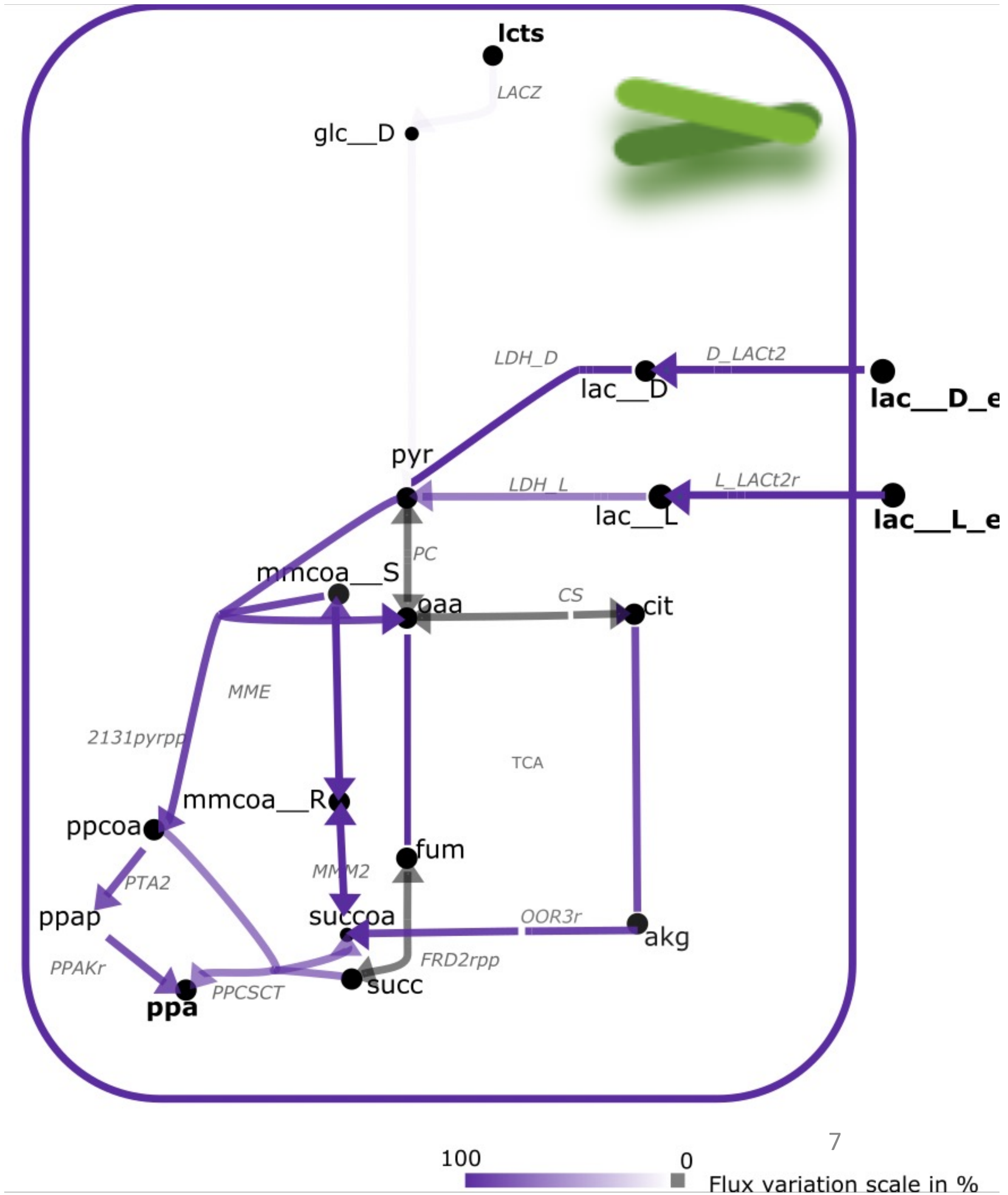
Flux distribution that maximizes biomass production



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King, Z. et al,2015. *PLoS Computational Biology*

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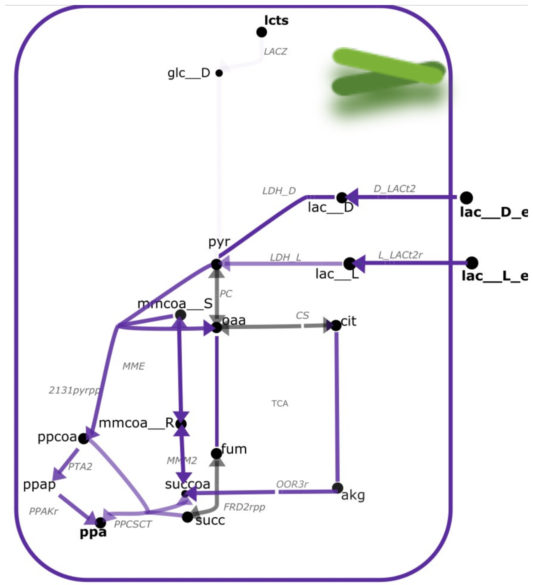
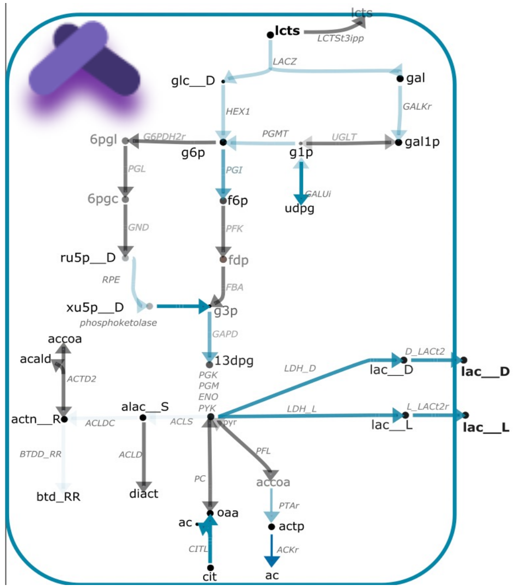
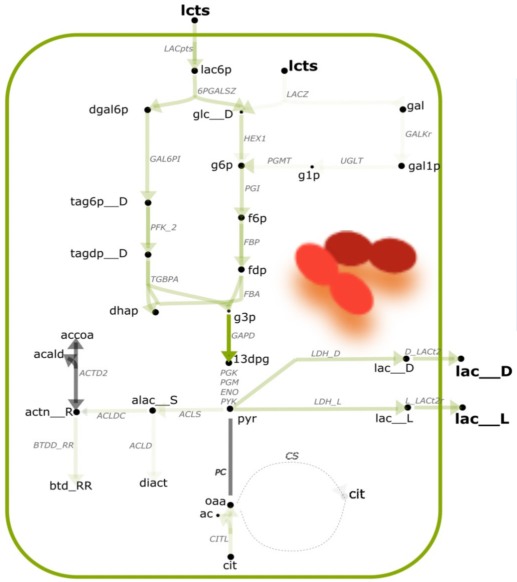






Individual models are in accordance with literature

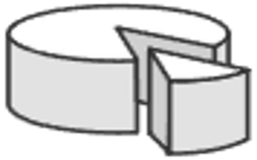
Orth, J. D. et al,2010,*Nature Biotechnology*
King, Z. et al,2015. *PLoS Computational Biology*

Thierry, A et al,2011, *International Journal of Food Microbiology*
Loux, V. et al, 2015, *BMC Genomics*

Cheese production: a dynamic and complex biological process

Liquid environment

solid environment

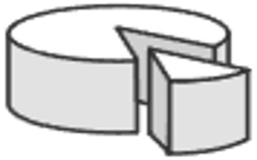


The consistency of the environment changes

Cheese production: a dynamic and complex biological process

Liquid environment

solid environment

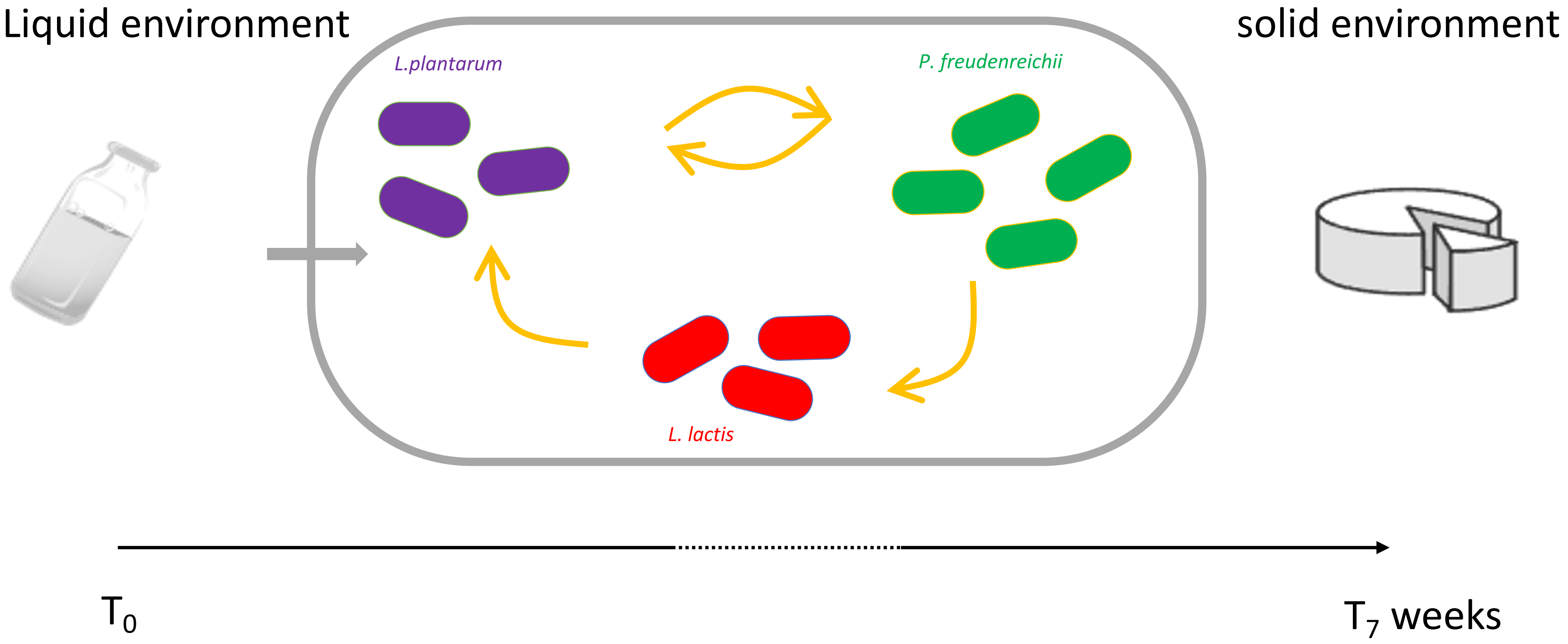


T_0

T_7 weeks

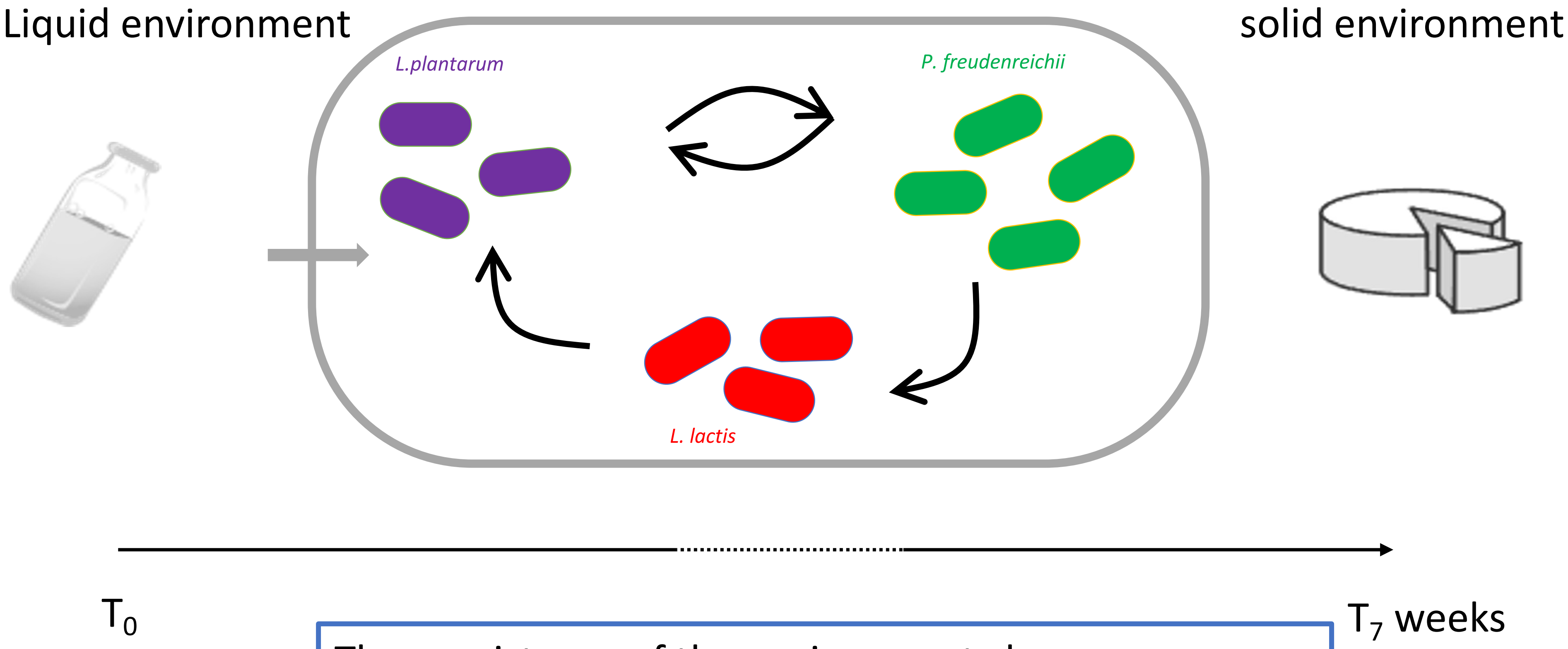
The consistency of the environment changes
A dynamic process

Cheese production: a dynamic and complex biological process



The consistency of the environment changes
A dynamic process
Interaction bacterium-bacterium

Cheese production: a dynamic and complex biological process



The consistency of the environment changes
A dynamic process
Interaction bacterium-bacterium

Dynamic modeling of metabolism (dFBA)

List of pre-defined interest compounds :

$$\partial_t m_j = \mu_{FBA_i}(c)_j b_i$$

Mahadevan et al,2002, *Biophysical Journal*

Dynamic modeling of metabolism (dFBA)

List of pre-defined interest compounds :

$$\partial_t m_j = \mu_{FBA_i}(c)_j b_i$$

Bacterial concentration :

$$\partial_t b_i = q_s(b_i)\mu_{FBA_i}(c)_i b_i$$

Mahadevan et al,2002, *Biophysical Journal*

Dynamic modeling of metabolism (dFBA)

List of pre-defined interest compounds :

$$\partial_t m_j = \mu_{FBA_i}(c)_j b_i$$

Bacterial concentration :

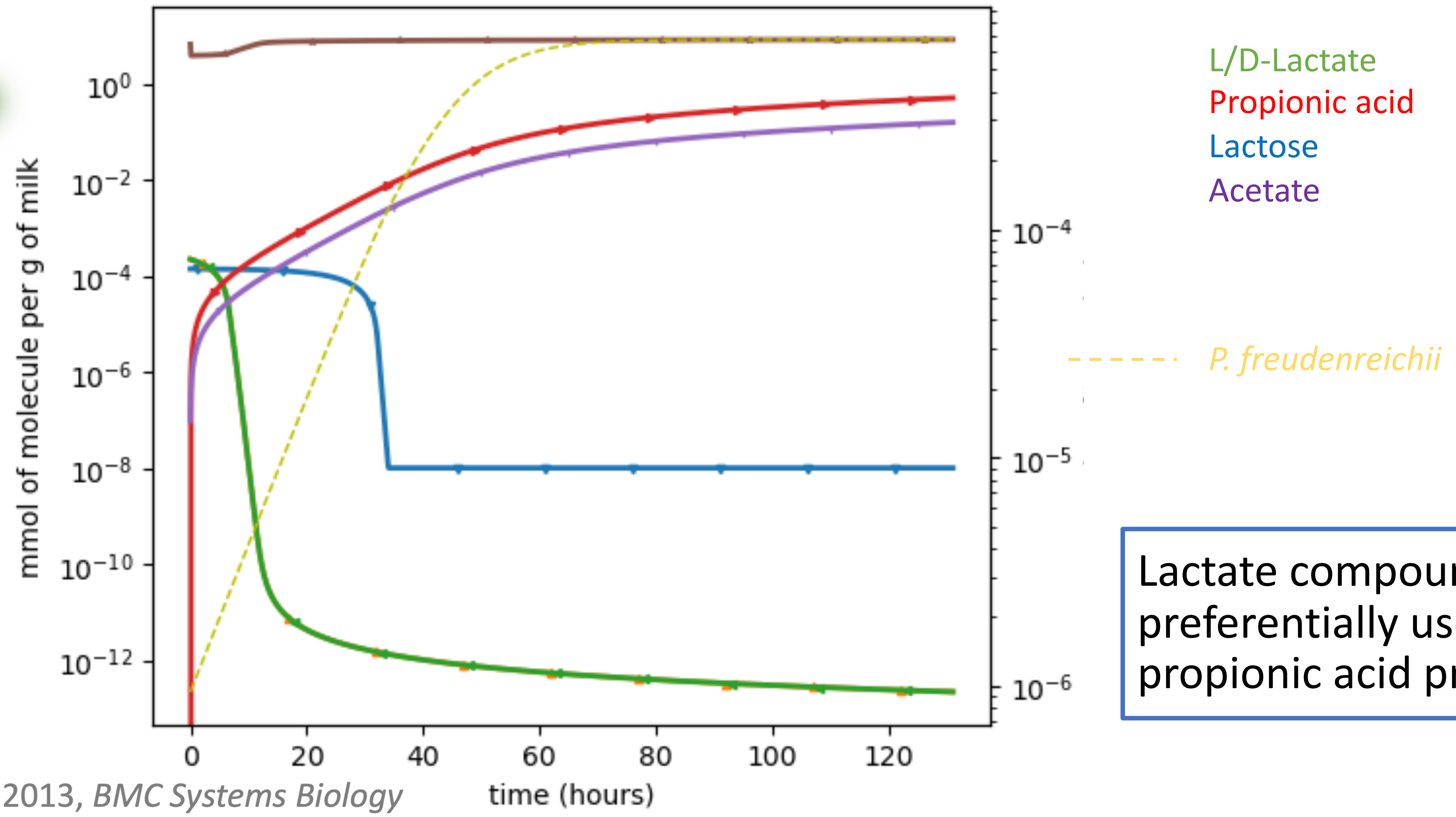
$$\partial_t b_i = q_s(b_i)\mu_{FBA_i}(c)_i b_i$$

q_S = Quorum sensing

$$q_s(b_i) = 1 - \frac{b_i}{\beta_i}$$

Mahadevan et al,2002, *Biophysical Journal*

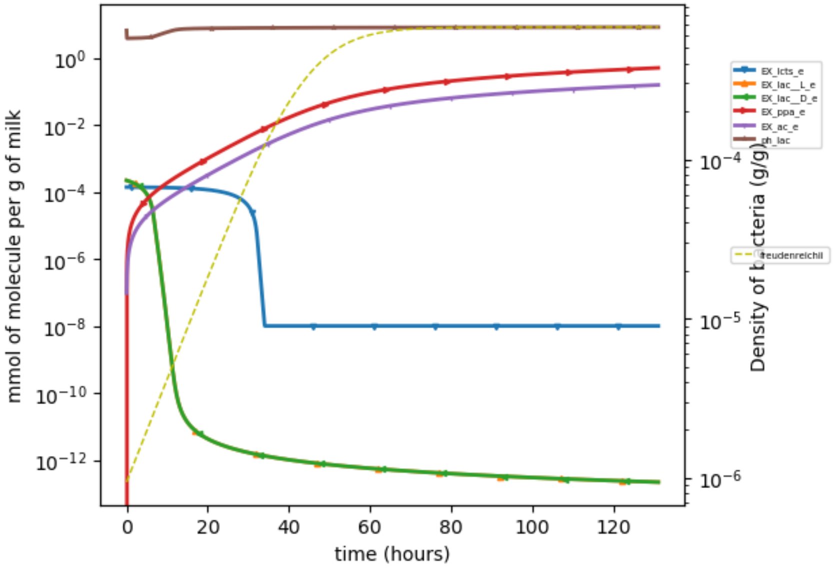
dFBA results of pure cultures

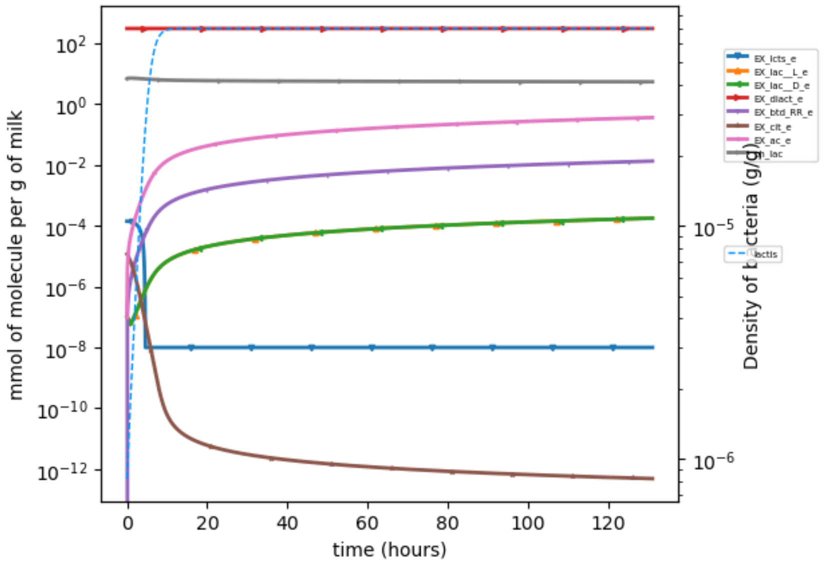


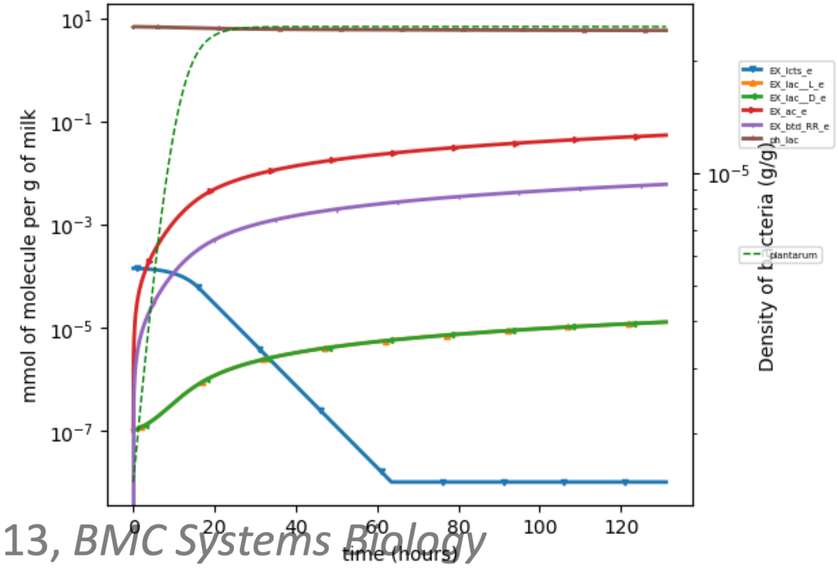
Lactate compounds preferentially used for propionic acid production

Ebrahim et al,2013, *BMC Systems Biology*

dFBA results of pure cultures







Individual dFBA simulations are performed

Fit with experimental data ? → growth and pH curves and metabolomics data

Ebrahim et al,2013, *BMC Systems Biology*

Optimizing dynamic models on pure cultures

$$J(b, pH|b_{exp}, pH_{exp}) = \|b - b_{exp}\|^2 + \alpha\|pH - pH_{exp}\|^2$$

Optimizing dynamic models on pure cultures

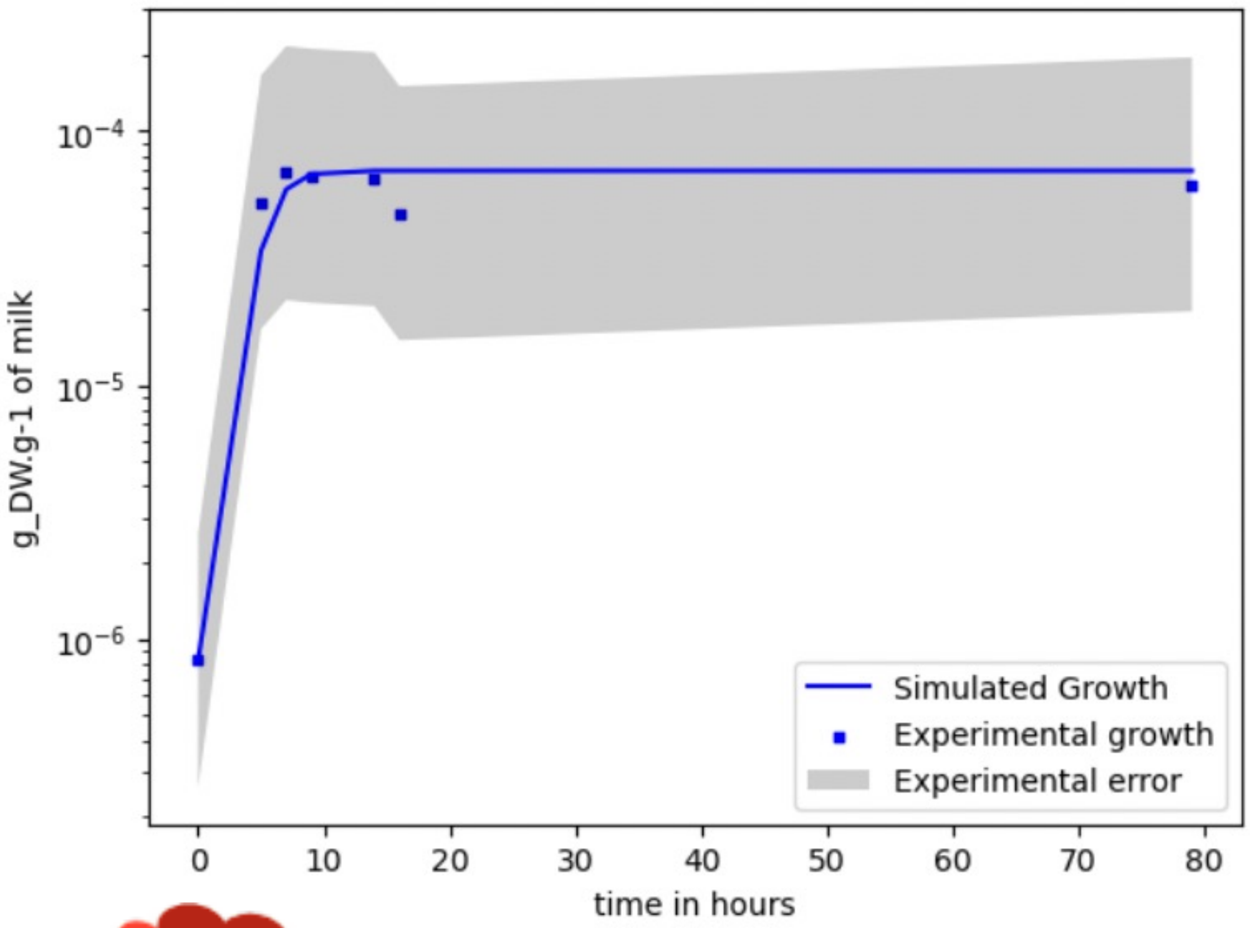
$$J(b, pH | b_{exp}, pH_{exp}) = \|b - b_{exp}\|^2 + \alpha \|pH - pH_{exp}\|^2$$

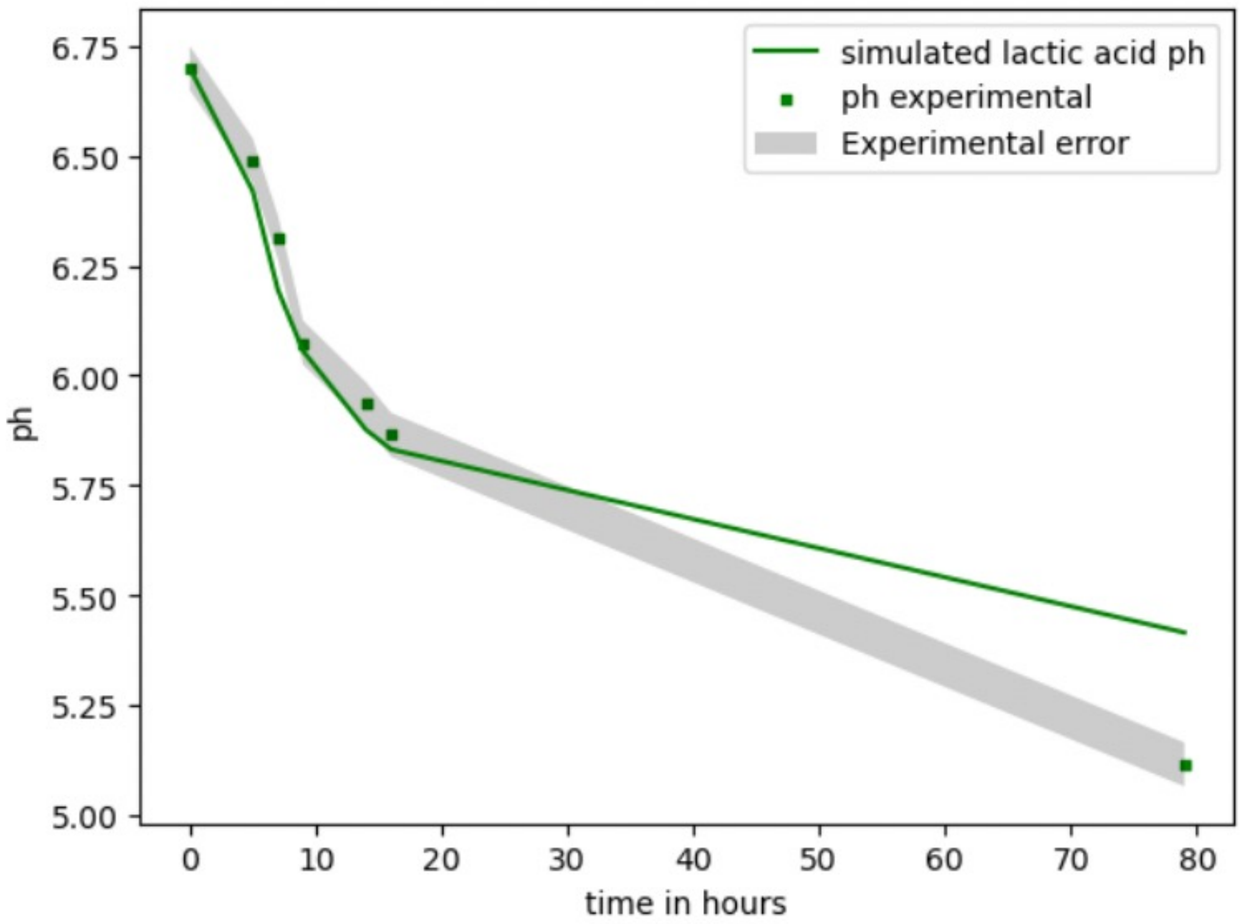
- Lambda 
- QS 
- K_lactate 
- C1 & C2 
- Vmin & Vmax lactose 
- Lactate_upper 

Identification of parameters to fit with experimental data

Virtanen. P, et al,2020, *Nature Methods*

dFBA results after optimization





Virtanen. P, et al,2020, *Nature Methods*
Ebrahim et al,2013, *BMC Systems Biology*

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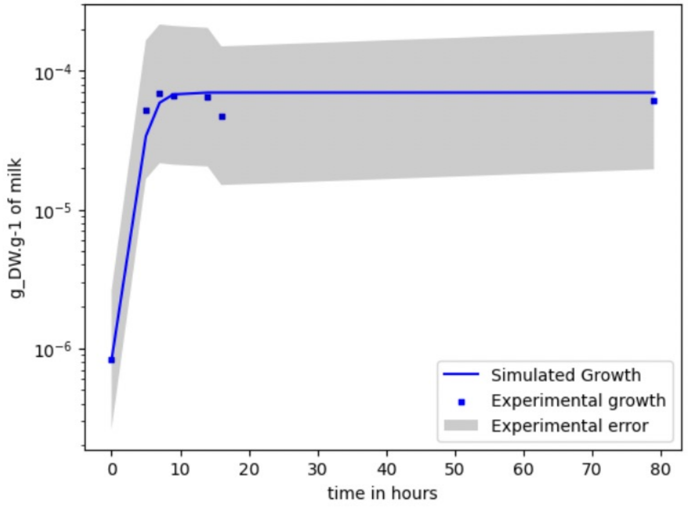
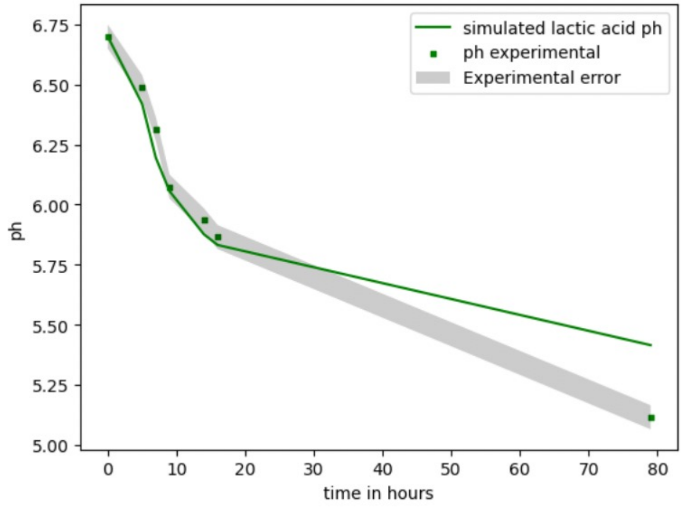
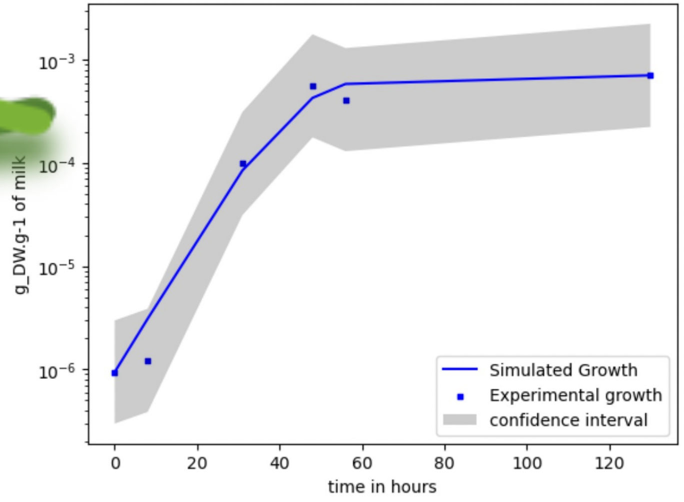
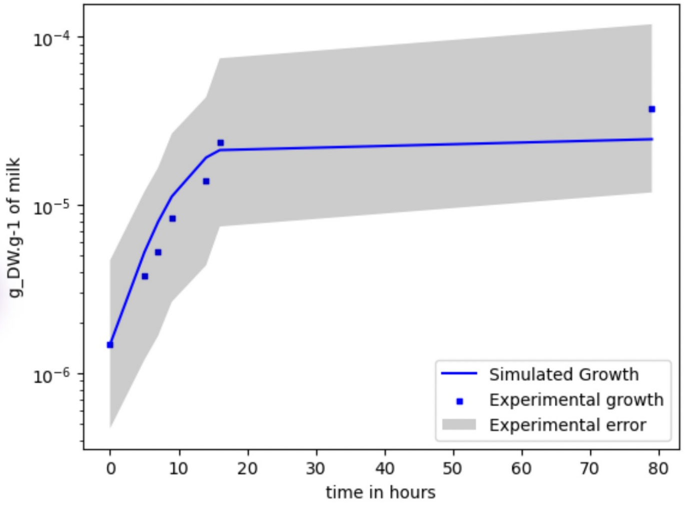
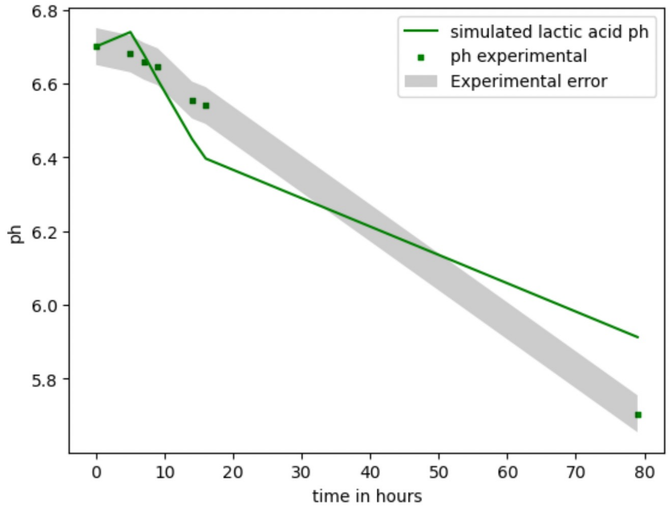










The small number of parameters is sufficient to explain the experimental data

What about metabolomics ?

Community dFBA

Community dFBA model

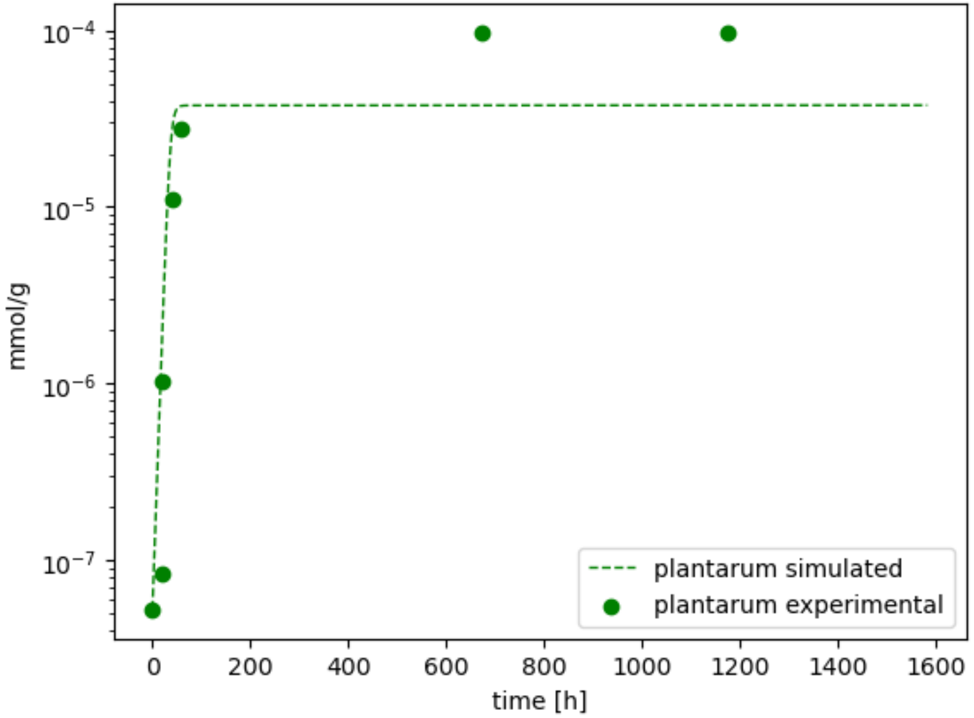
- Consider the change in medium volume
- Each bacterium optimizes its own biomass

Optimised dFBA models for each bacterium

$$\partial_t b_i = q_s(b_i)\mu_{FBA_i}(c)_i b_i + \mathcal{V}(t) b_i$$

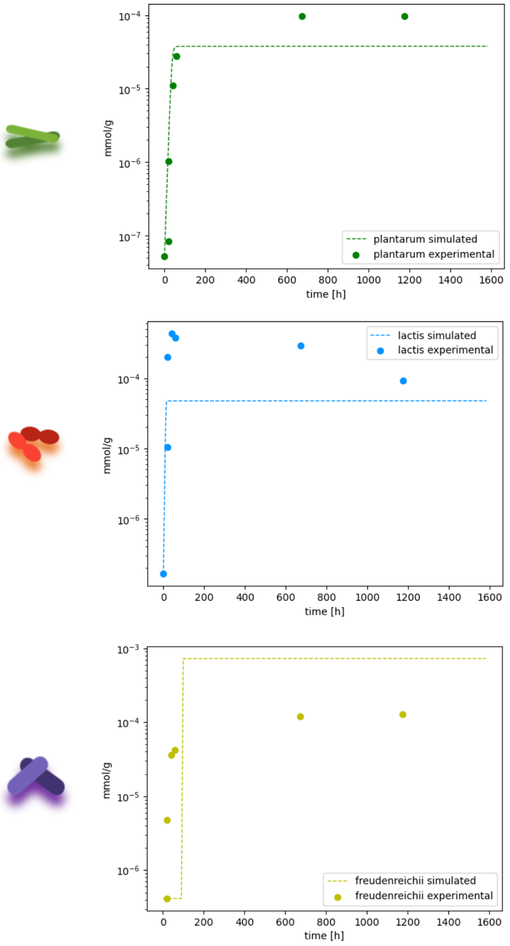
$$\mathcal{V}(t) = \frac{\partial_t v(t)}{V(t)} \qquad \text{where } v(t) = \begin{cases} V_M & \text{if} t \leq t_M \\ V_M + \frac{t-t_M}{t_D-t_M} V_D & \text{if} t_M \leq t \leq t_D \\ V_D & \text{if} t \geq t_D \end{cases}$$

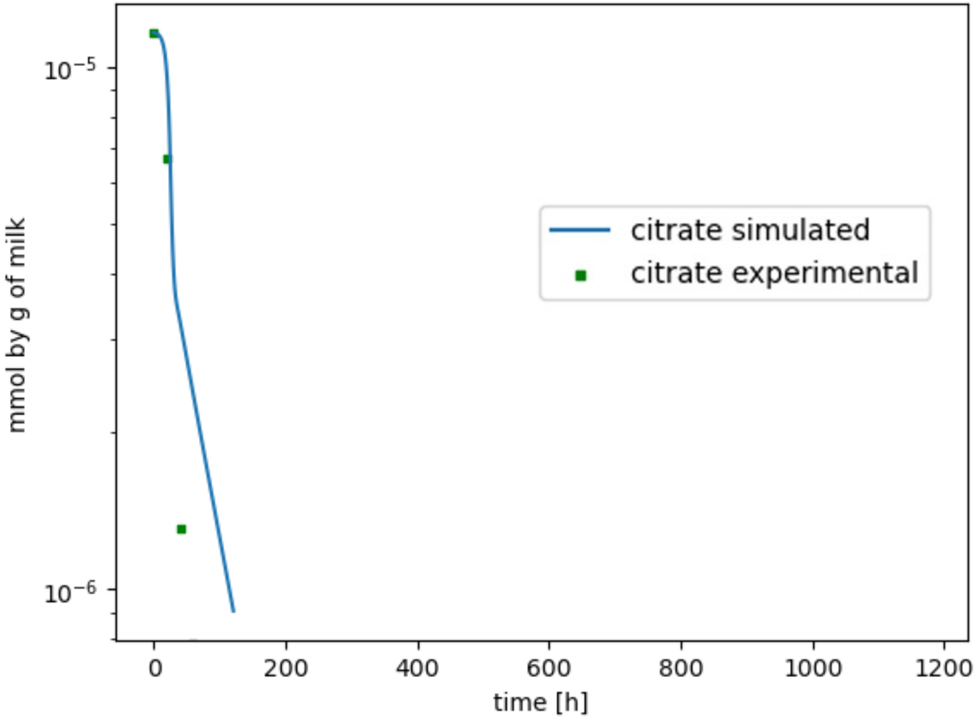
Community dFBA results



Bacterial growth computed with community dFBA fits with experimental data

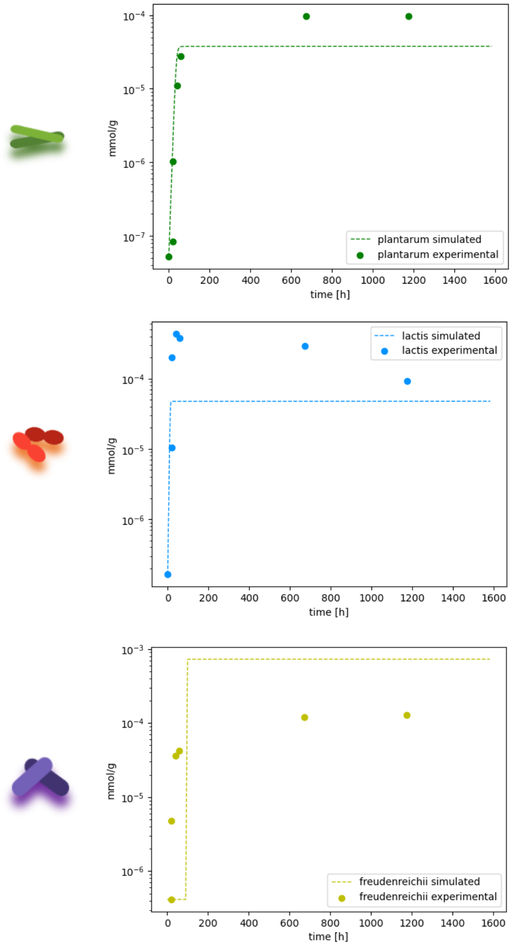
Community dFBA results

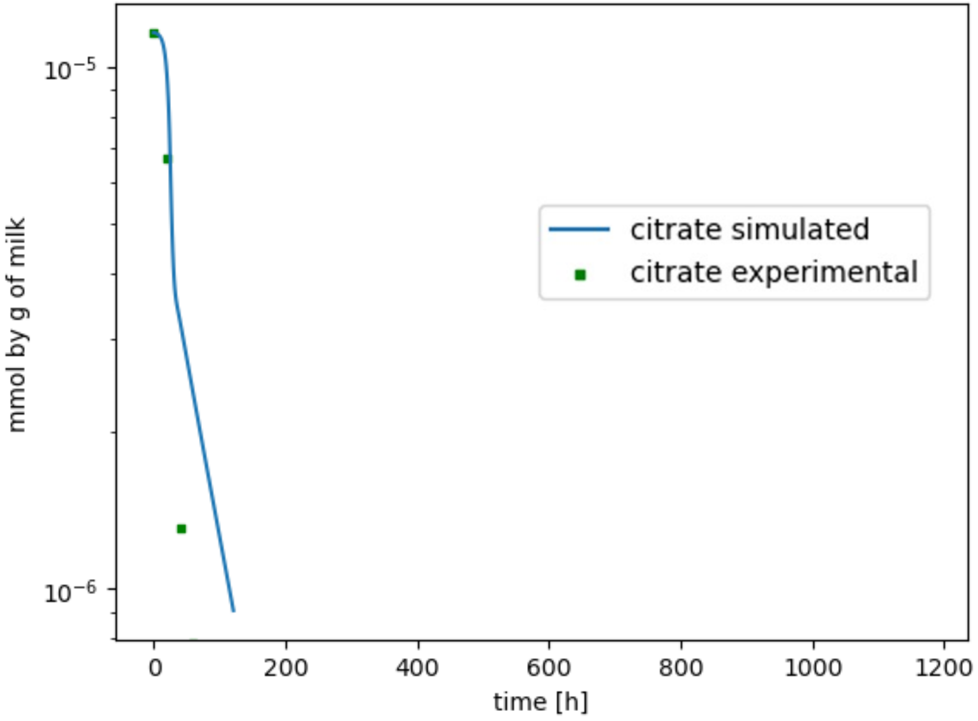


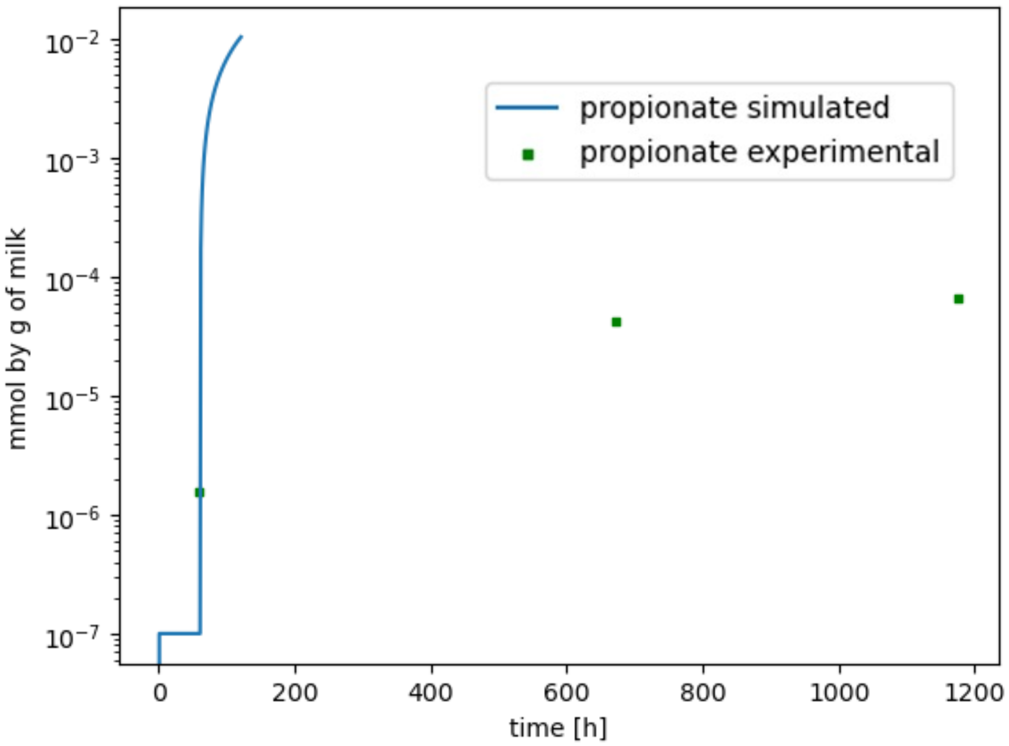


Some metabolites fit with metabolomics data …

Community dFBA results

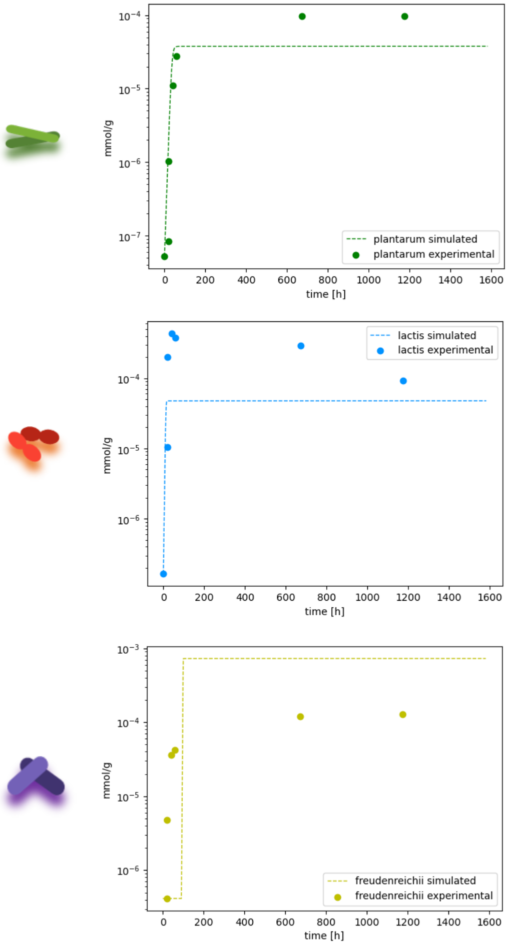


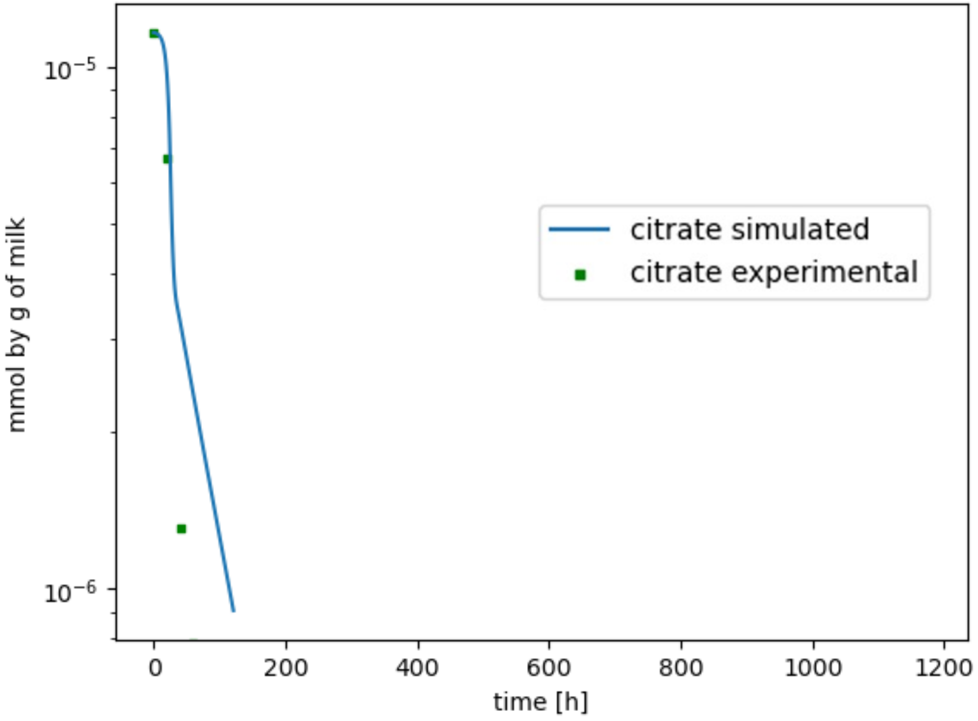


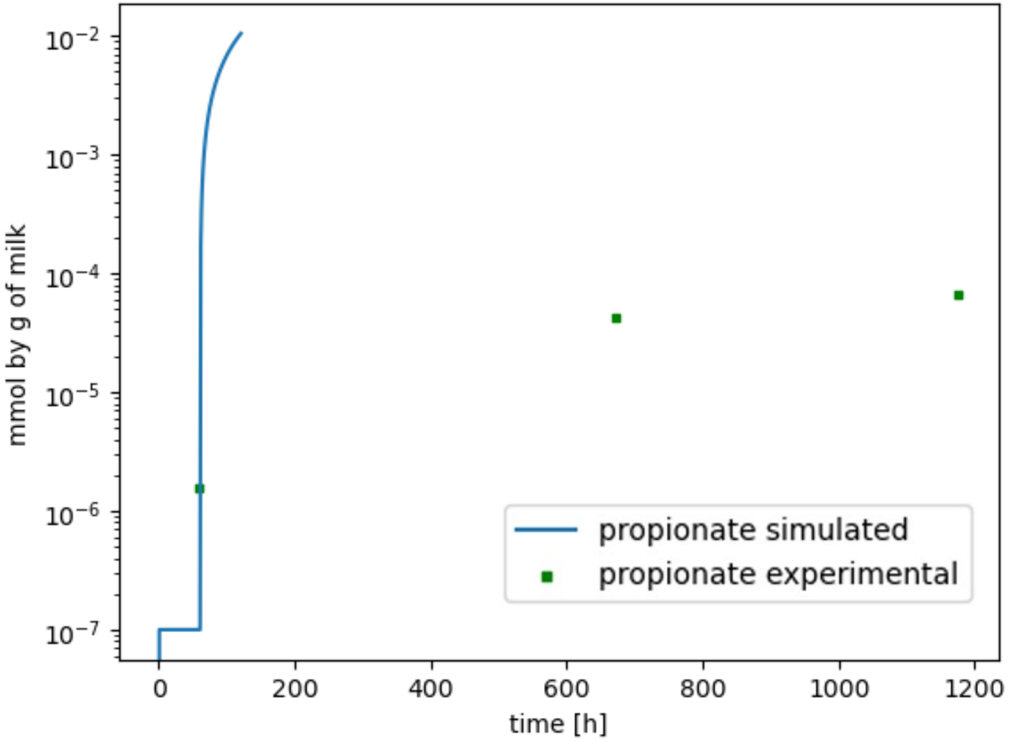


Not all of them

Community dFBA results

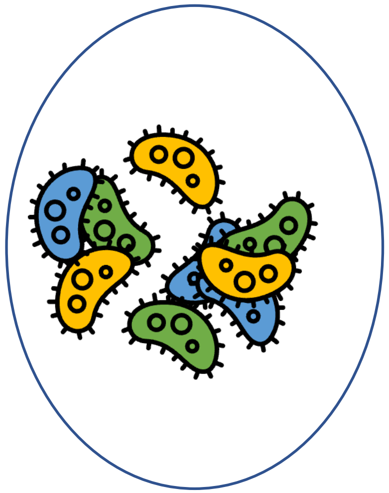




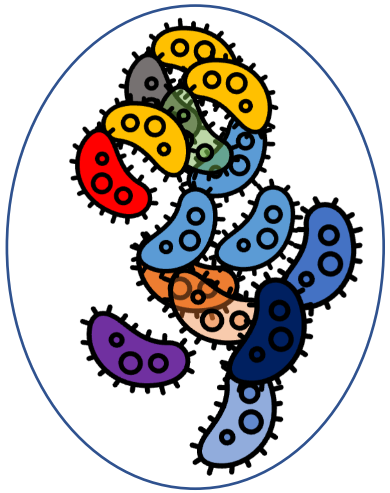


- The model do not capture all modifications brings by the community
- Integration of transcriptomic data is not significantly different
- Is qualitative result enough for characterizing communities ?

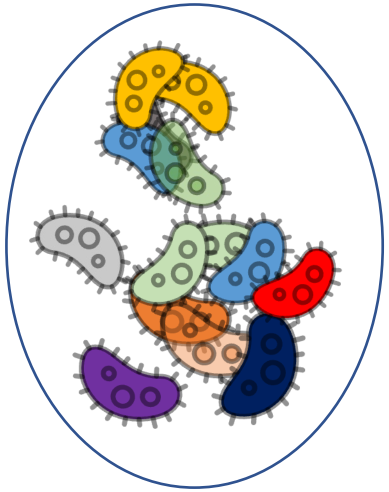
Characterizing natural communities (meta)genomics data



Community 1



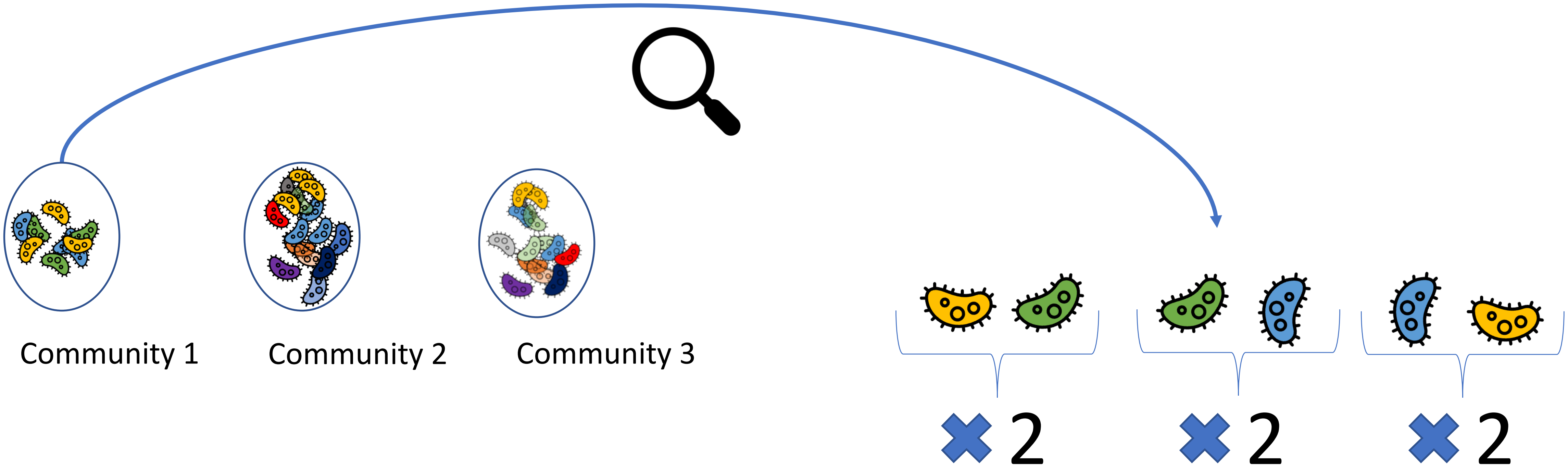
Community 2



Community 3

Calculating cooperation and competition potentials

Characterizing natural communities with (meta)genomics data



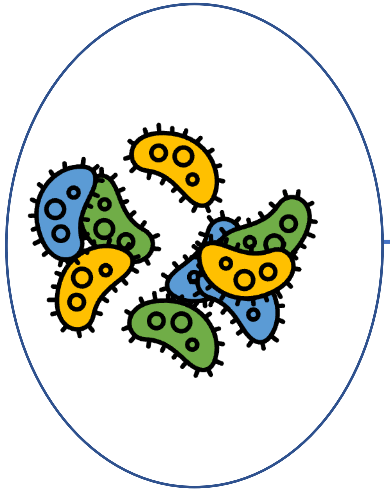
Not easily scalable

- Numerical methods
- Discrete methods

Freilich. S, et al,2011, *Nature Communications*
Zelezniak, A et al,2015,*Proceedings of the National Academy of Sciences of the United States of America*.

Levy et al, 2015.,*BMC Bioinformatics*.
Kreimer, A et al, 2012 *Bioinformatics*.

Discrete modelling of metabolism using metabolic potentials (work in progress)

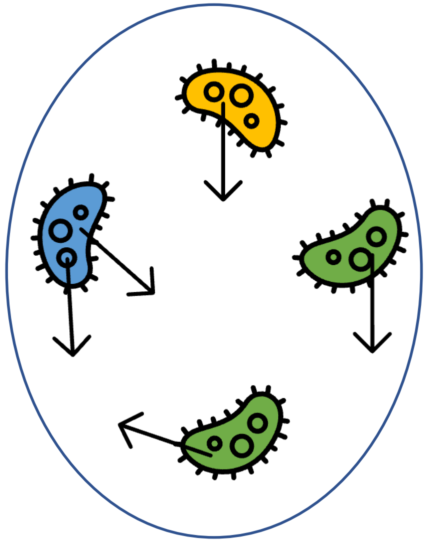


Descriptive

vocabular
using ASP

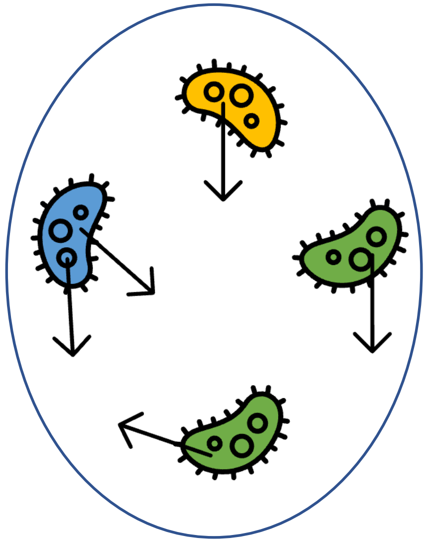
```
%%%%%% putative exchanged metabolites :
cooperation metric

escope(M,T,S) :- pscope(M,S),
reactant(M,_,T),not product(M,_,T),S!=T.
```

Cooperation and competition metrics

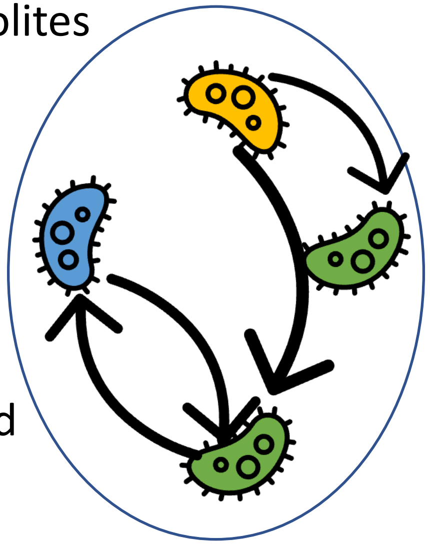


Number of metabolites produced

Cooperation and competition metrics

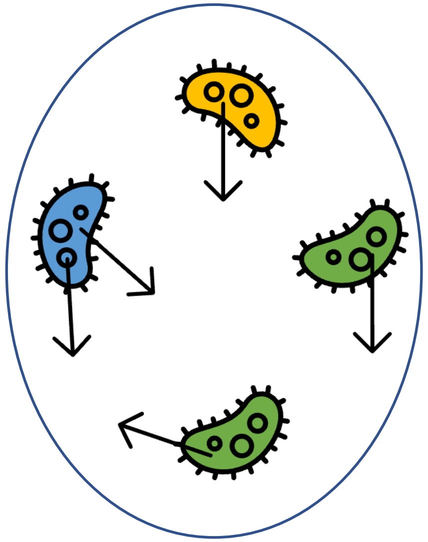


Number of metabolites produced

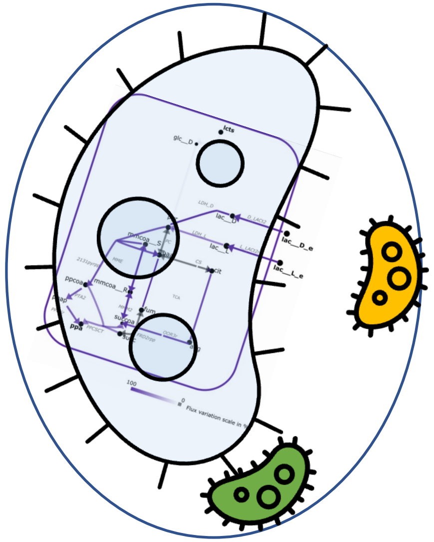


Number of exchanged metabolites

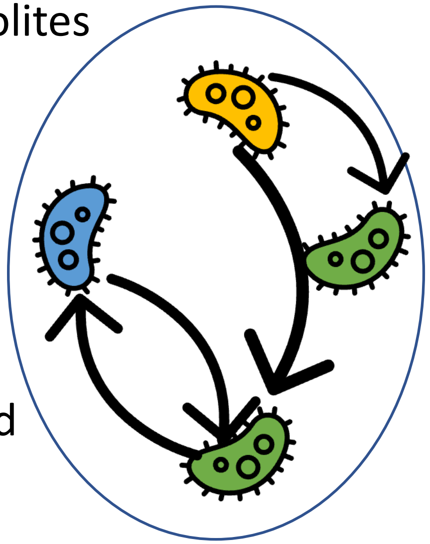
Cooperation and competition metrics



Number of metabolites produced

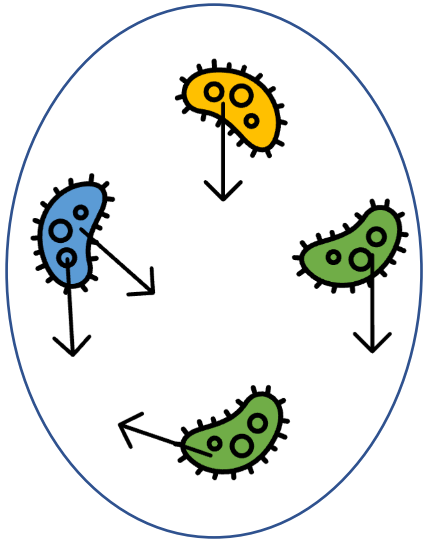


Number of activated reactions

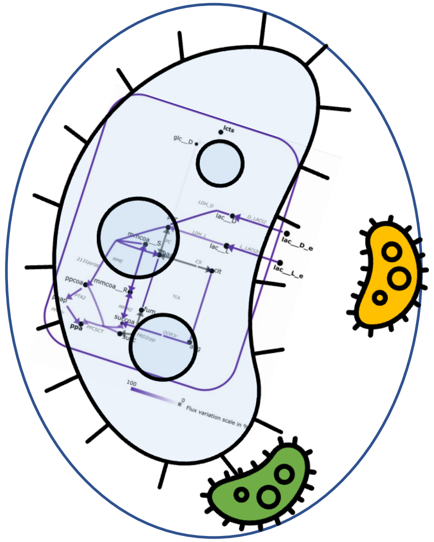


Number of exchanged metabolites

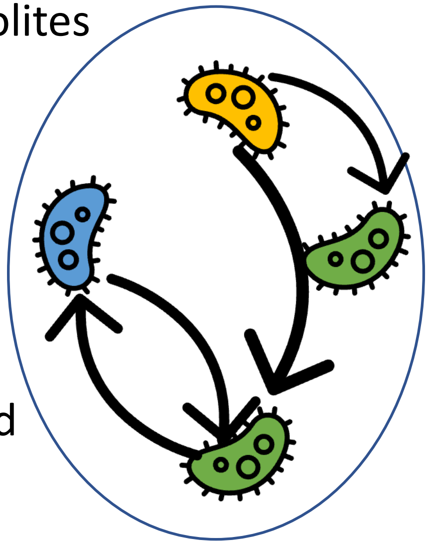
Cooperation and competition metrics



Number of metabolites produced



Number of activated reactions



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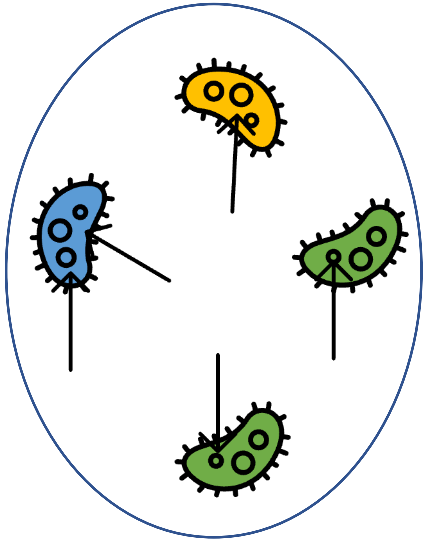
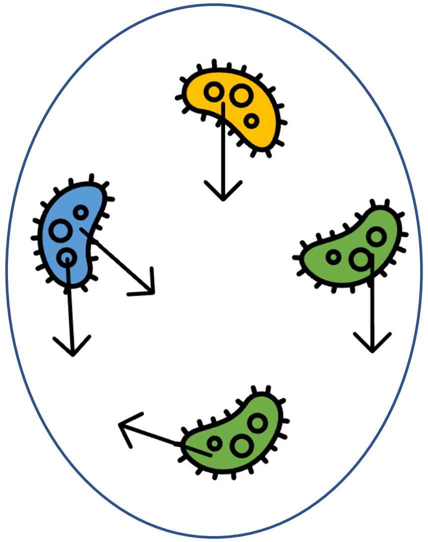
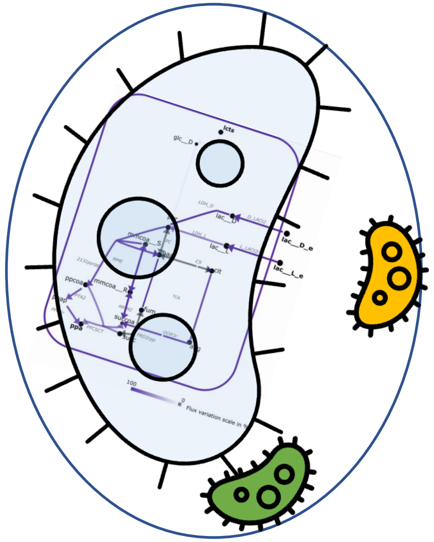


Related to limiting substrates consumed

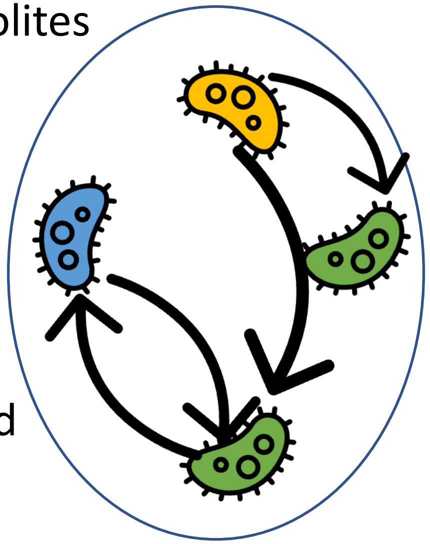
Cooperation and competition metrics



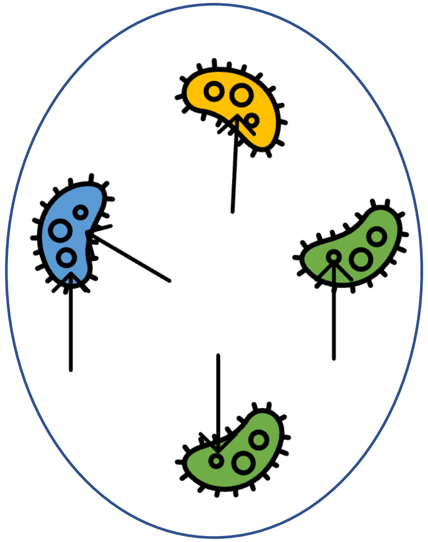
Number of metabolites produced



Number of activated reactions



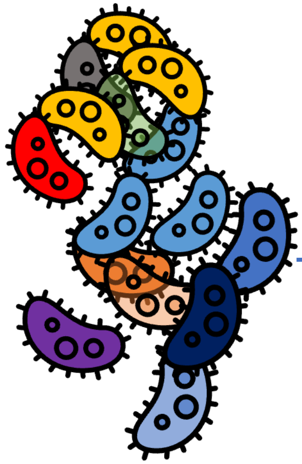
Number of exchanged metabolites



Related to limiting substrates consumed

Unify cooperation metrics to one unique score

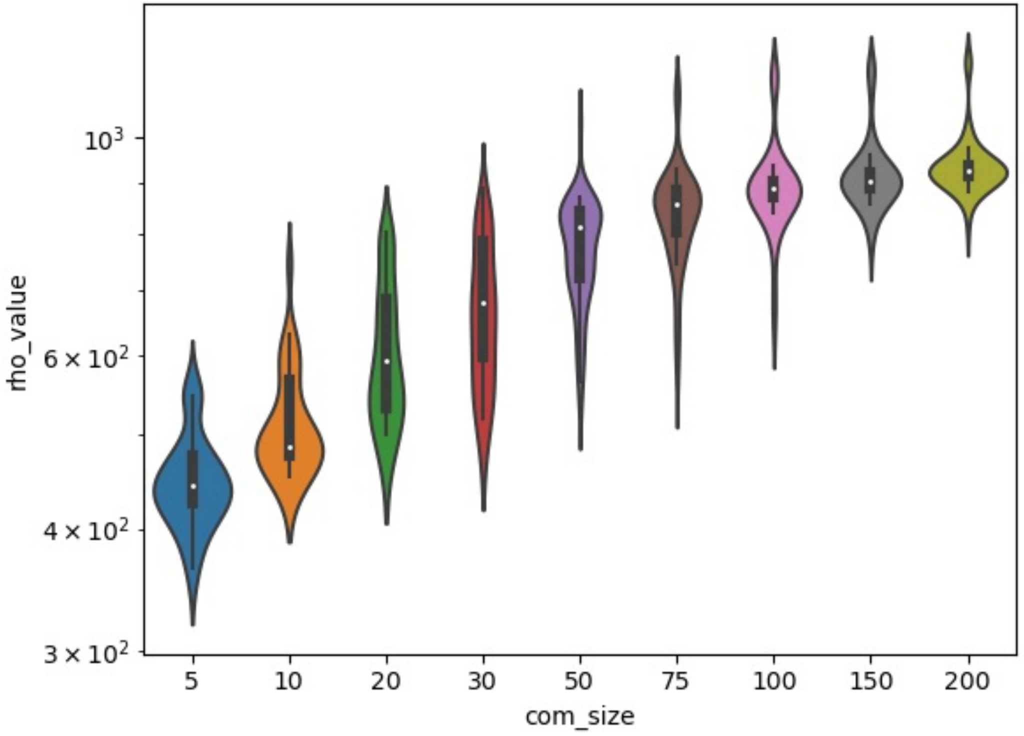
Model test on designed communities



1520 bacteria

50 random communities
of size 5 to 200

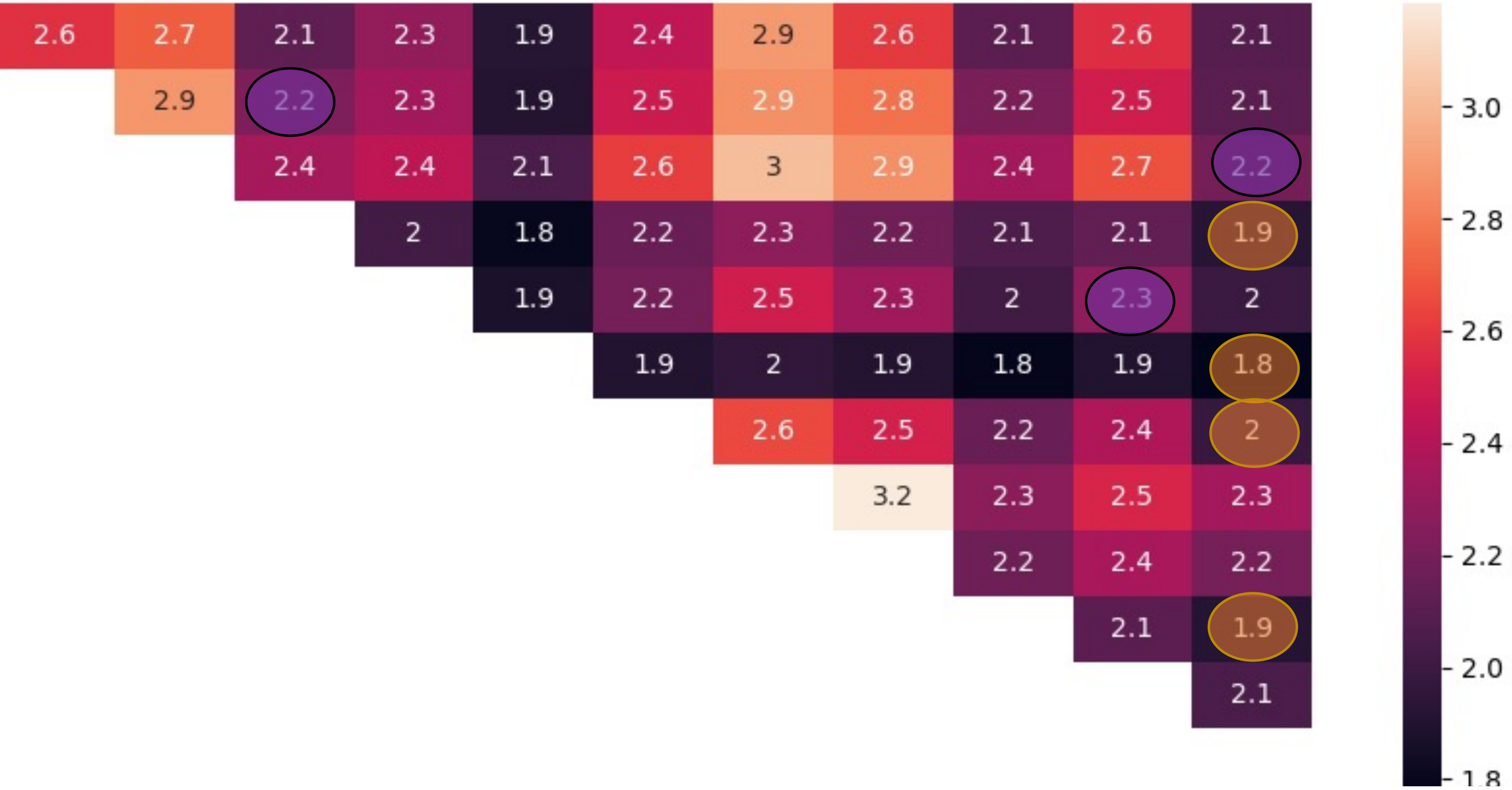
Calculation of
cooperation and
competition metrics

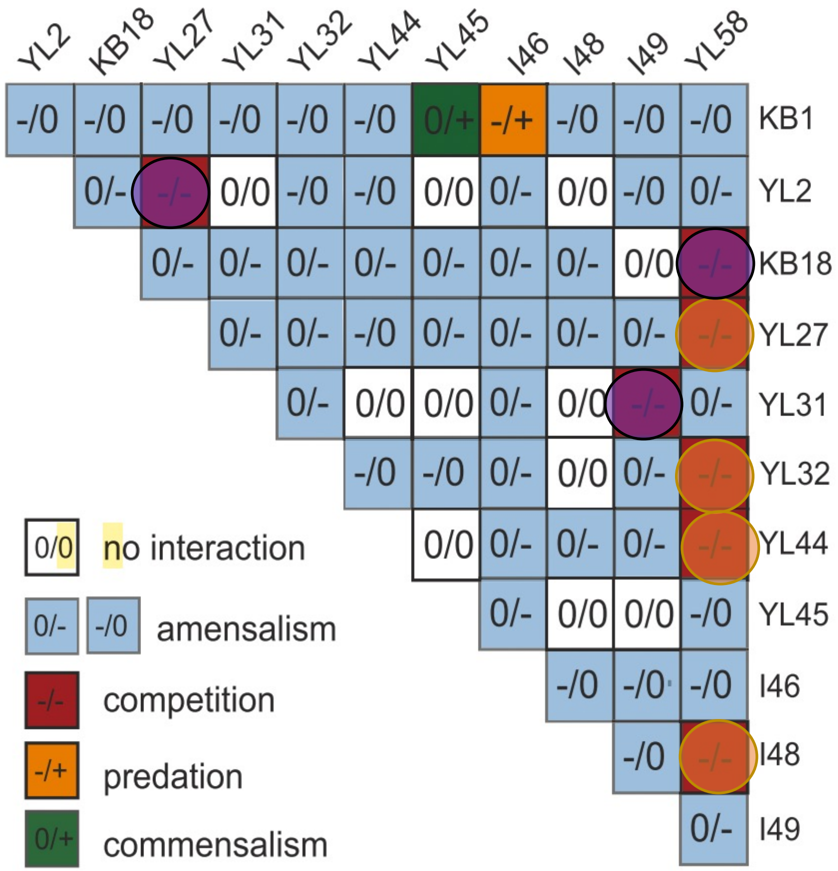


Metrics are not linearly correlate with the community size

Zou, Y., et al, 2019,*Nature Biotechnology*

Competition score test on real data





Identify some correlation between our score and the experimental data

Weiss, A. S., et al,2022, *ISME Journal*

Conclusion

- Numerical accuracy not mandatory to characterize community

- Our method based on discrete modeling seems to characterize natural community → improve cooperation and competition scores

- Test on different communities → Hiring data (genomic data)

- David Sherman
- Clémence Frioux
- Simon labarthe

- Hélène Falentin

Thanks for your attention

Dynamic modeling of metabolism (dFBA)

List of pre-defined interest compounds :

$$\partial_t m_j = \mu_{FBA_i}(c)_j b_i$$

$$c_k = \begin{cases} \lambda_i \max(-m_k/(\Delta_t \sum_{i \in \mathcal{B}} b_i, c_k) & \text{if } 1 \neq k \neq N_m \\ = e_k & \text{if } N_m + 1 \leq k \leq N_c \end{cases}$$

Mahadevan et al, 2002, *Biophysical Journal*

Summary of the interactions in the cheese ecosystem