

Approche hybride de modélisation explicable du métabolisme des écosystèmes microbiens

Hybrid approach for explainable metabolic modelling of microbial ecosystems'

PhD defense of Maxime LECOMTE

November 30, 2023

Membres du jury

Président: SIMON Laurent

Rapportrices: BAROUKH Caroline
COCAIGN-BOUSQUET Muriel

Examinateurs: COTTRET Ludovic
LAROCHE Béatrice
MARKOV Gabriel

Co-direction: David SHERMAN et Hélène FALENTIN
Encadrement: Clémence FRIOUX

École doctorale
Mathématiques
et informatique

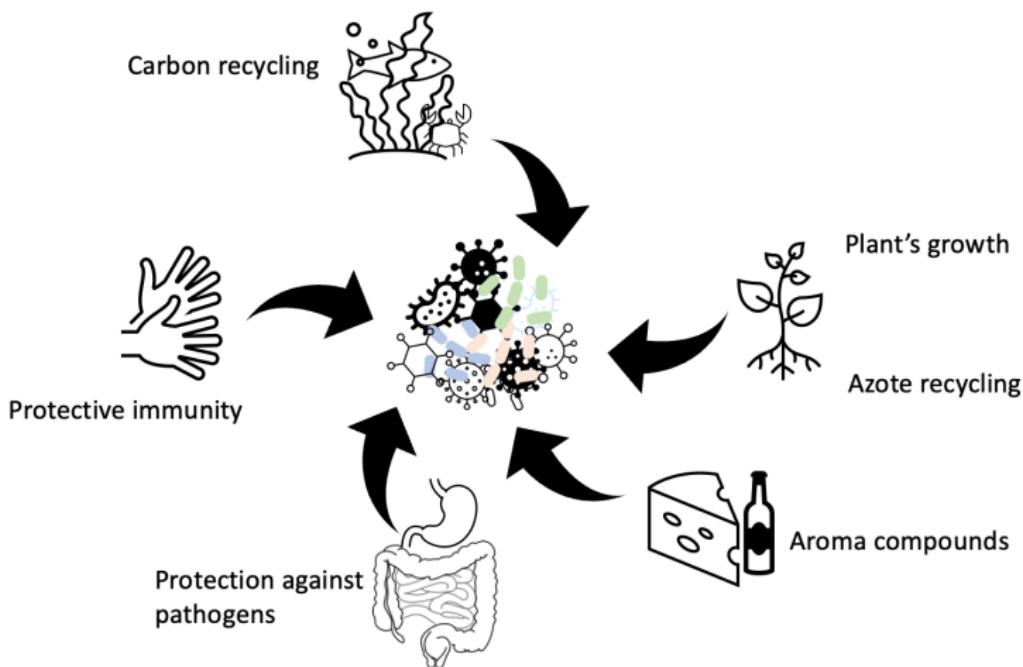
/Université
de BORDEAUX

INRAe

Inria

LaBRI

Why study microorganisms ?



- High diversity of microorganisms in all ecosystems
- Microorganisms roles specific to the environment (Royet and Plailly, 2004; Belkaid and Hand, 2014; Zhang et al., 2015; Hoorman, 2011; McSweeney and Sousa, 2000)

Bacterial interaction are responsible of the observed roles

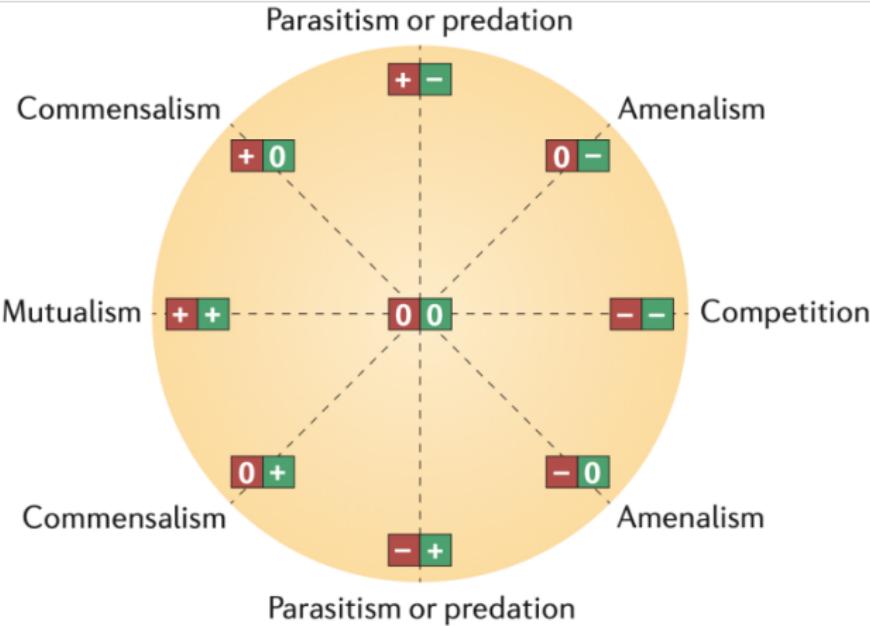


Figure 1: List of different types of bacterial interactions (Faust and Raes, 2012)

- Bacterial interactions are distinguishable within two species
- And within ecosystems composed of thousand of species ? → computational biology

How can we combine biological knowledge and infomatic program ?

Systems biology

System biology

Associate an organism to a system and study the all system (Kitano, 2002)

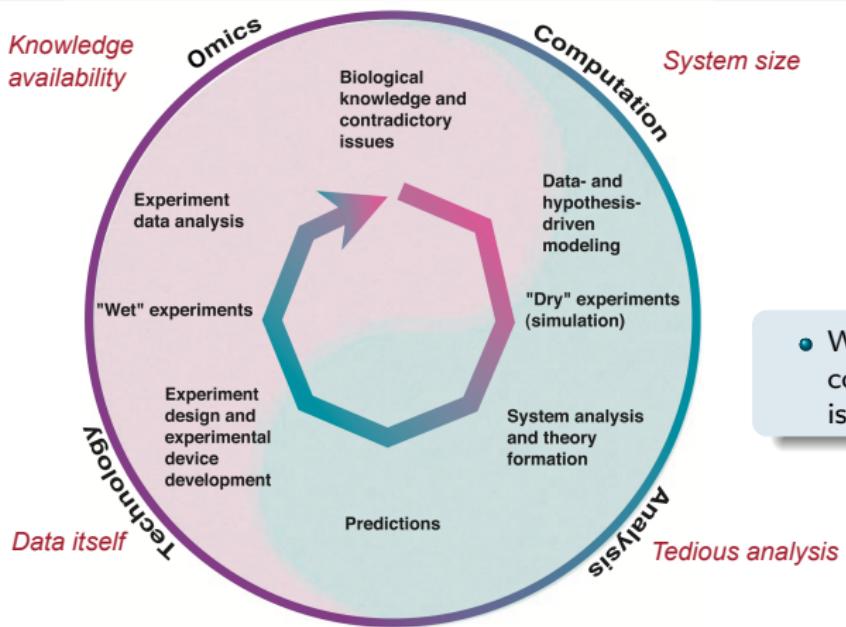


Figure 2: System biology modified from Kitano, 2002

Metabolism as a starter pack for analysing bacterial interactions

Metabolism

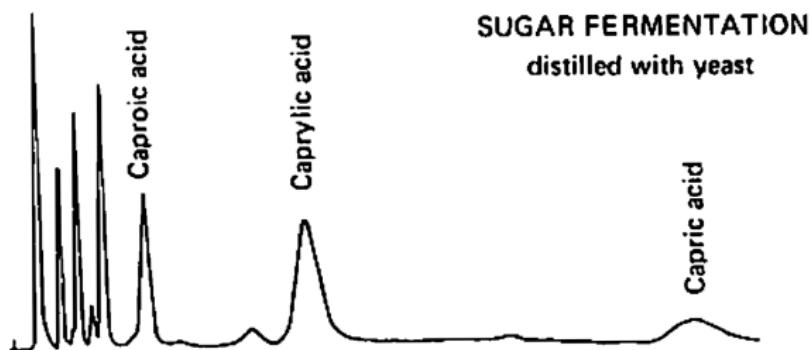


Figure 3: Gas chromatograms of the major aroma compounds isolated from rum (from Suomalainen and Lehtonen, 1978)

Metabolism as a starter pack for analysing bacterial interactions

Metabolism

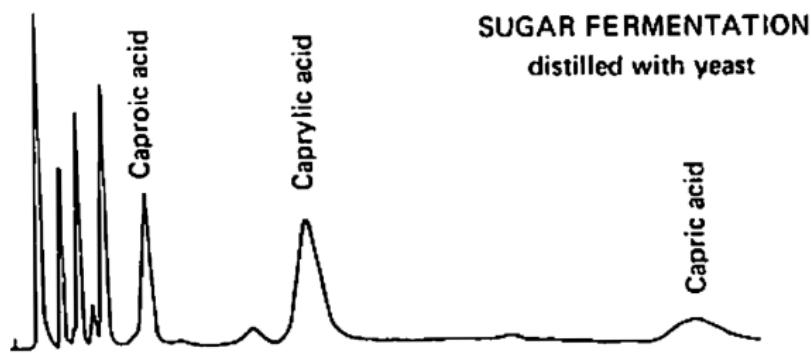


Figure 3: Gas chromatograms of the lower fatty acids produced by yeast in a nitrogen-free sugar fermentation (from Suomalainen and Lehtonen, 1978)

What is metabolism ?

Set of all biochemical reactions occurring in the cell of an organism that permit the production of energy and metabolic goods. (Sánchez López de Nava A, 2023)

What underlying mechanisms are responsible of the observed activity ?

Metabolism and Bacterial interactions

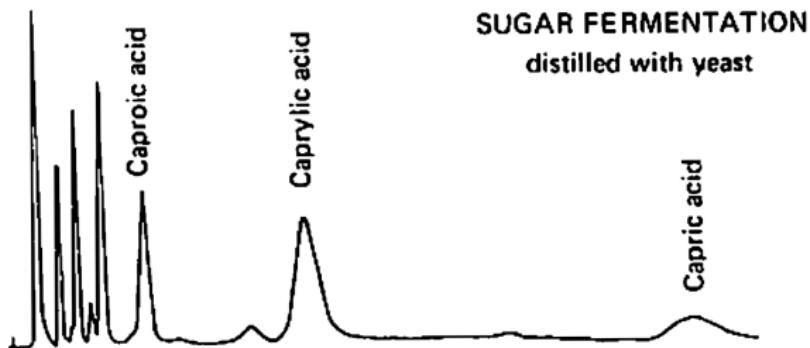
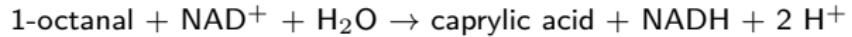


Figure 3: Gas chromatograms of the lower fatty acids produced by yeast in a nitrogen-free sugar fermentation (from Suomalainen and Lehtonen, 1978)

What is metabolism ?

Set of all biochemical reactions occurring in the cell of an organism that permit the production of energy and metabolic goods. (Sánchez López de Nava A, 2023)



What underlying mechanisms are responsible of the observed activity ?

Metabolism and Bacterial interactions

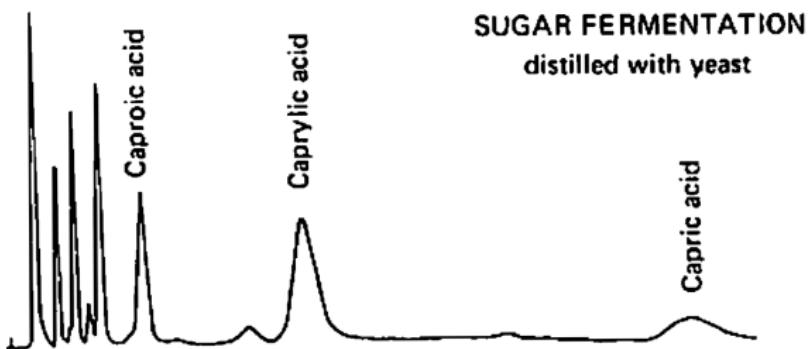
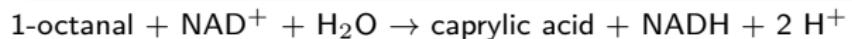


Figure 3: Gas chromatograms of the lower fatty acids produced by yeast in a nitrogen-free sugar fermentation (from Suomalainen and Lehtonen, 1978)

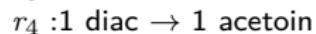
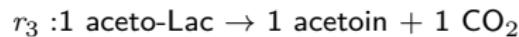
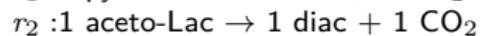
What is metabolism ?

Set of all biochemical reactions occurring in the cell of an organism that permit the production of energy and metabolic goods. (Sánchez López de Nava A, 2023)



- Metabolism of an organism explain observable phenotype
- Is impacted by bacterial interactions

How is the metabolism represented?



How is the metabolism represented?

- $r_1 : 2 \text{ pyr} \rightarrow 1 \text{ aceto-Lac} + 1 \text{ CO}_2$
 $r_2 : 1 \text{ aceto-Lac} \rightarrow 1 \text{ diac} + 1 \text{ CO}_2$
 $r_3 : 1 \text{ aceto-Lac} \rightarrow 1 \text{ acetoin} + 1 \text{ CO}_2$
 $r_4 : 1 \text{ diac} \rightarrow 1 \text{ acetoin}$
 $r_5 : 1 \text{ acetoin} \rightarrow 1 \text{ butanediol}$

Stoichiometry matrix

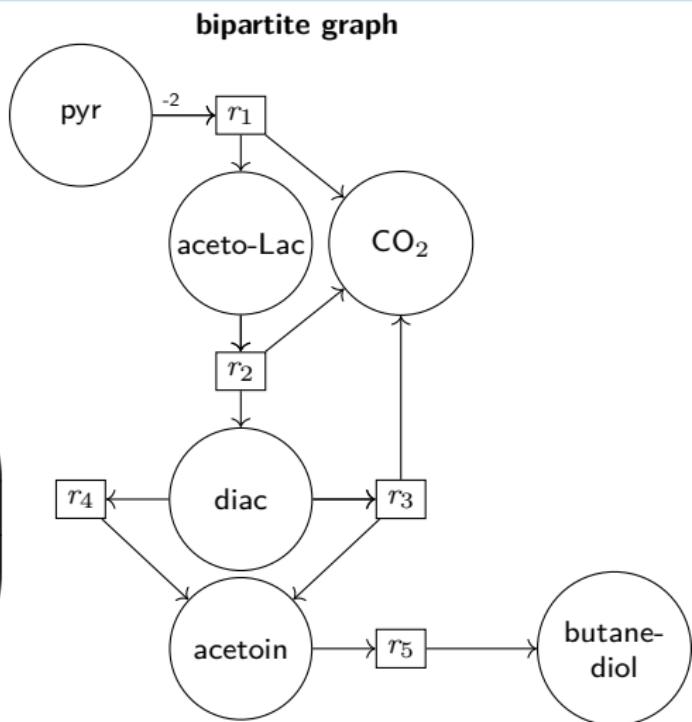
$$\begin{array}{c|ccccc} & r_1 & r_2 & r_3 & r_4 & r_5 \\ \text{pyr} & -2 & 0 & 0 & 0 & 0 \\ \text{aceto-Lac} & 1 & -1 & -1 & 0 & 0 \\ \text{diac} & 0 & 1 & 0 & -1 & 0 \\ \text{CO}_2 & 1 & 1 & 1 & 0 & 0 \\ \text{acetoin} & 0 & 0 & 1 & 1 & -1 \\ \text{butanediol} & 0 & 0 & 0 & 0 & 1 \end{array}$$

How is the metabolism represented?

$r_1 : 2 \text{ pyr} \rightarrow 1 \text{ aceto-Lac} + 1 \text{ CO}_2$
 $r_2 : 1 \text{ aceto-Lac} \rightarrow 1 \text{ diac} + 1 \text{ CO}_2$
 $r_3 : 1 \text{ aceto-Lac} \rightarrow 1 \text{ acetoin} + 1 \text{ CO}_2$
 $r_4 : 1 \text{ diac} \rightarrow 1 \text{ acetoin}$
 $r_5 : 1 \text{ acetoin} \rightarrow 1 \text{ butanediol}$

Stoichiometry matrix

$$\begin{array}{c} & r_1 & r_2 & r_3 & r_4 & r_5 \\ \text{pyr} & -2 & 0 & 0 & 0 & 0 \\ \text{aceto-Lac} & 1 & -1 & -1 & 0 & 0 \\ \text{diac} & 0 & 1 & 0 & -1 & 0 \\ \text{CO}_2 & 1 & 1 & 1 & 0 & 0 \\ \text{acetoin} & 0 & 0 & 1 & 1 & -1 \\ \text{butanediol} & 0 & 0 & 0 & 0 & 1 \end{array}$$



Stoichiometry matrix is commonly used for quantitative analysis instead of **graph**, more focused on topology analysis

How is the metabolism reconstructed?

Genome-scale metabolic network (GSMN) reconstruction

Genome-scale metabolic network (GSMNs)

Contain metabolic reactions predicted from the entire genomic content through gene-protein-reaction (GPR) relationships (Thiele and Palsson, 2010)

$$r1 : (g1 \wedge g2) \vee (g1 \wedge g3)$$

$$r2 : g1 \wedge (g2 \vee g3)$$

Example of trivial boolean GPR relationship

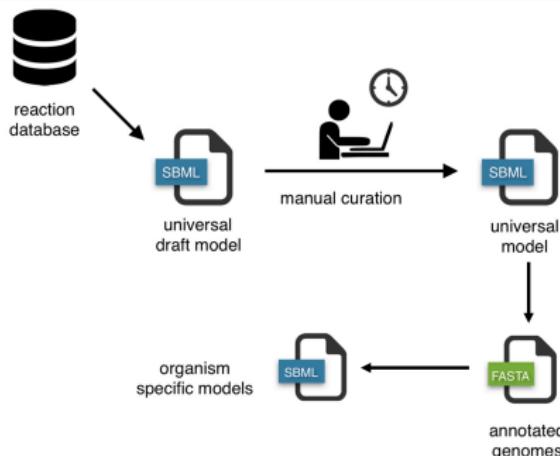


Figure 4: Top down genome-scale metabolic network reconstruction approach (modified from Machado et al., 2018)

- For bacteria: average of 2000 reactions, 1200 genes, 1000 metabolites
- In ecosystem, combinatorial problem occurs

Reasoning-based metabolic analysis

Definition

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

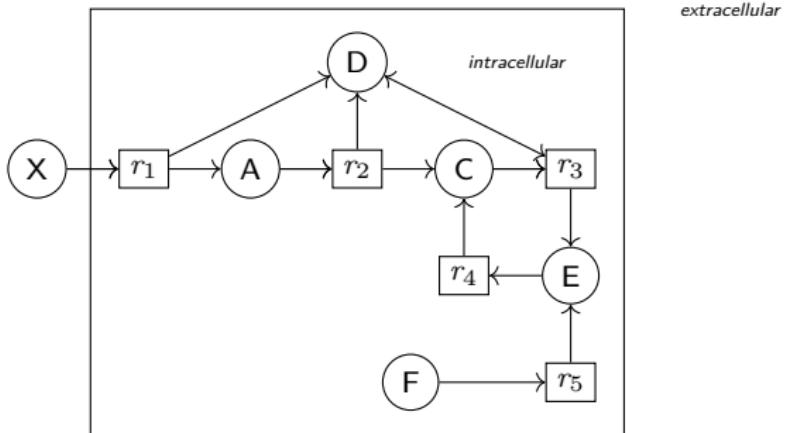
Reasoning-based metabolic analysis

Definition

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



How to compute metabolic capability ?

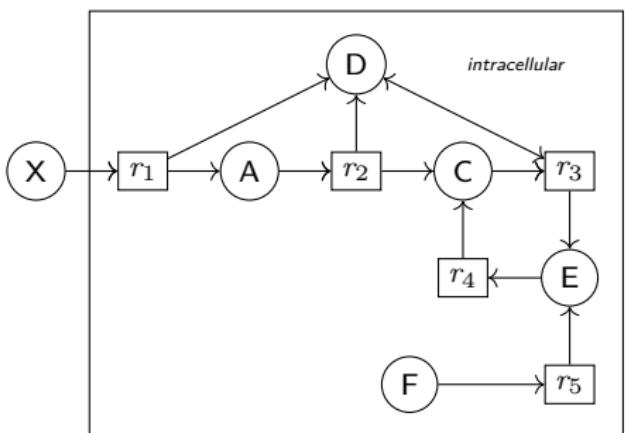
Reasoning-based metabolic analysis

Definition

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



extracellular

- Producibility is initiated by the presence of nutrients,
- The products of a reactions are producible if all reactants of this reaction are themselves producible

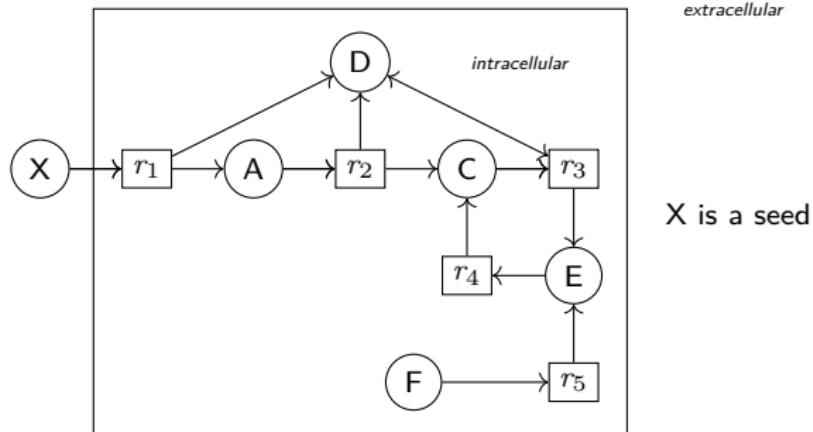
The scope, *i.e.* the metabolic capacity, a network is reached in 2 logical rules (Ebenhöh, Handorf, and Heinrich, 2004)

Reasoning-based metabolic analysis

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches

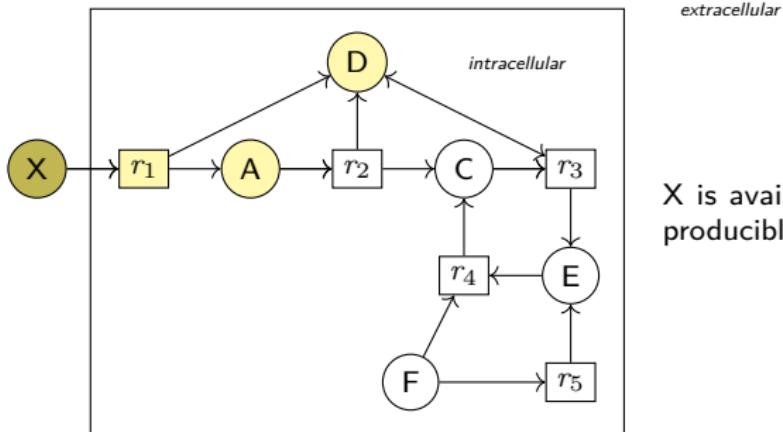


Reasoning-based metabolic analysis

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



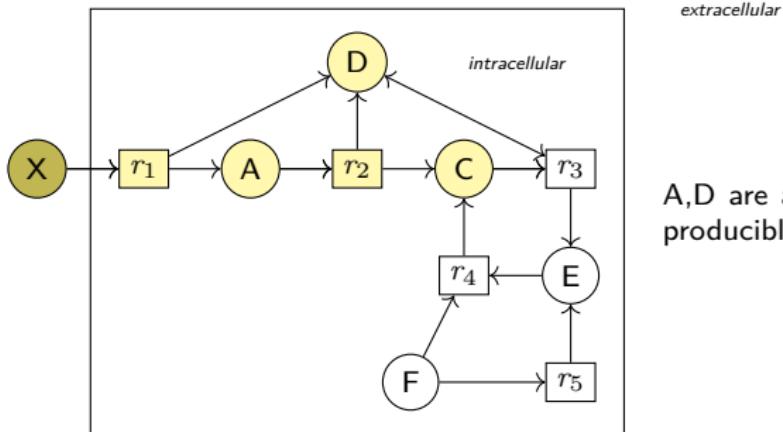
X is available; r_1 is activated and A,D are producible

Reasoning-based metabolic analysis

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



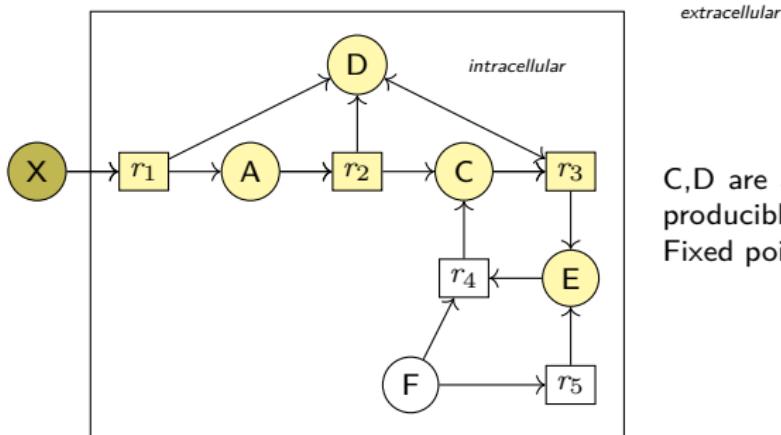
A,D are available; r_2 is activated and C is producible

Reasoning-based metabolic analysis

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



C,D are available; r_3 is activated and E is producible
Fixed point : E

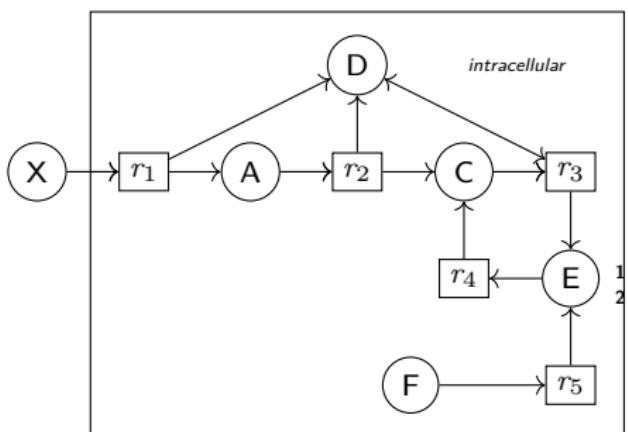
- The potential metabolic capability and topology dependant

Logical rules implementation

Reasoning-based

Allow us to infer qualitative models from logical rules based on biological knowledge

topological-based approaches



extracellular

- Producibility is initiated by the presence of nutrients,
- The products of a reactions are producible if all reactants of this reaction are themselves producible

```
scope(M) :- seed(M).
scope(M) :- bacteria(B), product(M,R,B),
reaction(R,B), scope(M2) : reactant(M2,R,
B).
```

Logical rules are embedded using Answer Set Programming (Lifschitz, 2008)

Why using Answer Set Programming

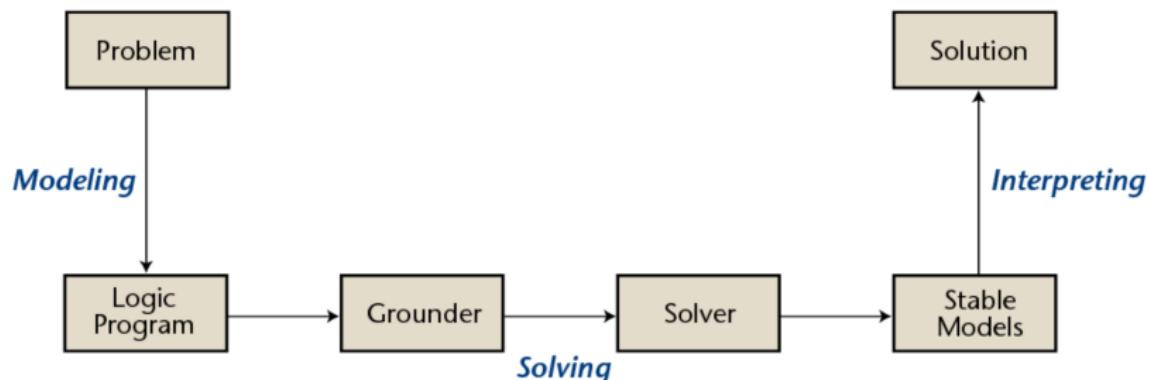


Figure 5: The workflow of Answer set programming (ASP) (Kaufmann et al., 2016)

- Close assumption
- Mechanistic model
- Solve combinatorial problems

Numerical metabolic model of the metabolism

definition

Metabolic model

From a GEM, a model metabolic has the capacity to simulate and to predict on the metabolic activity



Numerical metabolic model of the metabolism

Flux Balance Analysis (Orth, Thiele, and Palsson, 2010)

Metabolic model

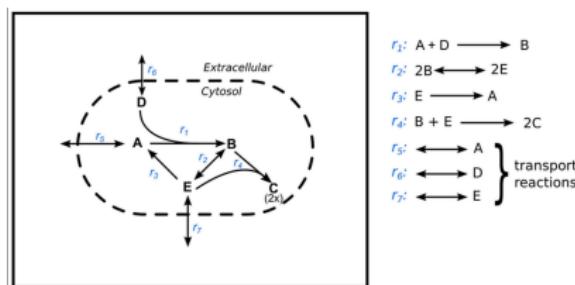
From a GEM, a model metabolic has the capacity to simulate and to predict on the metabolic activity

Constraint-based approaches

maximiser/minimiser f_{obj}

tel que $(S, y)_{int} = 0$

et $v_{j_{\min}} < v_j < v_{j_{\max}}$



	r_1	r_2	r_3	r_4	r_5	r_6	r_7
A	-1	0	1	0	1	0	0
B	1	-2	0	-1	0	0	0
S = C	0	0	0	2	0	0	0
D	-1	0	0	0	0	1	0
E	0	2	-1	-1	0	0	1

$$\vec{v} = \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ v_7 \end{bmatrix}$$

Metabolic flux values

Figure 7: A. Stoichiometry matrix representation and the flux vector \mathbf{v}

Figure 6: Example of metabolic network

Numerical metabolic model of the metabolism

Flux Balance Analysis (Orth, Thiele, and Palsson, 2010)

Metabolic model

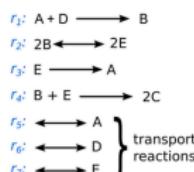
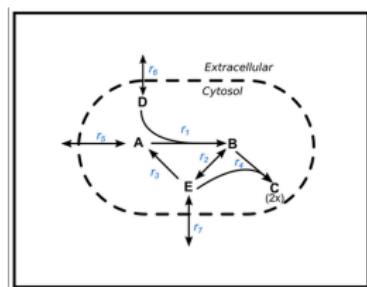
From a GEM, a model metabolic has the capacity to simulate and to predict on the metabolic content.

Constraint-based approaches

maximiser/minimiser f_{obj}

tel que $(S.v)_{int} = 0$

et $v_{i_{\min}} \leq v_i \leq v_{i_{\max}}$



Subject to

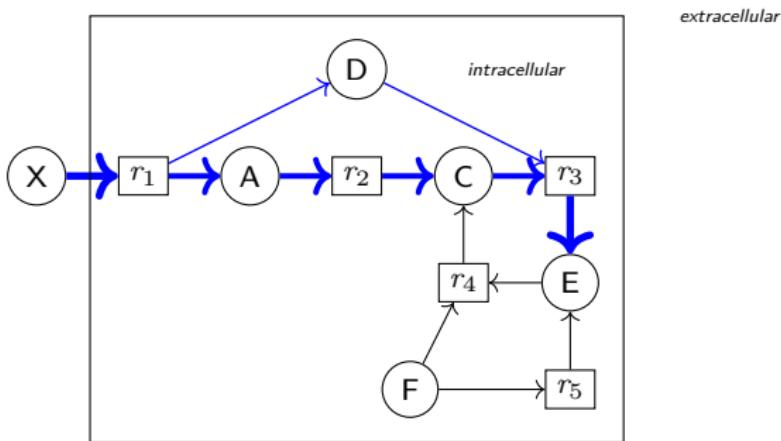
$$\mathbf{S}\vec{v} = \vec{0} = \begin{cases} \frac{dA}{dt} = -v_1 + v_3 + v_5 & 0 \leq v_1 < \infty \\ \frac{dB}{dt} = v_1 - 2v_2 - v_4 & -\infty < v_2 < \infty \\ \frac{dC}{dt} = 2v_4 & 0 \leq v_3 < \infty \\ \frac{dD}{dt} = -v_1 + v_6 & 0 \leq v_4 < \infty \\ \frac{dE}{dt} = 2v_2 - v_3 - v_4 + v_7 & 0 \leq v_5 \leq \infty \\ & -\infty < v_6 < \infty \\ & 0 \leq v_7 \leq \infty \end{cases}$$

(Steady state system) (Reaction bounds)

Figure 7: B. Linear programming problem.

Figure 6: Example of metabolic network

Flux application



- Reaction flux depending of the stoichiometry coefficient
- Can explain metabolic observations through reaction fluxes
- difficult to apply to large-scale: computational cost, cultivated species

Contributions and objective

Objective

Contribute to analyzing metabolic interactions of bacterial communities associated to two use cases: controlled and uncontrolled environment

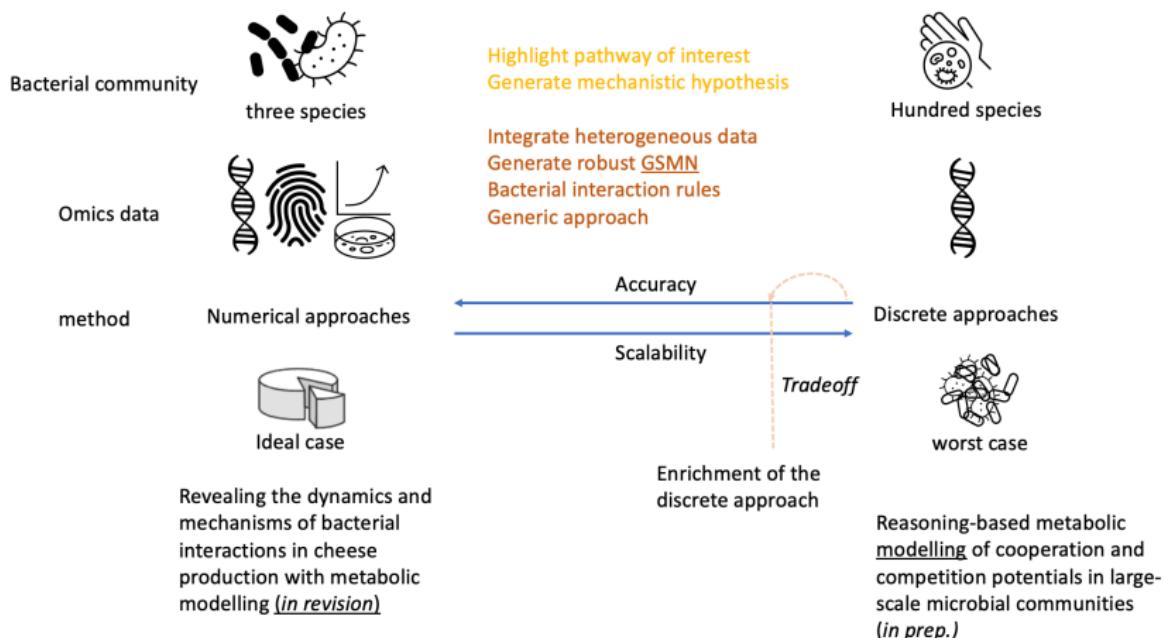
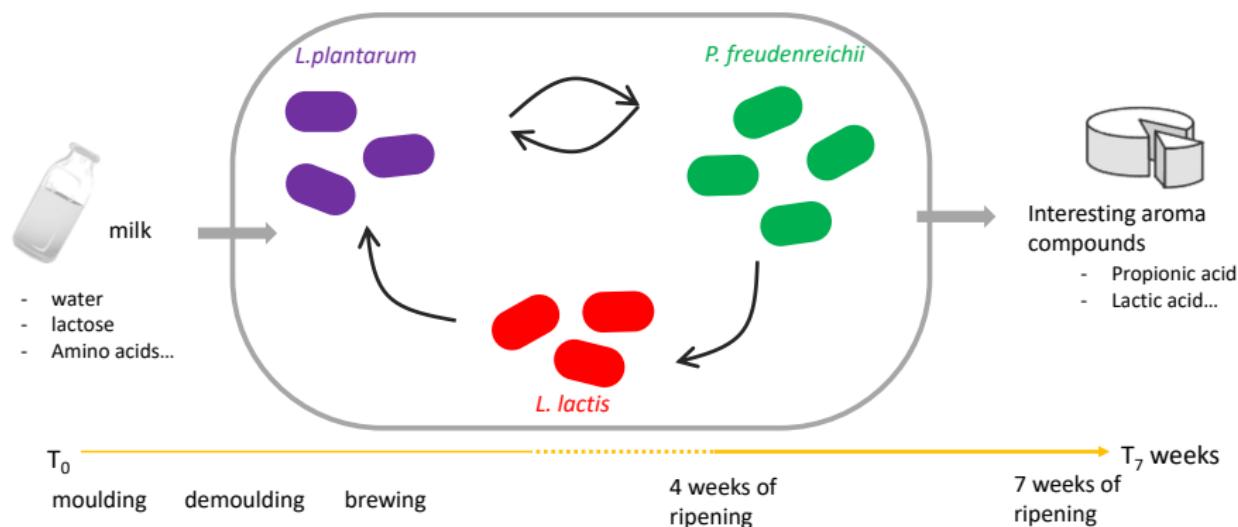


Figure 8: Contributions in my thesis. In yellow and brown-red are respectively biological and methodological contributions

Biological context: cheese bacterial fermentation



Heterogenous data are available for analysing bacterial fermentation

Multi-omics strategy



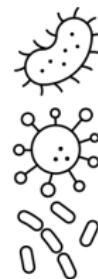
Annotated genomes



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

Dynamic and numerical model of the metabolism can integrate all the data

Refinement of metabolic networks - 1

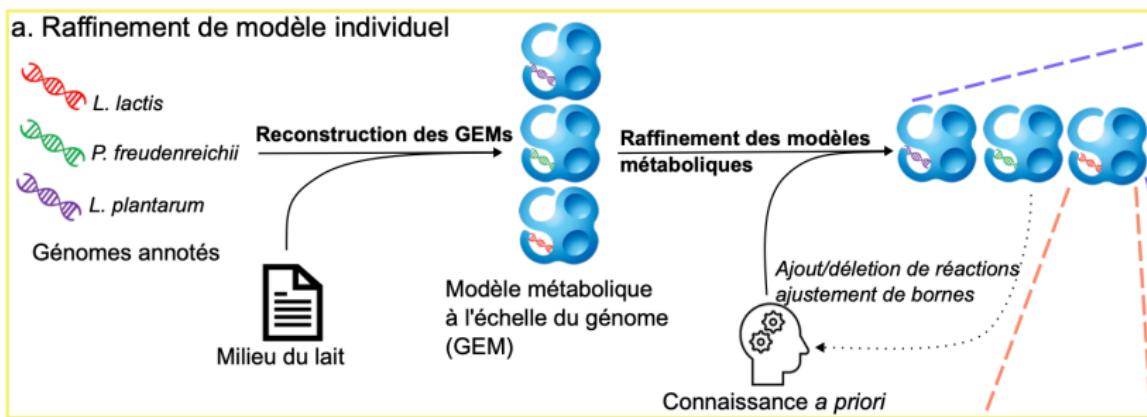
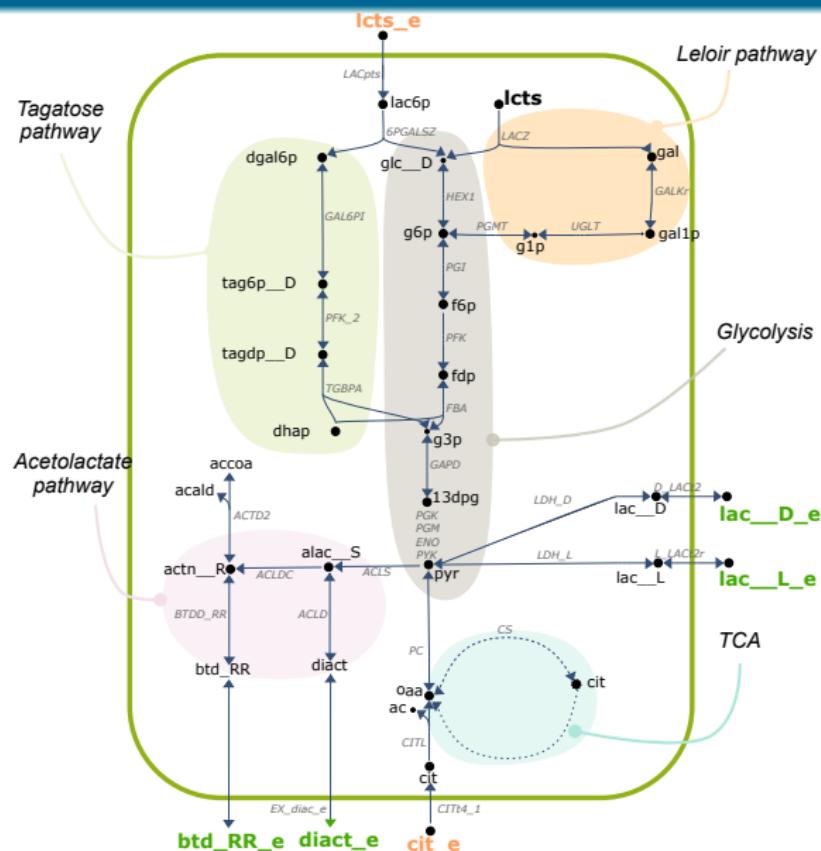


Figure 9: First part of the numerical workflow

- Adding/removing reactions
- Qualitative check of existing pathway and metabolic goods
- Tedious analysis

Refinement of metabolic networks - 2

L. lactis case

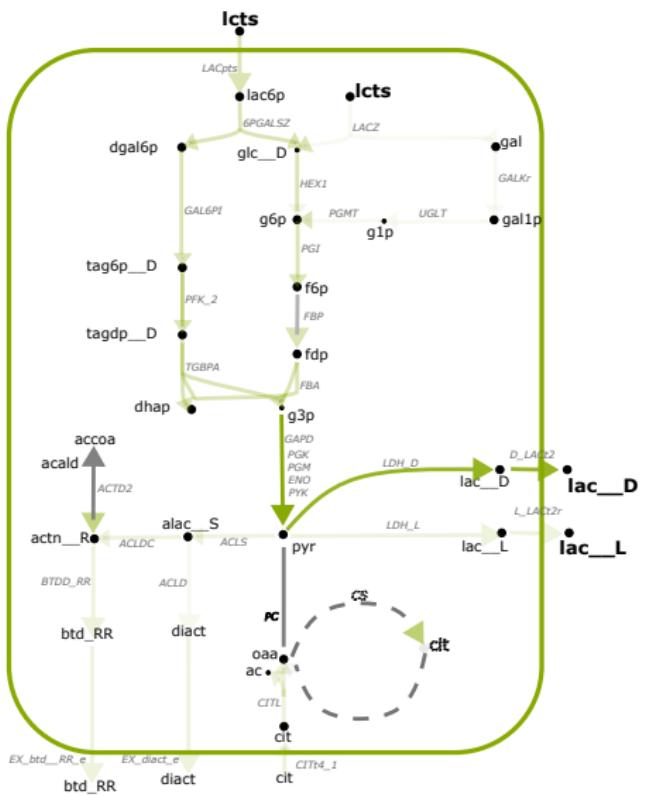


Objectif

- Production of diacetyl
- activation of acetolactate pathway

Refinement of metabolic networks - 2

L. lactis case



Objectif

- Production of diacetyl
- activation of acetolactate pathway

Refinement

- Acétoine-dehydrogenase was blocked (ACTD2)
- Modification of bounds: acétolactate decarboxylase (ACLDC) and acétolactate synthase (ACLS)

Dynamic individual calibration

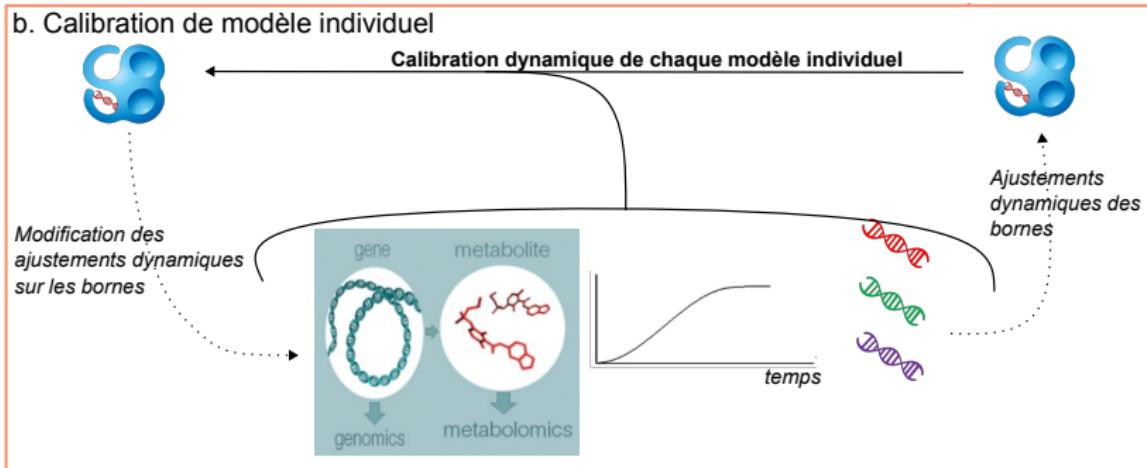
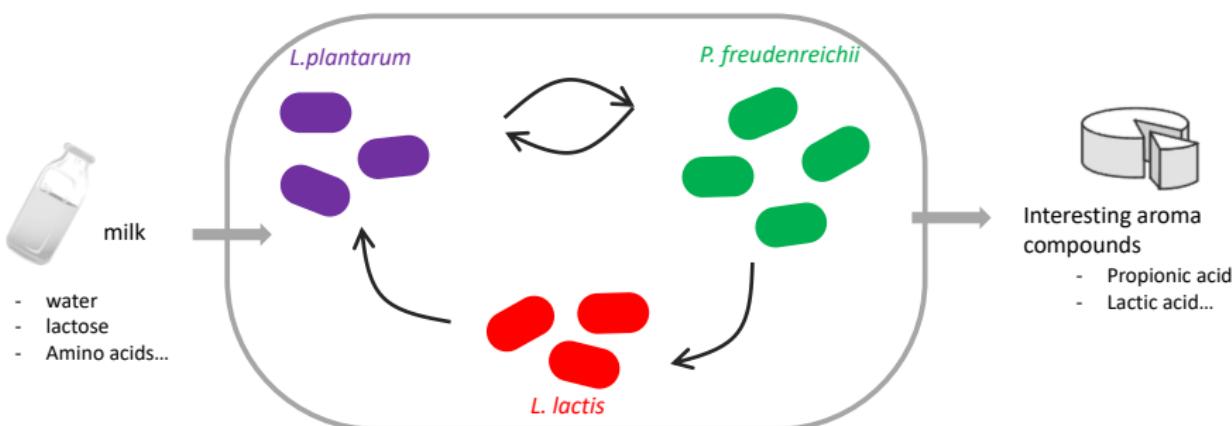


Figure 10: Second part of the numerical workflow

- Finding optimal parameters for explaining **individual** biological observations
- Quantitative check of metabolic goods and biomass density

Bacterial fermentation: a dynamic process



Challenge

- Nutrient concentration over time
- The dynamic of bacterial density
- Resource sharing

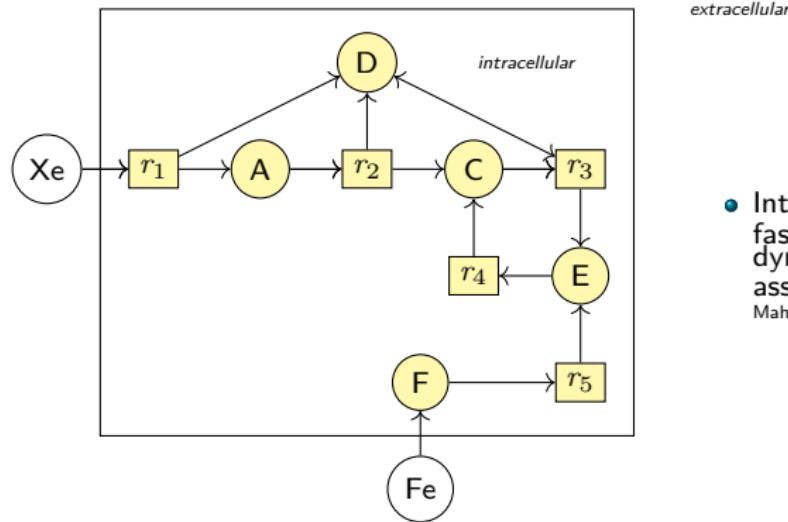
Solution

- Compute dynamic model of the metabolism (dFBA) (Mahadevan, Edwards, and Doyle, 2002)

Dynamic modeling of the metabolism (dFBA)

What is a dynamic version of the FBA

FBA is solved at each time step.

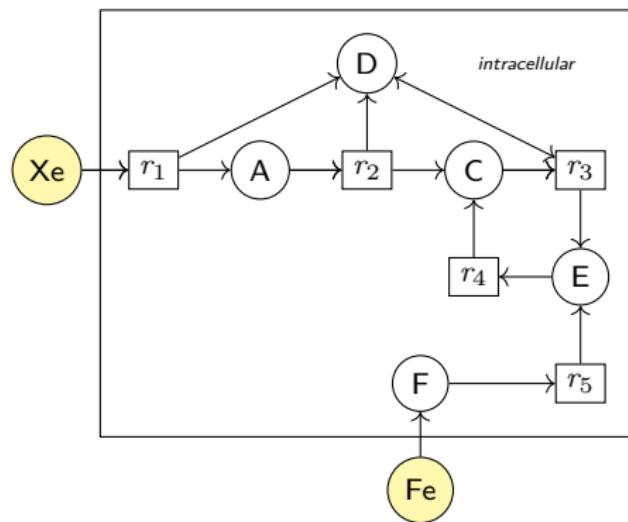


- Intracellular dynamics are faster than the extracellular dynamics → Steady-state assumption (de Oliveira, Le Roux, and Mahadevan, 2023)

Dynamic modeling of the metabolism (dFBA)

What is a dynamic version of the FBA

FBA is solved at each time step.



extracellular

- Intracellular dynamics are faster than the extracellular dynamics → Steady-state assumption (de Oliveira, Le Roux, and Mahadevan, 2023)
- Apply mass balance equations to describe the behavior of the extracellular metabolites (de Oliveira, Le Roux, and Mahadevan, 2023)

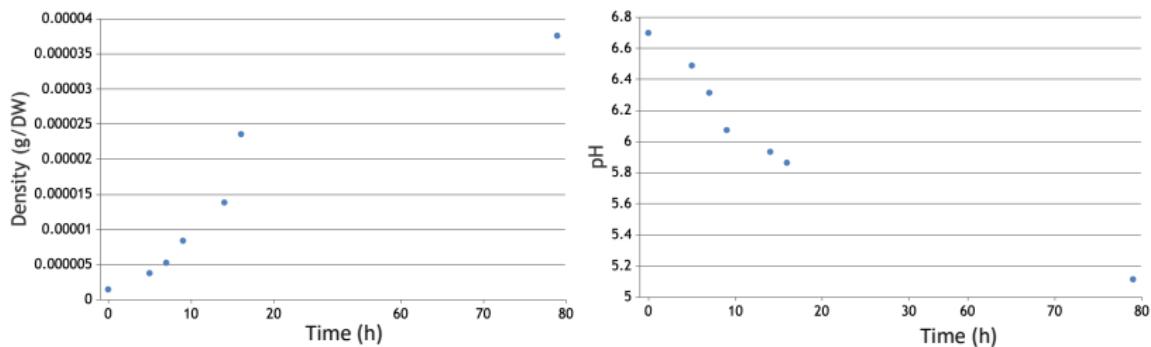
Objectif of the individual metabolic model calibration

Goal

Make individual GSMM accurate for inferring mechanistic bacterial behavior

LAB

$$J(b_i, pH | \theta_i, b_{i,exp}, pH_{exp}) = \left\| \frac{\log_{10}(b_i) - \log_{10}(b_{i,exp})}{\sigma_{\log, i, exp}} \right\|^2 + \alpha \left\| \frac{pH - pH_{exp}}{\sigma_{pH, exp}} \right\|^2 \quad (1)$$



- Without adding extra nutrient
- Experimental and simulated data must fit

Objectif of the individual metabolic model calibration

Goal

Make individual GSMM accurate for inferring mechanistic bacterial behavior

P. freudenreichii

$$J(b, m | \theta_i, b_{exp}, m_{exp}) = \left\| \frac{\log_{10}(b) - \log_{10}(b_{exp})}{\sigma_{\log, b, exp}} \right\|^2 + \alpha \left\| \frac{m - m_{exp}}{\sigma_{m, exp}} \right\|^2 \quad (1)$$

Acides	Concentration T_0	Concentration $T_f = 89$ h	Erreur standard
Lactate	16.5	7.88	0.08
Acetate	0	3.07	0.02
Succinate	0	0.371	0.063
Propionate	0	8.31	0.02

Table 1: Acids data in g/L

- Adding lactate in the nutritional environment
- Experimental and simulated data must fit

Dynamic modulations

Defining the usual consumption limitation in the model

available for a specific set of metabolites

$$c_{min,i,j}^{ex} = \max\left(-\frac{m_j}{\Delta t * \sum_{i \in \mathcal{M}_j} b_i}, v_{i,j}^{int}\right) \quad (2)$$

- \mathcal{M}_j Bacteria subset can metabolize j
- $v_{i,j}^{int}$ close to the FBA value
- Balanced resource sharing

Lactose consumption case for LAB

1- Calcul of pH

pH value from lactate concentration following the approach of Ozcan (Ozcan et al., 2020)

$$pH = pK_{lac} c_1 * (m_{lac_L_e} + m_{lac_D_e}) + c_2$$

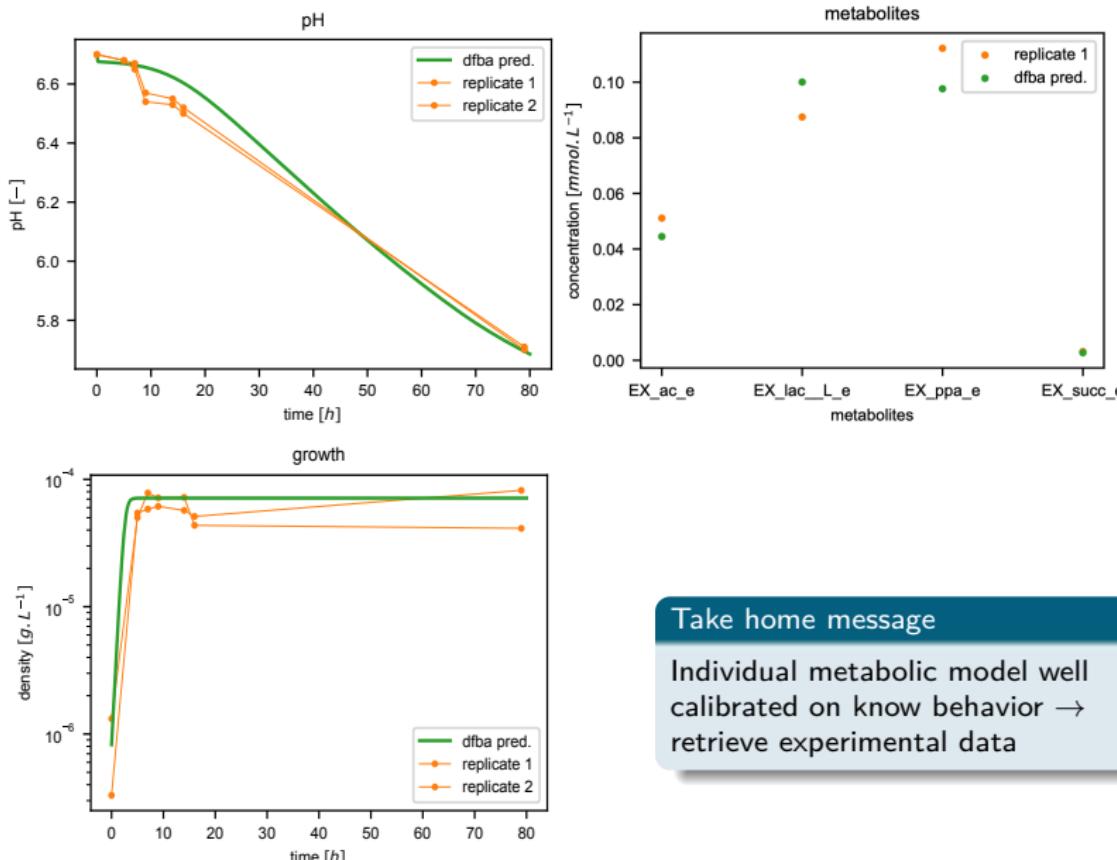
2- Regulate lactose consumption

Model the effect of pH on the lactose consumption

$$c_{min,i,j}^{ex} = \max\left(-\frac{m_{lcts_e}}{\Delta t * \sum_{i \in \mathcal{M}(lcts_e)} b_i}, -\mu_{max,lcts} * 10^{(-k_{lac} * \phi_{undiss})} - \mu_{min,lcts}\right) \quad (3)$$



Model validation

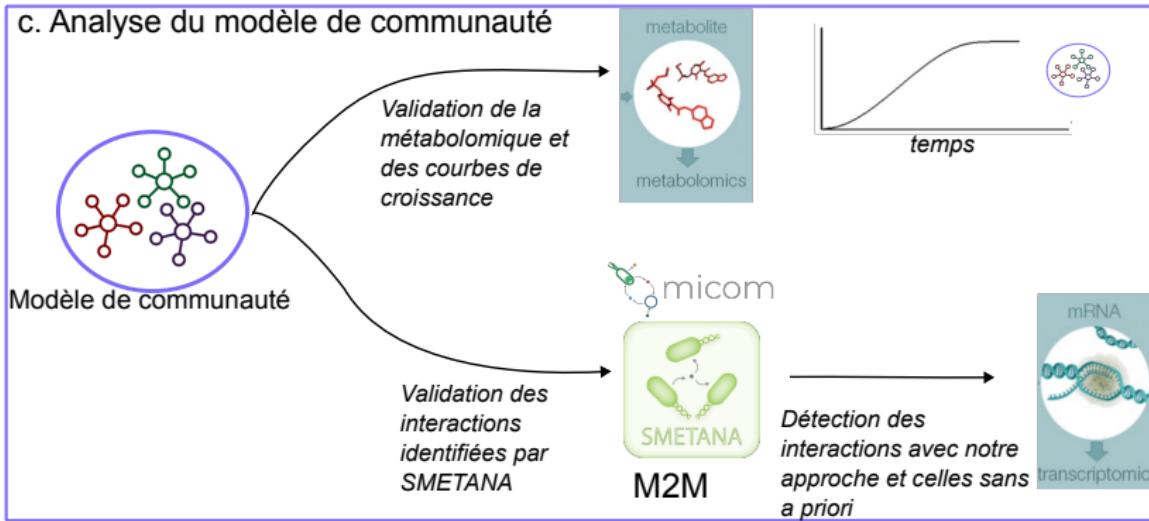


Take home message

Individual metabolic model well calibrated on known behavior → retrieve experimental data

Community validation

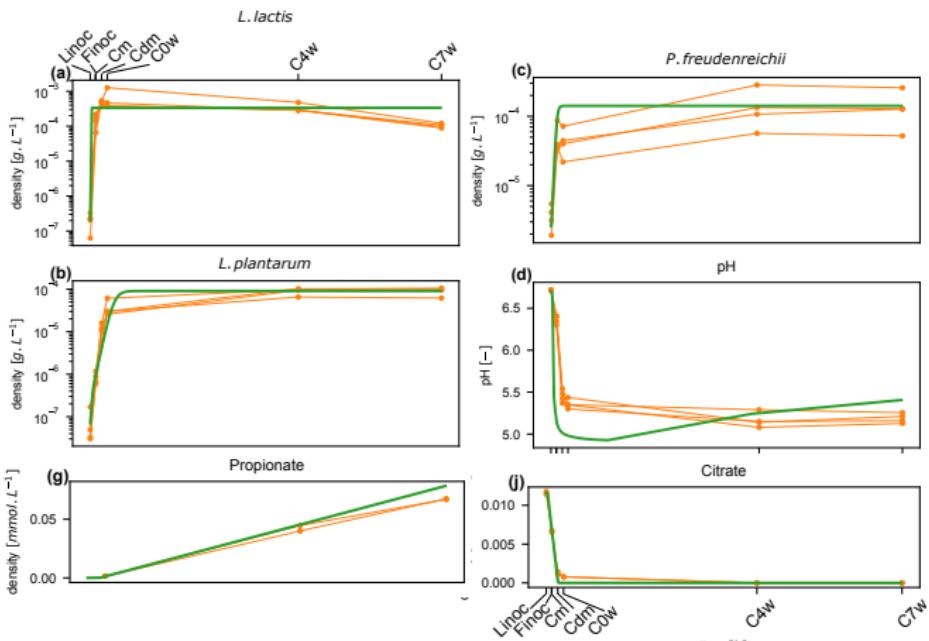
c. Analyse du modèle de communauté



- Bacterial interaction prediction
- Metabolic explanation of biological observations
- **No community calibration**

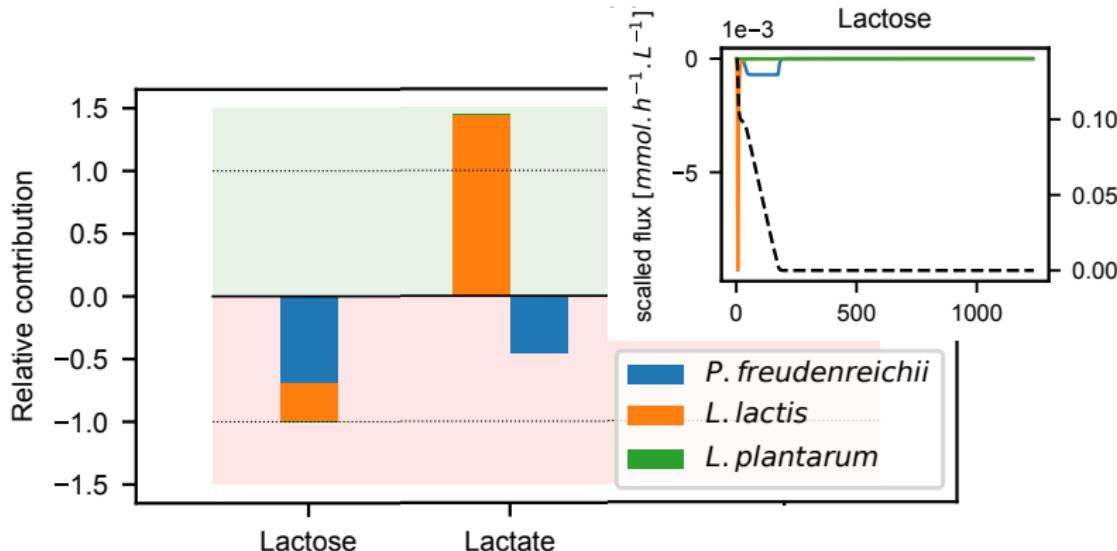
Community prediction

Growth and pH



- Growth well predicted for all bacteria
- Lactate proxy production can explain the observed pH
- Metabolomic is well predicted

Highlighted bacterial behavior



- No competition for lactose
- *L. lactis* main lactate producer
- Cooperation between *L. lactis* and *P. freudenreichii*
- 11 shared metabolites predicted with SMETANA, MiCOM (phénylalanine, succinate, xanthine..)

Added-value and limitations

Take home message

Originality

- High quality of refinement and well calibrated individual GSMM from cheese
- Individual calibrations based on community behavior permit the prediction metabolites concentrations

Scalability issue

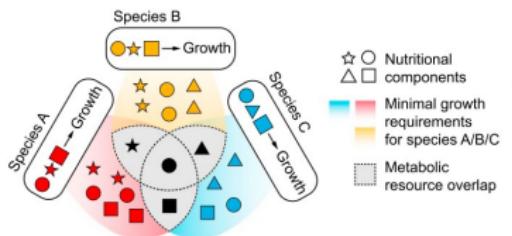
- Refinement process is time consuming
- The iterative methodology assume well documented GSMMs in literature
- Based on *a priori* knowledge for screening compounds

Solution

For screening large community, use of different formalism is required

How large-scale bacterial communities are characterized ?

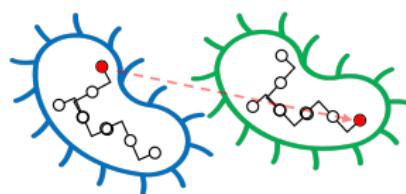
Numerical methods



Competition potentials

- Community size analysis up to 18 in a reasonable time (SMETANA Zeleznak et al., 2015)

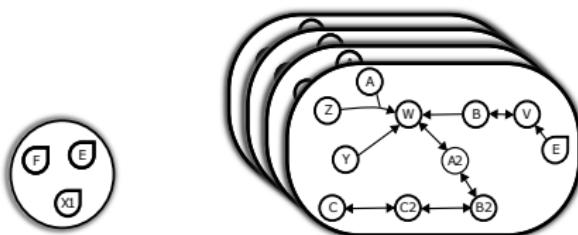
Graph based methods



Cooperation potentials

- Tedious pairwise analysis for graph base methods (NetCoop Levy et al., 2015)
- Discrete-based methods not limited by the size of community

Contribution 2: Discrete approach for characterizing large-scale bacterial



Description of
the environment

Collection of
metabolic networks

CoCoMiCo

Reasoning-based
model of interaction

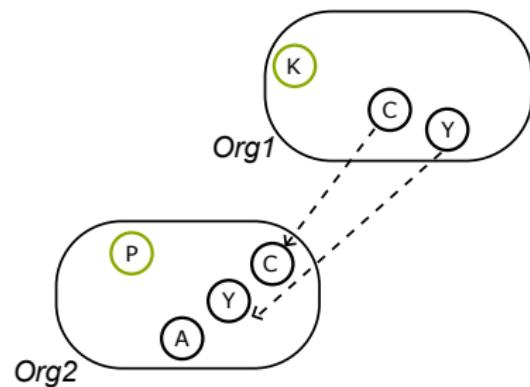
Potentials of
competition and
cooperation

- Combination of the extension of network expansion algorithm and answer set programming for the calculation of cooperation and competition potentials
- Which cooperation and competition properties am I interested in?

Contribution 2: Discrete approach for characterizing large-scale bacterial

Cooperation properties

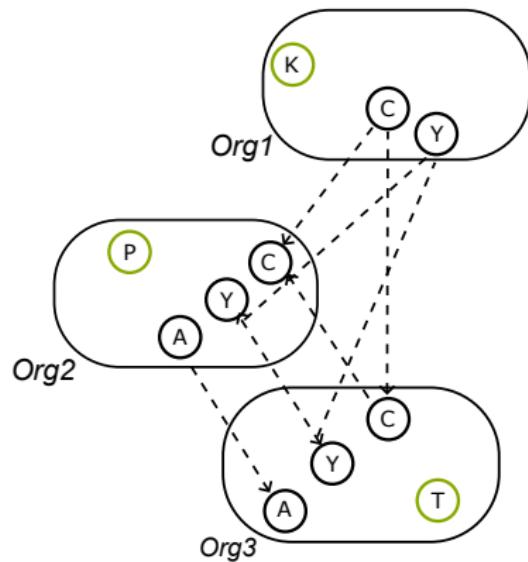
- Exchanged metabolites



Contribution 2: Discrete approach for characterizing large-scale bacterial

Cooperation properties

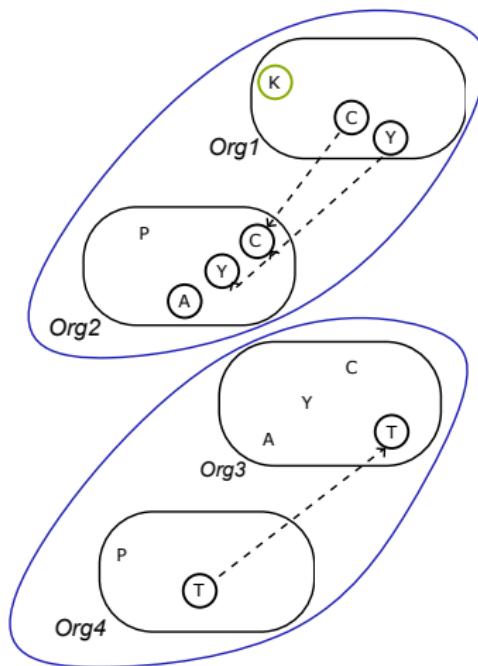
- Exchanged metabolites
- Can a species give to everyone in the system ?
- added-value of adding species



Contribution 2: Discrete approach for characterizing large-scale bacterial

Cooperation properties

- Exchanged metabolites
- Can a species give to everyone in the systeme ?
- Compare cooperation indexes



Contribution 2: Discrete approach for characterizing large-scale bacterial

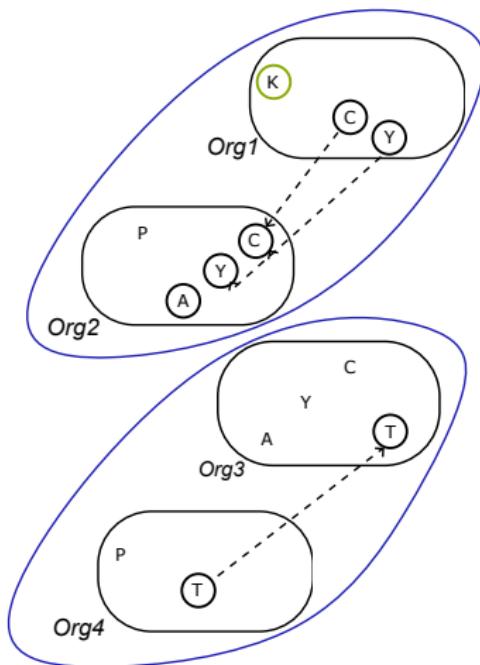
Cooperation properties

- Exchanged metabolites
- Can a species give to everyone in the systeme ?
- Compare cooperation indexes

Competition property

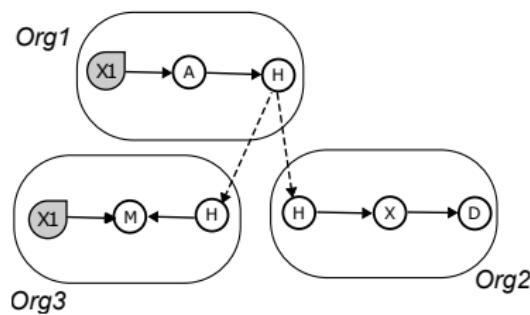
- Polyopsonic substrates

- How cooperation and competition rules are defined ?



Potential interactions rules

```
1 exchange(M,P,C) :- taxon(P),
2   taxon(C),
3   P != C,
4   reactant(M,_,C),
5   product(M,_,P),
6   scope(metabolite(M,P), all),
7   not scope(metabolite(M,C), self(C)).
```



Potential interactions rules

```

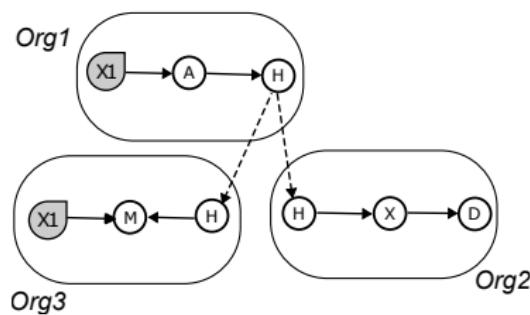
1 exchange(M,P,C) :- taxon(P),
2   taxon(C),
3   P != C,
4   reactant(M,_,C),
5   product(M,_,P),
6   scope(metabolite(M,P), all),
7   not scope(metabolite(M,C), self(
      C)).

```

```

1 % cas pour les metabolites
2   % échangeables
3 polyopsonist(M,N) :- N =# count{C,
4   M:exchange(M,_,C)},
5   exchange(M,_,_), N > 1.
6
7 % cas pour les graines
8 polyopsonist(S,N) :- N =# count{B:
9   seed_consumed_by_taxon(S,B)},
10  },
11  N > 1, seed(S).

```



Cooperation and polyopsonistic indexes

Cooperation weight

how to qualitatively model the contributions of exchange species?

Exchangeable metabolites

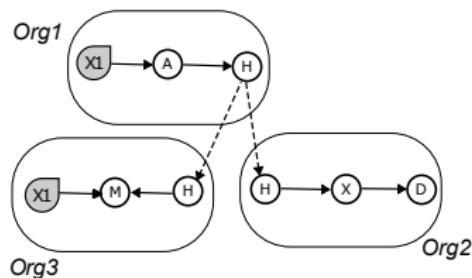
- 1 H:{Org1} % producer
- 2 H:{Org2,Org3} % consumers

$$w(k) = 2 - 0.5^{k-1}$$

- 1 H:{1} % producer
- 2 H:{1.5} % consumers

$$\text{CooP} = \sum_{m \in M} w(|P_m|) + \sum_{m \in M} w(|C_m|)$$

$$\text{CooP} = 2.5$$



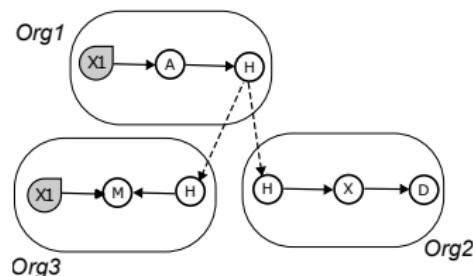
Cooperation and polyopsonistic indexes

Polyopsonistic

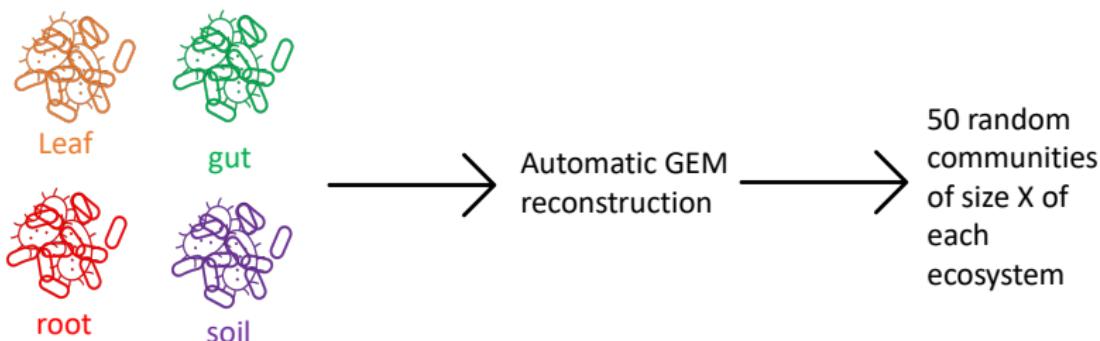
Number of consumers involve in exchangeable metabolites and seed > 1

```
1 H:{Org2,Org3} % exchanged  
metabolite  
2 H:{Org1,Org3} % the nutrient
```

```
1 Comp = sum(polyopsonist.values  
())) / len(community.taxa)  
2 Comp = 4/3
```



Workflow



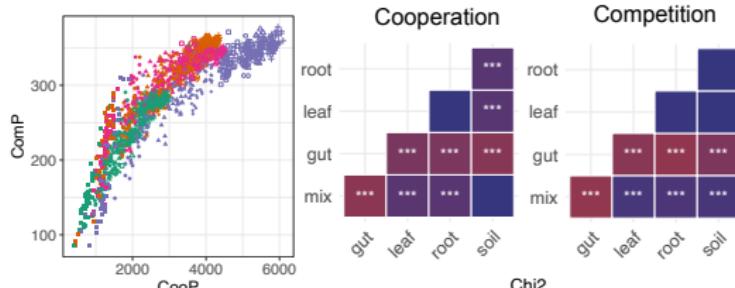
Reference
genomes of
cultivable species
from 4 ecosystems

Goal

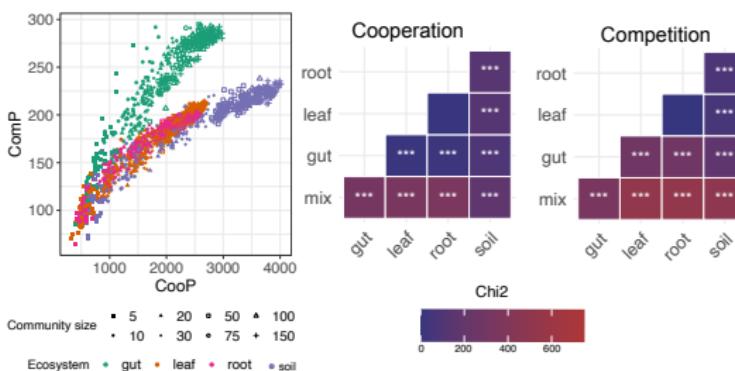
- Ecosystem consistency
- Added-value of removing/adding species
- Generic and specific seeds

Are scores ecosystem consistent ?

Generic seeds



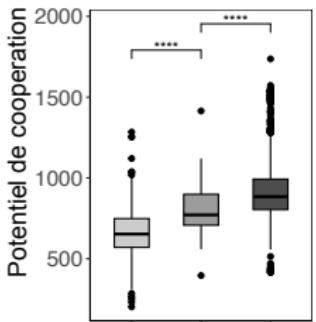
Specific seeds



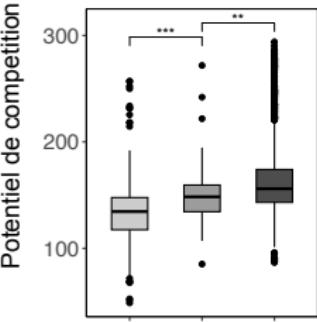
- Overlap between leaf and root
- Better ecosystem consistency with specific seeds

added-value of removing/adding species on the gut microbiota

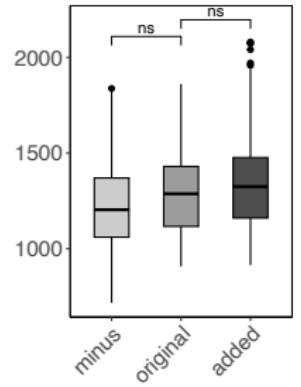
Communautés de taille 5



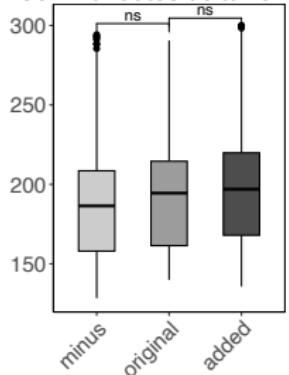
Communautés de taille 5



Communautés de taille 10



Communautés de taille 10



- Small communities are impacted by species modifications
- Changes in the nutritional environment do not impact the taxonomic disturbance

Comparison with quantitative state-of-the-art tools for deciphering cooperation and competition in microbial communities



Up-to community size of 30

Cooperation and competition scores

Up to 2 days



Up-to community size of 50

No scores --> deduced from all pair combinations of KO process

From 1h to a days



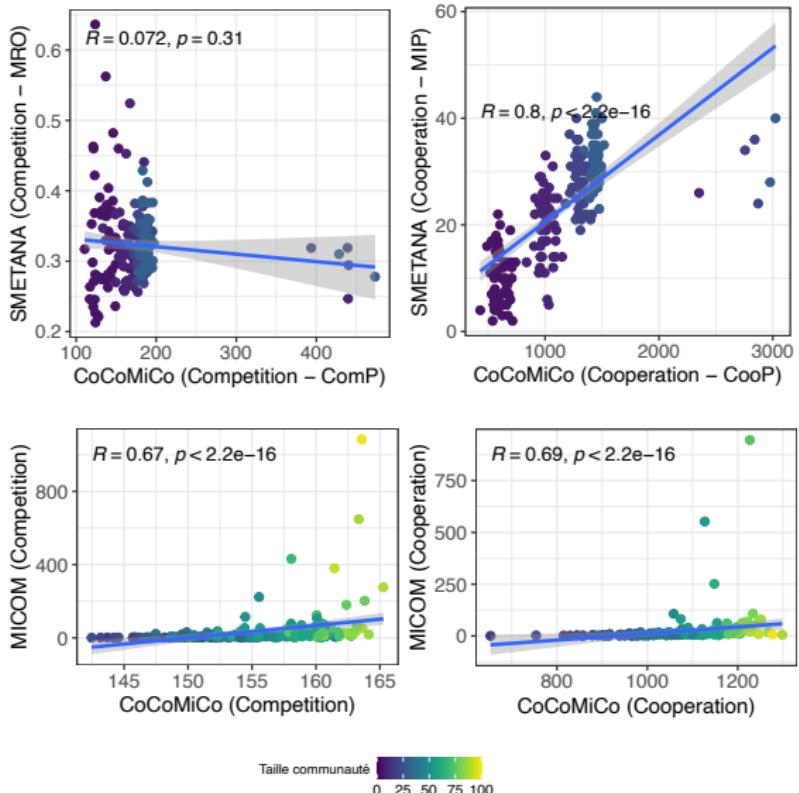
Up-to community size of 800

Cooperation and competition scores

From 1s to hours

- Our approach is faster than SMETANA (Zelezniak et al., 2015) and MiCOM (Diener, Gibbons, and Resendis-Antonio, 2020)
- Compare cooperation and competition potentials

Cooperation and competition potentials



● Competition and cooperation coherent with MiCOM

Discrete model added-value and limitations

Take home message

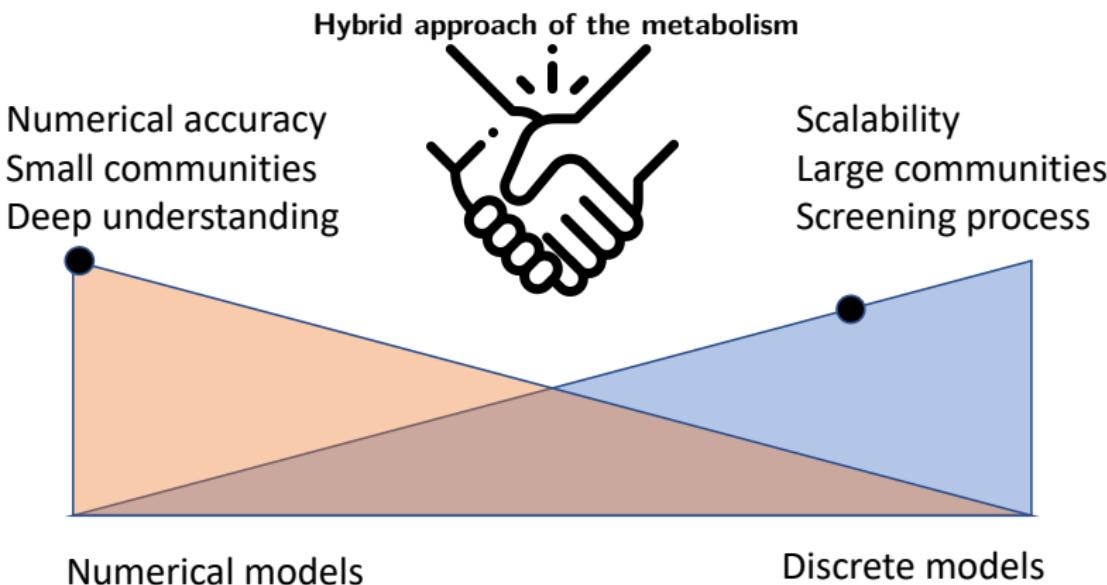
Originality

- Using ASP for inferring competition-like potentials
- Enrichment of previous ASP metabolism-based models
- Mechanistic models

Qualitative issue

- Bacterial interactions
- State of the cell

Can we go further with discrete models?



- No need to create hybrid model for inferring community behaviors
- Can we add accuracy to discrete model ?

Conclusion



Perspective and discussion



Dynamic modeling of the metabolism (dFBA)

General dynamic model



$$F_j = \sum_{i \in \mathcal{B}} \mu_{i,j} ((c_{min,i}^{ex}, c_{max,i}^{ex})(b^n, m^n)) b_i \quad (4)$$

$$b_i^{n+1} = b_i^n + \Delta t * F_{b_i} \quad (5)$$

$$m_j^{n+1} = \begin{cases} m_j^n + \Delta t * F_j & \text{si } F_j > 0 \text{ (cas explicite)} \\ m_j^n / (1 - \Delta t * F_j / m_j^n) & \text{sinon (cas implicite)} \end{cases} \quad (6)$$

$$\partial_t b_i = \mathcal{R}_i(b_i) \mu_{i,i} ((c_{min,i}^{ex}, c_{max,i}^{ex})(b, m)) b_i \quad (7)$$

$$\partial_t m_j = \sum_{i \in \mathcal{B}} \mu_{i,j} ((c_{min,i}^{ex}, c_{max,i}^{ex})(b, m)) b_i \quad (8)$$

Bacterial densities and metabolites concentrations are therefore computed in $g.L^{-1}$ and $mmol.L^{-1}$

-  Belkaid, Yasmine and Timothy W. Hand (2014). "Role of the Microbiota in Immunity and Inflammation". In: *Cell* 157.1, pp. 121–141. ISSN: 0092-8674. DOI: <https://doi.org/10.1016/j.cell.2014.03.011>. URL: <https://www.sciencedirect.com/science/article/pii/S0092867414003456>.
-  de Oliveira, Rafael D., Galo A.C. Le Roux, and Radhakrishnan Mahadevan (2023). "Nonlinear programming reformulation of dynamic flux balance analysis models". In: *Computers & Chemical Engineering* 170, p. 108101. ISSN: 0098-1354. DOI: <https://doi.org/10.1016/j.compchemeng.2022.108101>. URL: <https://www.sciencedirect.com/science/article/pii/S0098135422004343>.
-  Diener, Christian, Sean M Gibbons, and Osbaldo Resendis-Antonio (2020). "MICOM: Metagenome-Scale Modeling To Infer Metabolic Interactions in the Gut Microbiota". In: *mSystems* 5.1. Ed. by Nicholas Chia. DOI: [10.1128/mSystems.00606-19](https://doi.org/10.1128/mSystems.00606-19). URL: <https://msystems.asm.org/content/5/1/e00606-19>.
-  Ebenhöh, Oliver, Thomas Handorf, and Reinhart Heinrich (2004). "Structural analysis of expanding metabolic networks.". In: *Genome informatics. International Conference on Genome Informatics* 15.1, pp. 35–45. ISSN: 09199454.
-  Faust, Karoline and Jeroen Raes (2012). "Microbial interactions: From networks to models". In: *Nature Reviews Microbiology* 10.8, pp. 538–550. ISSN: 17401526. DOI: [10.1038/nrmicro2832](https://doi.org/10.1038/nrmicro2832). URL: <http://dx.doi.org/10.1038/nrmicro2832>.
-  Hoorman, James J (2011). "The Role of Soil Bacteria". In: *The Ohio State University Extension*, pp. 1–4.

-  Kaufmann, Benjamin et al. (2016). "Grounding and Solving in Answer Set Programming". In: *AI Mag.* 37, pp. 25–32. URL: <https://api.semanticscholar.org/CorpusID:30782765>.
-  Kitano, Hiroaki (2002). "Systems biology: A brief overview". In: *Science* 295.5560, pp. 1662–1664. ISSN: 00368075. DOI: 10.1126/science.1069492.
-  Levy, Roie et al. (2015). "NetCooperate: A network-based tool for inferring host-microbe and microbe-microbe cooperation". In: *BMC Bioinformatics*. ISSN: 14712105. DOI: 10.1186/s12859-015-0588-y.
-  Lifschitz, Vladimir (2008). "What is answer set programming?" In: *Proceedings of the National Conference on Artificial Intelligence* 3, pp. 1594–1597.
-  Machado, Daniel et al. (2018). "Fast automated reconstruction of genome-scale metabolic models for microbial species and communities". In: *Nucleic Acids Research*. ISSN: 13624962. DOI: 10.1093/nar/gky537.
-  Mahadevan, Radhakrishnan, Jeremy S Edwards, and Francis J Doyle (2002). "Dynamic Flux Balance Analysis of Diauxic Growth in Escherichia coli". In: *Biophysical Journal* 83.3, pp. 1331–1340. ISSN: 0006-3495. DOI: 10.1016/s0006-3495(02)73903-9.
-  McSweeney, Paul L.H. and Maria José Sousa (2000). "Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review". In: *Lait* 80.3, pp. 293–324. ISSN: 00237302. DOI: 10.1051/lait:2000127.
-  Orth, Jeffrey D., Ines Thiele, and Bernhard O. Palsson (2010). *What is flux balance analysis?* DOI: 10.1038/nbt.1614.

-  Ozcan, Emrah et al. (2020). "Dynamic co-culture metabolic models reveal the fermentation dynamics, metabolic capacities and interplays of cheese starter cultures". In: *Biotechnology and Bioengineering* 118.1, pp. 223–237. ISSN: 0006-3592. DOI: 10.1002/bit.27565.
-  Royet, Jean-Pierre and Jane Plailly (Oct. 2004). "Lateralization of Olfactory Processes". In: *Chemical Senses* 29.8, pp. 731–745. ISSN: 0379-864X. DOI: 10.1093/chemse/bjh067. eprint: <https://academic.oup.com/chemse/article-pdf/29/8/731/930410/bjh067.pdf>. URL: <https://doi.org/10.1093/chemse/bjh067>.
-  Suomalainen, By Heikki and Matti Lehtonen (1978). "JJu It". In: 85, pp. 149–156.
-  Sánchez López de Nava A, Raja A (2023). *Physiology, Metabolism*. URL: www.ncbi.nlm.nih.gov/books/NBK546690/.
-  Thiele, Ines and Bernhard O Palsson (2010). "A Protocol for Generating a High-Quality Genome-Scale Metabolic Reconstruction". In: *Nature Protocols* 5.1, pp. 93–121. ISSN: 1754-2189. DOI: 10.1038/nprot.2009.203.
-  Zelezniak, Aleksei et al. (2015). "Metabolic dependencies drive species co-occurrence in diverse microbial communities". In: *Proceedings of the National Academy of Sciences of the United States of America*. ISSN: 10916490. DOI: 10.1073/pnas.1421834112.
-  Zhang, Yu Jie et al. (2015). "Impacts of gut bacteria on human health and diseases". In: *International Journal of Molecular Sciences* 16.4, pp. 7493–7519. ISSN: 14220067. DOI: 10.3390/ijms16047493.