Metabolic modelling applied to plant pathogens

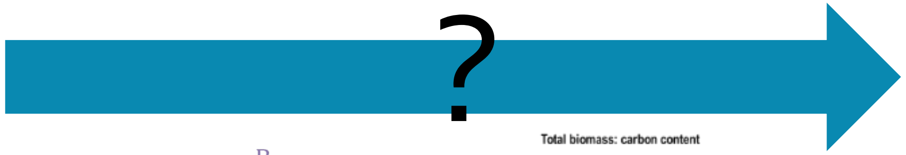
Caroline Baroukh

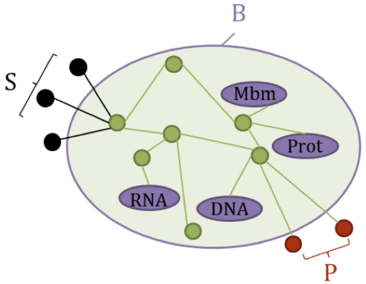
Laboratoire des Interactions Plantes Microbes Environnement

My main goal

Use mathematical modeling to predict phenotype from genotype…

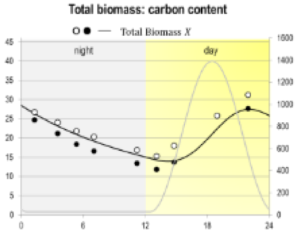
Genome





Metabolism

+



$$\frac{dM}{dt} = K.v.B$$

Mathematical models

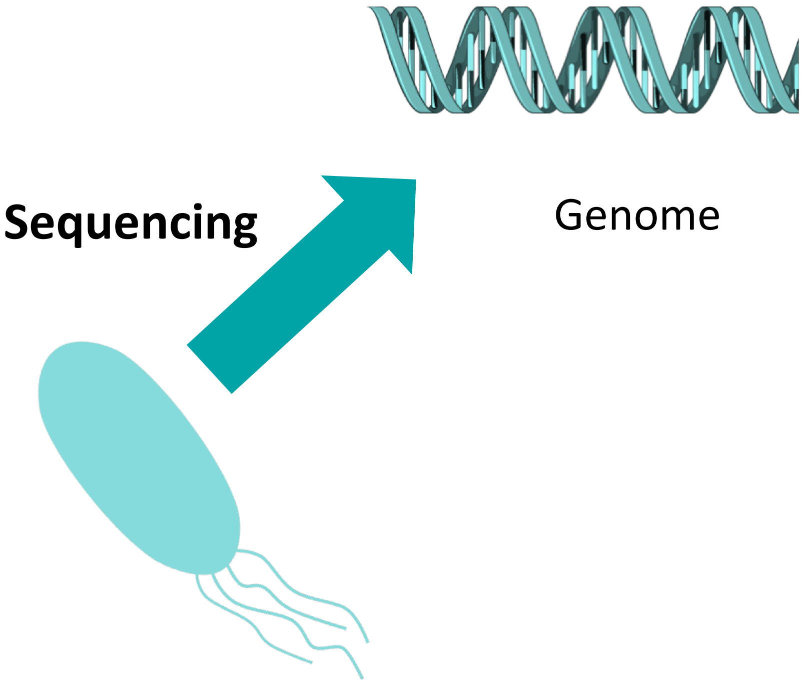
Pathogenicity

… to understand how biological systems works and better control them

Why use metabolic modelling ?

- Make a link between genotype (omics data) and phenotype (growth rate, production of molecule of interest, etc.)
- Quantitative modelling
- Study emergent properties of the system
- Optimize systems' behaviour

Metabolic modeling in a nutshell



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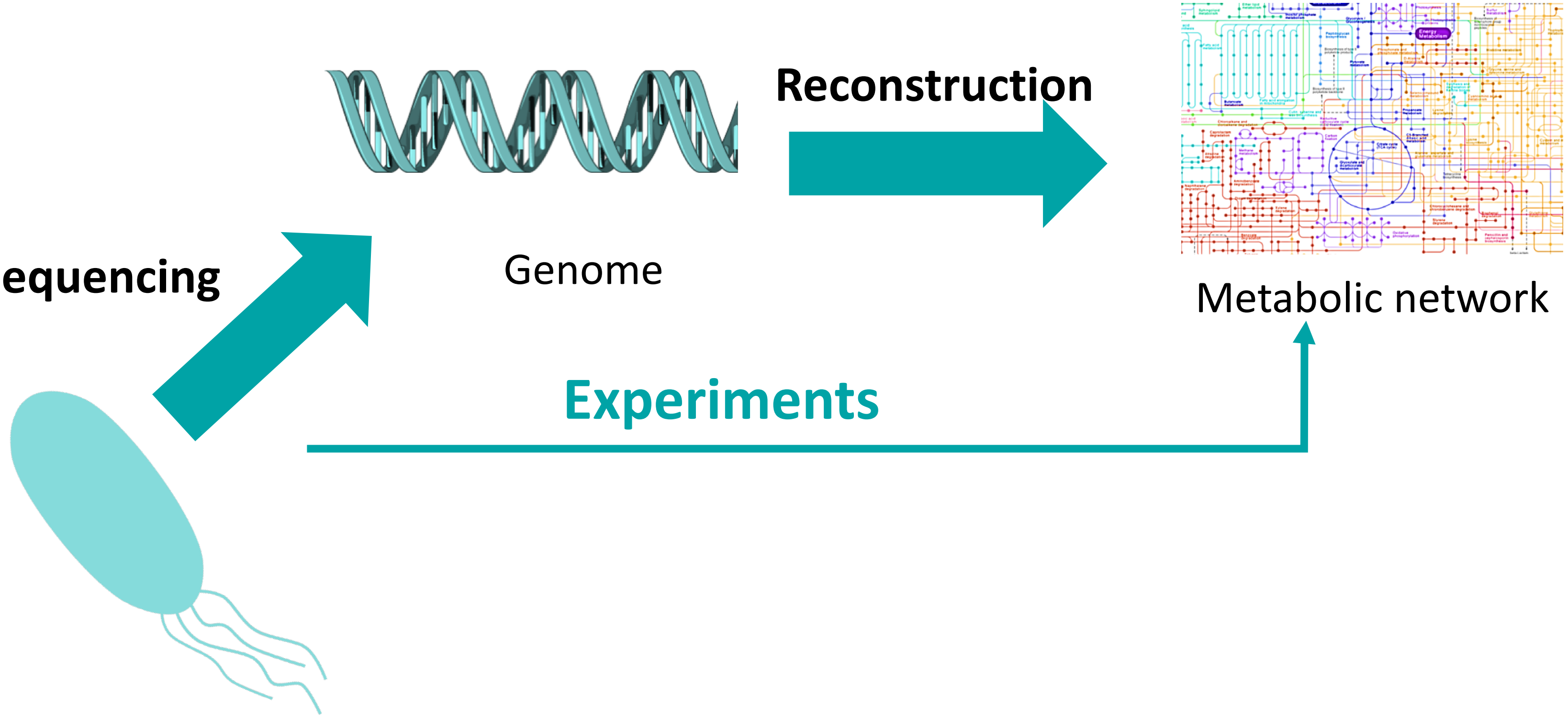


Reconstruction

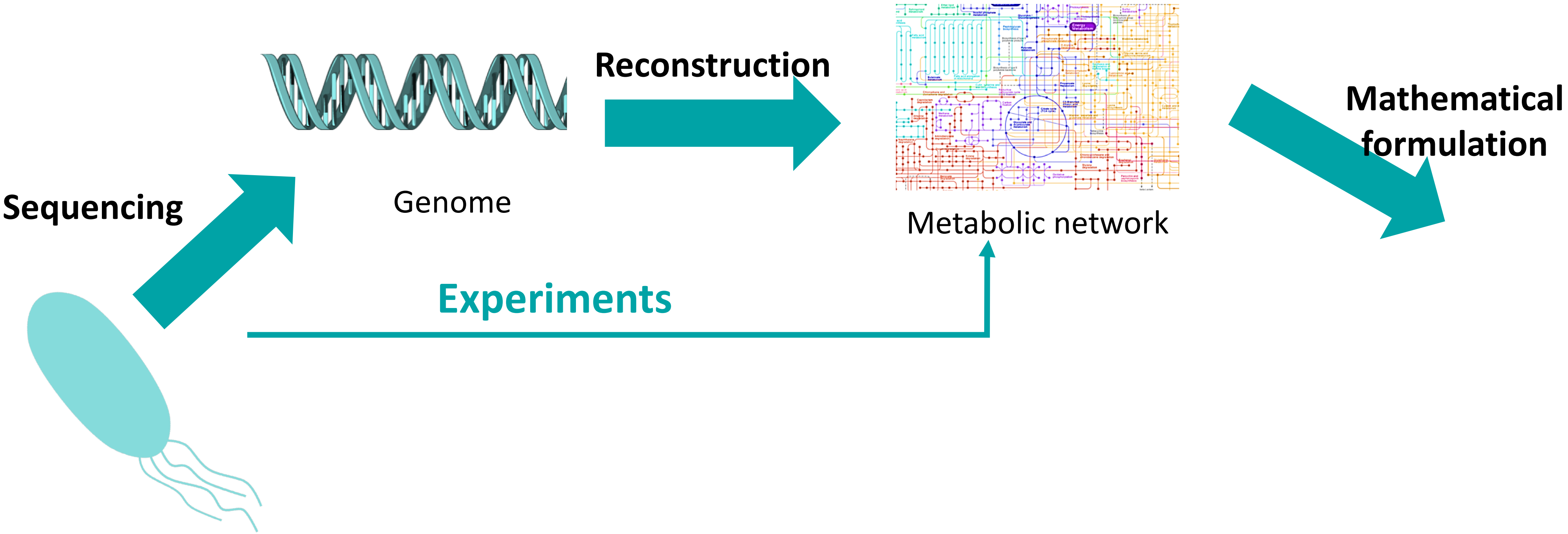
Sequencing

Genome

Metabolic network

Experiments

Metabolic modeling in a nutshell



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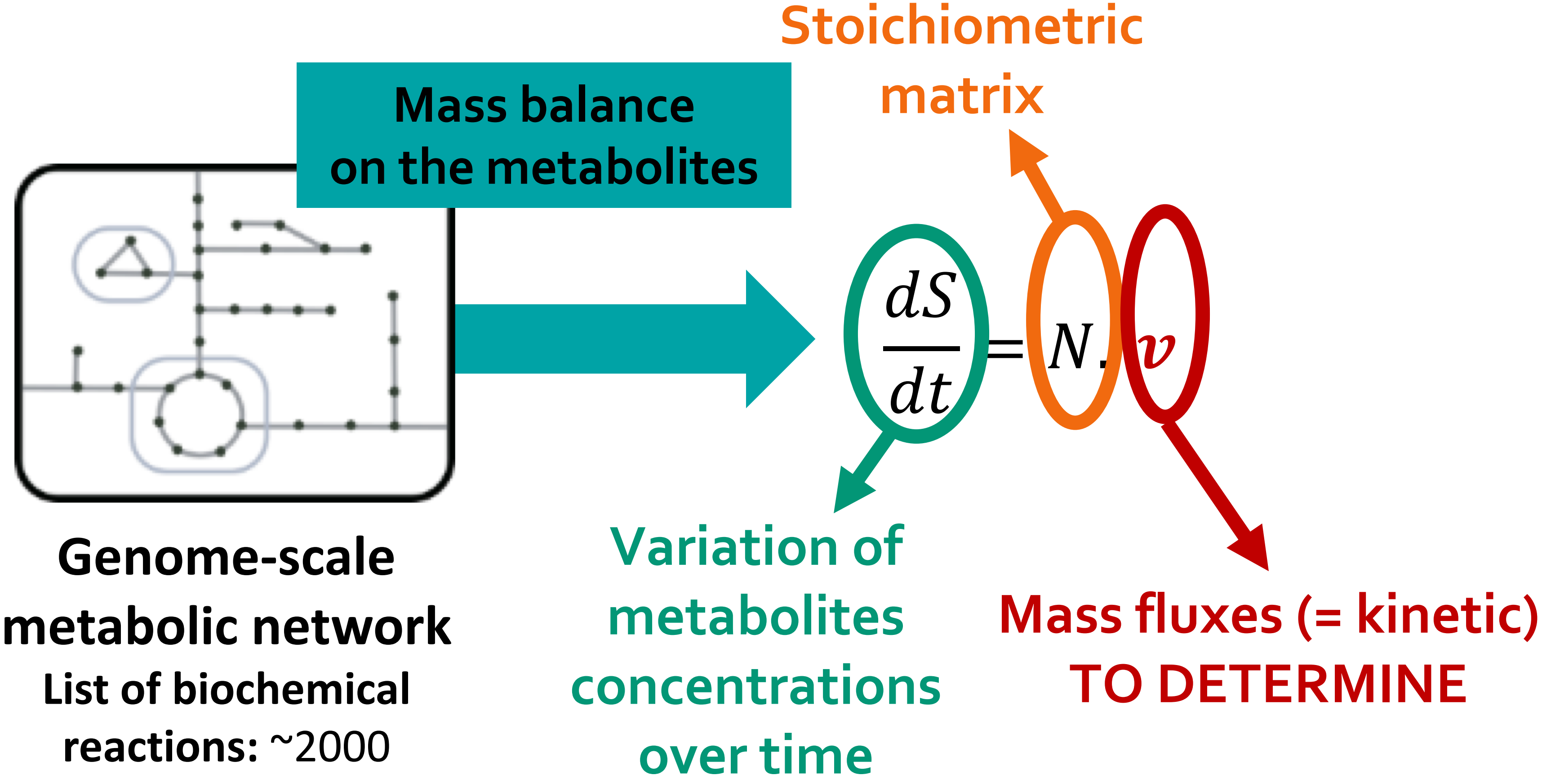
Mathematical formulation

Mass balance on a metabolic system



Mass balance on the metabolites

Stoichiometric matrix

$$\frac{dS}{dt} = N.\boldsymbol{v}$$

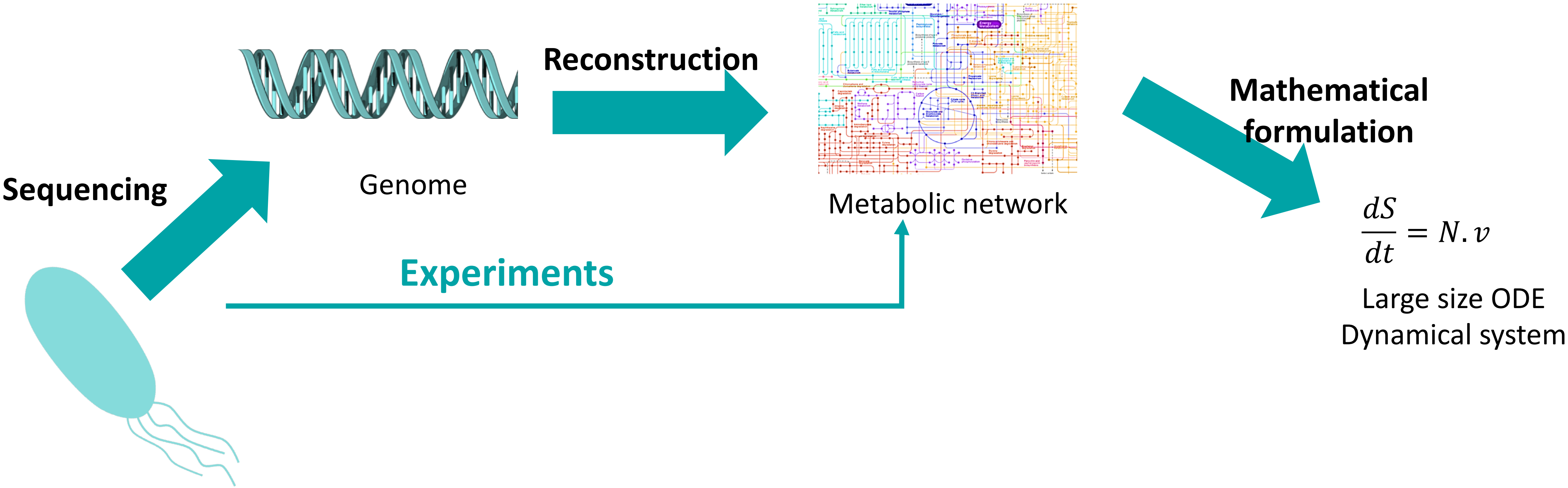
Variation of metabolites concentrations over time

Mass fluxes (= kinetic) TO DETERMINE

Genome-scale metabolic network

List of biochemical reactions: ~2000

Metabolic modeling in a nutshell



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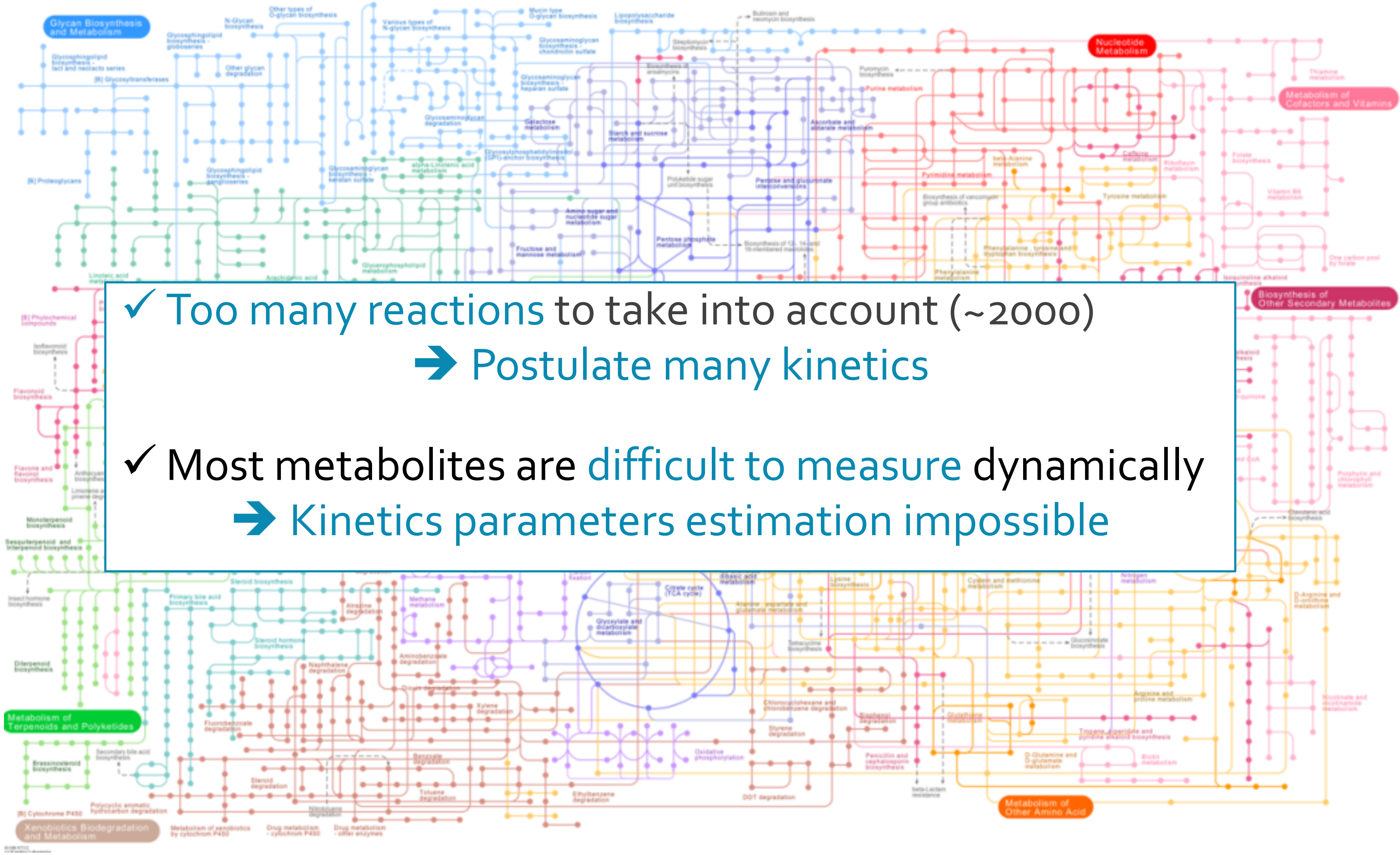
Large size ODE
Dynamical system

Metabolic modeling in a nutshell



✓ Too many reactions to take into account (~2000)

➔ Postulate many kinetics

✓ Most metabolites are difficult to measure dynamically

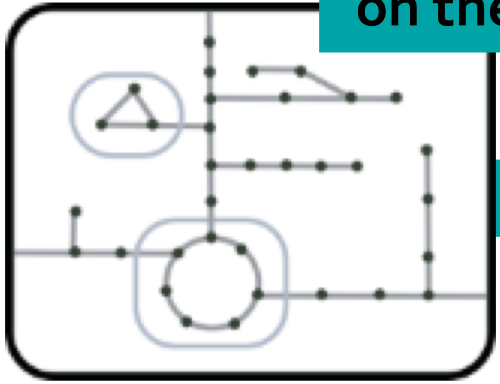
➔ Kinetics parameters estimation impossible

Mathematical formulation

$$\frac{dS}{dt} = N.v$$

Large size ODE
Dynamical system

Quasi-steady state assumption



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Genome-scale metabolic network

List of biochemical reactions: ~2000

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$$\frac{dS}{dt} = N.\boldsymbol{v}$$

Variation of metabolites concentrations over time

Mass fluxes of reactions TO DETERMINE

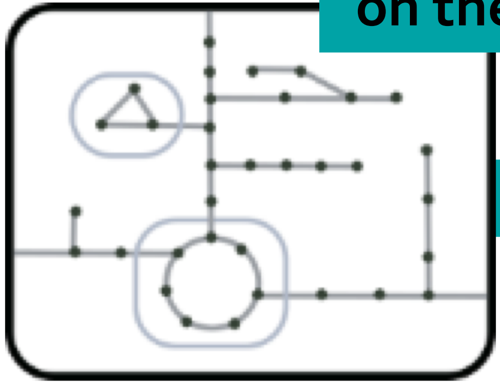
First hypothesis:
Quasi-steady-state assumption
Internal metabolites consumed once they are produced

$$\begin{cases} \frac{dS_{ext}}{dt} = N_{S_{ext}}.\,\boldsymbol{v} \\ N_{S_{int}}.\,\boldsymbol{v} = 0 \end{cases}$$

TO DETERMINE

Quasi-steady state assumption

Mass balance on the metabolites



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TO DETERMINE

BUT indeterminate system !!!!

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

➔ Solve an optimization problem

Flux Balance Analysis

First hypothesis: internal metabolism are assumed at quasi-steady-state

Second hypothesis: metabolism is assumed optimal by evolution

Optimization problem

Objective (maximization or minimization)

e.g. Biomass synthesis maximization

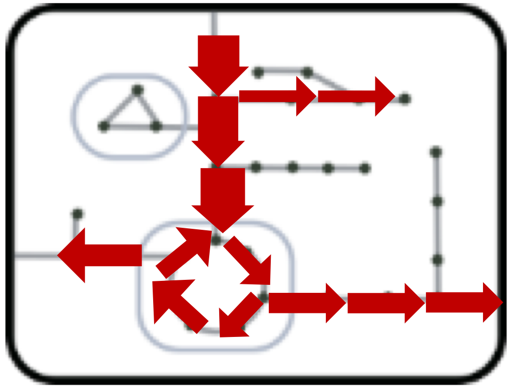
Constraints

$N_{S_{int}} . \boldsymbol{v} = 0$ **and**

e.g Limitation of substrate assimilation

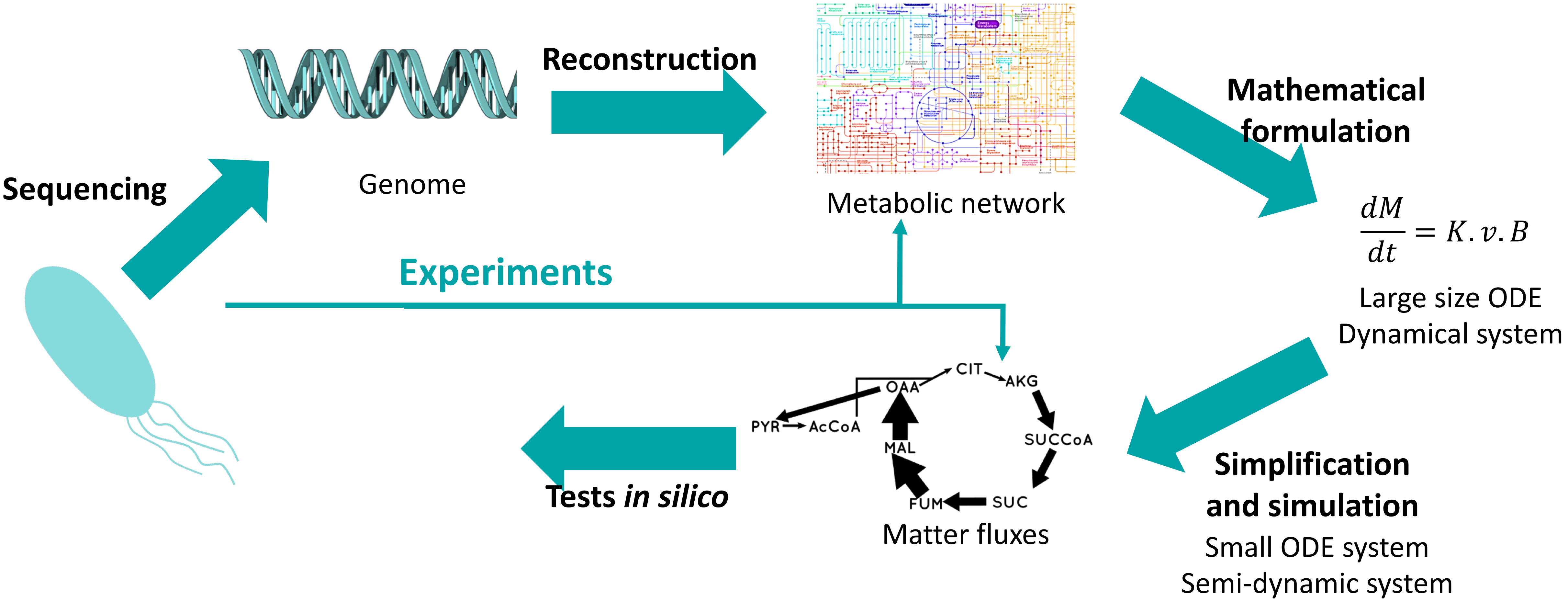
e.g. Irreversible reactions fluxes ≥ 0

e.g. Energetic cost for maintenance processes



Fluxes of matter (v)

Metabolic modeling in a nutshell



Sequencing

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Metabolic network

Mathematical formulation

$$\frac{dM}{dt} = K.v.B$$

Large size ODE
Dynamical system

Simplification and simulation

Small ODE system
Semi-dynamic system

Matter fluxes

Tests *in silico*

Experiments

Metabolic modeling - use

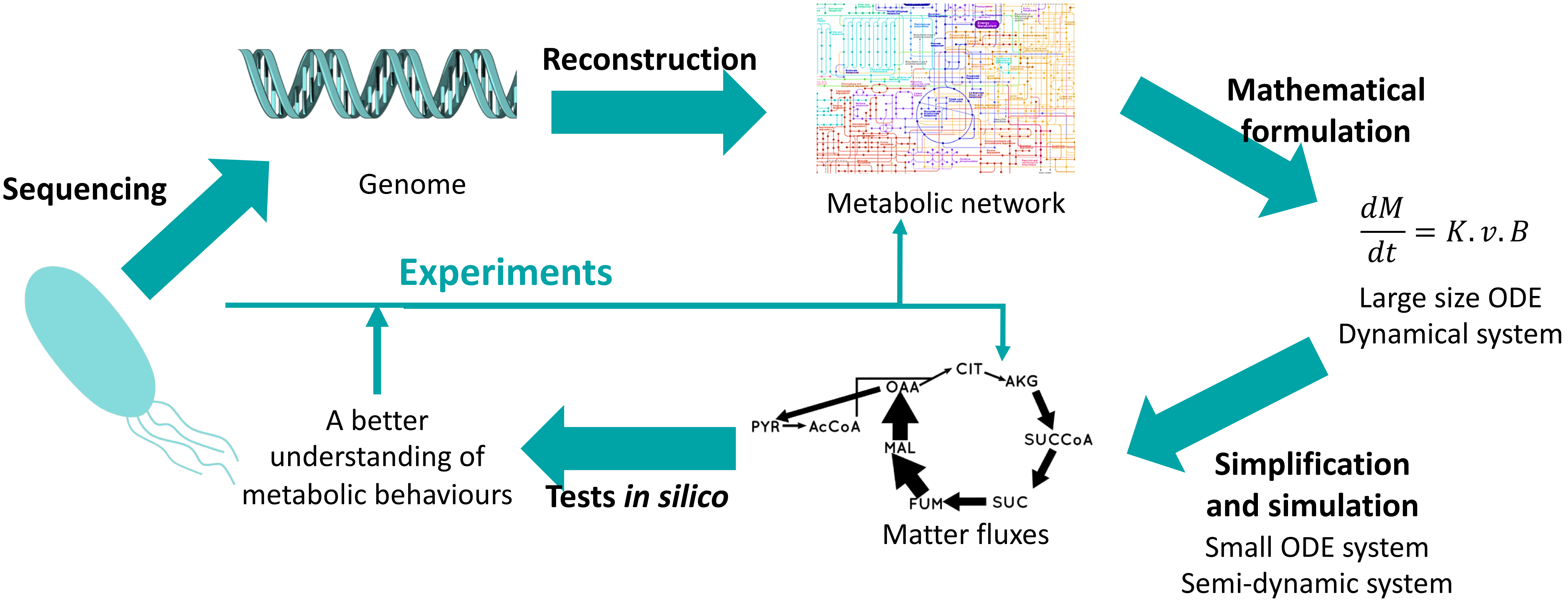
- Quantitative prediction of intracellular fluxes

- Comparison of fluxes distribution between
  - Several environmental conditions
  - Several strains

- Study the impact of
  - The deletion of one or genes
  - The inhibition or catalysis of a metabolic reaction

- Study the metabolic/genetic modifications to perform so as to, e.g., optimize a bioprocess

Metabolic modeling in a nutshell



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$$\frac{dM}{dt} = K.v.B$$

Large size ODE
Dynamical system

Simplification and simulation

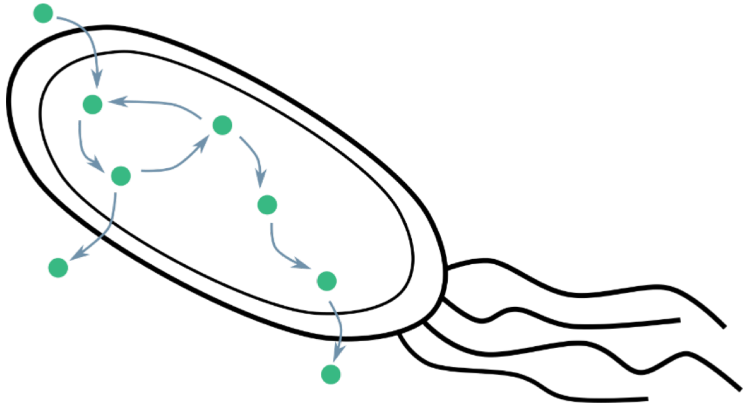
Small ODE system
Semi-dynamic system

Matter fluxes

Tests *in silico*

A better understanding of metabolic behaviours

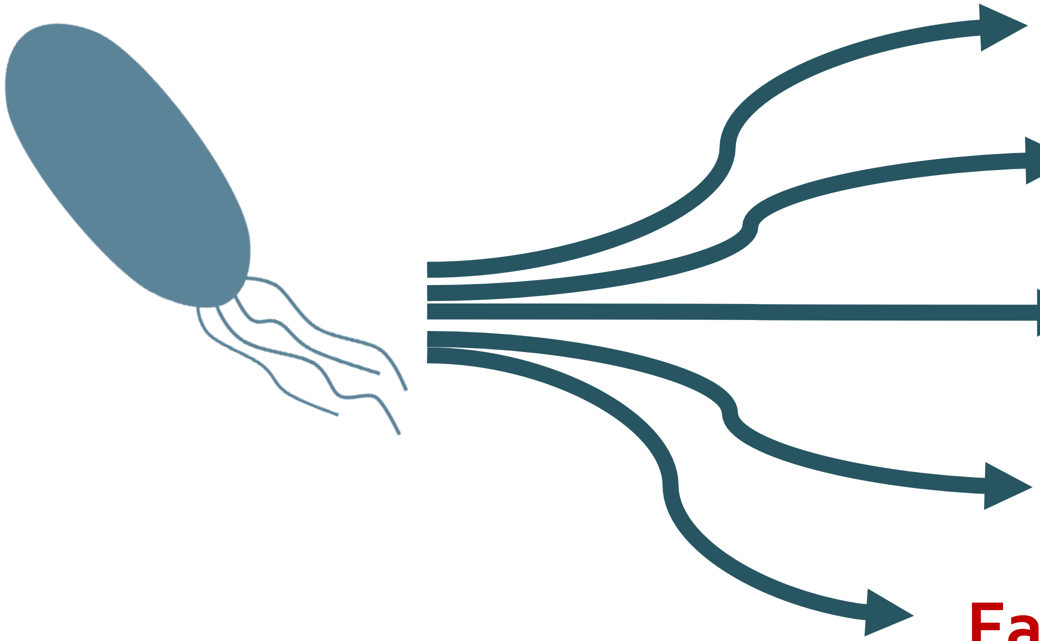
Experiments



$$\frac{dM}{dt} = K.v.B$$

Example 1: *Xylella fastidiosa*

Xylella fastidiosa



Infection of over a 100 plant species

Grapevine, Olive tree, …

Leaf necrosis, leaf drying

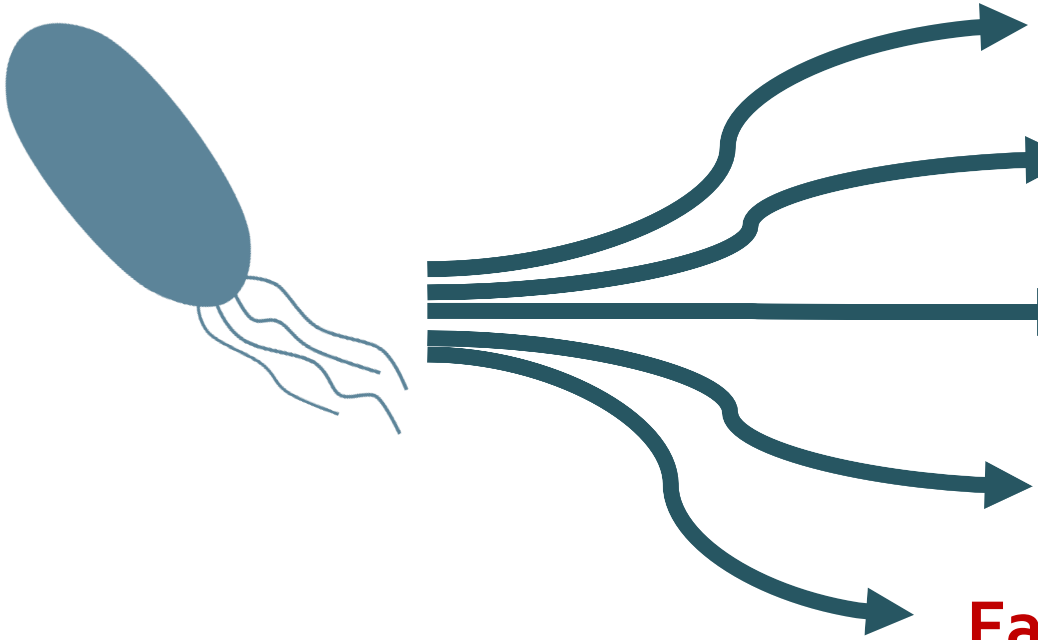
Insect-transmitted

Xylem sap pathogen

Fastidious bacteria :

isolation and *in vitro* cultures difficult

Xylella fastidiosa



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Xylem sap pathogen

Fastidious bacteria :
isolation and *in vitro* cultures difficult

➔ Biological question: what makes this bacteria fastidious ?

Metabolic reconstruction process

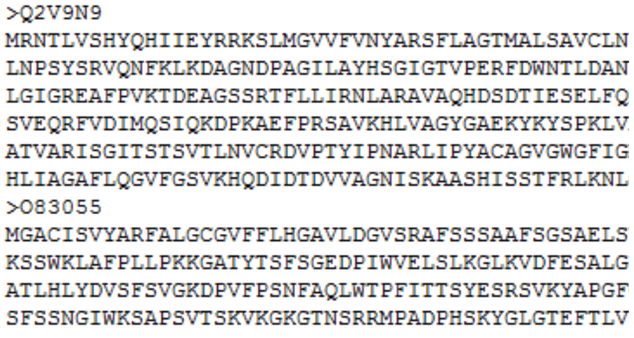
Séquences protéiques

Automatic by orthology

« Draft model »

Intensive manual curation

Réseau métabolique



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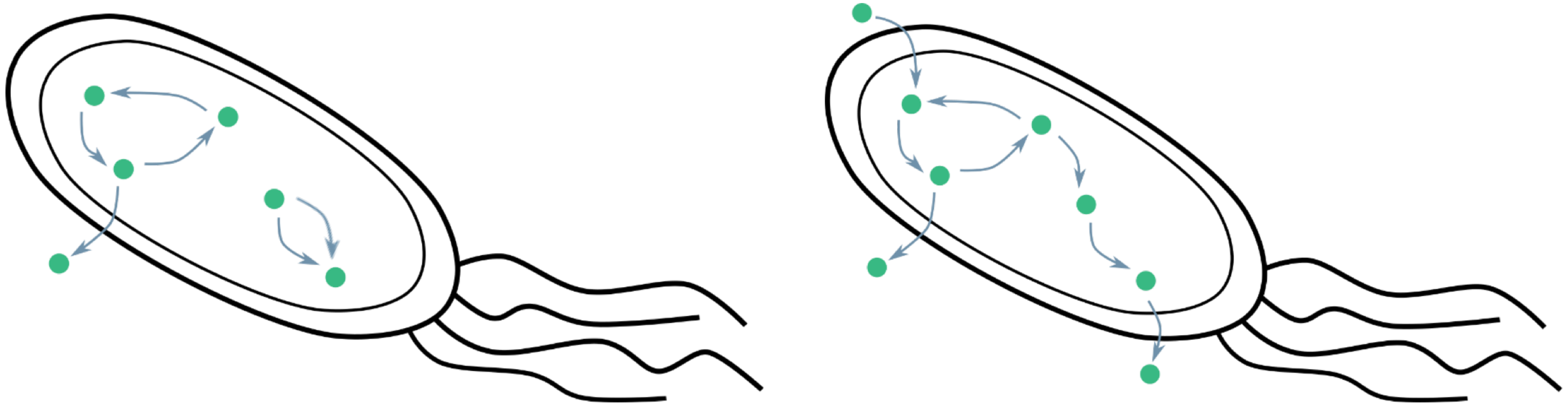
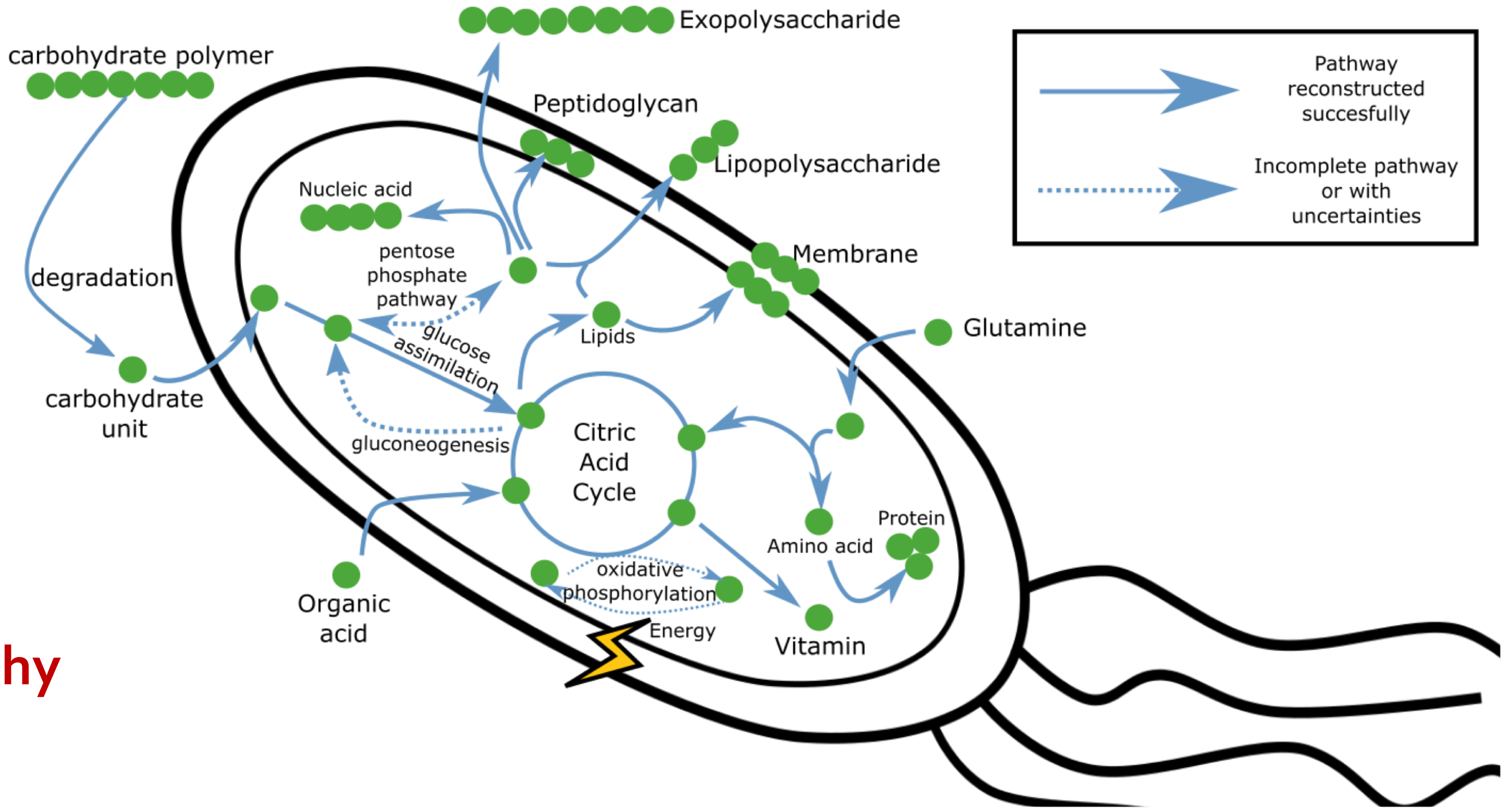


Reference metabolic networks used

1. *E. coli*
2. *R. solanacearum*
3. *P. aeruginosa*
4. *R. eutropha*
5. *B. subtilis*

Gerlin et al. 2020

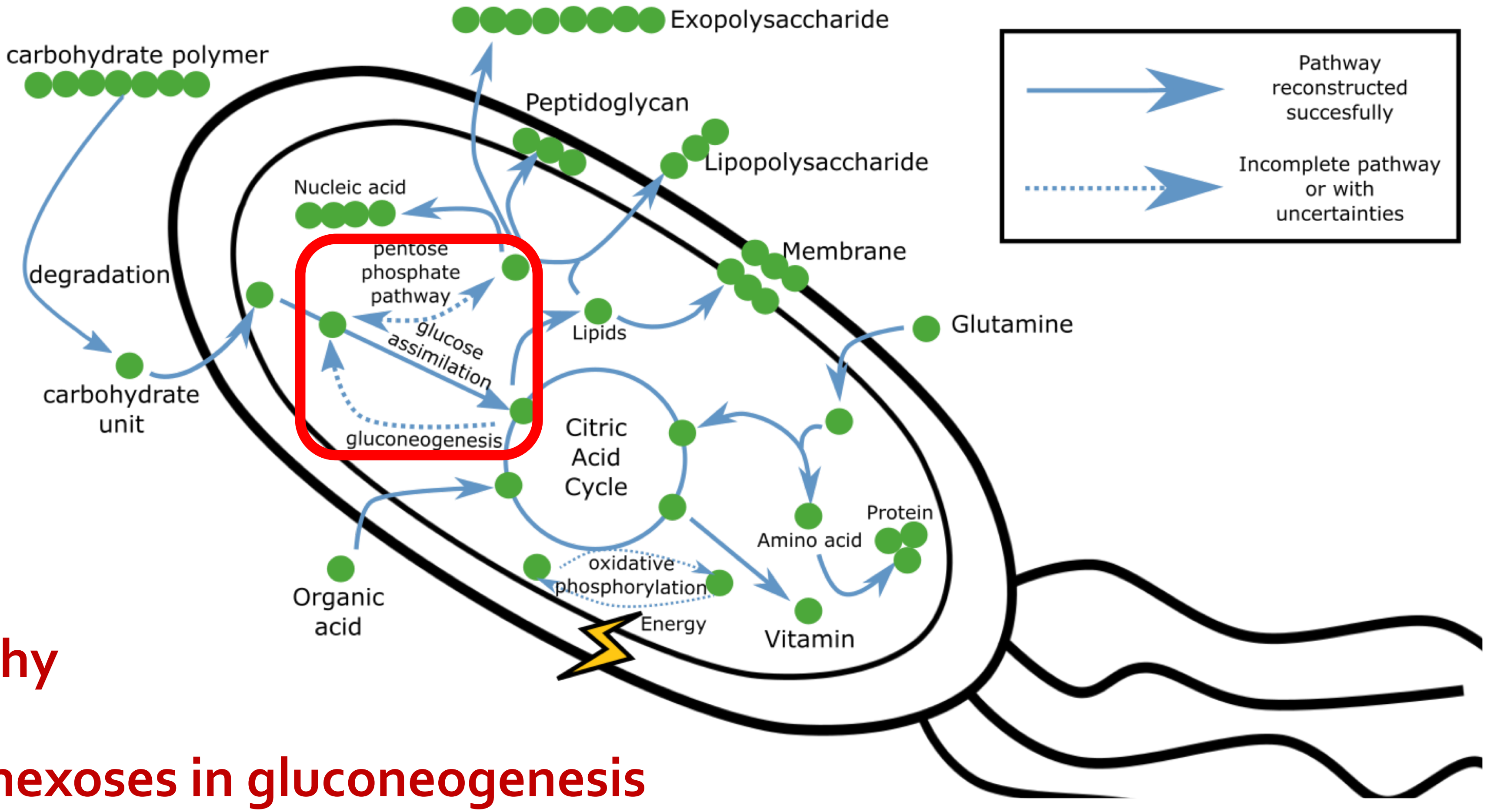
A complete network, but minimal



No auxotrophy

Gerlin et al. 2020

A complete network, but minimal

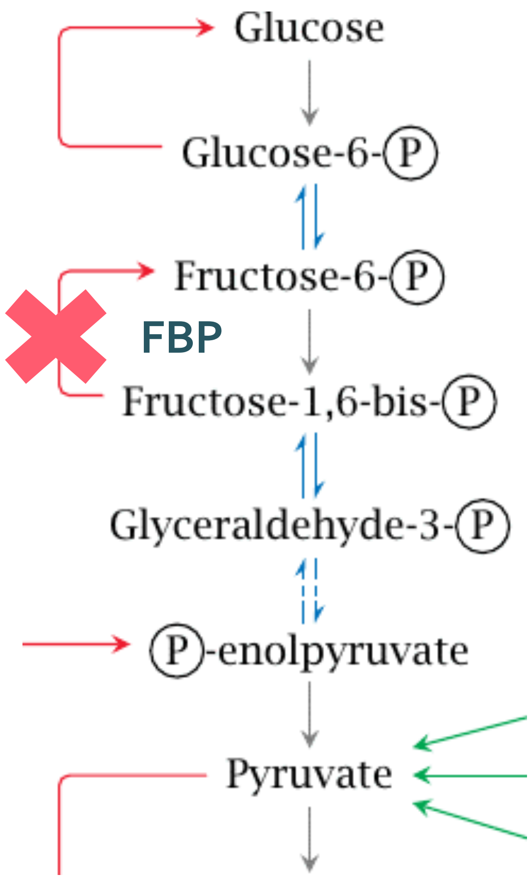


No auxotrophy

Except 1 for hexoses in gluconeogenesis

Gerlin et al. 2020

Where is the fructose-1,6-bisphosphatase?


Glucose

Glucose-6-Ⓟ

Fructose-6-Ⓟ

FBP

Fructose-1,6-bis-Ⓟ

Glyceraldehyde-3-Ⓟ

Ⓟ-enolpyruvate

Pyruvate

Glycolysis / Gluconeogenesis

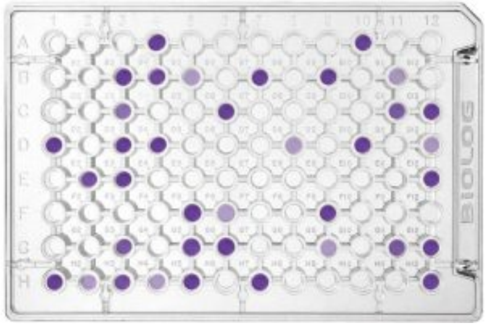
MISSING **FBP**

Fructose-1,6-bisphosphatase
EC 3.1.3.11

- A key enzyme of the gluconeogenesis pathway : essential to generate hexose and grow, as experimented on *S. cerevisiae* and *C. glutamicum* (Sedivy et al., 1985; Rittmann et al., 2003)
- An enzyme conserved in « all » living organisms:
« Same » enzyme for animals/plants/bacteria/etc
- Loss in all *Xylella* strains sequenced and some *Xanthomonas strains*

Gerlin et al. 2020

Is *Xylella* auxotroph for hexoses ?



- Biolog PMs: growth assay (on/off) on 190 carbon sources

➔*Xylella* has no auxotrophy

Gerlin et al. 2020

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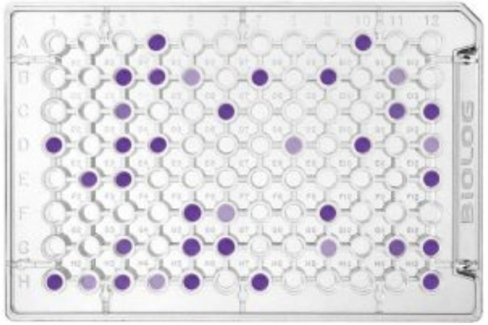

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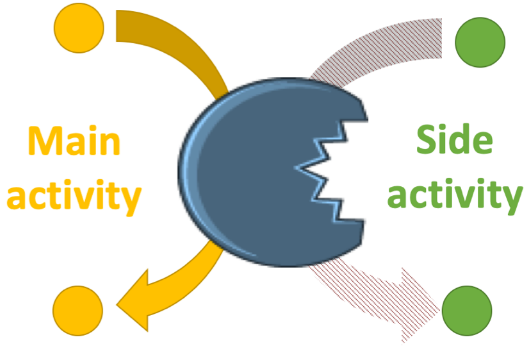
  ➔ *Xylella* has no auxotrophy

- A side activity of an enzyme is different from its main activity and is **not optimal** (100 times slower according to Notebaart et al., 2014)



Gerlin et al. 2020

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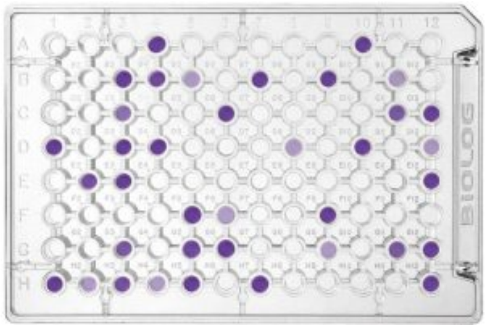

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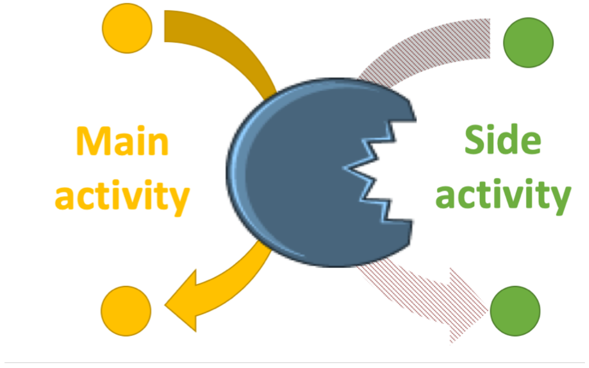

In silico growth assay (FBA)

Main activity → Growth rate: 0.39 h^{-1} — **2h doubling time**
similar to *fast growing bacteria*

Side activity
+ non-optimized FBP (optimal flux/3) → Growth rate: 0.016 h^{-1}
24 times slower — **50h doubling time**
FASTIDIOUS

Gerlin et al. 2020

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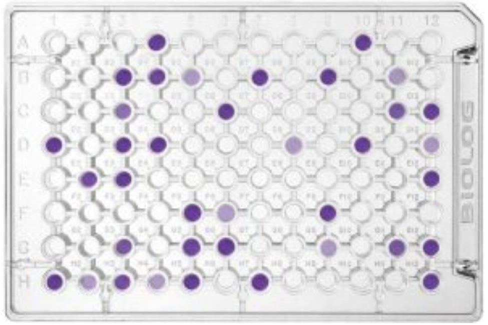

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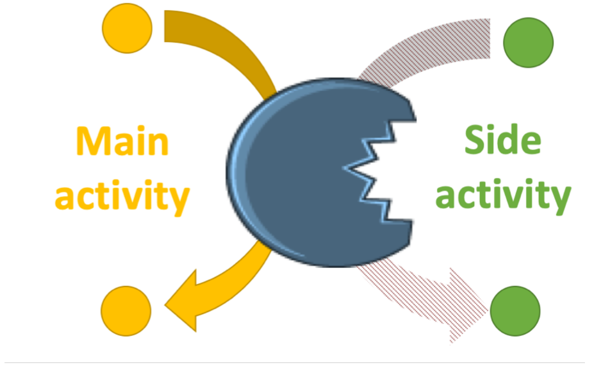

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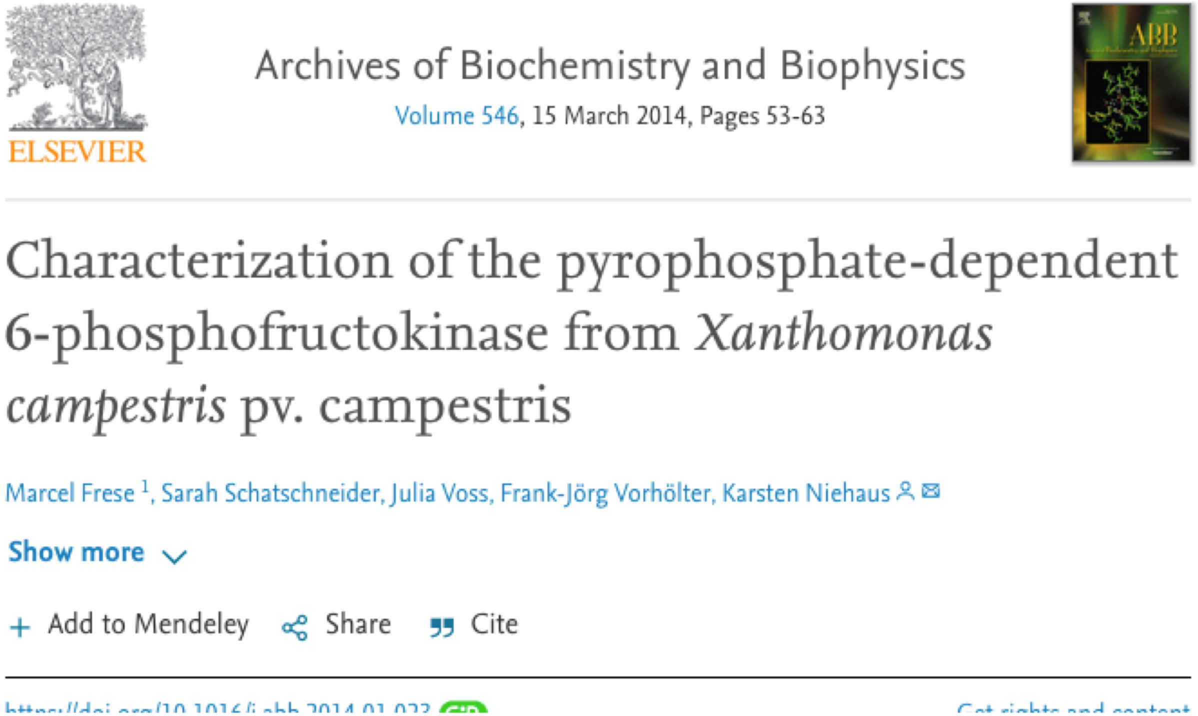
➔ **Fastidious growth reproduced in silico with a non-optimal (side activity) FBP.**

Gerlin et al. 2020

In fact, is the missing enzyme PFK ?

- In *Xanthomonas campestris*, Frese et al. (2014) has shown that the enzyme that catalyze the reaction F6P + PPi <--> F16P + Pi is exceptionnally reversible



ELSEVIER

Archives of Biochemistry and Biophysics

Volume 546, 15 March 2014, Pages 53-63

Characterization of the pyrophosphate-dependent 6-phosphofructokinase from *Xanthomonas campestris* pv. campestris

Marcel Frese [1], Sarah Schatschneider, Julia Voss, Frank-Jörg Vorhölter, Karsten Niehaus

https://doi.org/10.1016/j.abb.2014.01.023

Gerlin et al. 2020

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Gerlin et al. 2020

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- *X. campestris* (and probably the common ancestors between *X. campestris* and *X. fastidiosa*) possesses both the FBP and this enzymes
- *Xylellas* could thus loose the FBP without being auxotroph to hexoses

Gerlin et al. 2020

A network lacking robustness

Gene deletion study (*in silico, multiple FBAs*)

Organism	Total number of genes in the network	Proportion of essential genes	Total number of genes
Xylella fastidiosa	537	53,8%	2 782
Ralstonia solanacearum	1474	18,8%	5 194

Gerlin et al. 2020

➔ Lack of robustness and flexibility

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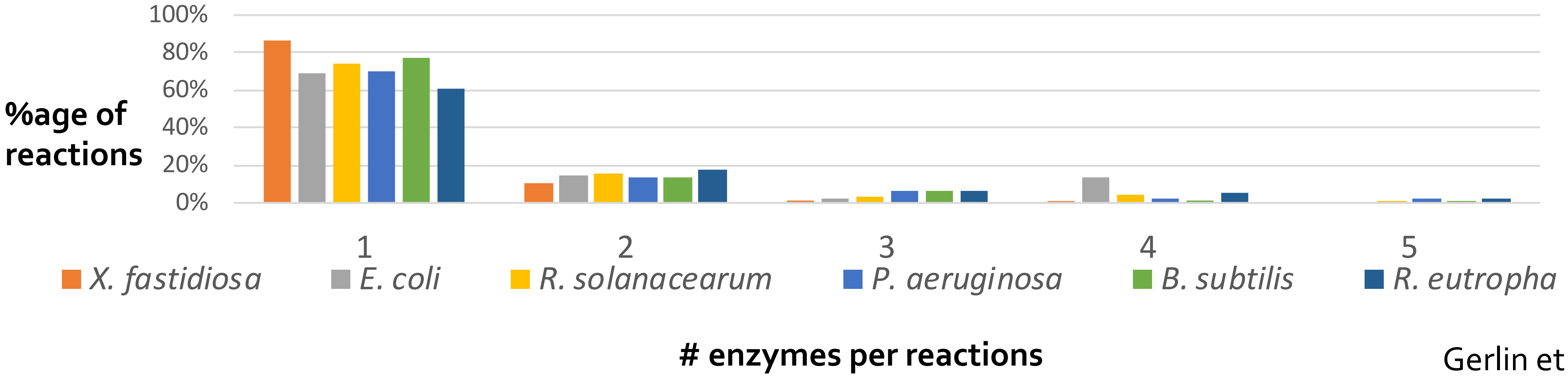

Gerlin et al. 2020

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Xylella fastidiosa	537	53,8%	[illegible]32
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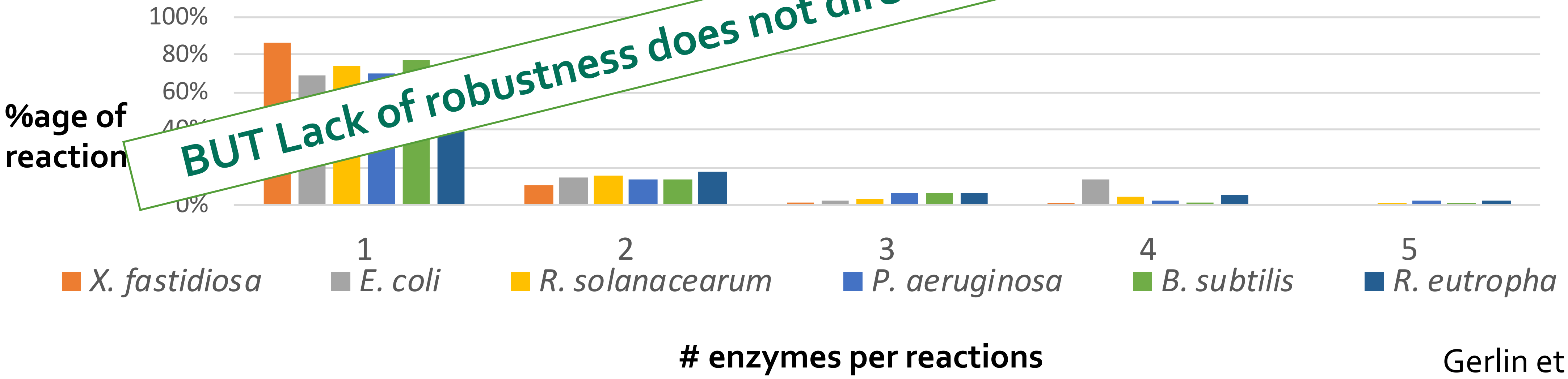
BUT Lack of robustness does not directly explain fastidious growth.

Isoenzyme study (*in silico*)



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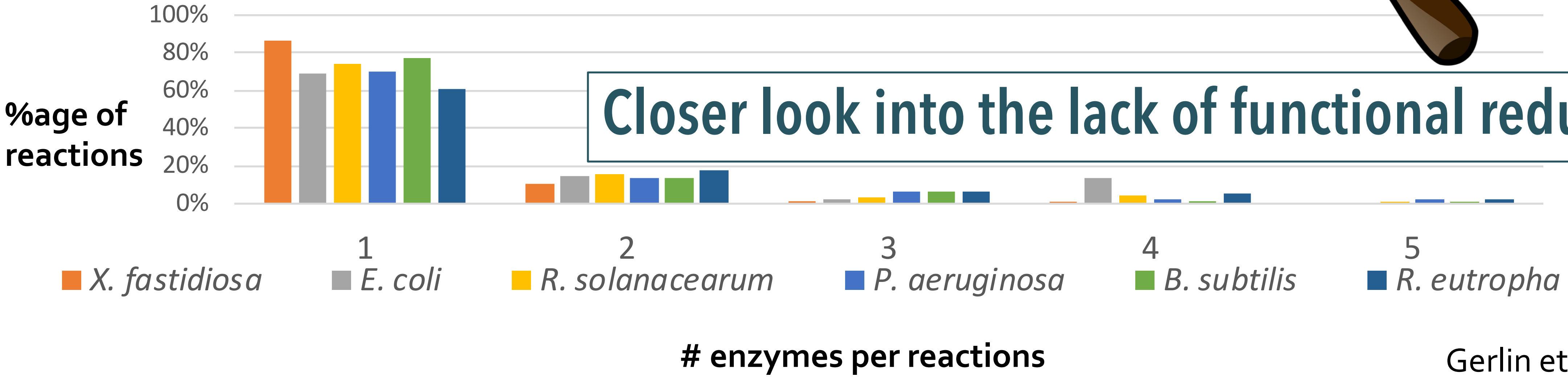


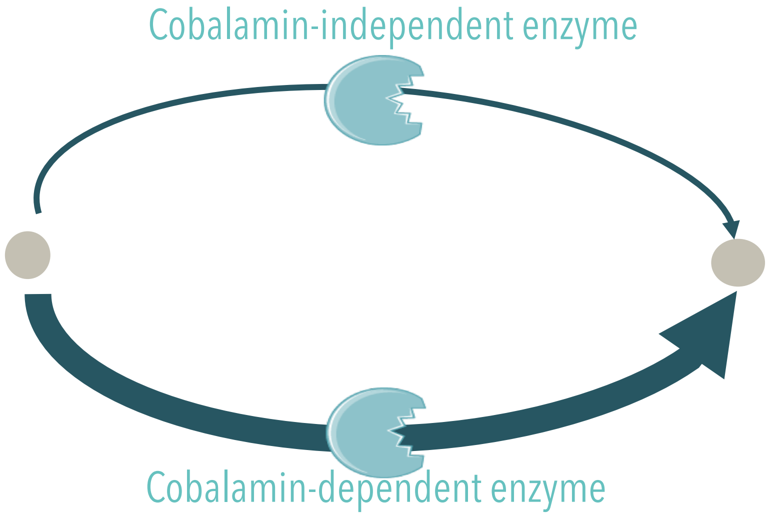
Closer look into the lack of functional redundancy

Gerlin et al. 2020

➔ Lack of robustness and flexibility

Where are efficient enzymes ?

- Cobalamin (B12) vitamin example
  - The same metabolic reaction can be performed by cobalamin-dependent / cobalamin-independent enzymes.
  - Cobalamin-dependent enzymes are more efficient.



X. fastidiosa:

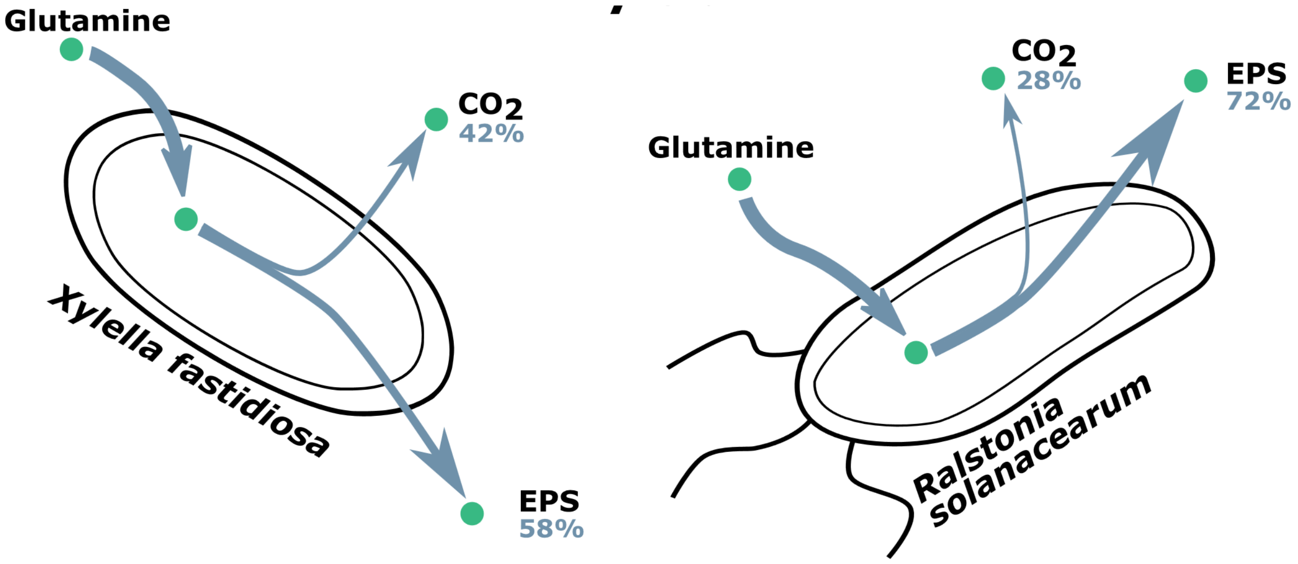
- ○ Cannot synthesize cobalamin
- ○ Does not possess cobalamin-dependent enzymes
- ○ This is true for other cofactors such as molybdopterin

➔**Global absence of efficient enzymes.**

➔ **Reduced fluxes which could decrease growth rate.**

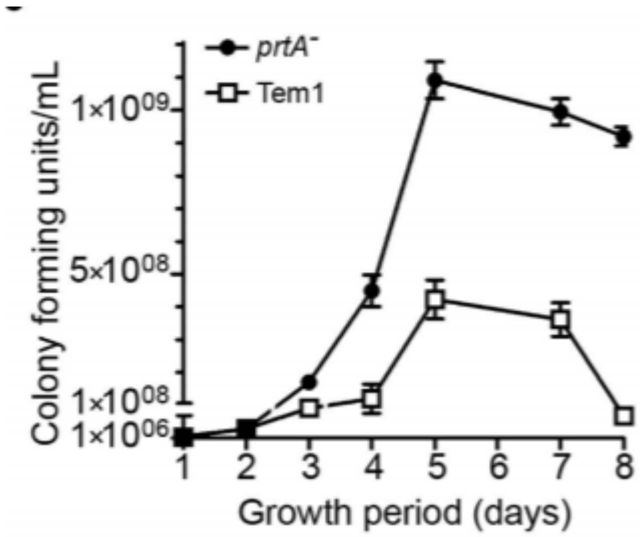
Gerlin et al. 2020

Efficiency study: exopolysaccharides (EPS) production



➔ **Inefficient EPS production in *Xylella***

(Gouran et al., 2016)

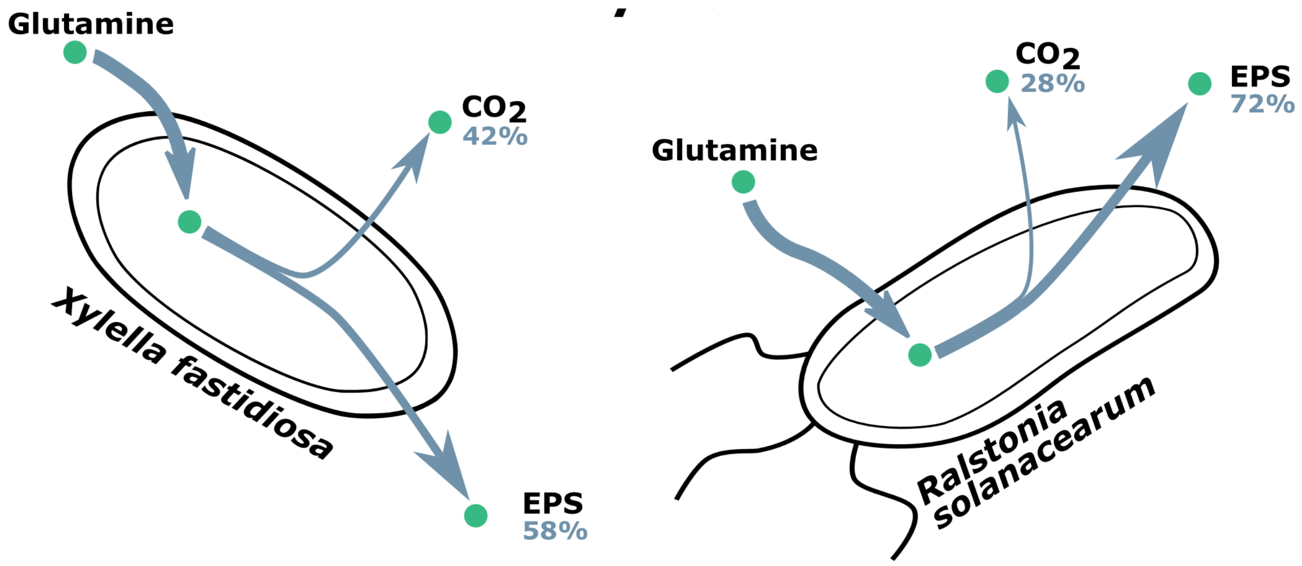


prtA$^-$: reduced biofilm (EPS)/protein secretion

➔ Enhanced growth

Gerlin et al. 2020

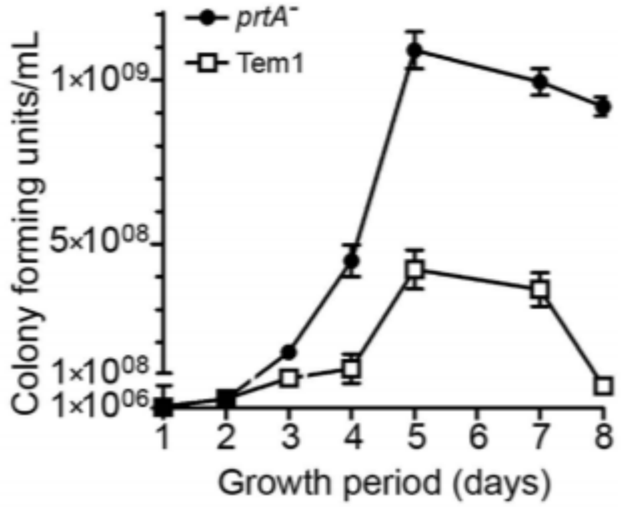
Efficiency study: exopolysaccharides (EPS) production



➔ **Inefficient EPS production in *Xylella***

➔ **Evolution drove *Xylella* to slow growth: strategy of virulence ? (low population level to remain undetected by the host)**

(Gouran et al., 2016)



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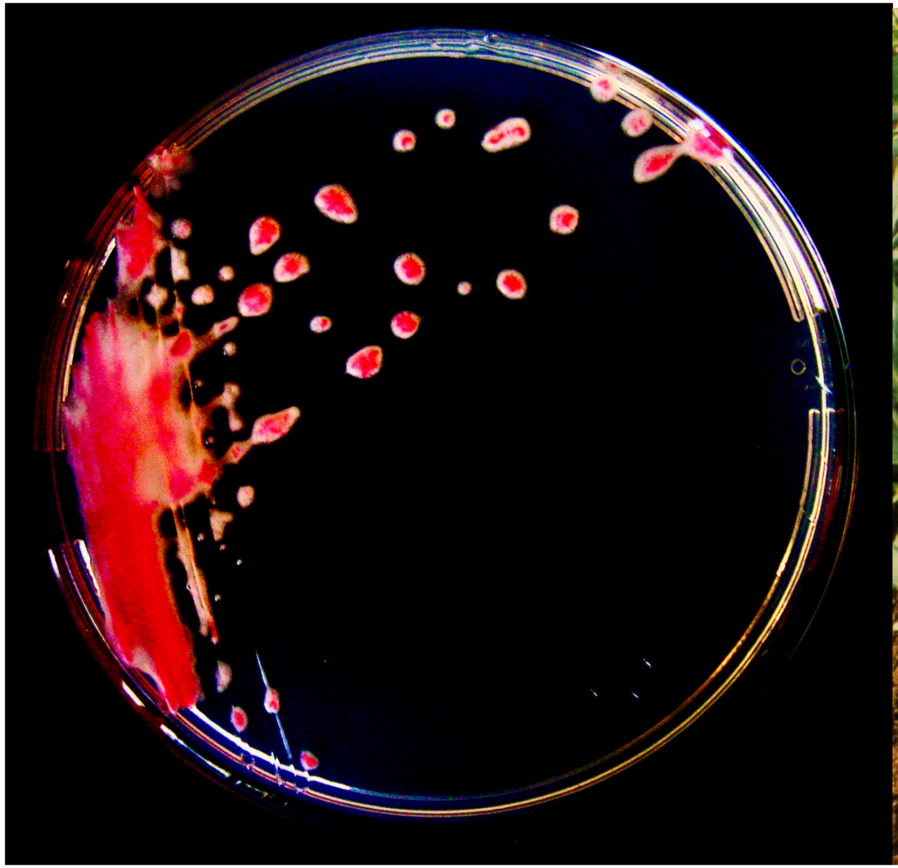
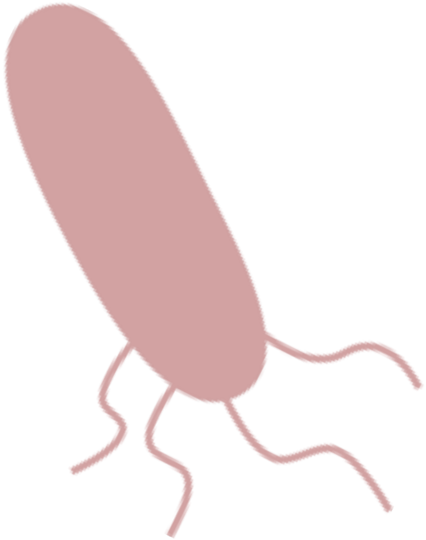
Gerlin et al. 2020

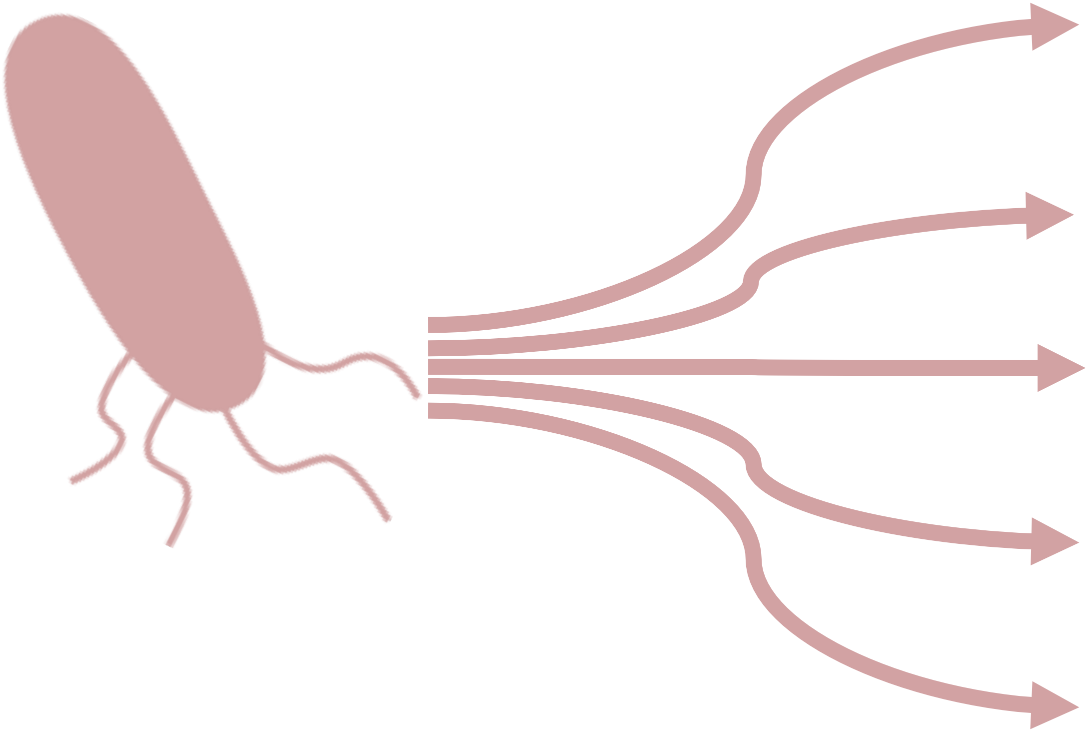




$$\frac{dM}{dt} = K.v.B$$

Example 2 & 3: *Ralstonia solanacearum* in tomato plants

Ralstonia solanacearum



Infection of over a 200 plant species
Tomato, Banana, Ginger,…

Bacterial wilting

Soil bacteria

Xylem sap pathogen

Relatively fast growth

Biological questions

How *R. solanacearum* thrive inside the plant, since xylem is often cited as a poor environment ?

Biological questions

How *R. solanacearum* thrive inside the plant, since xylem is often cited as a poor environment ?

What impact on plant metabolism and physiology ?

Biological questions

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What impact on plant metabolism and physiology ?

Is *R. solanacearum* able to modify plant metabolism to support its growth ?

Biological questions

How *R. solanacearum* thrive inside the plant, since xylem is often cited as a poor environment ?

What impact on plant metabolism and physiology ?

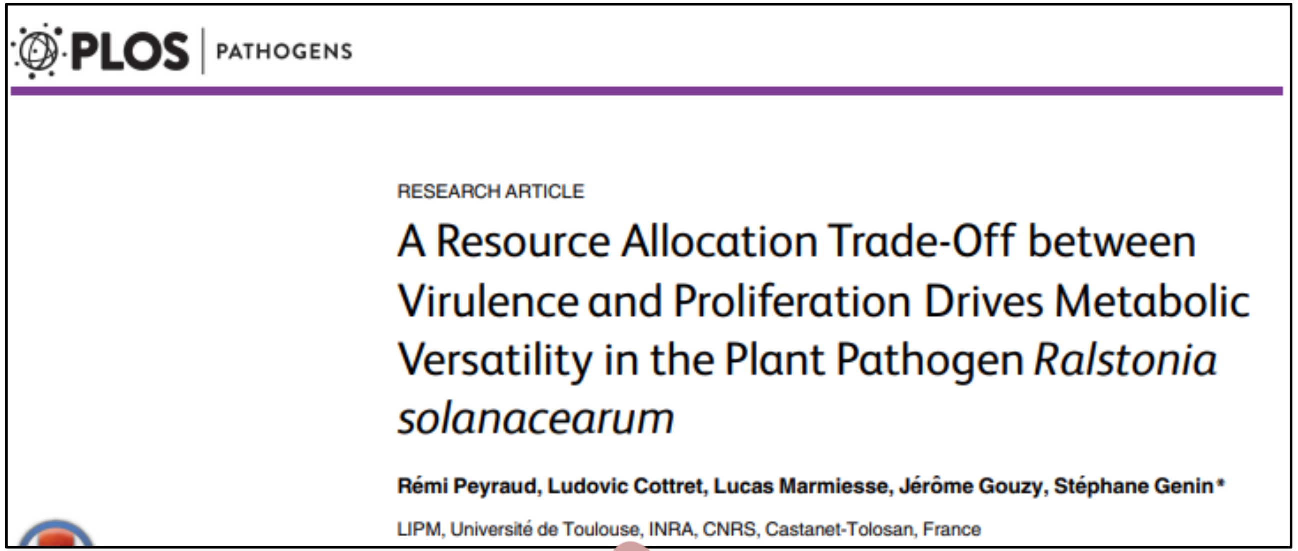
Is *R. solanacearum* able to modify plant metabolism to support its growth ?

➔ Need to study plant and pathogen metabolism

Metabolic models already developed

Ralstonia solanacearum GMI1000
(Peyraud et al., 2016)

Tomato: natural host of *Rs* GMI1000
leaf cell (Yuan et al., 2016)



PLOS | PATHOGENS

RESEARCH ARTICLE

A Resource Allocation Trade-Off between Virulence and Proliferation Drives Metabolic Versatility in the Plant Pathogen *Ralstonia solanacearum*

Rémi Peyraud, Ludovic Cottret, Lucas Marmiesse, Jérôme Gouzy, Stéphane Genin*

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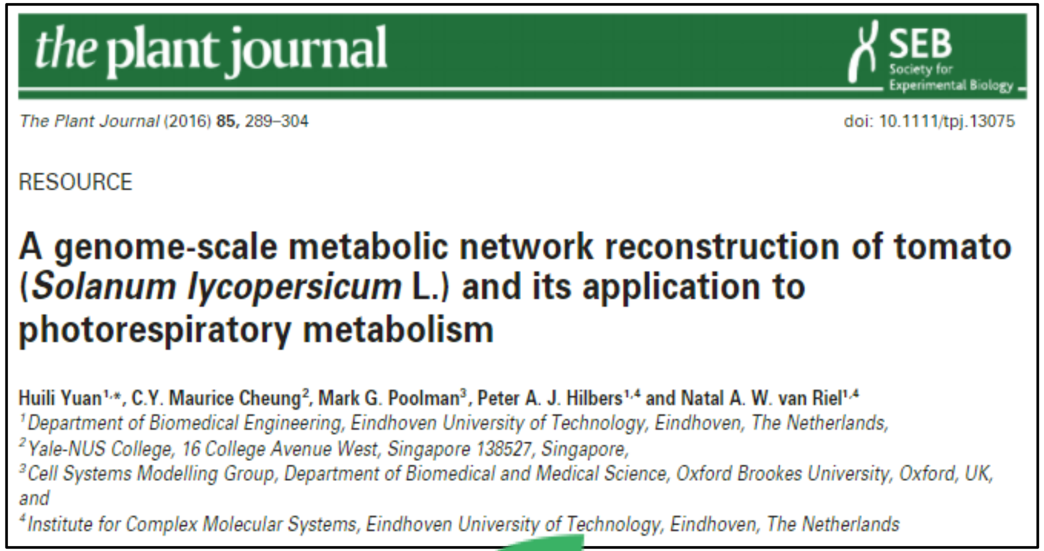

the plant journal — SEB Society for Experimental Biology

The Plant Journal (2016) **85**, 289–304 — doi: 10.1111/tpj.13075

RESOURCE

A genome-scale metabolic network reconstruction of tomato (*Solanum lycopersicum* L.) and its application to photorespiratory metabolism

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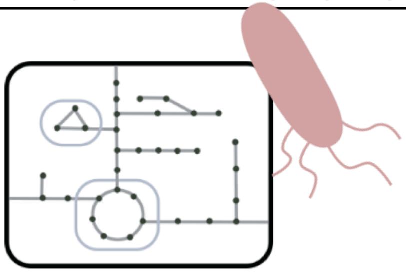

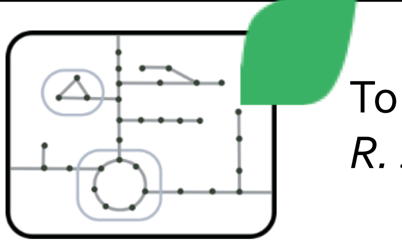

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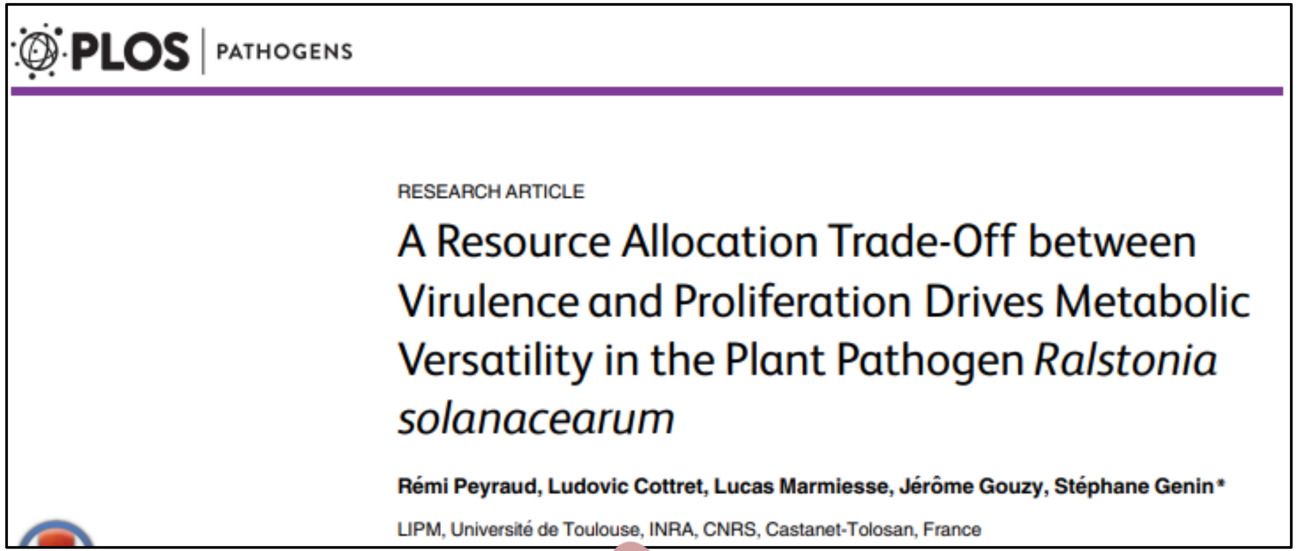

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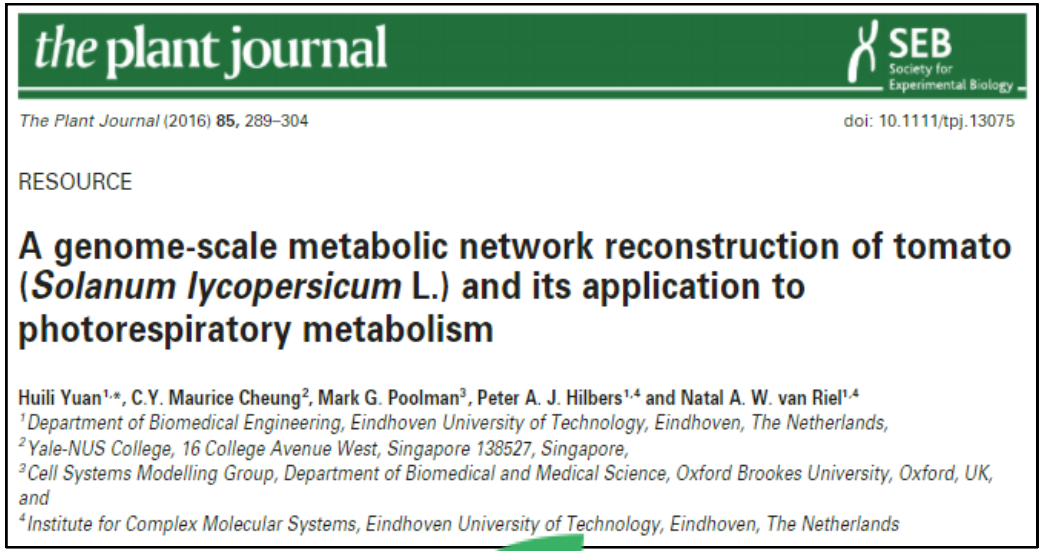

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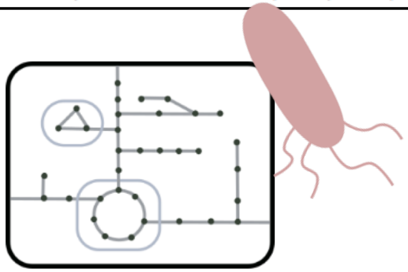
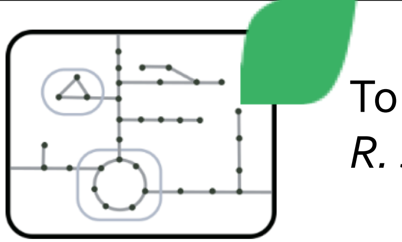


Next step



Tomato: natural host of
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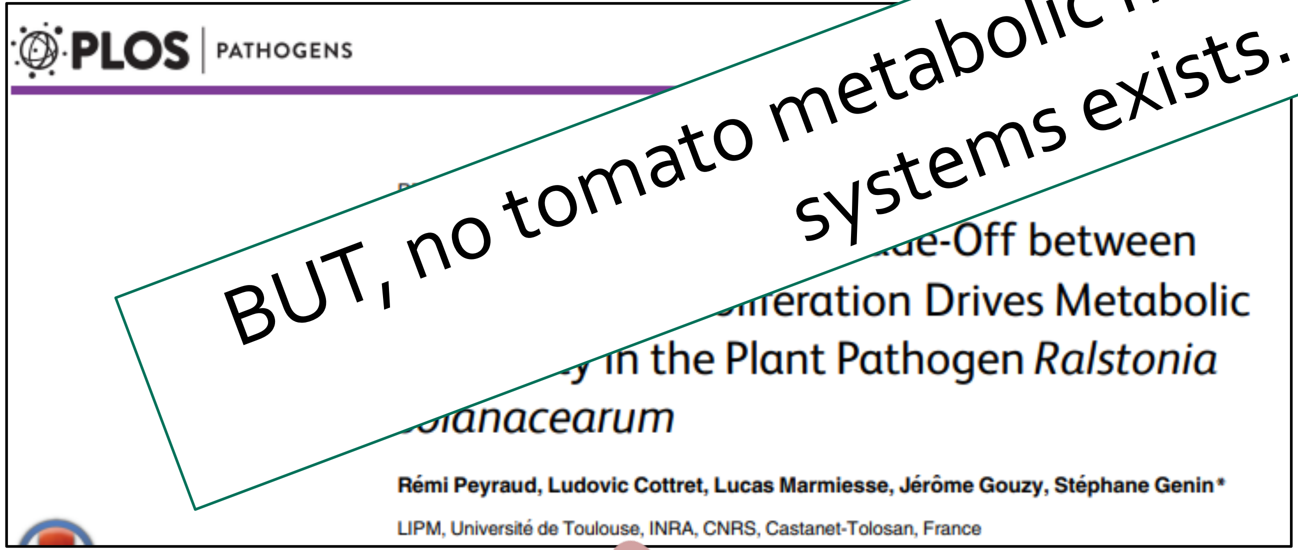
BUT, no tomato metabolic model with a vascular systems exists...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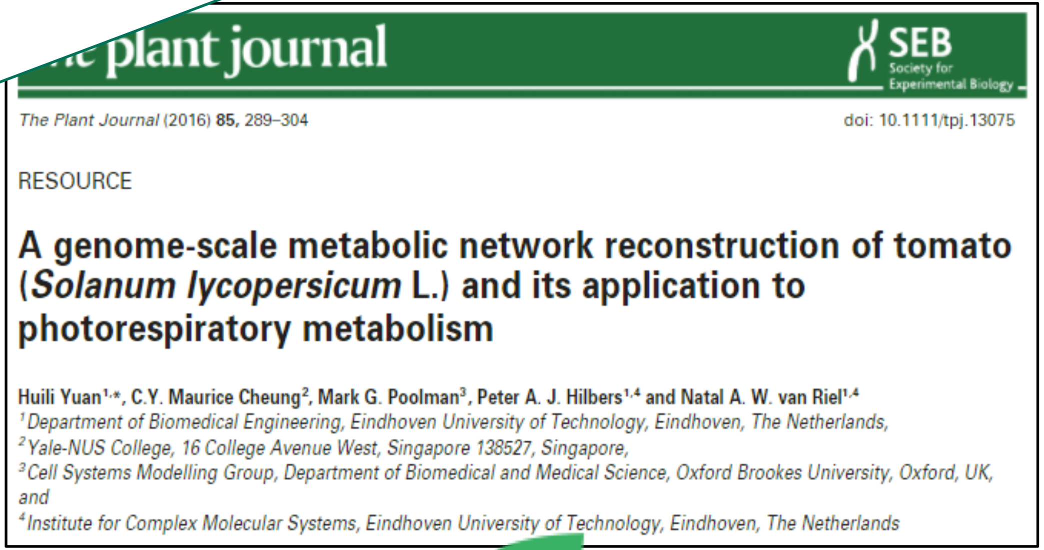
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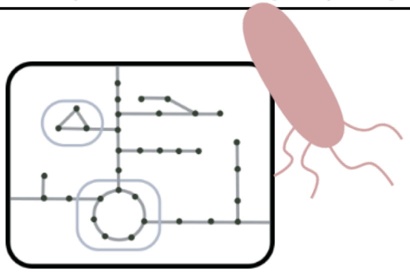

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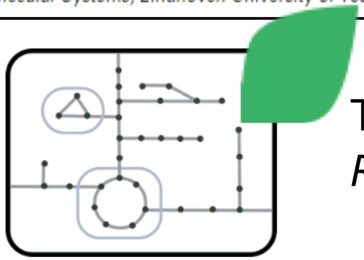

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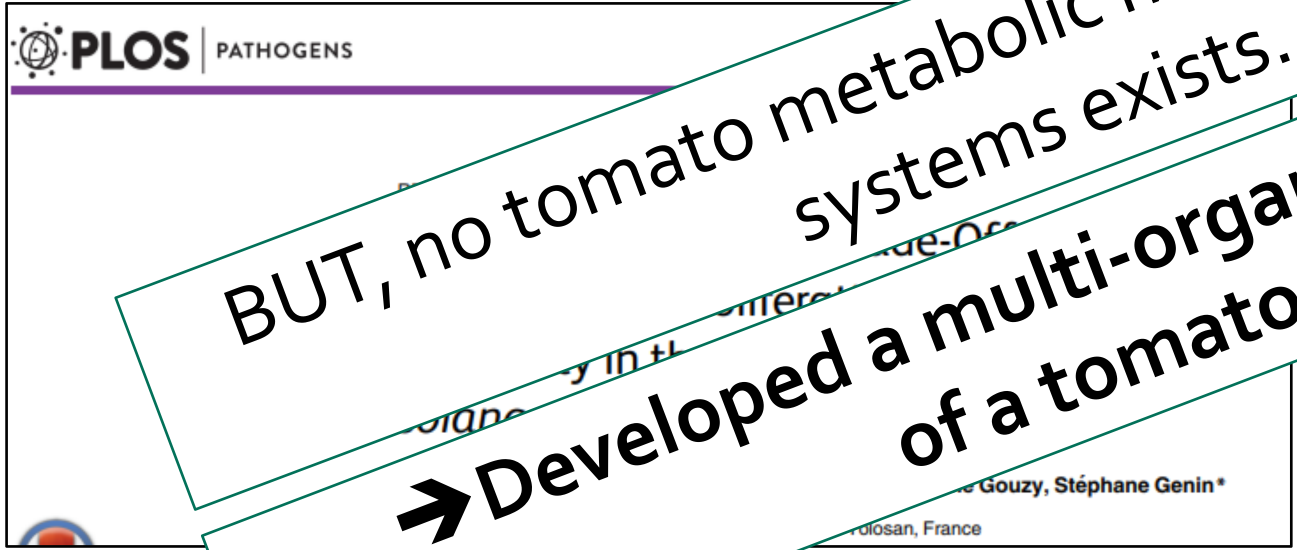
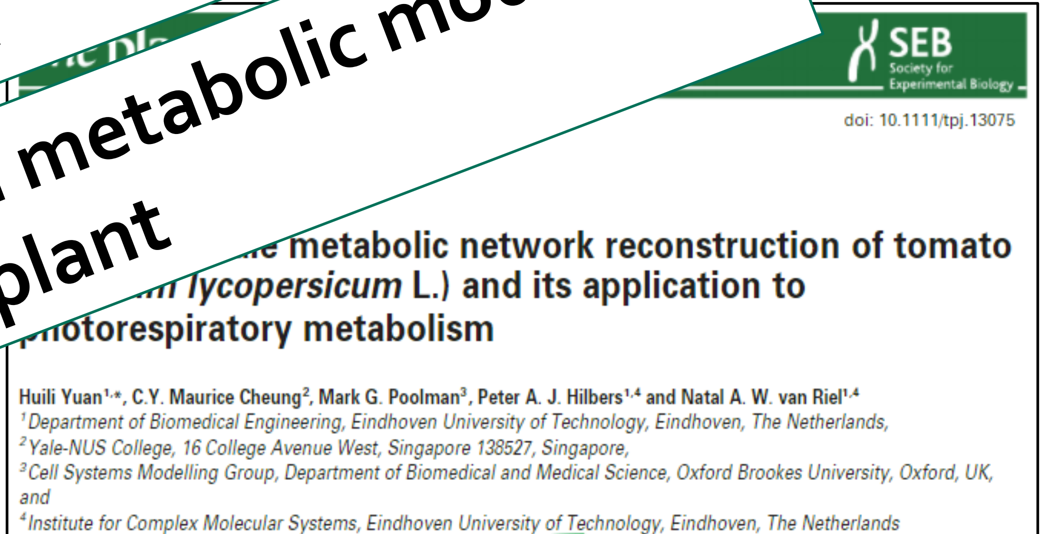


Gouzy, Stéphane Genin*

olosan, France



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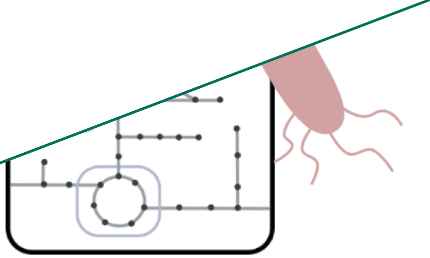
BUT, no tomato metabolic model with a vascular systems exists…

➔ Developed a multi-organ metabolic model of a tomato plant



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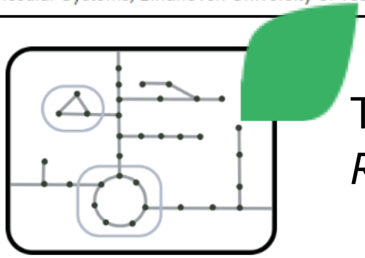
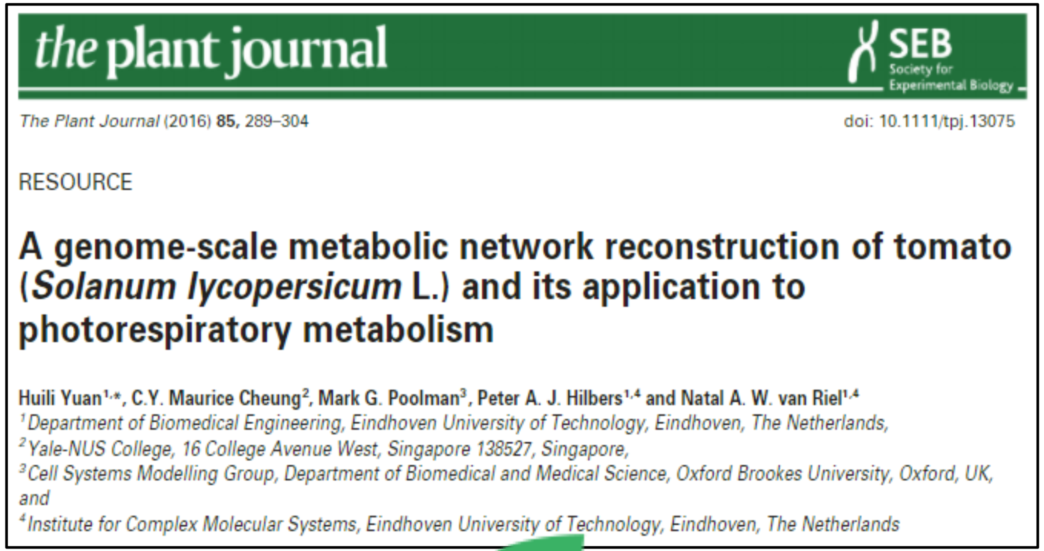


Tomato: natural host of *R. solanacearum*

VYTOP: a multi-organ tomato model

1. Curation of the leaf model

Tomato: natural host of *Rs* GMI1000 leaf cell (Yuan et al., 2016)



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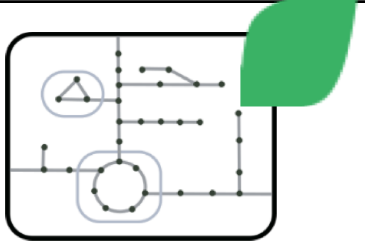


Gerlin et al. 2022

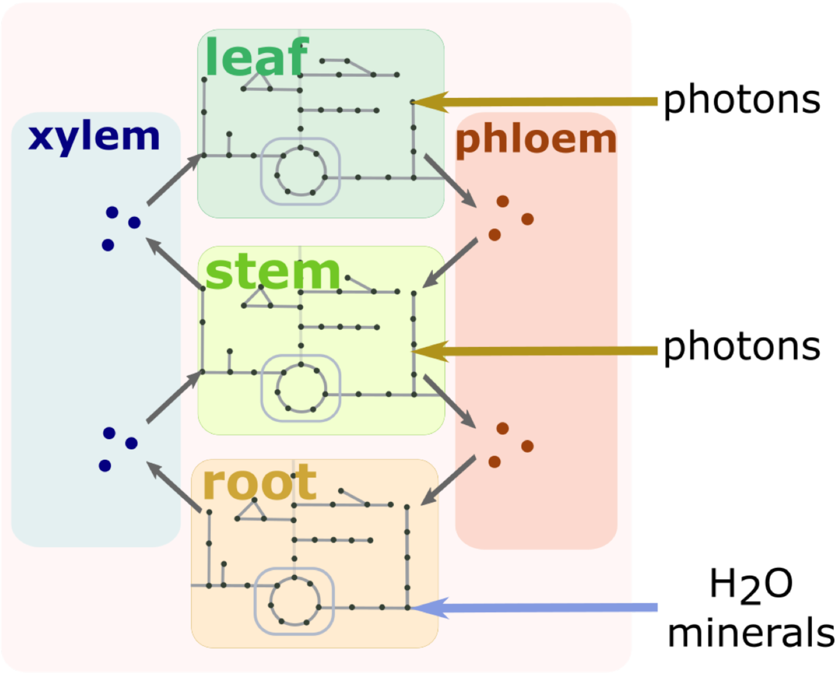
VYTOP: a multi-organ tomato model

1. Curation of the leaf model
2. Construction of a multi-organ model based on the physiological role of each organ

leaf
photosynthetic activity: generation of organic matter

stem
exchanges between root and leaf
architectural structure
limited photosynthetic activity

root
uptake of nutrients and water from the soil



Gerlin et al. 2022

VYTOP: a multi-organ tomato model

1. Curation of the leaf model
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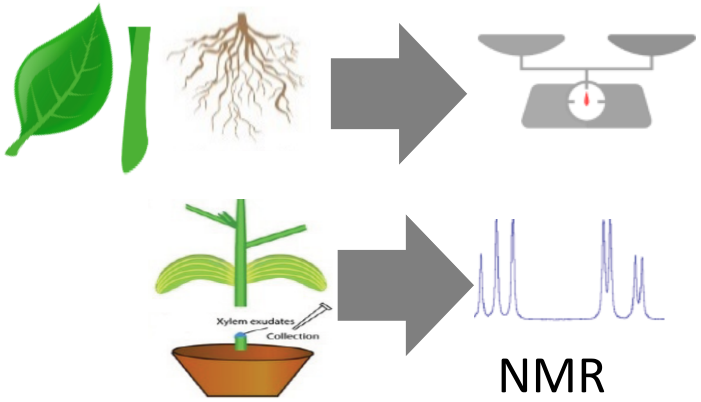
3. Calibration on experimental data



Experimental calibration on 90 tomato plants

Gerlin et al. 2022

VYTOP: a multi-organ tomato model

1. Curation of the leaf model
2. Construction of a multi-organ model based on the physiological role of each organ
3. Calibration on experimental data
4. Simulation

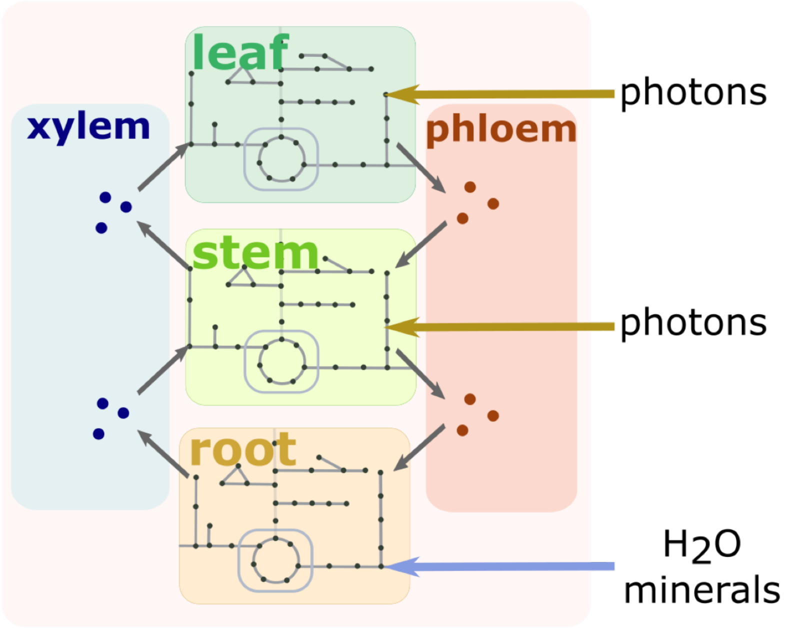
2 successive FBA

1. Minimization of photon uptake
2. Minimization of |flux| sum

Biomass growth set as a constraint

Gerlin et al. 2022

5. Validation of the model



- ✓ Recovered the physiological role of each organ
- ✓ Analyzed the stem as a sink of matter/energetical burden
- ✓ Predicted xylem fluxes of matter
- ✓ Predicted the impact of nitrogen limitation in the soil
- ✓ Predicted the behavior of transgenic tomato lines

Gerlin et al. 2022

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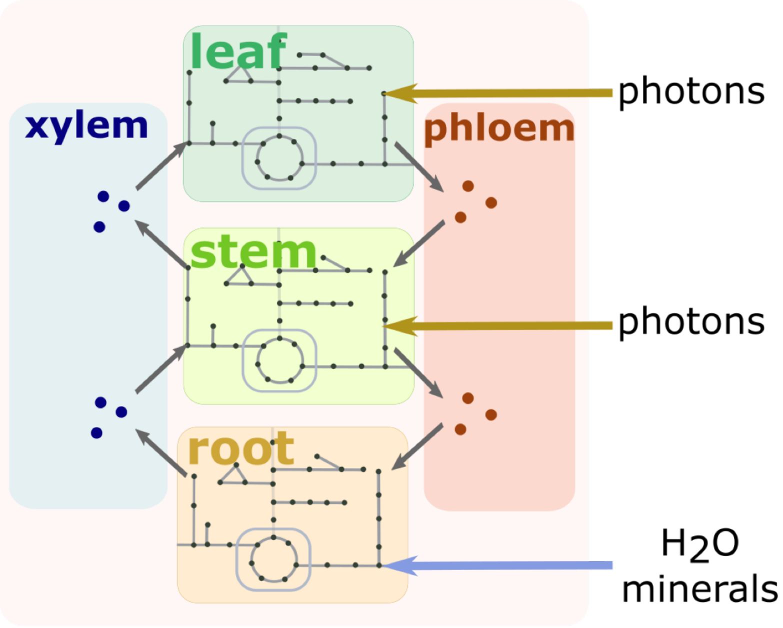

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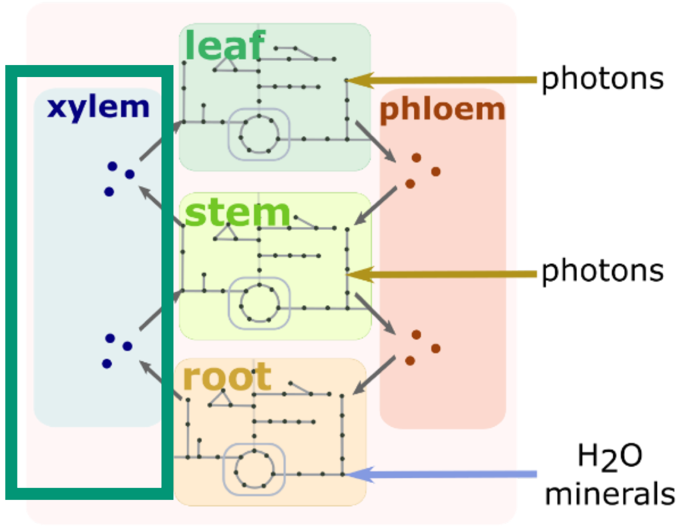
Gerlin et al. 2022

Tomato xylem sap composition



metabolite	concentration (mM)
glutamine	4.019 ± 1.419
asparagine	0.213 ± 0.170
lysine	0.177 ± 0.080
ethanol	0.113 ± 0.094
threonine	0.106 ± 0.092
proline	0.099 ± 0.052
leucine	0.095 ± 0.036
valine	0.079 ± 0.026
isoleucine	0.054 ± 0.018
sucrose	0.041 ± 0.039
phenylalanine	0.027 ± 0.015
tyrosine	0.025 ± 0.011
glucose	0.024 ± 0.045
fumarate	0.001 ± 0.002

High ↑

↓ **Low**

Gerlin et al. 2022

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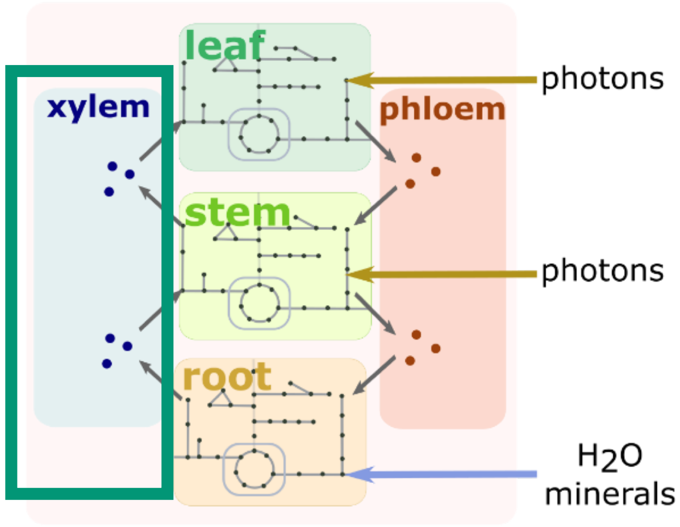

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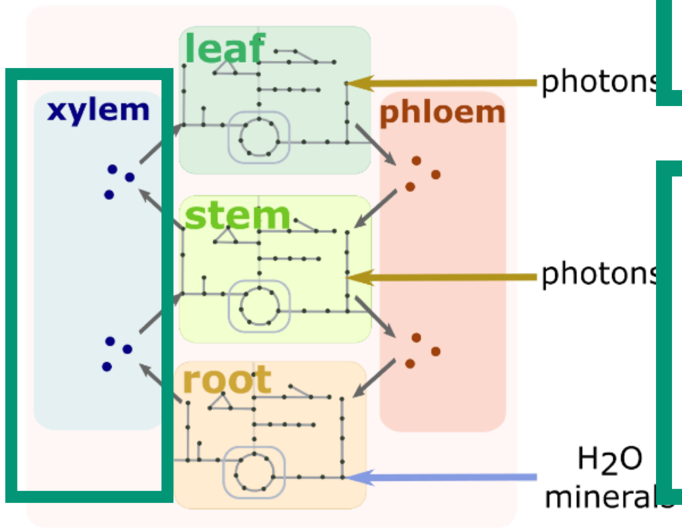
High

Low

predominance of glutamine (75% of organic carbon)

Gerlin et al. 2022

Tomato xylem sap composition



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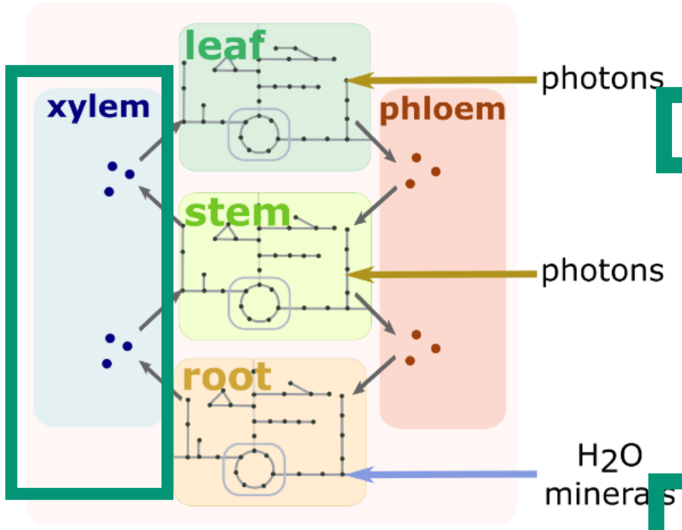
High

Low

presence of several amino acids at +/- diluted concentrations

Gerlin et al. 2022

Tomato xylem sap composition



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tyrosine	0.025 ± 0.011
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fumarate	0.001 ± 0.002

Low

presence of ethanol

very diluted concentrations of sucrose, glucose, fumarate

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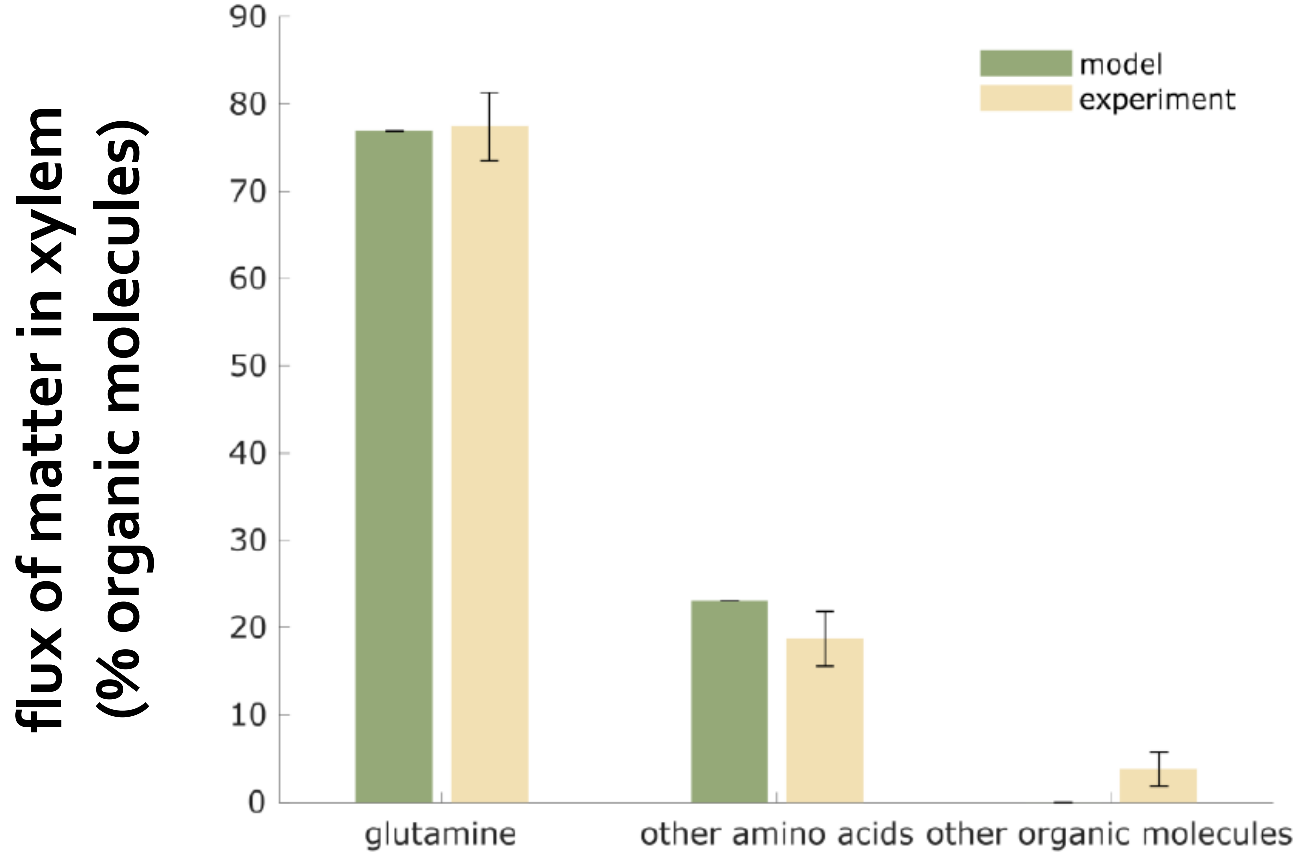
Prediction of tomato xylem sap composition



Constraint: energetical cost (ATP) per molecule exchanged

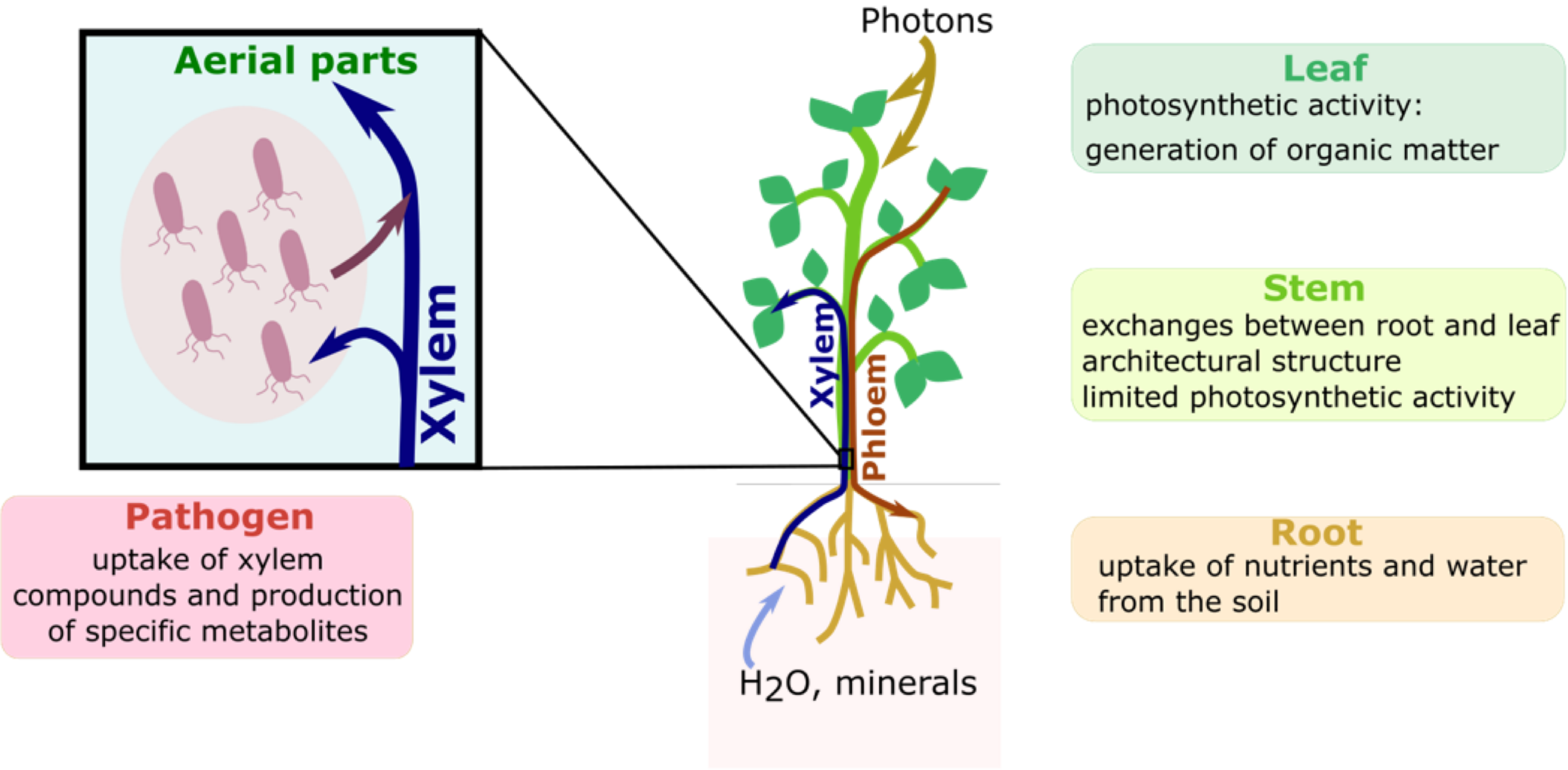
Predominance of glutamine + presence of additional amino acids predicted by the model

➔ **Driven by plant physiological constraints and optimality**

Gerlin et al. 2022

Modeling a plant pathogen interaction

➔ **Next step: modelling the pathogen interaction with the plant**



Pathogen
uptake of xylem compounds and production of specific metabolites

Leaf
photosynthetic activity:
generation of organic matter

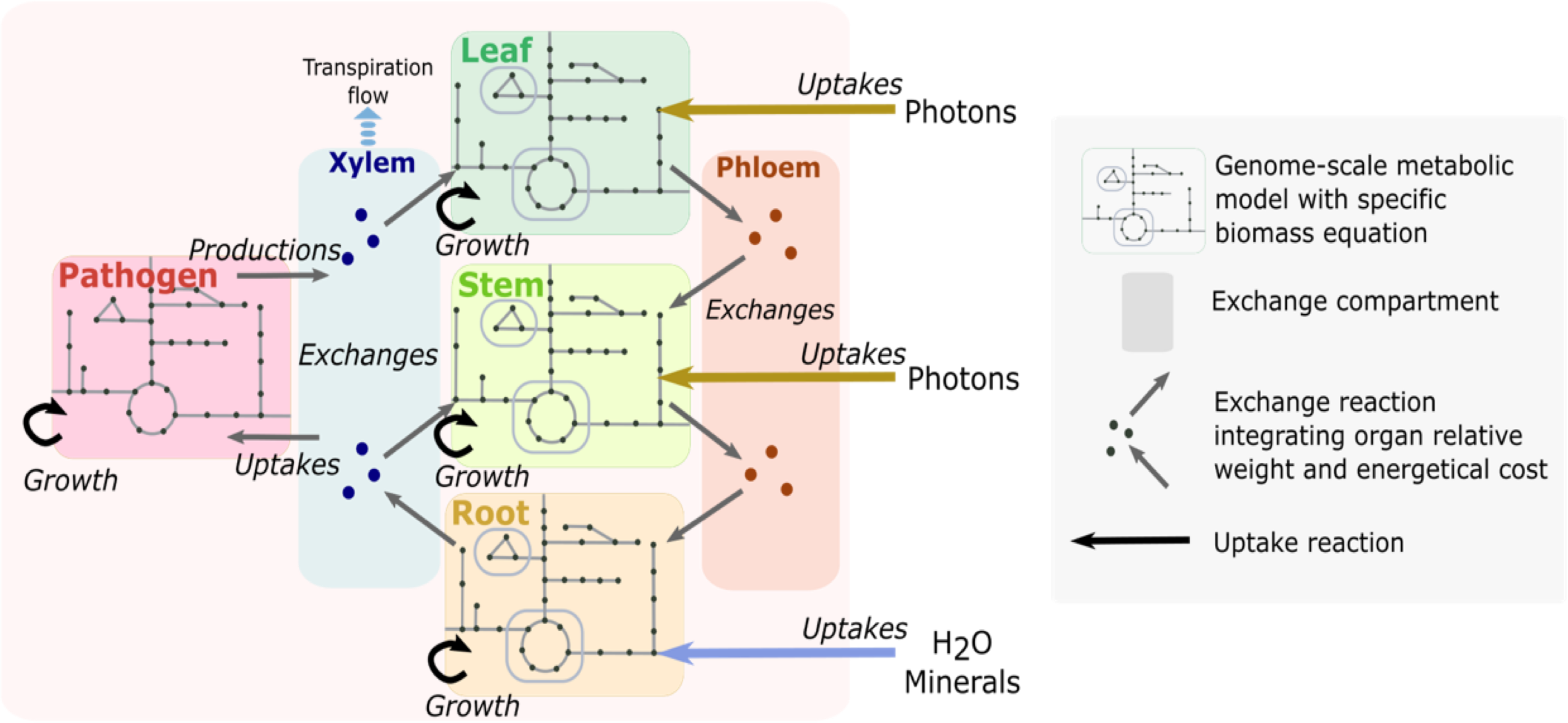
Stem
exchanges between root and leaf
architectural structure
limited photosynthetic activity

Root
uptake of nutrients and water from the soil

Gerlin et al. submitted

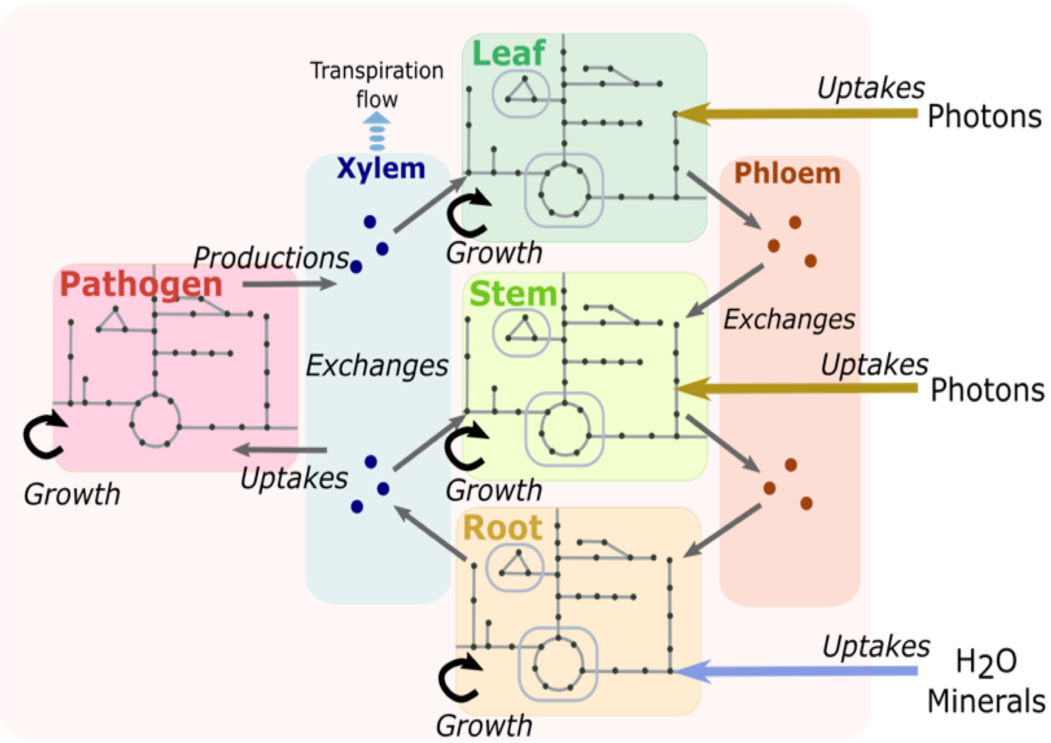
Modeling a plant pathogen interaction

➔ **Next step: modelling the pathogen interaction with the plant**



Gerlin et al. submitted

Modeling a plant pathogen interaction: FBA 1



Constraints on *R. solanacearum* assimilation rate deduced from experiments and a macroscopic model

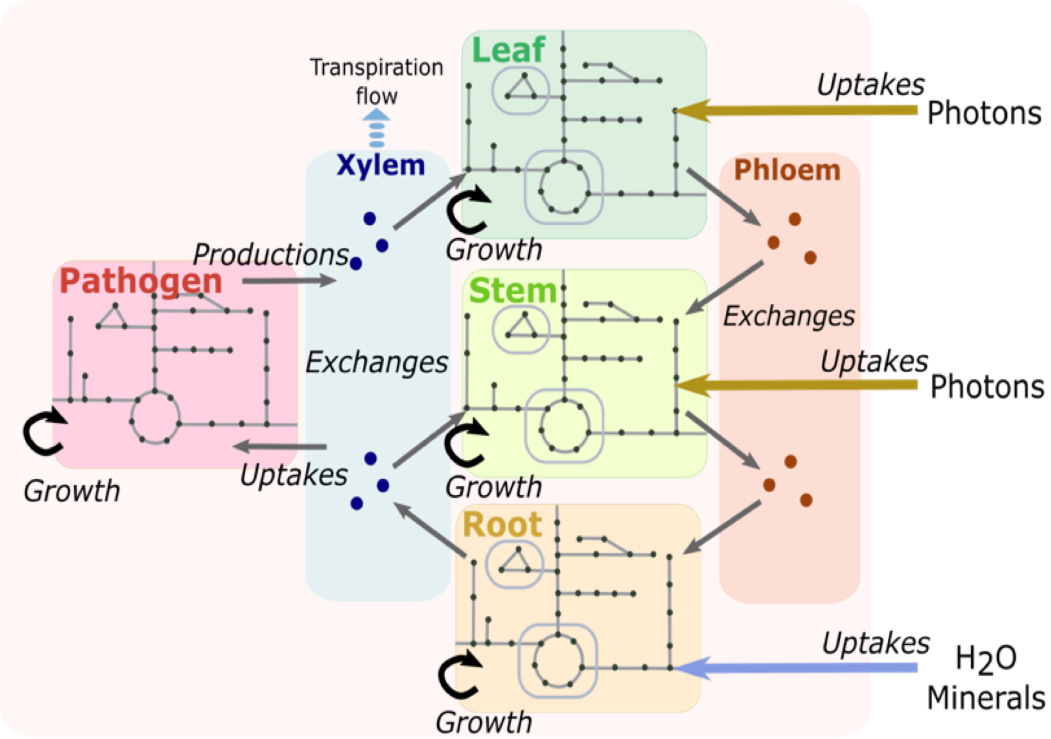
4 successive FBA

1. Minimization of photon uptake with leaf, stem and root growth set as constraint.

➔ To know the minerals and photon requirements of an healthy plant.

Gerlin et al. submitted

Modeling a plant pathogen interaction: FBA 2



Constraints on *R. solanacearum* assimilation rate deduced from experiments and a macroscopic model

4 successive FBA

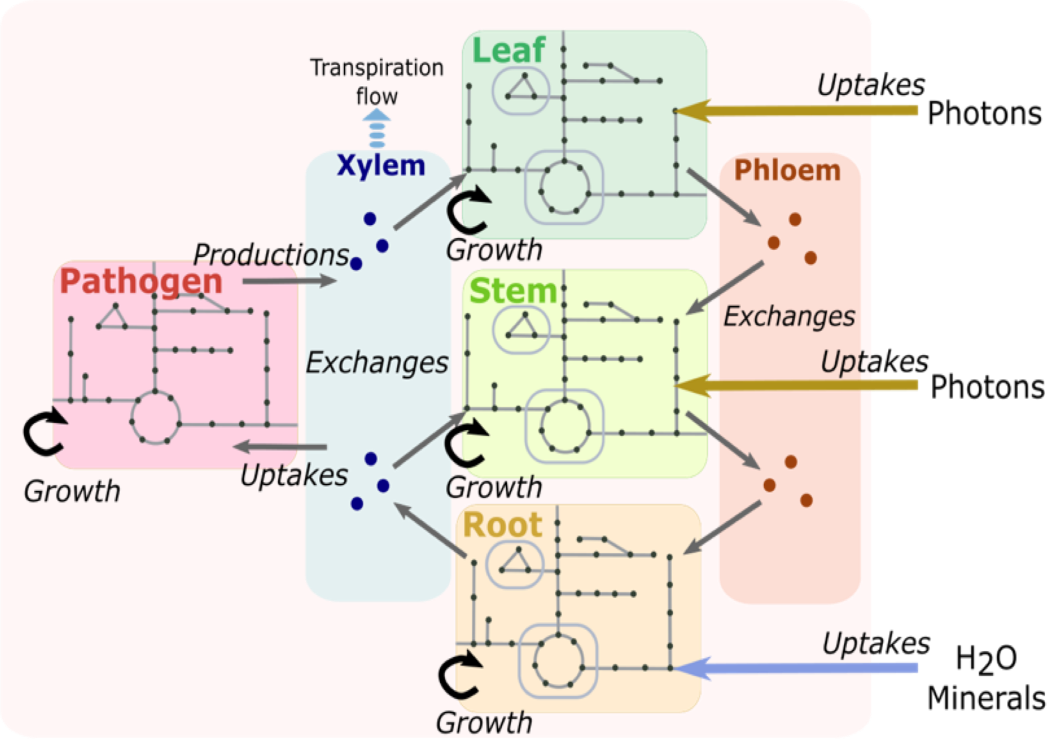
1. Minimization of photon uptake
2. Maximization of pathogen biomass with max photons assimilation set from FBA 1 and root growth set from exp. data.

➔ To predict the pathogen's growth

Optionally, can be set: nitrogen or iron requirements, effect of a decrease in transpiration flow.

Gerlin et al. submitted

Modeling a plant pathogen interaction: FBA 3



4 successive FBA

1. Minimization of photon uptake
2. Maximization of pathogen biomass
3. Maximization of aerial parts biomass with Ralstonia growth rate set from FBA 2.

➔ To predict the aerial parts growth

Constraints on *R. solanacearum* assimilation rate deduced from experiments and a macroscopic model

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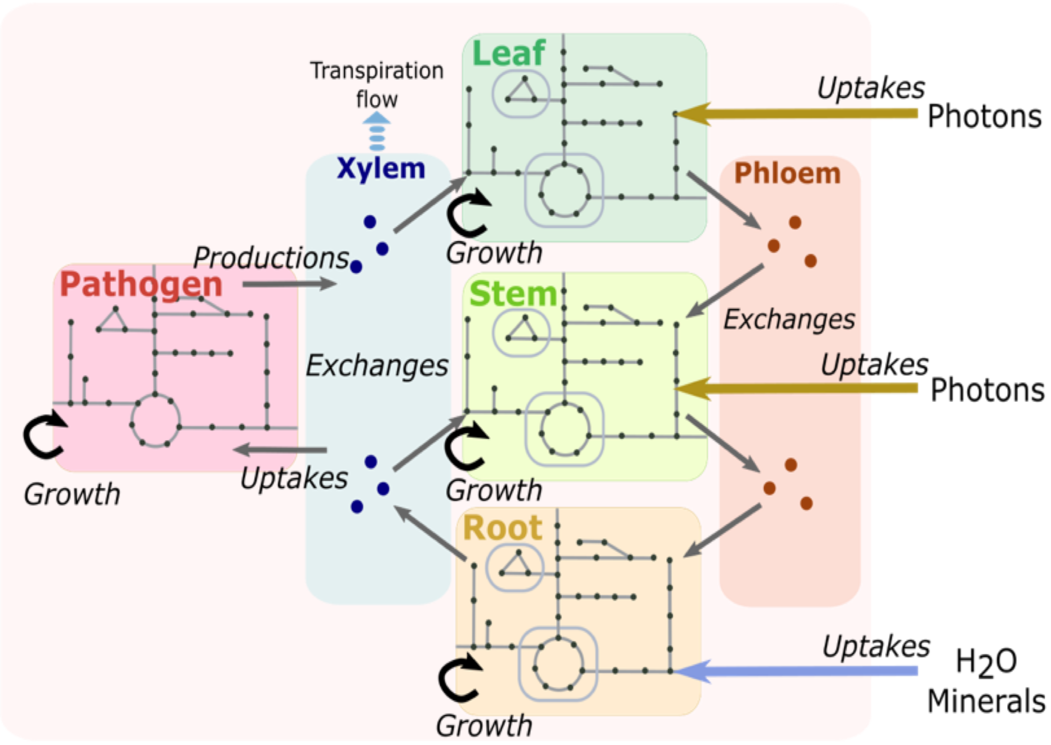
Modeling a plant pathogen interaction: FBA 4



4 successive FBA

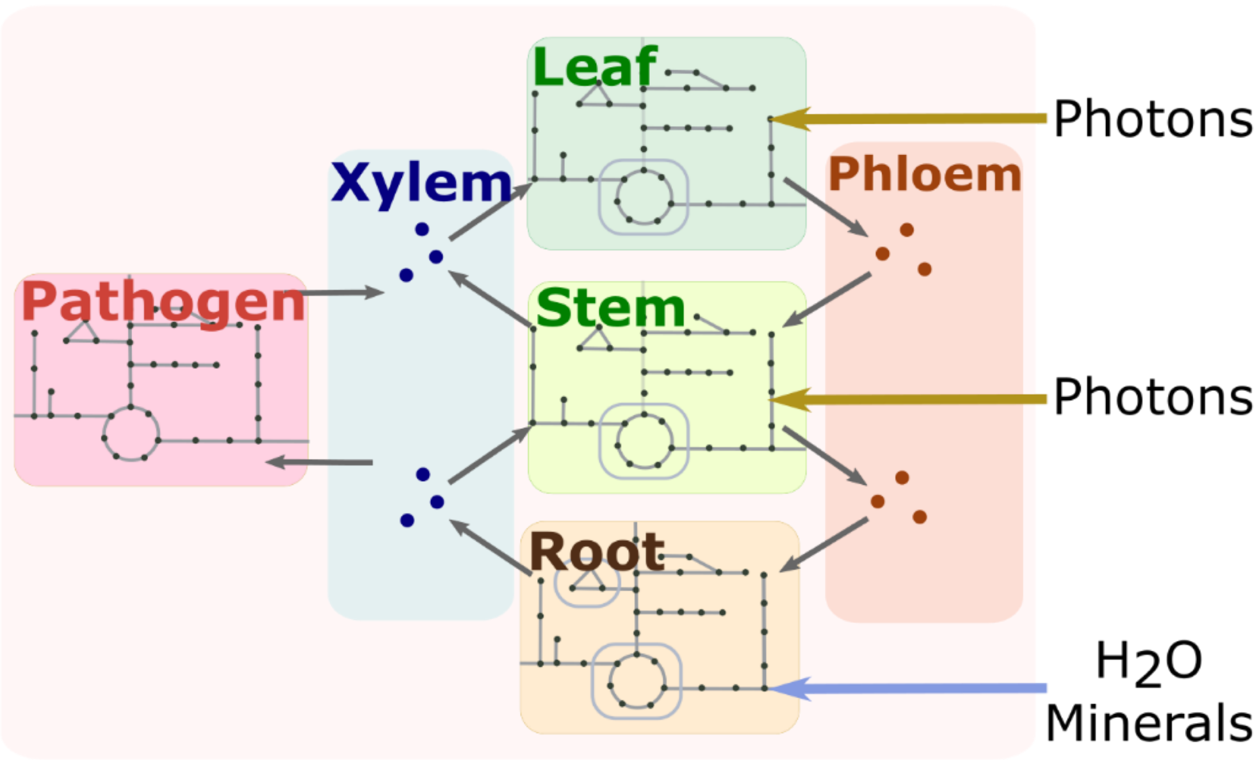
1. Minimization of photon uptake
2. Maximization of pathogen biomass
3. Maximization of aerial parts biomass
4. Minimization of |flux| sum with aerial growth rate set from FBA 3

➔ To consider a parsimonious enzyme usage

Constraints on *R. solanacearum* assimilation rate deduced from experiments and a macroscopic model

Gerlin et al. submitted

Global simulation approach



Constraints on *R. solanacearum* assimilation rate deduced from experiments and a macroscopic model

4 successive FBA

1. Minimization of photon uptake
2. Maximization of pathogen biomass
3. Maximization of aerial parts biomass
4. Minimization of | flux | sum

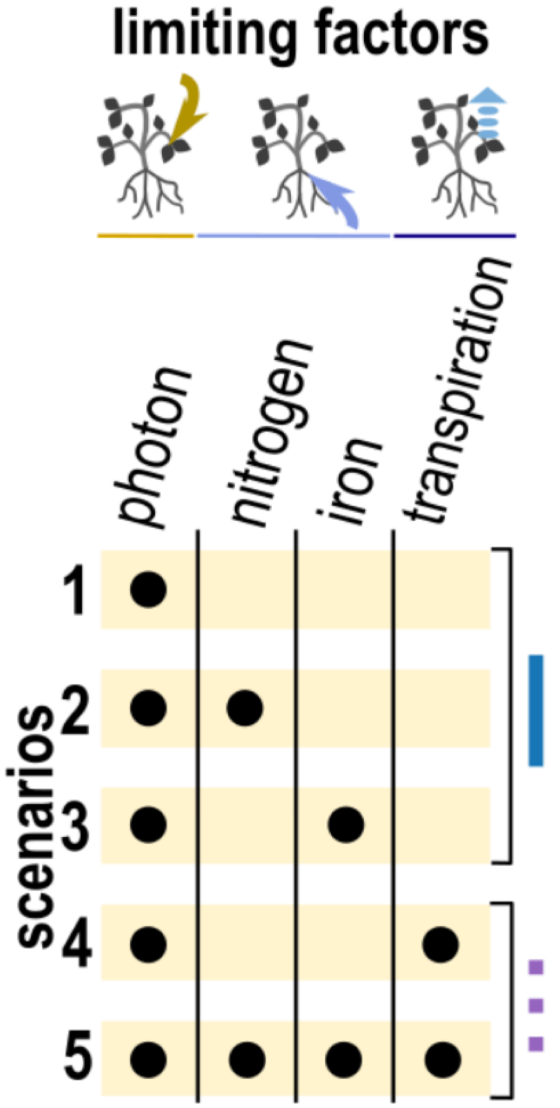
Simulations for different plant/pathogen weight ratio

$10^6 – 10^{12}$ cells/g stem FW

Gerlin et al. submitted

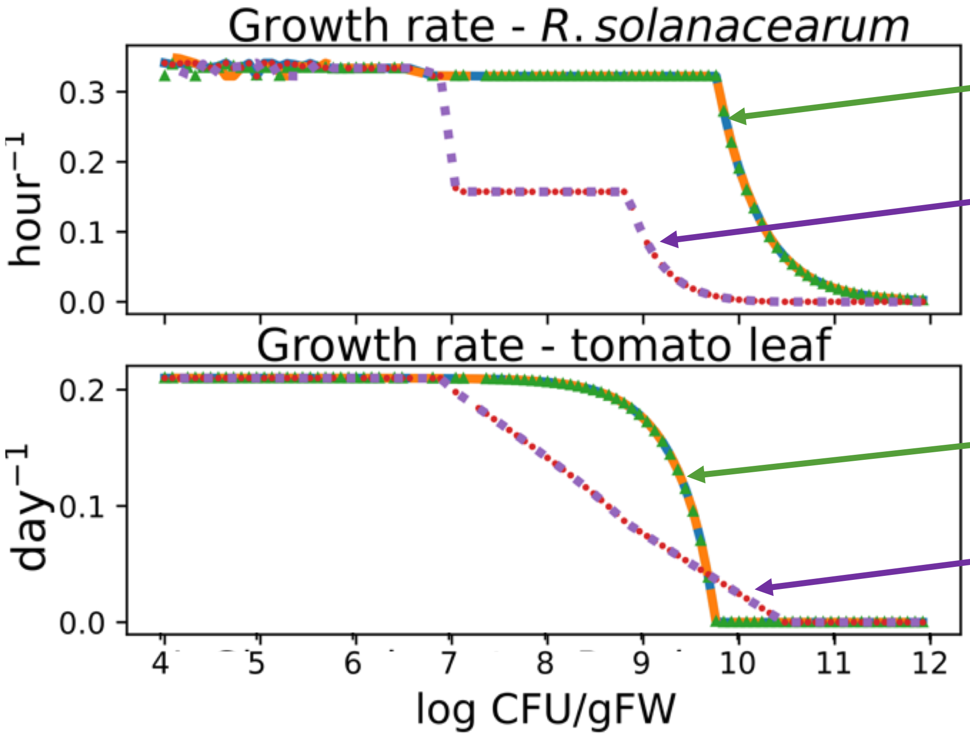
What limits pathogen's growth in the plant ?

5 scenarios of simulations to understand what constrains *in planta* bacterial growth during an infection



Gerlin et al. submitted

What limits pathogen’s growth in the plant ?



No transpiration limit

Transpiration limit

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Transpiration limit

- Photosynthesis contrains Ralstonia to a max density of 10^{11} CFU/gFW

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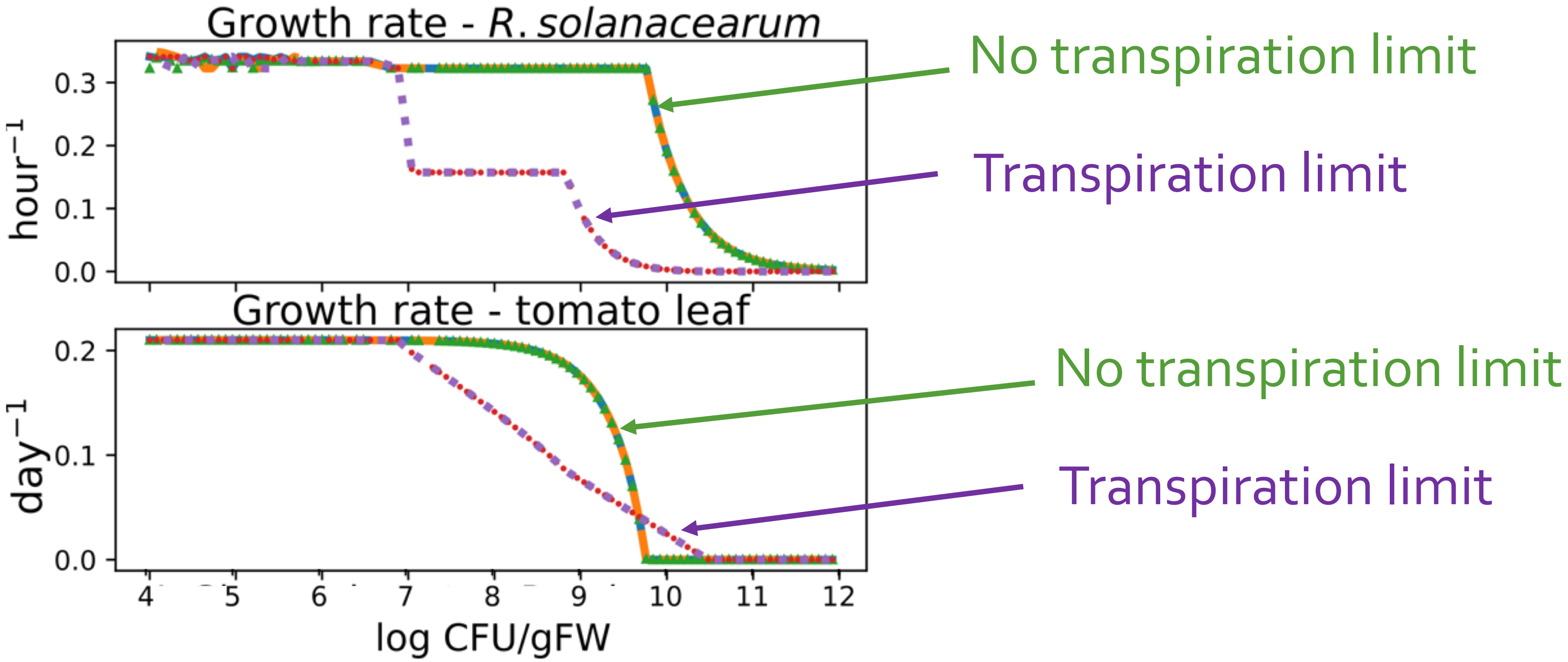

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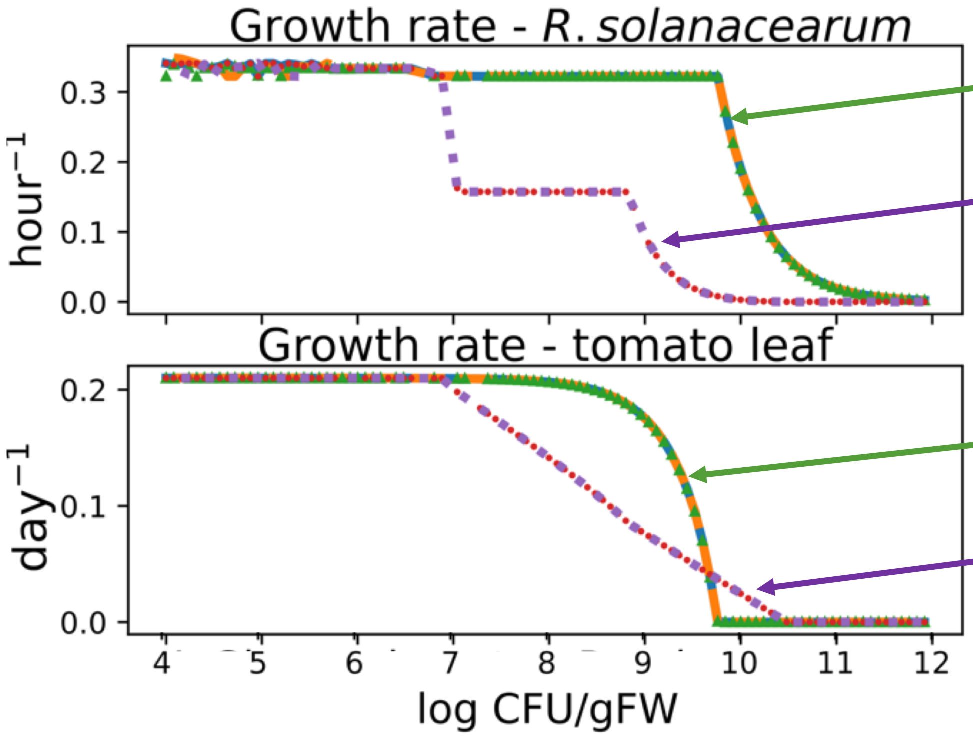
Transpiration limit

- Photosynthesis contrains Ralstonia to a max density of 10^{11} CFU/gFW
- With no transpiration limit, aerial plant growth rate decline at 10^{9} CFU/gFW and Ralstonia growth rate decline at 10^{10} CFU/gFW
- With transpiration limit, aerial plant growth rate decline at 10^{7} CFU/gFW and Ralstonia growth rate decline at 10^{9} CFU/gFW

What limits pathogen’s growth in the plant ?



No transpiration limit

Transpiration limit = in agreement with exp data

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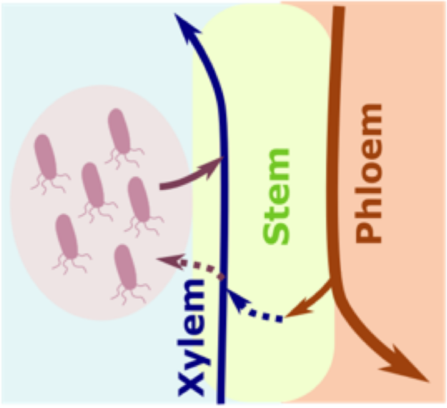
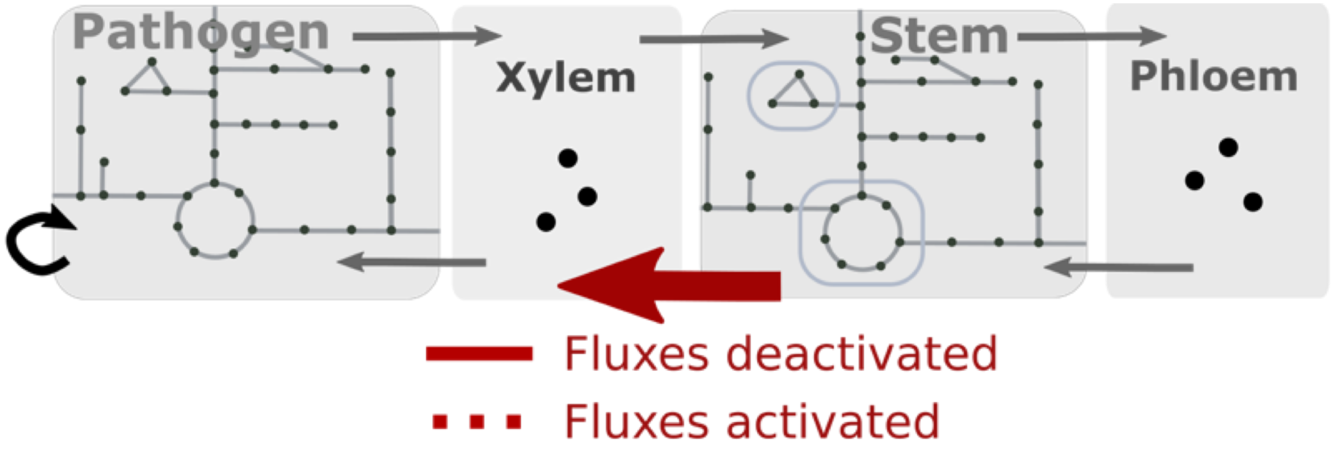
- ➔ Xylem sap is not poor since it can sustain Ralstonia growth up to 10^{11} CFU/gFW
- ➔ Transpiration is the most limiting factor for Ralstonia growth
- ➔ Plant growth arrest is due to transpiration decline
- ➔ Transpiration has a stronger effect than resource competition on xylem sap

Stem resource highjacking, a good strategy for Ralstonia ?

- Stem contribution to xylem sap was forbidden so far
- But the pathogen could have a hijacking resource strategy which makes the phloem contribute to the xylem via the stem
- We assessed with the model if this hypothesis is plausible

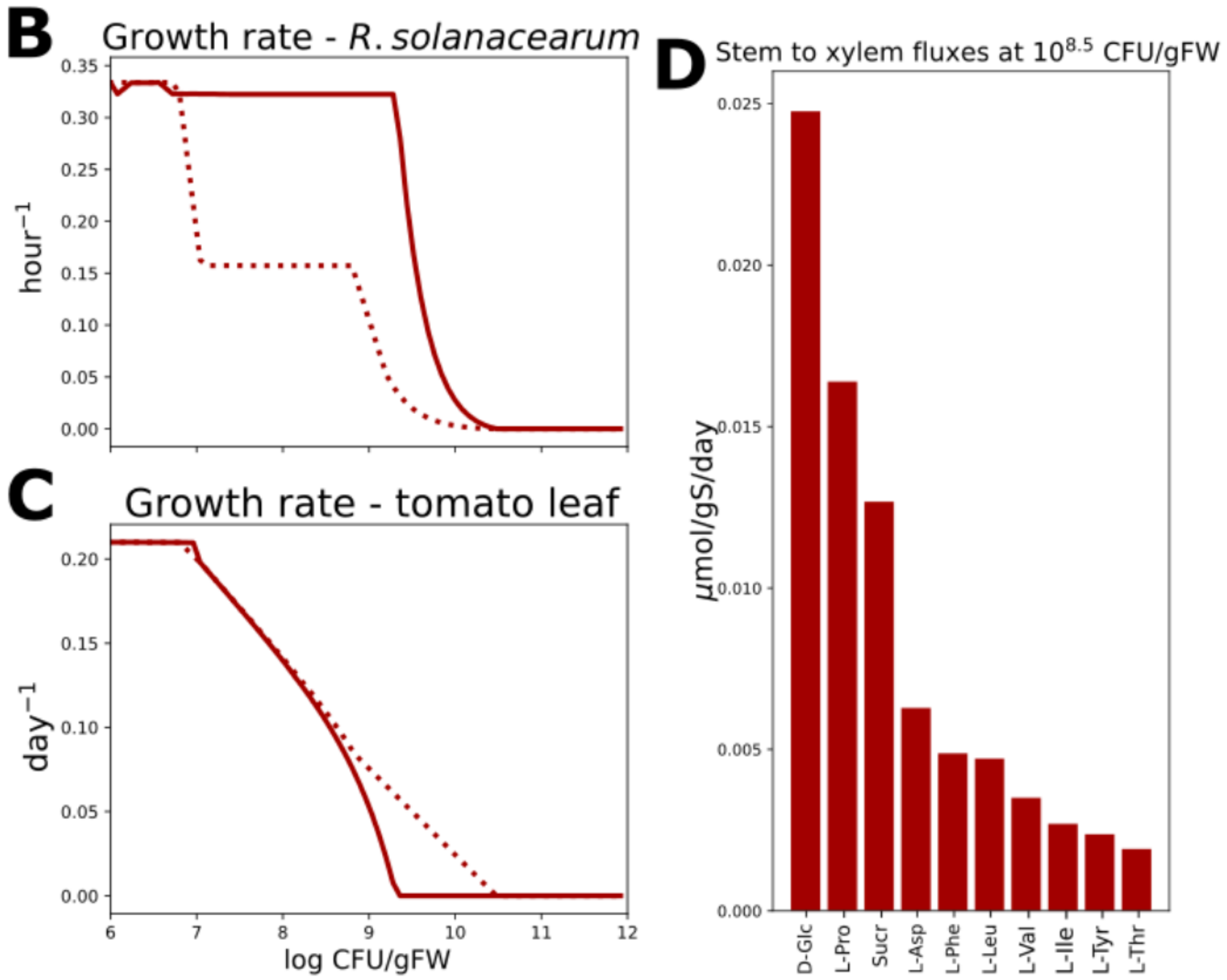




Stem resource highjacking, a good strategy for Ralstonia ?

If stem can contribute to xylem sap (plain line):

- Aerial part growth slightly impacted
- Max growth rate of Ralstonia sustained up to 10^{10} CFU/gFW
- Xylem sap mainly enriched in glucose, proline, sucrose
- Xylem sap enrichment by stem bypass transpiration limit for the pathogen.



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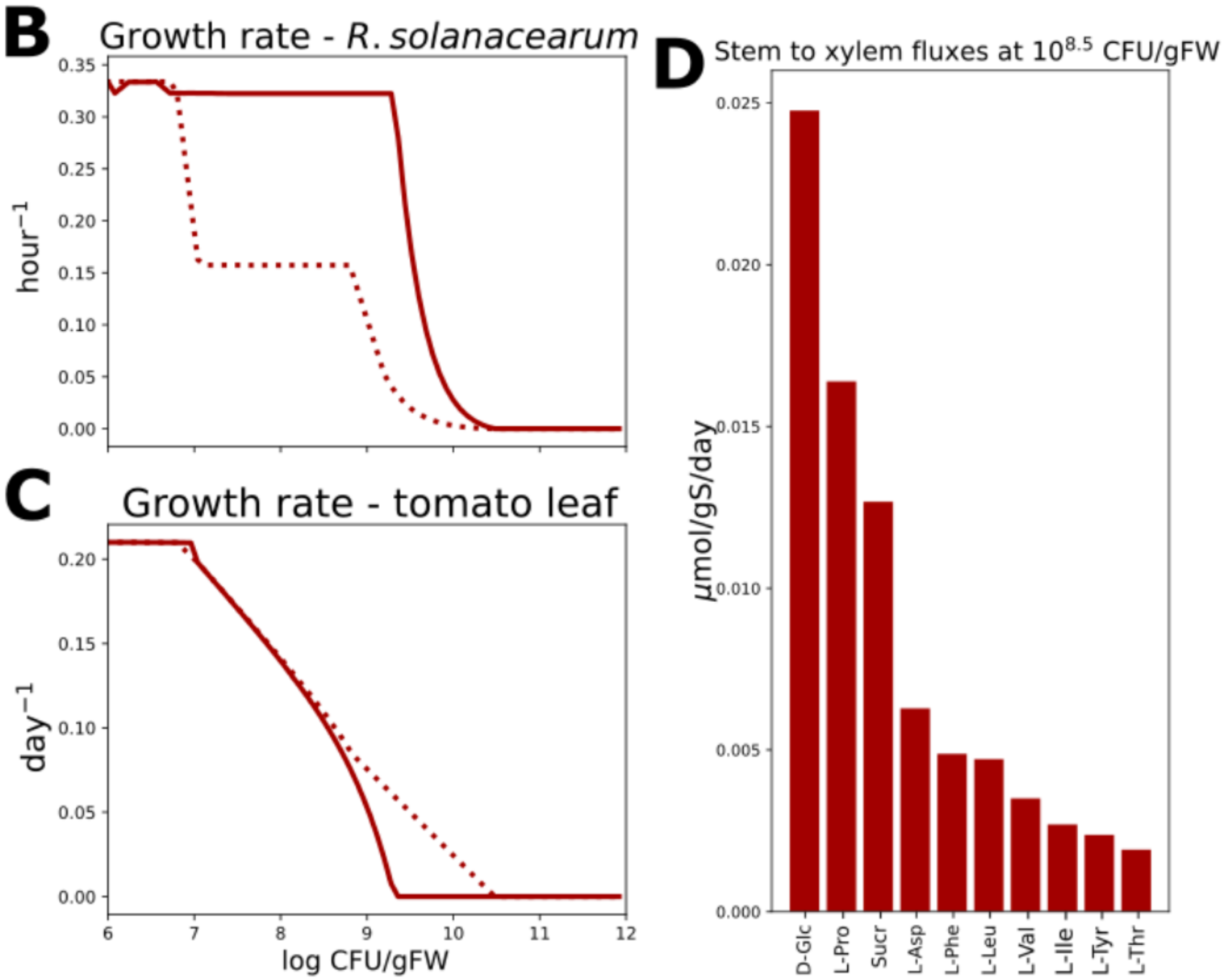

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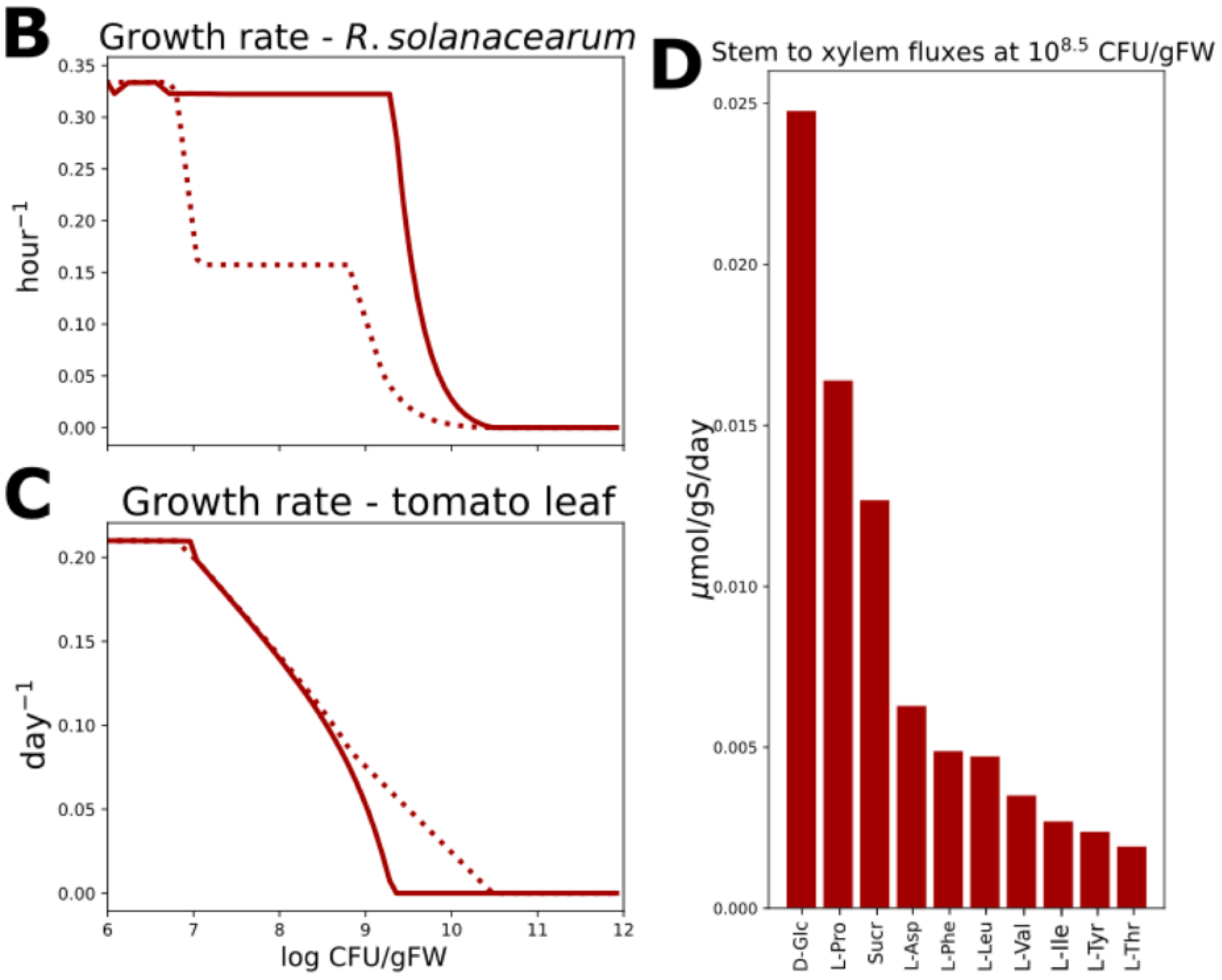

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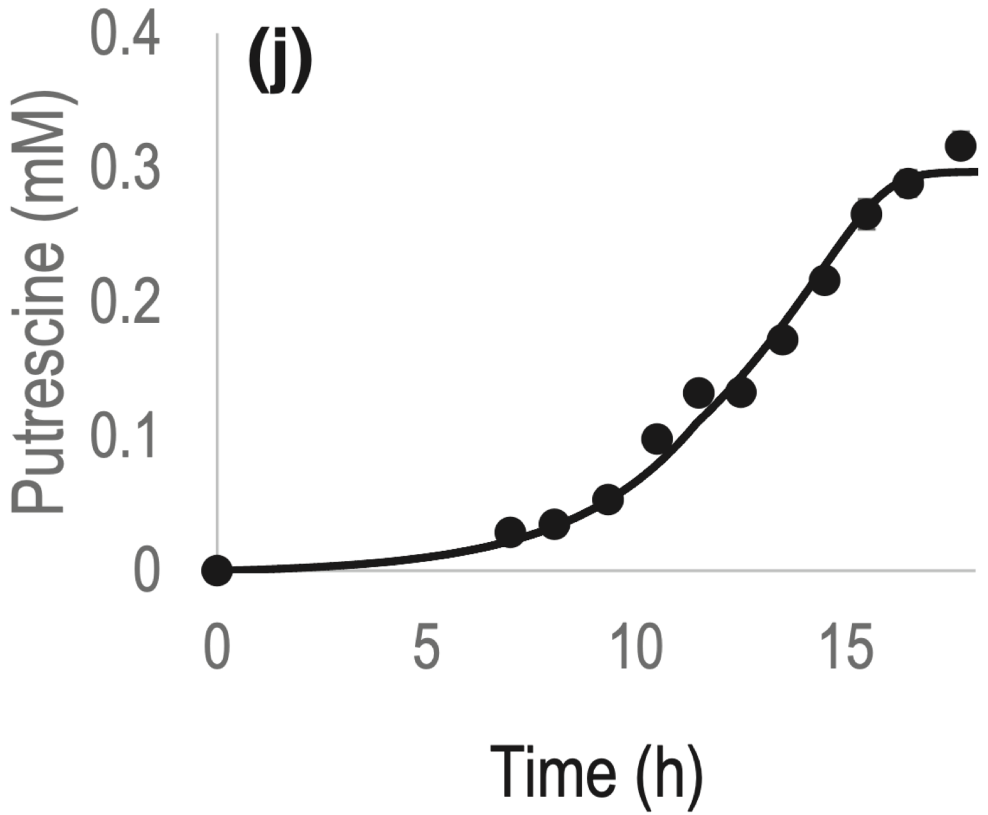
➔ Strategy could be employed by the pathogen (Hamilton et al. 2022, Gerlin et al 2021), but in a very limited manner quantitatively speaking

Fate of excreted putrescine

- Ralstonia excretes large amount of putrescine

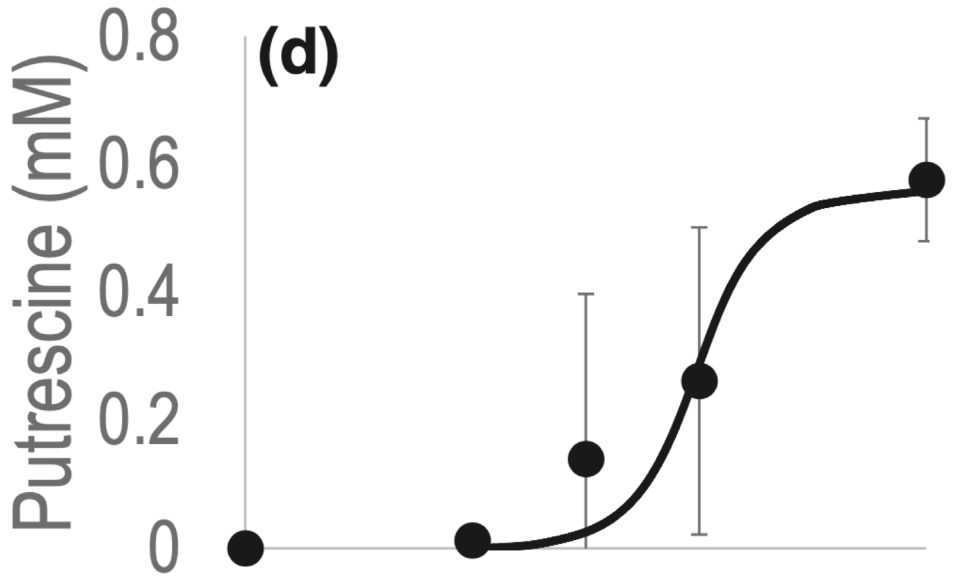


Ralstonia putrescine excretion in xylem mimicking medium

Fate of excreted putrescine

- Ralstonia excretes large amount of putrescine
- What happens to the putrescine excreted ?

  → A macroscopic model trends to show it is assimilated by the plant



Ralstonia putrescine in the plant xylem sap

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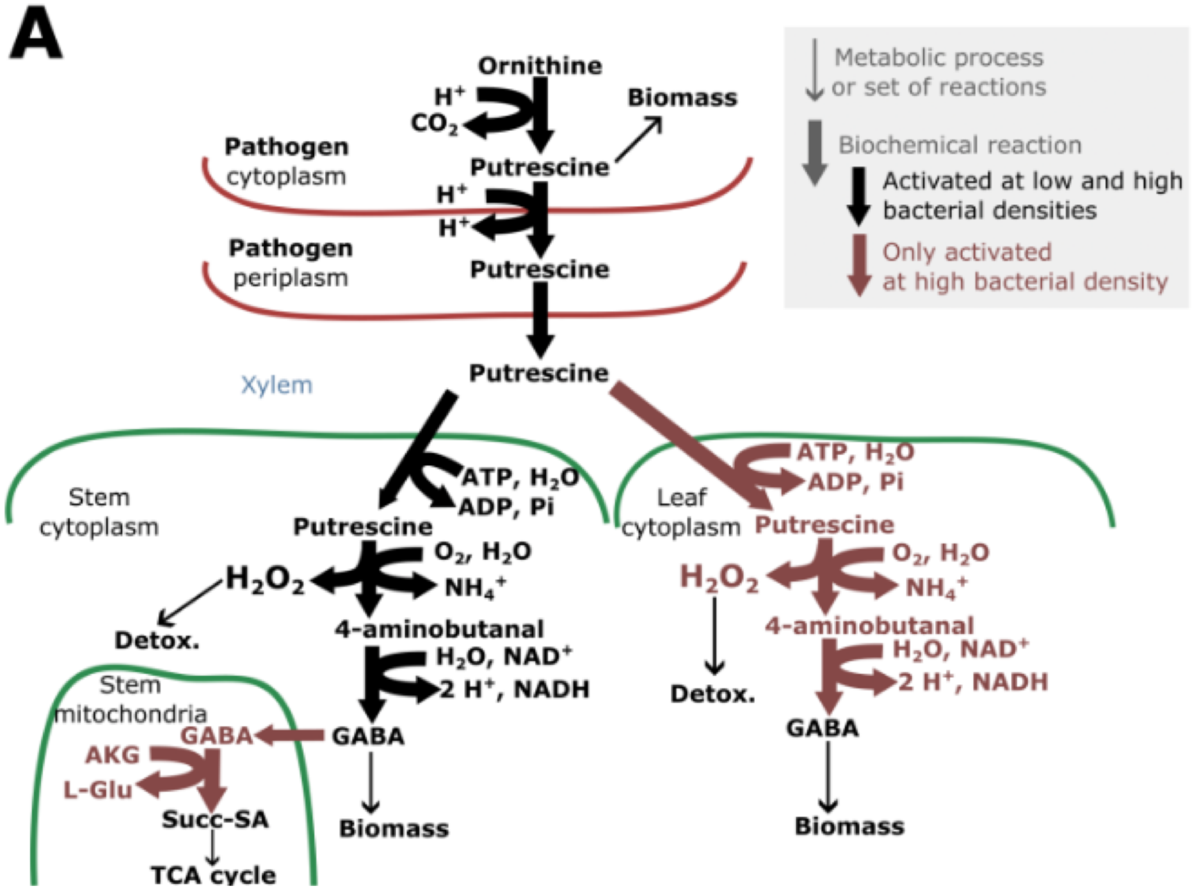
- At low bacterial densities, all putrescine ends up directly into biomass equation via GABA



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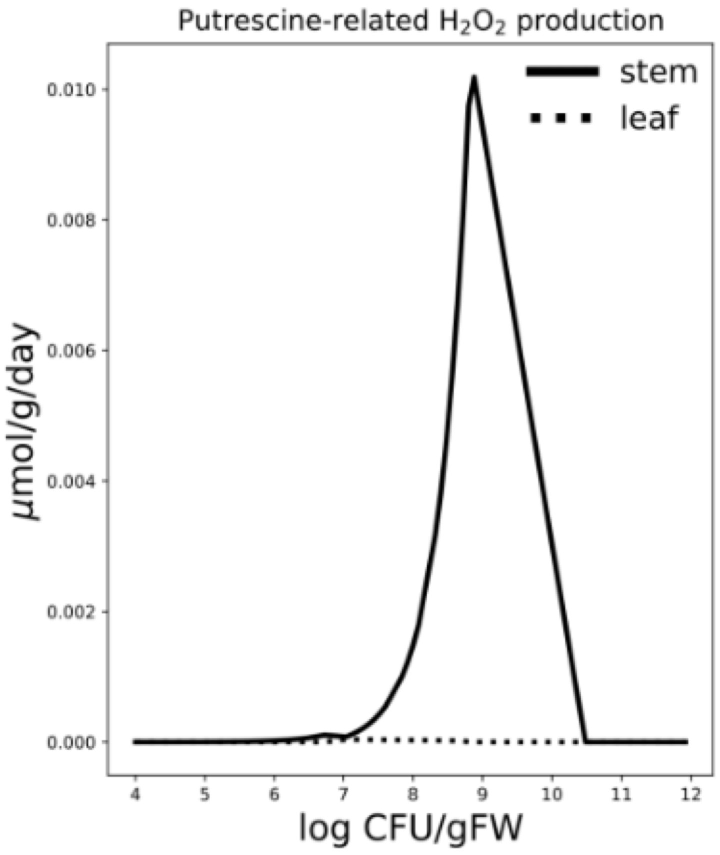
- At high bacterial densities, putrescine is catabolized into the TCA cycle, perturbing H_2O_2 production



In conclusion

- Used metabolic modeling on several biological systems

- The model:
  - reproduced the behavior of the system quantitatively
  - allowed to have an integrative view of the system
  - gave new insight in its functioning
  - and gave new assumptions

- These assumptions were/will be verified experimentally

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➔A virtuous cycle between modeling and experimental work

➔A complementary approach to conventional biological approaches, which gives a different view of the biological system

Thank you for your attention !

$$\frac{dM}{dt} = K.v.B$$

*Remember that all models are wrong; the practical question is how wrong do they have **to be to not be** useful.*

George E. P. Box (1987)

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