The multi-level relationship between heterotrophic bacteria and nutrients

Diauxie and co-utilization are not exclusive during growth in nutritionally complex environments

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The classic view of microbial growth strategy when multiple carbon sources are available states that they either metabolize them sequentially (diauxic growth) or simultaneously (co-utilization).

”**All you can eat**” *vs.* ”**a la cart**” strategies result in different growth dynamics (Monod, 1942).

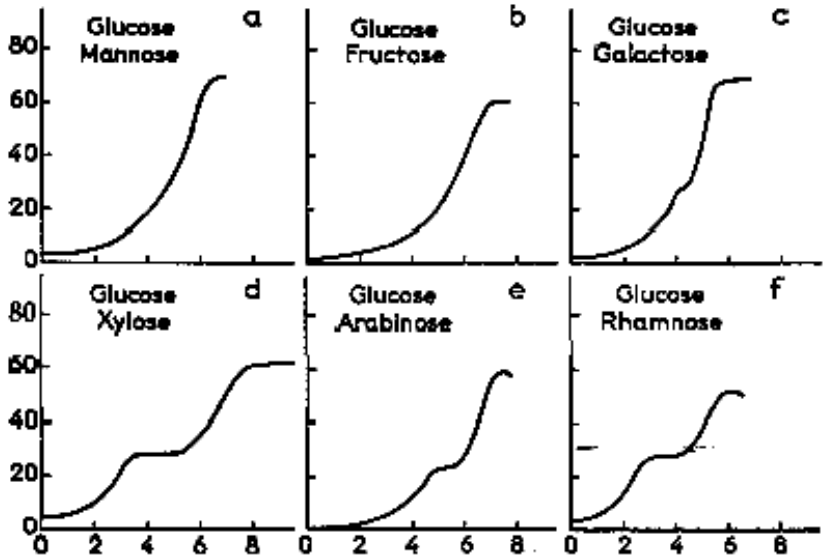
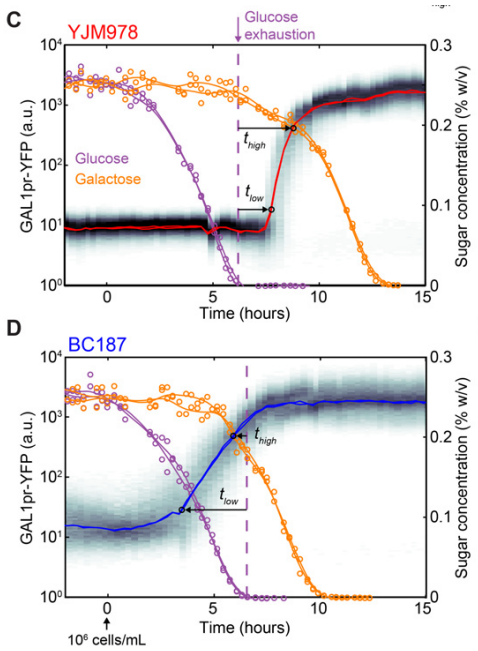
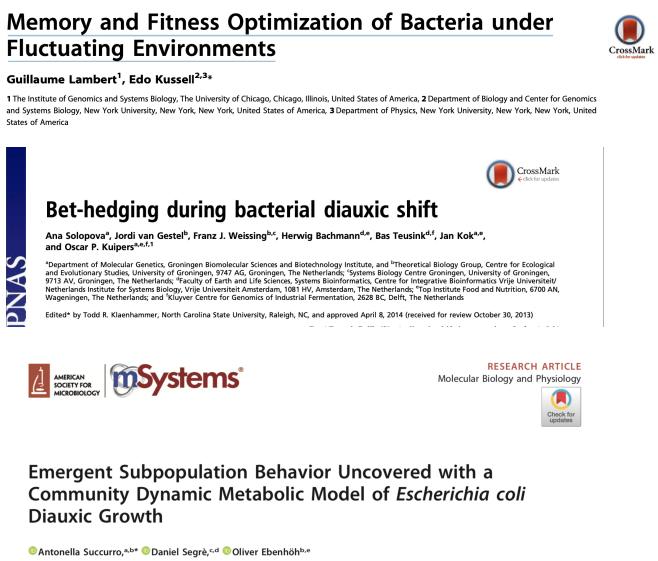


Fig.1. Growth of *Escherichia coli* in the presence of different carbohydrate pairs serving as the only source of carbon in a synthetic medium[30].

Diauxic shift is commonly seen as a phase in which the bacteria prepare themselves to use the second sugar. The existence of two stable cell types with alternative metabolic strategies emerge and coexist in a culture of the bacterium has been demonstrated.

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PLOS | GENETICS

Memory and Fitness Optimization of Bacteria under Fluctuating Environments

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Bet-hedging during bacterial diauxic shift

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RESEARCH ARTICLE
Molecular Biology and Physiology

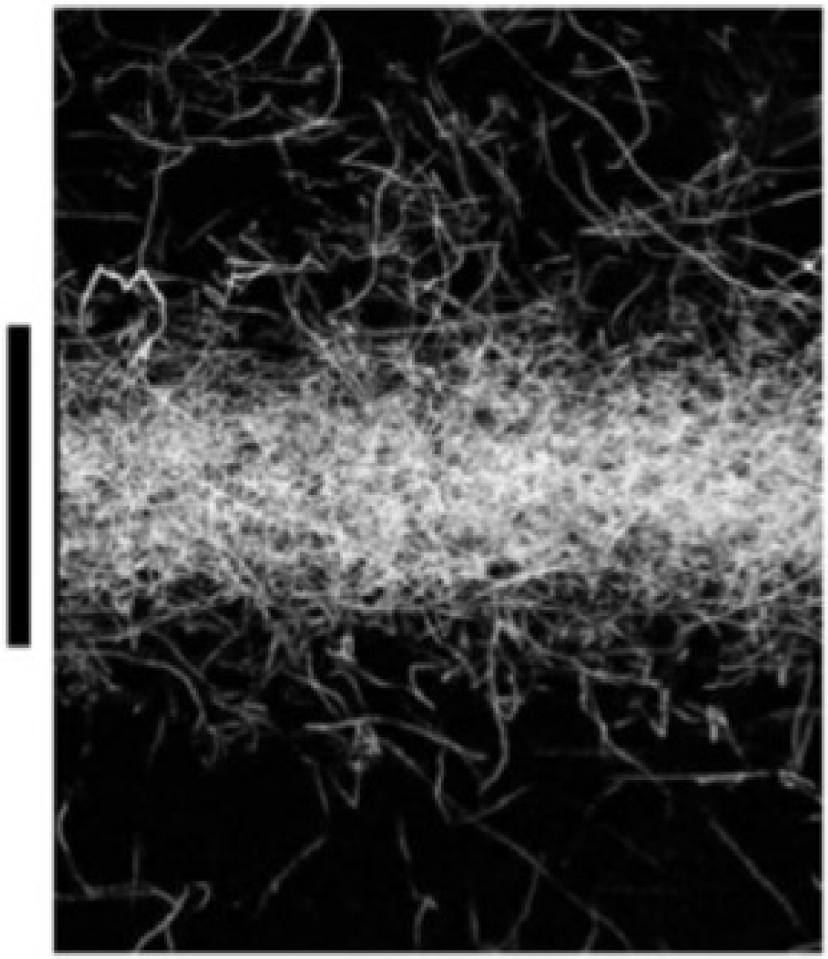
Emergent Subpopulation Behavior Uncovered with a Community Dynamic Metabolic Model of *Escherichia coli* Diauxic Growth

Antonella Succurro,[a,b]* Daniel Segrè,[c,d] Oliver Ebenhöh[b,e]

Our knowledge is biased by the fact that this process has been mainly analyzed in **over-simplified laboratory settings,** i.e. using a few model microorganisms and growth media containing only two alternative compounds.

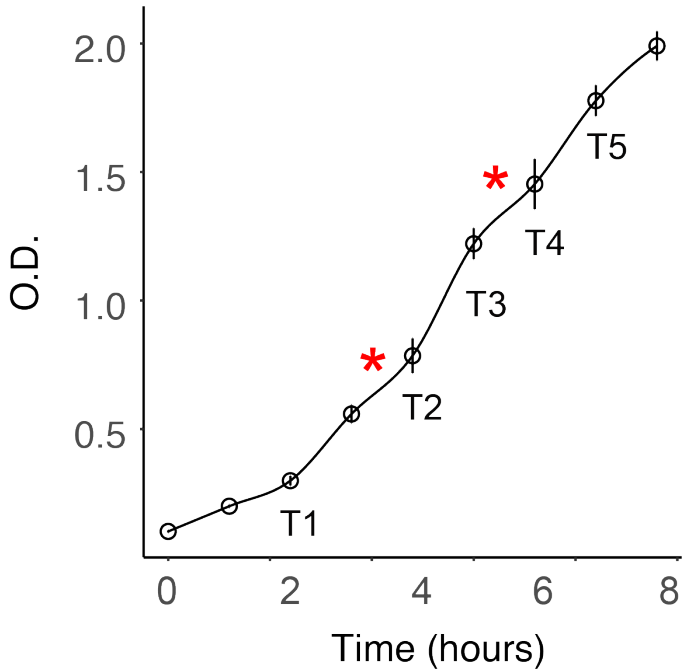
The marine environment:

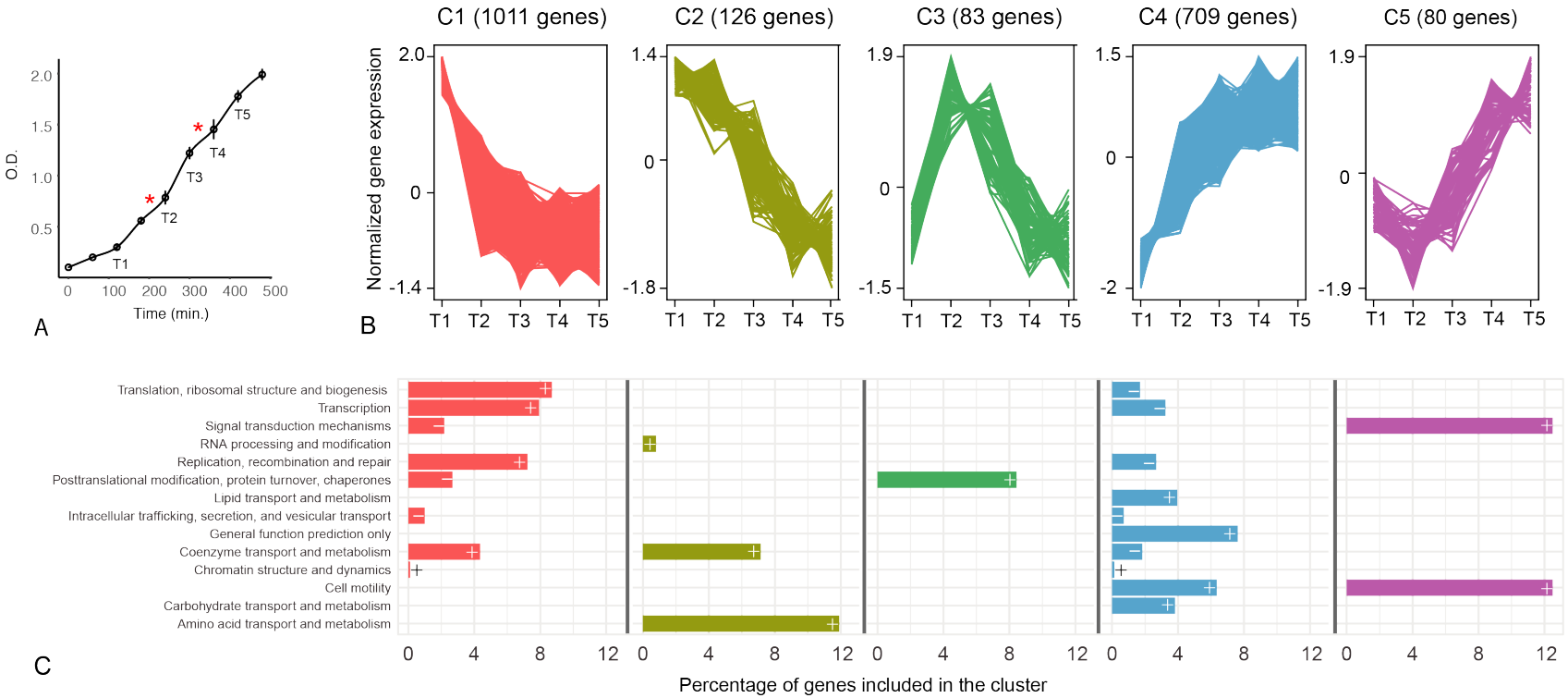
- Low average nutrient level (e.g., the concentration of amino acids is in the range of about 10^{-9} M)
- Nutrients appear and disappear in a sporadic fashion.
- DOM as a complex medium



- Chemotaxis of *Pseudoalteromonas haloplanktis* TAC125 (PhTAC125) toward a pulse of phytoplankton exudates (Stocker et al. 2010, Science)
- What happens once the patch is colonized?

We grew PhTAC125 in a complex medium (peptone, 7 replicates) and analysed its growth features.

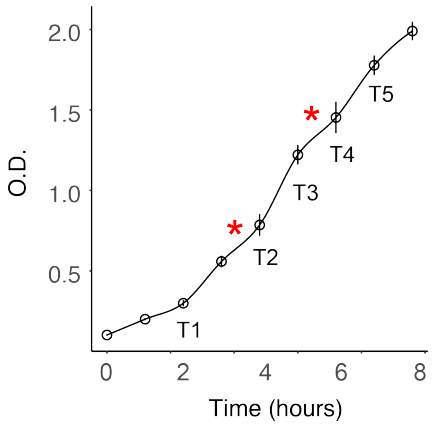


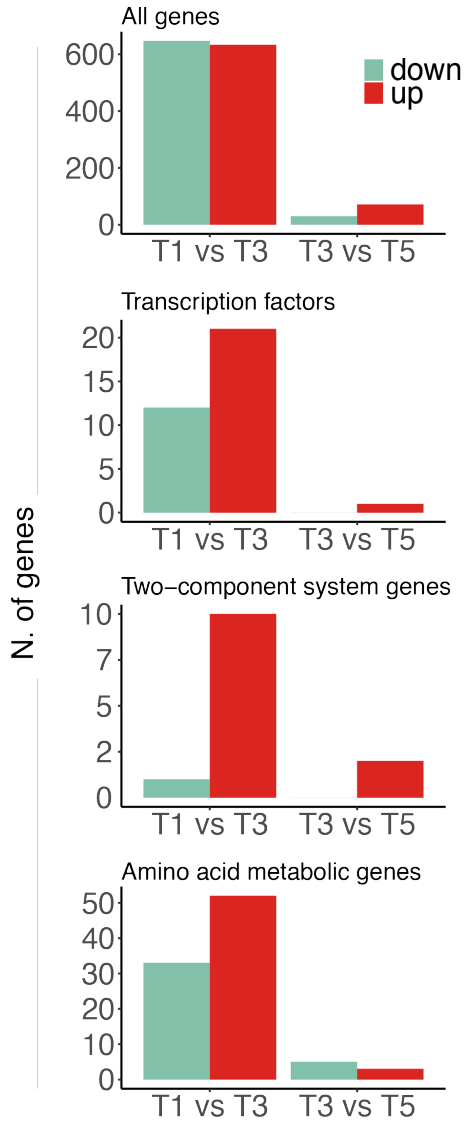


- High expression of growth-related genes in the first hours
- Stress related genes up between T1 and T2
- Motility-related genes up at the end of the growth

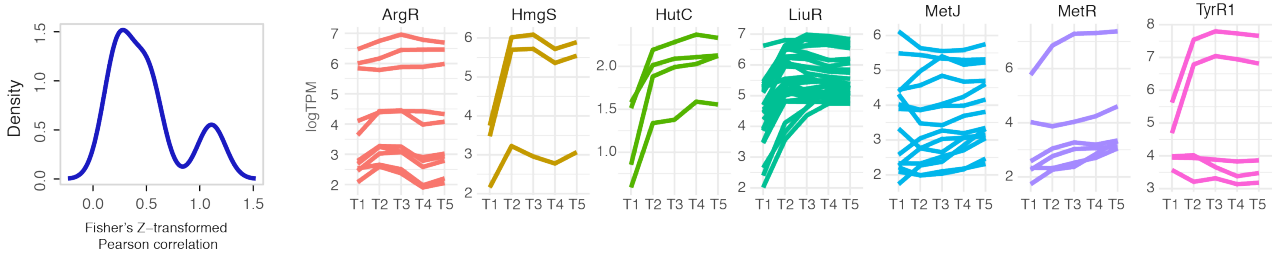
A non *E. coli*-like response to growth lag

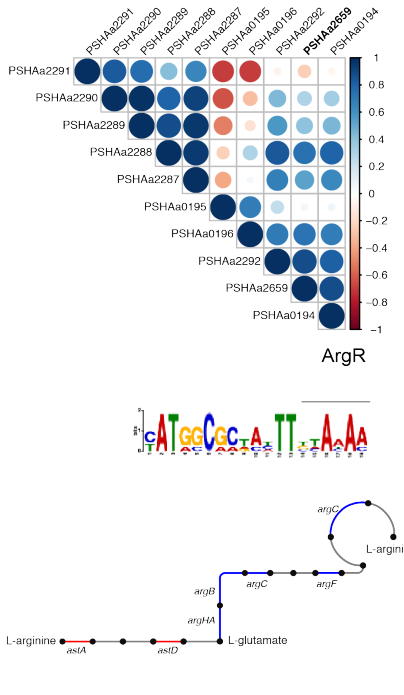
- 81 TF known to dcontrol central metabolic enzymes in *E. coli*
- Homologs for 34 of them in PhTAC125, 8 of them differentially expressed (10%)
- Only RpoS and RpoD are differentially expressed among 8 selected *E. coli* global regulators

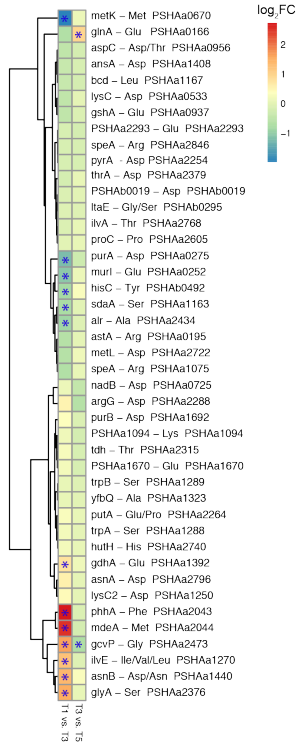




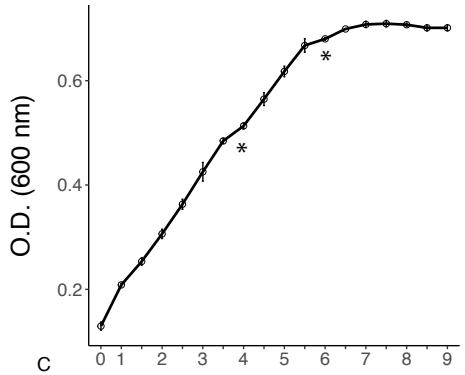
A focus on amino acids degradation. PhTAC125 is know to thrive preferentially on amino acids (Wilmes et al. 2010).







We assembled a defined, nutritionally rich medium including 19 amino acids ([0.2 mM], Cys not included) and analysed growth and metabolic features.



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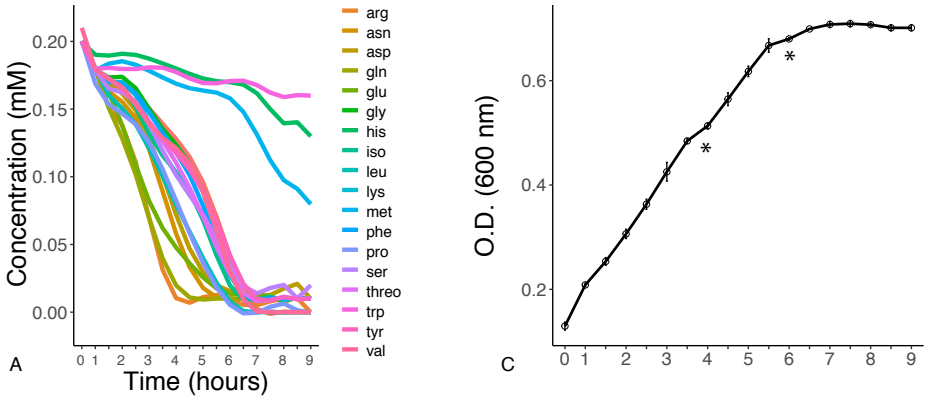

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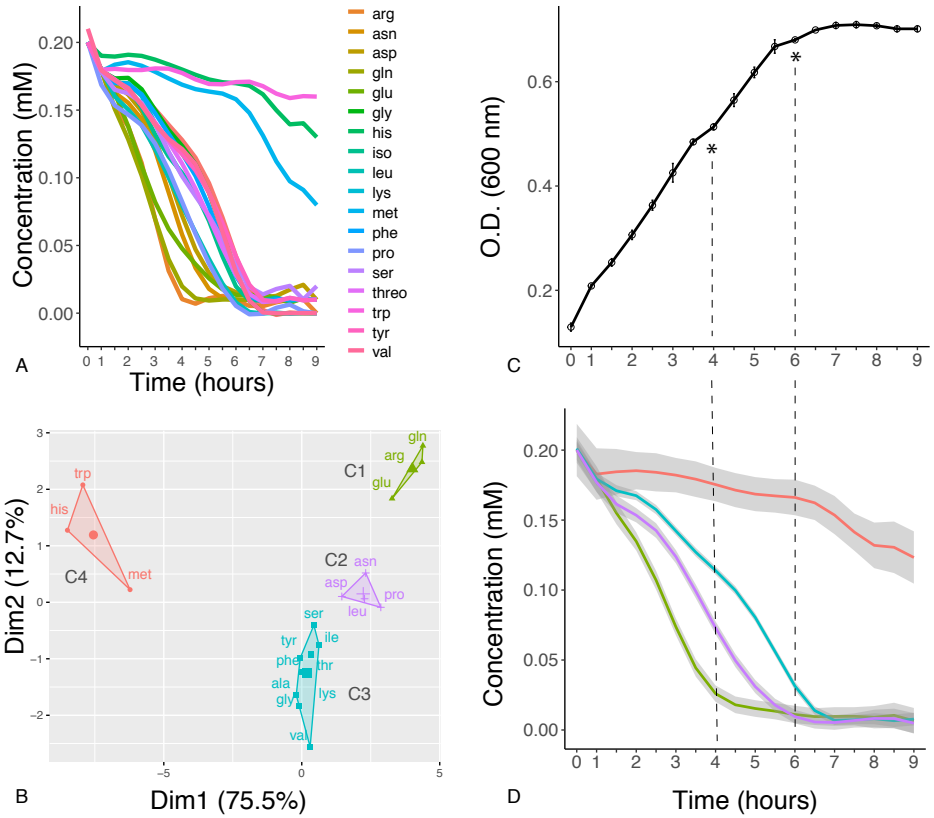

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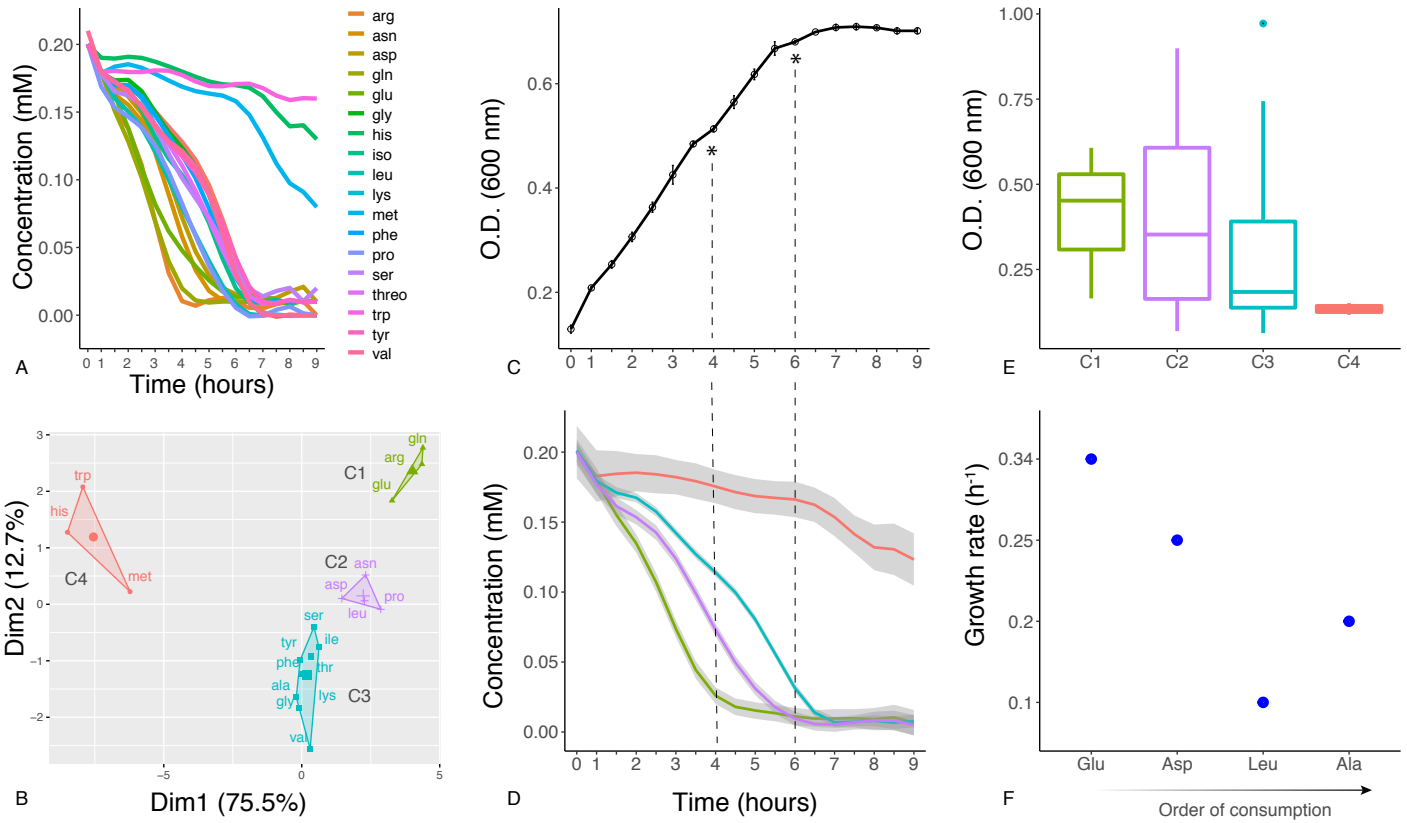


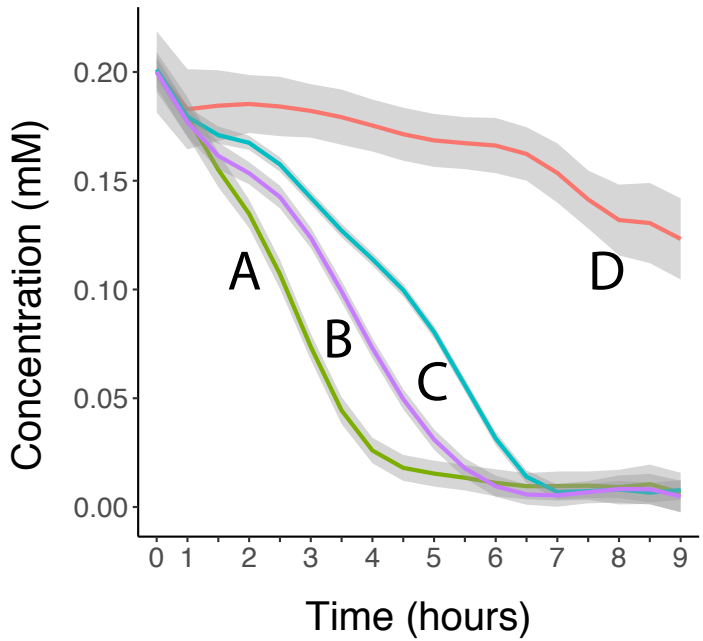
What is regulating the system? Uptake kinetics or gene regulation? A simple model accounting for bacterial (P) duplication thanks to nutrients (A, B, C and D) available in the medium.

$$P + A \xrightarrow{\frac{\beta_A \cdot \phi_A}{k_A + \phi_A}} 2P$$

$$P + B \xrightarrow{\frac{\beta_B \cdot \phi_B}{k_B + \phi_B}} 2P$$

$$P + C \xrightarrow{\frac{\beta_C \cdot \phi_C}{k_C + \phi_C}} 2P$$

$$P + D \xrightarrow{\frac{\beta_D \cdot \phi_D}{k_D + \phi_D}} 2P$$



$$\mathrm{P} + \mathrm{A} \xrightarrow{\frac{\beta_\mathrm{A} \cdot \phi_\mathrm{A}}{k_\mathrm{A} + \phi_\mathrm{A}}} 2\,\mathrm{P}$$
$$\mathrm{P} + \mathrm{B} \xrightarrow{\frac{\beta_\mathrm{B} \cdot \phi_\mathrm{B}}{k_\mathrm{B} + \phi_\mathrm{B}}} 2\,\mathrm{P}$$
$$\mathrm{P} + \mathrm{C} \xrightarrow{\frac{\beta_\mathrm{C} \cdot \phi_\mathrm{C}}{k_\mathrm{C} + \phi_\mathrm{C}}} 2\,\mathrm{P}$$
$$\mathrm{P} + \mathrm{D} \xrightarrow{\frac{\beta_\mathrm{D} \cdot \phi_\mathrm{D}}{k_\mathrm{D} + \phi_\mathrm{D}}} 2\,\mathrm{P}$$

$$\frac{d\phi_P}{dt} = \frac{\beta_A * \phi_A^2}{k_A + \phi_A} + \frac{\beta_B * \phi_B^2}{k_B + \phi_B} + \frac{\beta_C * \phi_C^2}{k_C + \phi_C} + \frac{\beta_D * \phi_D^2}{k_D + \phi_D}$$

$$\frac{d\phi_A}{dt} = -\frac{\beta_A * \phi_A^2}{k_A + \phi_A} * \phi_P$$

$$\frac{d\phi_B}{dt} = -\frac{\beta_B * \phi_B^2}{k_B + \phi_B} * \phi_P$$

$$\frac{d\phi_C}{dt} = -\frac{\beta_C * \phi_C^2}{k_C + \phi_C} * \phi_P$$

$$\frac{d\phi_D}{dt} = -\frac{\beta_D * \phi_D^2}{k_D + \phi_D} * \phi_P$$

$$
\begin{aligned}
P + A &\xrightarrow{\frac{\beta_A \cdot \phi_A}{k_A + \phi_A}} 2P \\
P + B &\xrightarrow{\frac{\beta_B \cdot \phi_B}{k_B + \phi_B}} 2P \\
P + C &\xrightarrow{\frac{\beta_C \cdot \phi_C}{k_C + \phi_C}} 2P \\
P + D &\xrightarrow{\frac{\beta_D \cdot \phi_D}{k_D + \phi_D}} 2P
\end{aligned}
$$

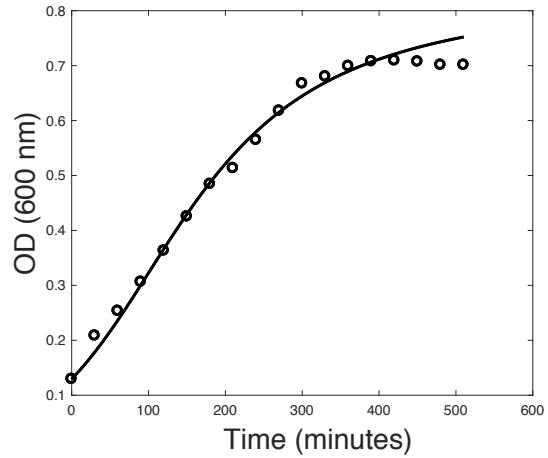
$$\frac{d\phi_P}{dt} = \frac{\beta_A * \phi_A^2}{k_A + \phi_A} + \frac{\beta_B * \phi_B^2}{k_B + \phi_B} + \frac{\beta_C * \phi_C^2}{k_C + \phi_C} + \frac{\beta_D * \phi_D^2}{k_D + \phi_D}$$

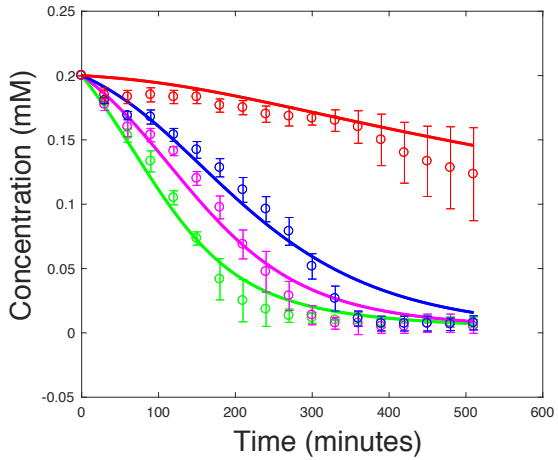
$$\frac{d\phi_A}{dt} = -\frac{\beta_A * \phi_A^2}{k_A + \phi_A} * \phi_P$$

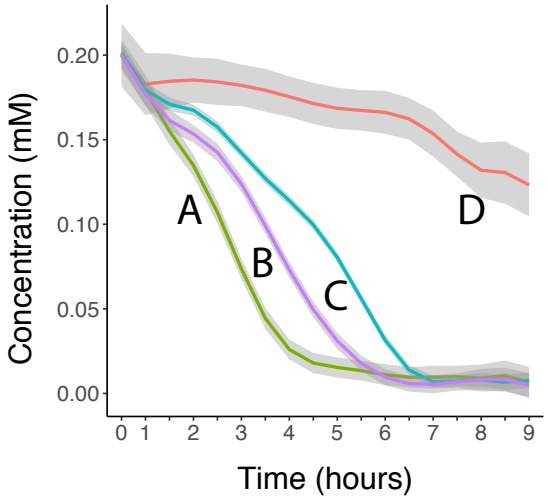
$$\frac{d\phi_B}{dt} = -\frac{\beta_B * \phi_B^2}{k_B + \phi_B} * \phi_P$$

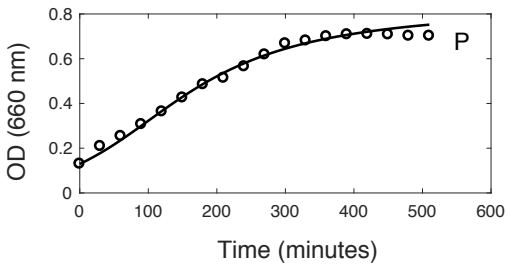
$$\frac{d\phi_C}{dt} = -\frac{\beta_C * \phi_C^2}{k_C + \phi_C} * \phi_P$$

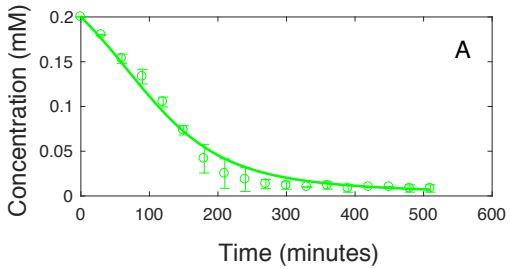
$$\frac{d\phi_D}{dt} = -\frac{\beta_D * \phi_D^2}{k_D + \phi_D} * \phi_P$$

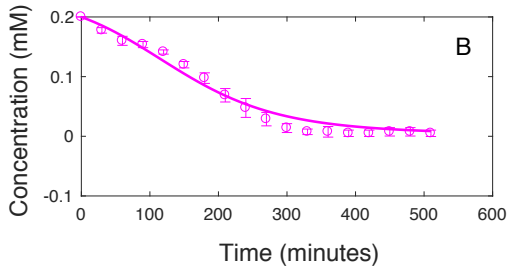


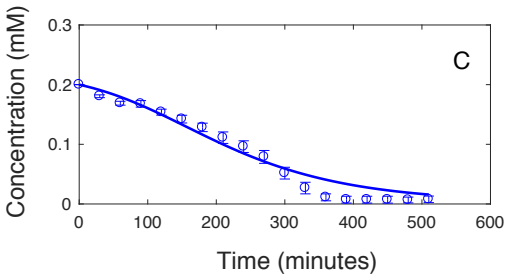


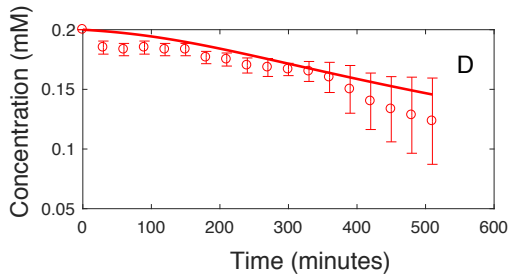

Concentration (mM)
Time (hours)
A
B
C
D


OD (660 nm)
Time (minutes)
P


Concentration (mM)
Time (minutes)
A


Concentration (mM)
Time (minutes)
B


Concentration (mM)
Time (minutes)
C


Concentration (mM)
Time (minutes)
D

- PhTAC125 growth on complex media results in a triauxic growth with a regulation response to nutrient starvation that does not resemble the model one.
- Growth lag phases are due to nutrient switching.
- Sequential and co-utilization are not exclusive. Most efficient C sources are consumed first.
- A simple mathematical model based on Michaelis-Menten kinetics uptake does not result in a perfect fit with the data. Regulatory mechanisms must be included to explain this growth dynamic at the single cell level
- Emergent sub-populations?

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- Prof. Renato Fani
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- Camilla Fagorzi
- Michele Giovannini

CERM, Unifi

- Veronica Ghini
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CSDC, Unifi

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- Lisa Carraro