Dynamic optimization of resource allocation in microorganisms

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Bacterial growth

- Bacteria are unicellular organisms geared towards growth. 
  *E. coli* cells have doubling times up to 20 min.

- Metabolism fuels growth by production of energy and building blocks for macromolecules.

  Different growth rates depending on medium (carbon, nitrogen, ...)

Stewart et al. (2005), *PLoS Biol.*, 3(2): e45
Growth and macromolecular composition

- Macromolecular composition of cell varies with growth rate
  Quantity of DNA, RNA, protein, … per cell or per volume

<p>| Table 3 | Parameters pertaining to the macromolecular synthesis rates in exponentially growing E. coli B/r as a function of growth rate at 37°C |
|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Symbol</th>
<th>Units</th>
<th>At ( t ) (min) and ( \mu ) (doublings per h):</th>
<th>Observed parameter(s)</th>
<th>Footnote</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA polymerase protein/total protein</td>
<td>( \alpha_P )</td>
<td>%</td>
<td>0.90 1.10 1.30 1.45 1.55</td>
<td>( \alpha_P ), ( \beta_P )</td>
<td>a</td>
</tr>
<tr>
<td>RNA polymerase molecules/cell</td>
<td>( N_P )</td>
<td>10^7 RNAP/cell</td>
<td>1.5, 2.1, 2.5, 3.0, 3.5</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>RNA polymerase activity</td>
<td>( \beta_P )</td>
<td>%</td>
<td>17 20 21 24 30</td>
<td>( \alpha_P ), ( \beta_P ), ( N_P )</td>
<td>c</td>
</tr>
<tr>
<td>Active RNA polymerase per cell</td>
<td>( N_{AP} )</td>
<td>RNAP/cell</td>
<td>105 503 993 1,929 3,298</td>
<td></td>
<td>c</td>
</tr>
<tr>
<td>Active RNA synthesized per cell</td>
<td>( \psi )</td>
<td>%</td>
<td>24 36 56 68 79</td>
<td>( r_r ), ( r_f )</td>
<td>e</td>
</tr>
<tr>
<td>Active RNA polymerase synthesizing stable RNA</td>
<td>( c_f )</td>
<td>Nuc/hr</td>
<td>85 85 85 85 85</td>
<td>Indirect</td>
<td>f</td>
</tr>
<tr>
<td>tRNA chain elongation</td>
<td>( c_m )</td>
<td>Nuc/hr</td>
<td>30 45 50 52 55</td>
<td>Indirect</td>
<td>g</td>
</tr>
<tr>
<td>Rate of stable RNA synthesis/cell</td>
<td>( r_f )</td>
<td>10^4 nuc/min/cell</td>
<td>3.0 9.9 29.0 66.4 132.5</td>
<td>( R_G )</td>
<td>h</td>
</tr>
<tr>
<td>Rate of mRNA synthesis/cell</td>
<td>( r_m )</td>
<td>10^4 nuc/min/cell</td>
<td>4.3 9.2 13.7 18.7 23.4</td>
<td>( r_r ), ( r_m ), ( r_f )</td>
<td>i</td>
</tr>
<tr>
<td>ppGpp concentration</td>
<td>( ppGpp/M )</td>
<td>pmol/O2/g</td>
<td>55 38 22 15 10</td>
<td>( ppGpp/M )</td>
<td>j</td>
</tr>
<tr>
<td>r-Protein per total protein</td>
<td>( \alpha_r )</td>
<td>%</td>
<td>9.0 11.4 14.6 17.5 21.1</td>
<td>( P_m ), ( P_d )</td>
<td>k</td>
</tr>
<tr>
<td>Ribosomal activity</td>
<td>( \beta_r )</td>
<td>%</td>
<td>80 80 80 80 80</td>
<td>Indirect</td>
<td>l</td>
</tr>
<tr>
<td>Ribosomes/cell</td>
<td>( N_r )</td>
<td>10^7 ribosomes/cell</td>
<td>6.8 13.5 26.3 45.1 73.2</td>
<td>( R_G ), ( R_f )</td>
<td>o</td>
</tr>
</tbody>
</table>

Bremer and Dennis (1996), *Escherichia Coli and Salmonella*, ASM Press, 1553-69
Growth and macromolecular composition

- Phenomenological **growth laws** capture variation of macromolecular composition with growth rate
  
  Distribution of proteins over different categories


- Explanation of growth laws (implicitly or explicitly) based on optimization principle

  Bacteria have evolved so as to distribute limited resources over cellular processes in order to optimize growth (biomass)
Growth and optimization

“The aim of the RESET project is to break with these classical approaches and propose a novel strategy for improving product yield and productivity.”

- Optimization: models and optimal control
- How does the cell optimize its growth?
- How can we optimize production with external inducer?
- A nice blend of biology, modelling, and mathematics…”
Steady-state and dynamic optimization

- Most growth laws and data concern **steady state** (balanced growth)
  
  Well-controlled and reproducible in laboratory

- However, most bacteria evolve in **dynamic** environment

  Example: *E. coli* in human colon

Towards dynamic growth laws

- **Aim**: study optimal allocation of resources to gene expression machinery and metabolism during growth-phase transitions. Which allocation is optimal for sustaining maximal growth (biomass)?

- Simple model of cell: bacteria as **self-replicators**


- Tools from **optimal control** theory
Self-replicator model of cell

- Reaction scheme:
  \[
  S \xrightarrow{V_M} P \\
  nP \xrightarrow{V_R} \alpha R + (1 - \alpha)M
  \]

- Stochiometry model with **extensive** variables:
  \[
  \frac{d}{dt} \begin{bmatrix} P \\ M \\ R \end{bmatrix} = \begin{bmatrix} 1 & -n & 0 \\ 0 & 1 & -\alpha \\ 0 & 0 & \alpha \end{bmatrix} \cdot \begin{bmatrix} V_M \\ V_R \end{bmatrix} = N \cdot V
  \]

- Volume and growth rate:
  \[
  Vol = \beta(M + R) \\
  \mu = \frac{1}{Vol} \frac{dVol}{dt} = \frac{1}{M + R} \frac{d(M + R)}{dt}
  \]
Reformulated self-replicator model of cell

- Definition of intensive variables:
  \[ p = \frac{P}{Vol}, \quad m = \frac{M}{Vol}, \text{ and } r = \frac{R}{Vol} \]

- Kinetics:
  \[ v_M = \frac{k_M}{K_M + s} m = e_M (1/\beta - r), \]
  \[ v_R = \frac{k_R}{K_R + p} r, \]
  \[ \mu = \frac{V_R}{R + M} = \beta v_R. \]

- Model with intensive (dimensionless) variables:
  \[
  \begin{align*}
  \frac{dp}{dt} &= E_M \cdot (1 - r) - (1 + p) \frac{p}{K+p} r, \\
  \frac{dr}{dt} &= (\alpha - r) \frac{p}{K+p} r.
  \end{align*}
  \]
Steady-state analysis of model

- Control parameter $\alpha$ determines fractional distribution of resources over metabolic and gene expression subsystems.
- **Result:** for constant $\alpha$, the system has a single steady state with growth rate $\mu^*(p^*, r^*, \alpha)$.

\[
\begin{align*}
\frac{dp}{dt} &= E_M \cdot (1 - r) - (1 + p) \frac{p}{K+p} r, \\
\frac{dr}{dt} &= (\alpha - r) \frac{p}{K+p} r.
\end{align*}
\]
Steady-state analysis of model

- **Result**: system admits single maximum growth rate for value $\alpha = \alpha_{opt} \in [0, 1]$

  Maximum varies with medium quality, represented by parameter $e_M$
Dynamic optimal control problem

• Bacterial cell has to reallocate resources after change in environment to reach optimal growth rate (change $\alpha$)

• What is the best dynamic resource allocation strategy?

• Optimal control problem for biomass

$$\max_{\alpha \in U} J(\alpha) := \int_{0}^{+\infty} \mu(p, r, \alpha, t) dt$$

with set of admissible controls: $U = \{\alpha : \mathbb{R}^+ \to [0, 1]\}$
Pontryagin Maximum Principe

\[ H := \lambda_p E_M (1 - r) - \frac{p}{K + p} r [\lambda_p (1 + p) + \lambda_r r + \lambda_0] + \alpha \lambda_r \frac{p}{K + p} r, \]

\[ \dot{\lambda}_p = \frac{K}{(K + p)^2} r \left[ \lambda_p (1 + p) + \lambda_r (r - \alpha) + \lambda_0 \right] + \frac{p}{K + p} r \lambda_p, \]

\[ \dot{\lambda}_r = \lambda_p E_M + \frac{p}{K + p} \left[ \lambda_p (1 + p) + \lambda_r (2r - \alpha) + \lambda_0 \right]. \]

The maximization condition is given by:

\[ \alpha(t) \in \operatorname{argmax}_{\nu \in [0,1]} H(x(t), \lambda(t), \lambda_0, \nu), \]

a.e. \( t \in [0, +\infty) \).

The switching function:

\[ \phi := \lambda_r \frac{p}{K + p} r \quad \begin{cases} \alpha = 1 & \iff \phi > 0, \\ \alpha = 0 & \iff \phi < 0. \end{cases} \]
Characterization of singular arcs

• The singular arc corresponds to the optimal steady-state:

\[ \phi(t) = \dot{\phi}(t) = 0, \forall t \in [t_1, t_2] \quad \Rightarrow \quad (\hat{p}(t), \hat{r}(t)) = (\hat{p}_{opt}, \hat{r}_{opt}) \]

• Kelley condition:

\[ (-1)^q \frac{\partial}{\partial \alpha} \frac{d^2q}{dt^2} \phi(t) < 0 \quad \text{for } q = 2 \quad \Rightarrow \quad \text{Chattering arc (Fuller’s phenomena)} \]

• Optimal strategy: Turnpike?
Optimal strategy

- Numerical solutions by a direct method (bocop)
How to compute the switching curve?

- The tangent of the switching curve at \((p_{opt}, r_{opt})\) is vertical.
- Backward integration starting from \((p_{opt}, r_{opt} + \varepsilon)\)
- Validation on Fuller’s problem:

\[
\begin{align*}
\text{minimize} & \quad \int_0^T x_1^2(t) \, dt \\
\text{given} & \quad \begin{cases} \dot{x}_1 &= x_2, \\ \dot{x}_2 &= u, \end{cases}
\end{align*}
\]
Simple feedback control strategies

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Precursor</th>
<th>Switch</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha = f(E_M) )</td>
<td>( \alpha = g(\hat{\rho}) )</td>
<td>( \alpha = \begin{cases} 0, &amp; \text{if } \hat{\rho} &gt; g(\hat{\rho}) \ 1, &amp; \text{if } \hat{\rho} &lt; g(\hat{\rho}) \end{cases} )</td>
</tr>
</tbody>
</table>

\[
\frac{E_M}{1.00 + E_M}
\]

\[
\frac{\hat{\rho}^2}{0.19^2 + \hat{\rho}^2}
\]

\[
f(E_M)\]

\[
g(\hat{\rho})\]

\[
\alpha = 0 \quad \alpha = 1
\]
Comparison of control strategies
Biological implementation?

• Regulation of resource allocation via ppGpp (Bosdriesz et al., 2015)
Biological implementation?

- Regulation of resource allocation via ppGpp (Bosdriesz et al., 2015)
- Quasi steady-state approximation:
  - Fast variables: ppGpp, tRNA
Conclusions and perspectives

• Study of resource allocation in bacteria from first principles
  Self-replicator model derived from two macro-reactions and some common assumptions on reaction kinetics

• Optimal strategy: turnpike with chattering

• Near-optimal strategy: switch depending on the imbalance between precursors and ribosomes

• Implementation via ppGpp

• Experimental test of control strategy using fluorescent reporters
Conclusions and perspectives

- Some generalizations: degradation and recycling
- Similar results (paper submitted to OCAM)
Conclusions and perspectives

• Some generalizations: maximum of production with an inducer (Reset)
• Growth or production?
• Limited amount of substrate? ANR Maximic, new…
Collaborators (RESET)
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H. Geiselmann
F. Mairet
J.-L. Gouzé
I. Egorov