DOCTORAL THESIS

A computational study of bacterial growth in complex environments

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A Computational Study of Bacterial Growth in Complex Environments

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ABSTRACT

The biochemical processes underlying growth of the model bacterium *Escherichia coli* have been carefully studied experimentally for more than half a century, with vast knowledge collected on the properties of enzymes and pathways that make up its metabolic system. Yet there is limited understanding on how the cell orchestrates this metabolic repertoire to achieve fast growth under a variety of external conditions. This thesis work aims at developing systematic computational methodologies to i) identify the regulatory interactions at work from genome-scale metabolic profiling data that are becoming routinely available in recent years; ii) examine the performance of regulatory schemes that incorporate the observed interactions through model studies; iii) assist experimental investigations where bacteria growth is an important component in the system dynamics.

To extract patterns of metabolic regulation in the growth phase of *Escherichia coli*, selected datasets of metabolite concentration, protein abundance and enzymatic parameters were collected and analysed. By comparing the metabolite concentration data with the respective Michaelis constants, rate-limiting compounds for metabolic flow can be identified. For cases where the data is available, it is found that nearly all substrates of reactions in the active pathways are present at levels above or close to saturation. This is especially so for metabolites NADH, ATP, Glutamine, etc., which are known as carriers of redox potential, energy and supplier of nitrogen. Thus it appears that, at least for the growth condition under which the metabolite concentration data is collected, the cell seems to be in a "well-charged" state, leaving regulation of pathway flux to enzyme abundance and activity.

The end-product inhibition mechanism is examined for the amino acid synthesis. By comparing the metabolite concentration data with the inhibition constants, it is found that the two are generally within a factor of 5 from each other. Therefore such interactions can play a role in modulating enzyme activity even in steady-state growth. There are interesting patterns in the order of the inhibitory constants along a pathway that deserve further exploration.

Single-cell measurements of genome-scale enzyme copy numbers provide a very realistic and detailed view of the pathway activities under a given growth condition. Combined with information on enzyme turnover rate, the enzyme copy number can be used to assign a maximal flow capacity for a given reaction. In the few cases examined, we found that this maximum flow capacity matches well with the flow needed to sustain growth at the observed doubling rate. The observation confirms the widely-held belief that enzyme copy numbers are tightly regulated by the cell. At the same time, it points to a possible strategy to reconstruct the metabolic flow pattern from protein copy number data.

To gain a better understanding of the interplay between reaction flux and metabolite concentrations to reach "optimal growth", we study a dynamic version of a toy model for central carbon and energy metabolism introduced previously by Covert *et al.* Steady-state flow patterns of the model are first examined from the flux balance point of view. The "optimal" solution from the Flux Balance Analysis (FBA) is identified and its property as a function of the oxygen availability in the growth medium discussed. A detailed analysis of the solution structure reveals the regulatory targets, i.e., a key set of reactions that need to be regulated to achieve optimal flux distribution. With this understanding, a concentration-based dynamic model that incorporates proper end product-inhibitions is constructed. Solution of the model yields near-optimal flux distribution at reasonable metabolite concentrations across a wide range of ambient oxygen concentrations.

Bacterial infiltration of a solid tumor can significantly attenuate its growth. Although the underlying biology for the observed phenomenon can be complex, we consider here a model where the death of cancer cells is triggered by depletion of an essential nutrient by bacteria. Taking this nutrient as glutamine, we construct a mean-field model of cell and nutrient densities using realistic parameters. Approximate analytic solutions are obtained in a spherical geometry and checked against numerical simulations. Furthermore, the improved efficacy of drug-releasing bacteria on tumor development is investigated. Under proper boundary conditions, when the secretion rate or drug efficacy reaches a sufficiently high level, the tumor mass shrinks and eventually disappears altogether. The computational methodology established here allows for the exploration of a very large parameter space. This helps in the experimental design and optimization of bacteria-based cancer therapy using synthetic biology techniques.

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