Optimal control of a bacterial growth switch for the maximization of metabolite production

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January 12, 2018

The fitness of microorganisms is defined by their capacity to propagate in environments hosting a variety of competitors for available resources. The situation faced by these single-cell organisms can be seen as an optimization problem: the allocation of resources to different cellular functions so as to maximize growth or another fitness criterion. So-called self-replicator models provide a simple, but fruitful description of microbial growth (Figure 1A) and have been used to formulate resource allocation in microorganisms as an optimal control problem [1, 2, 3, 4, 5, 6]. The optimal solutions obtained may give new insights into the functioning of microorganisms, by comparing them with control strategies that microorganisms have evolved to distribute available resources over cellular functions, involving complex networks of regulatory interactions on the molecular level.

In addition to gaining a better understanding of naturally-evolved resource allocation, optimal control theory is also beneficial in biotechnology, where the objective is not to optimize microbial growth, but to exploit the synthetic capacities of microorganisms for maximizing the production of compounds of interest. Control theory has contributed a rich variety of mathematical and computational tools for achieving this, notably through on-line estimation, adaptive control and optimization of process conditions. Recent advances in biology have provided new tools for the implementation of control strategies, by increasing our understanding of the functioning of regulatory networks on the molecular level and facilitating their (re)engineering.

Self-replicator models provide a useful conceptual framework for studying the dynamic reallocation of resources in microorganisms which is central to biotechnology [7]. In this study we aim at exploiting this potential by reformulating the redirection of resources from growth to metabolite production as a dynamic optimal control problem. We take inspiration from the recent development of an *E. coli* strain that allows growth to be switched on and off externally by adjusting the availability of RNA polymerase, a major component of the gene expression machinery [8]. It was shown that by arresting the expression of RNA polymerase, resources are shuttled away from protein synthesis (growth) to the production of a metabolite of interest (glycerol). In particular, we hope to answer the question which strategy for the dynamic regulation of RNA polymerase expression maximizes metabolite production and how this strategy compares with the approach followed in the original publication.

We first formulate a model of a self-replicator extended with a pathway for the heterologous expression of a metabolite as well as external control of the synthesis of components of the gene

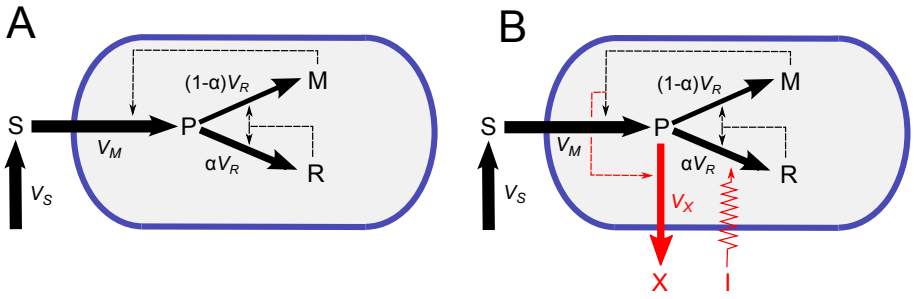

Figure 1: Self-replicator models of bacterial growth and metabolite production. *A:* Original self-replicator model from [2], describing the synthesis of precursor metabolite P from external substrate S, and the utilization of P for the synthesis of gene expression machinery R and metabolic machinery M, at proportions α and $1-\alpha$, respectively. V_M and V_R denote the rates of precursor and protein synthesis, respectively. M catalyzes precursor synthesis and R protein synthesis. *B:* Extended self-replicator model in which a heterologous pathway allows the cell to produce metabolite X from precursor P, at a rate V_X. Moreover, following [8], the synthesis of gene expression machinery R can be modulated by an external signal I.

expression machinery (Figure 1B), and consider this problem in the case of limited and unlimited nutrient supply, corresponding roughly to batch and continuous cultivation modes, respectively. Given the control input for these models, modulation of the synthesis rate of components of the gene expression machinery, we then formulate two dynamic optimal control problems, one involving maximization of the production of the heterologous metabolite and one involving maximization of the production of biomass. We solve the optimal control problems by analytical and numerical means [9, 10, 11], and notably find that the optimal solutions for product maximization and biomass maximization are very similar in the case of the model with unlimited nutrient supply, but become different when the available nutrients are limited.

The solutions thus obtained provide gold standards in the sense that they represent the optimum that can be theoretically achieved, under the assumption that external control of the gene expression machinery completely overrides natural feedback growth control in bacteria. Since the two are expected to interfere in biotechnological applications, we adapt the model, in a final step, to account for natural feedback growth control. We show that the optimal solution for the models without this feedback control can be closely approximated when assuming a simple two-step control scheme modulating natural feedback control: a first phase with maximal expression of the gene expression machinery (and low production), followed by a second phase with no expression of the gene expression machinery (and high production). The practical relevance of this control scheme is assessed by comparing it with established strategies of dynamic process control [12].

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