

RECONSTRUCTION OF A GENOME-SCALE MODEL OF *B. SUBTILIS* TO FACILITATE DEVELOPMENT OF A MINIMAL ORGANISM

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INTRODUCTION

Bacillus subtilis is a gram positive bacteria often utilized in industry as a producer of high quality enzymes and proteins (1). One of the primary challenges involved in the use of *B. subtilis* in industry is the extensive regulatory pathways in the cell, making the flux through the metabolic reactions of the cell extremely resistant to alteration by genetic manipulation (2). It has already been demonstrated that removing a portion of the regulatory genes in *B. subtilis* results in significantly enhanced protein production by the cell (3). Now, we are endeavoring to produce a minimal strain of the *B. subtilis* genome. This minimal strain will lack every dispensable alternative metabolic pathway and every dispensable regulatory gene, making the strain much more amenable to alteration for industrial use.

METHODS

B. subtilis was selected as the platform organism for the construction of a minimal strain because the natural competence of *B. subtilis* allows for rapid knockouts of single genes and intervals of genes (4). Additionally, the extensive information available about *B. subtilis* allows for a systematic and planned approach to be used during the construction of the minimal strain. To facilitate the development of a minimal organism, we constructed a new genome-scale metabolic model of *B. subtilis* based on the annotations available in the SEED subsystems-based annotation environment (5) and supplemented by data included in two previously developed *B. subtilis* models (6, 7). The new model includes elements of the *B. subtilis* regulation when necessary to properly predict the effect of gene knockouts. The thermodynamic properties of the model reactions were estimated to predict the reversibility of model reactions (8). The new model also includes numerous genes and pathways not found in any previous models. The new model was validated against a variety of experimental observations including Biolog Phenotyping Array results (7), gene essentiality data (9), 60 published gene interval knockout experiments (3), and over 300 new gene-interval knockout experiments. When model predictions did not match experimental observations, a variety of methods were developed and applied to improve the model accuracy.

RESULTS

As a result of these efforts and the inclusion of features from currently published models, this is the most complete and accurate metabolic model of *B. subtilis* constructed to date. We will discuss the methods used to assemble and correct this new genome-scale model; we will compare the accuracy and content of the new model with previously published models; and we will discuss the application of the model to the design of a systematic and

optimized strategy for combining gene interval knockouts to produce a minimal strain of *B. subtilis*. Through this work, we demonstrate how mixed integer optimization, metabolic flux analysis, and genome-scale metabolic models may be applied in combination for the design of a minimal organism. Using the tools and model developed in this work, we explore the trade-offs that exist between genome size and maximum growth rate (Figure 1) and genome reduction and the number of deletions required for reduction (Figure 2).

Figure 1: Trade off between genome size and growth

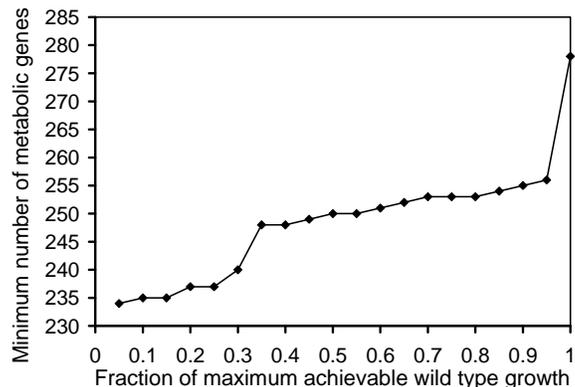
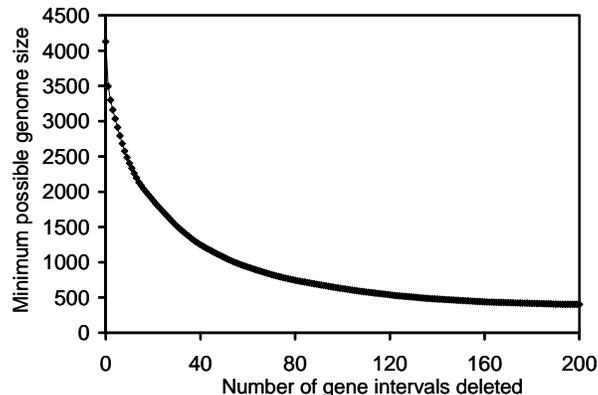


Figure 2: Tradeoff between genome size and deletions



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