Metabolic Modeling and Optimization of B. subtilis for Surfactin Production from Xylose-Rich substrates

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Lipopeptides produced by *Bacillus subtilis* have emerged as sustainable alternatives to their synthetic counterparts, offering superior biodegradability and reduced environmental impact. However, the high cost of substrates continues to pose a major challenge to their commercial viability. To address this issue, genome optimization of the production strain is essential for developing efficient microbial factories able to grow on cheap agro-resources. Nevertheless, the vast design space can hinder the timely identification of optimal genetic modifications. This study aims to model *B. subtilis* metabolism and its regulation, with the goal of computing suitable genetic modification to engineering a lipopeptide overproducing strain capable of utilizing cost-effective xylose-rich renewable substrates.

B. subtilis metabolic pathways were modeled using Partial Reaction Networks (PRNs). Knock-Out and Knock-Up predictions on PRNs were conducted based on constraint solving and abstract interpretation. BioComputing's reaction network prediction tool was used to for this purpose. CRISPR-Cas9 was used to edit the genome. The resulting mutant strains were cultivated in shake flasks to evaluate the accuracy of *in-silico* predictions.

Pentose Phosphate pathway was formally modeled as a PRN. This model made possible the prediction of *araE* overexpression as suitable target for enhancing xylose conversion into G3P. Overexpression of *araE* combined with the Knock-Up of *ilvBH* operon from BCAA PRN was experimentally validated by assessing the mutant strain growth and surfactin production. *B. subtilis* mutants demonstrated increased production of surfactin in accordance with the bioinformatic predictions. These findings show that formal reasoning can guide and provide explanation in support of *B. subtilis* metabolic engineering.

Keywords: Metabolic Engineering, Biotechnology, Bacillus, Lipopeptides, Model based prediction