Modeling Lactococcus lactis for synthetic biology

The number of methods tailored to address microbial metabolic engineering issues has grown considerably in recent years, in particular the development of microbial cell factories for the production of diverse classes of plant natural products. Hitherto, the gram-negative Escherichia coli has been the sole bacterium used as host for expression of plant phenolics. However, the redesign of microbial metabolism is a challenging step in the biotechnology industry for the production of commercially-valuable compounds.

Here, we take advantage of beneficial properties of a gram-positive bacterium Lactococcus lactis, such as easier expression of eukaryotic membrane proteins, to generate bacterial factories that excrete high-value phenolics with titers, yields and productivities enabling further commercial production. In this work, we adapt a previous published model of L. lactis with non-native flavonoid pathways in order to further investigate the L. lactis metabolism with heterologous metabolic reactions, and suggest important considerations for design in synthetic biology. We first compiled existing knowledge and identified plant metabolic pathways of interest to embed them in the L. lactis model, and subsequently modified and coupled with experimental data that will then be tested into in silico model of the bacterial host.

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It includes tools for the identification and annotation of transport proteins encoding genes and for generating transport reactions for such carriers. Also, merlin includes tools for the compartmentalisation of the model that predict the localisation of the proteins encoded in the genome, and thus the localisation of the metabolites involved in the reactions induced by such proteins. Finally, merlin expedites the transition from genome-scale data to SBML metabolic models.

merlin has already been used to perform re-annotations and to reconstruct GSMMs for several organisms. For instance, merlin was used to perform the genome-scale metabolic re-annotations of K. lactis, A. gossypii and H. pylori. All of the above annotations were used as a basis for the reconstruction of GSMMs of the respective organisms.

For instance, the iOD907 K. lactis model was assessed to experimental data, performing very well. Moreover, the model proved accurate when predicting the biomass, oxygen and carbon dioxide yields and in silico knockouts accurately predicted in vivo phenotypes. Also, the iTR (H. pylori) model complies better than previous models with in vivo data. It shows the same specific growth rate verified in vivo whilst being the only in silico model able to use glutamate as carbon source and performs quite well on the essentiality tests.

Thus, merlin proved being useful for the reconstruction of these models.

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How can the multitude of intracellular flux profiles that correspond to the same physiology influence our metabolic design strategies?

The size and complexity of metabolic systems hinders the exact experimental quantification of every intracellular metabolic flux. Here we demonstrate that the uncertainty in metabolic flux quantification leads to a multitude of estimated intracellular flux profiles that satisfy the same physiology. The aim of this study is to analyse the impact of alternative flux profiles on the metabolic responses to perturbations in enzyme activities and environmental conditions.

To perform this analysis we used ORACLE (Optimization and Risk Analysis of Complex Living Entities) framework and generated a population of kinetic models of cellular metabolism that satisfy thermodynamic and physico-chemical constraints, and that are consistent with the physiology of a succinate producing E. coli. With the generated kinetic models we computed the control coefficients to characterize the metabolic responses to changes in enzyme activities. We found that, depending on the analyzed flux profile, we could draw different conclusions about possible metabolic engineering strategies. We were also able to determine the sets of the enzymes whose control over the production fluxes was unaltered by alternative intracellular flux profiles. Furthermore, we showed that the ther-
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