Metabolic modeling of Griseorhodin A production in Streptomcetes

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Overview

Genome-scale metabolic modeling is a **powerful tool** that allows for an in-depth understanding of **biochemical networks** of organisms. These comprehensive **mathematical models** can be used to **simulate the fluxes** of metabolites and energy equivalents such as ATP or NADPH. Based on these models, it is possible to predict the pathways involved in the generation of biomass as well as in the production of the secondary metabolite Griseorhodin A, a promising telomerase and viral reverse transcriptase inhibitor. Here, we use this method to **analyze the correlation** of cell-growth and the production of the heterologously expressed Griseorhodin A within the metabolic network of the genomically minimized host strain *Streptomyces avermitilis* SUKA22 in order to identify **key reactions** that can be modified for the generation of an optimized producer of natural drugs.

Materials & Results

Griseorhodin A belongs to the group of **Rubromycines** which have been known for their vivid red colors since the 60‘s and are characterized by a **spiroketal moiety**. They show promising activity against **HIV reverse transcriptase** and human telomerase. Although extensive efforts towards their total synthesis have been undertaken, the available synthetic routes are **still limited**[1].

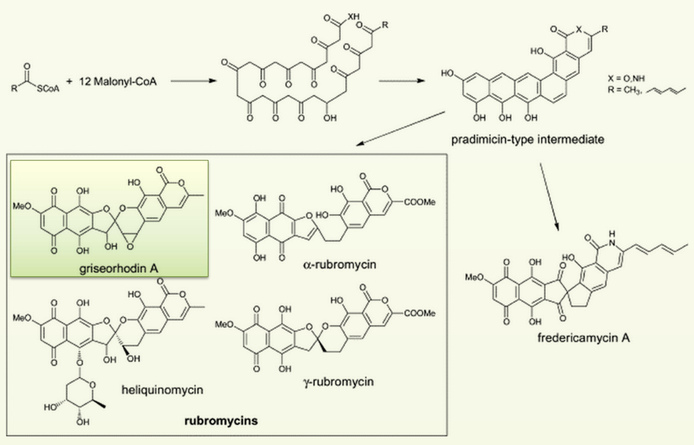

Fig. 1: *Rubromycines biosynthesis*[2]

Streptomycetes are excellent hosts for **heterologous expression** of secondary metabolites. The group of Prof. H. Ikeda has developed a **genomically optimized** mutant of the industrially used strain *S. avermitilis*. These SUKA strains have proven superior to many natural hosts in terms of **secondary metabolite** production[3].

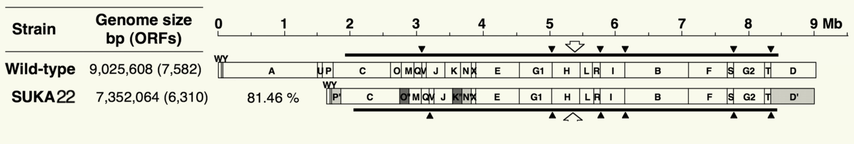

Fig. 2 (top): *S. avermitilis wild-type genome compared to SUKA 22 strain modified from Komatsu et al.*[3]

Fig 3 (right): *Streptomyces avermitilis (left), S. avermitilis SUKA22(right) and S. avermitils SUKA22 expressing Griseorhodin A (top) on minimal medium*

We have generated a **genome-scale** metabolic model of *S. avermitilis*, including 1632 reactions and 1697 metabolites. **Flux Balance Analysis** is used to calculate the flux rates within this huge network and to simulate the generation of biomass as well as the production of Griseorhodin A.

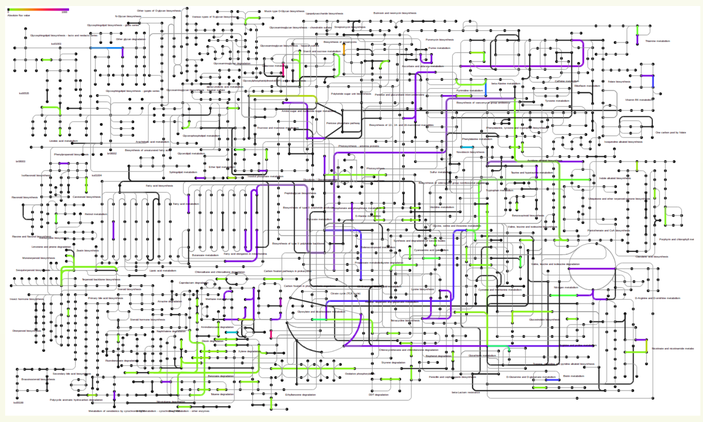

Fig. 4: *Flux rates during biomass production projected on an overview map of all metabolic processes. Simulation was performed with FAME*[4]*.*

Metabolic models have to be **validated iteratively** to increase the significance of their predictions. We are cultivating *Streptomyces avermitilis* SUKA22 and its Griseorhodin A producing variant in **various conditions**. The limiting factors of the experiments are determined and **integrated** into the model to match the experimental results.

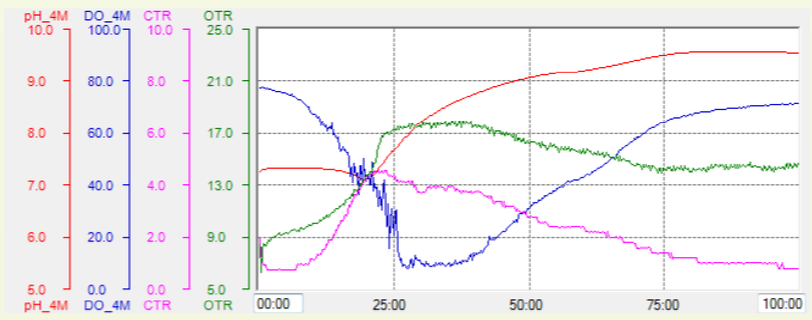

Fig. 5: *Online measurement of pH-Value, Dissolved oxygen [%], Carbon Transfer Rate and Oxygen Transfer Rate during Griseorhodin A production in TSB medium over 100 hours.*

Primary and secondary metabolism compete for resources such as carbon building blocks or cofactors. The predicted **correlation** between secondary metabolite production and growth shows **linear dependence** in four stages. This indicates the presence of four **key metabolites** within the metabolic network which are distributed between these two objectives. The boundary values of the correlation show that optimal secondary metabolite production leads only to a **5% loss** in biomass production.

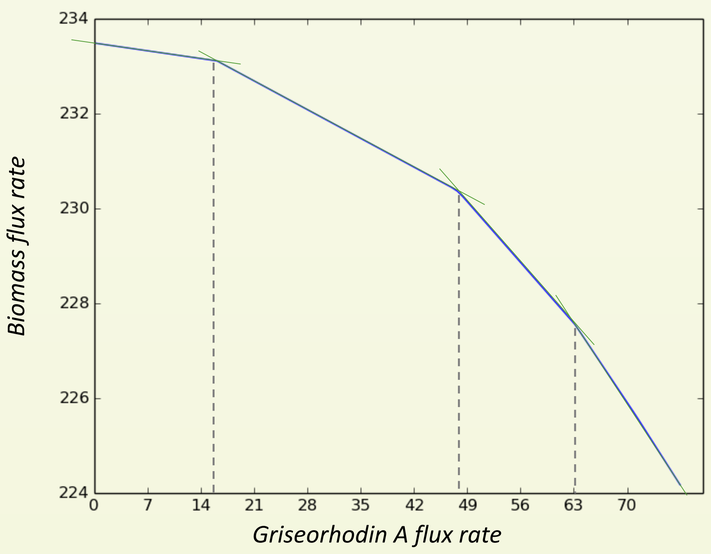

Fig. 6*: In silico correlation between biomass- and Griseorhodin A production. Flux rates are calculated in mmol / (g(dry weight) * h)*

Outlook

The presented experiments indicate the existence of different key metabolites which are distributed among primary and secondary metabolism. The metabolic network can be optimized towards productions of natural drugs with only slightly reducing the generation of biomass. The effects of these manipulations have to be elucidated in further *in silico* and *in vitro* experiments.

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