The biosynthesis of the antibiotic lugdunomycin in *Streptomyces*

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CELL BIOLOGY

INTRODUCTION

Antibiotic resistance is a quickly growing threat with a huge global impact. Bacteria of the genus *Streptomyces* have an enormous potential as a source of antibiotics, as they produce a wide variety of bioactive secondary metabolites, many of which are still to be identified.[1]

Lugdunomycin, an angucycline-derived polyketide, is a structurally unique antimicrobial compound that represents a novel class of antibiotics. The biosynthesis is, however, still partially unknown.[2] Understanding the mechanism of the chemically challenging formation of lugdunomycin is essential, as it would greatly benefit designing unique antibiotic structures.

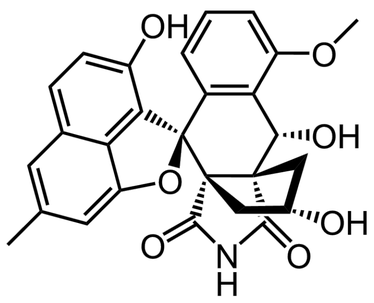

Figure 1. Structure of lugdunomycin.

METHODOLOGY

The lugdunomycin pathway intermediates were produced by constructing a plasmid of the *lug*-genes. The plasmids were assembled using the BioBricks method, which allowed constructing a library of the *lug*-gene building blocks. The inserts were digested with *Xba*I/*Pst*I and ligated with the backbone digested with *Spe*I/*Pst*I forming a plasmid pictured below in figure 2.

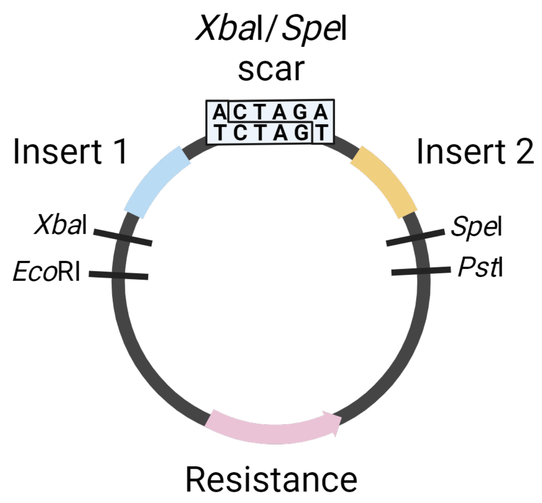

Figure 2. The BioBricks assembly. The ligation of an insert to the backbone leads to the formation of a scar site. Inserts are flanked by the cut sites for the restriction enzymes.

The assembled genes were conjugated from *E. Coli* into *S. coelicolor* already containing another plasmid coding for the early steps of the lugdunomycin biosynthesis. The strains were cultured with an adsorbent resin to collect wanted compounds. Production analysis was performed on UPLC with an UV-Vis detector. Produced compounds were purified from the production cultures by extracting them from resin with methanol, after which they were analyzed with preparative HPLC. The workflow is presented in figure 3.

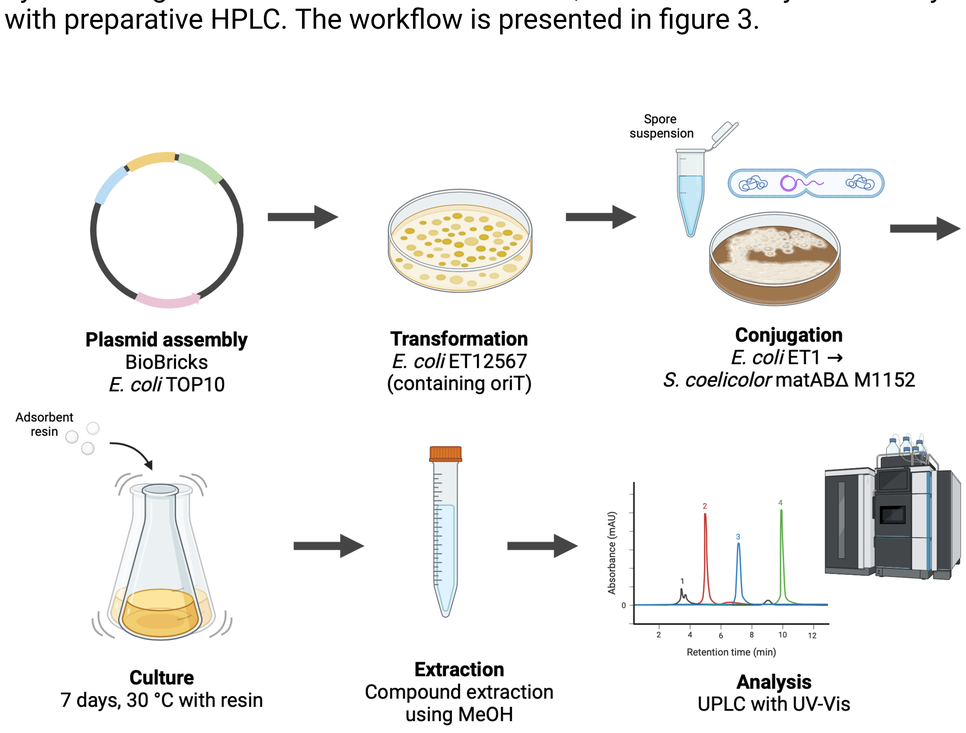

Figure 3. Compound production workflow.

RESULTS

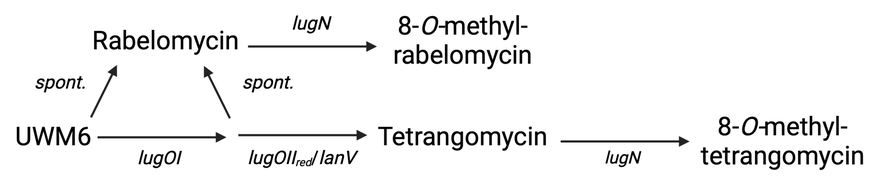

Figure 4. Biosynthesis of lugdunomycin pathway intermediates. Rabelomycin (R) and 8-*O*-methylrabelomycin are formed as shunt products from UWM6, whereas *lugOII*$_{red}$ or *lanV* guide the pathway towards tetrangomycin (T) and 8-*O*-methyltetrangomycin (MT).

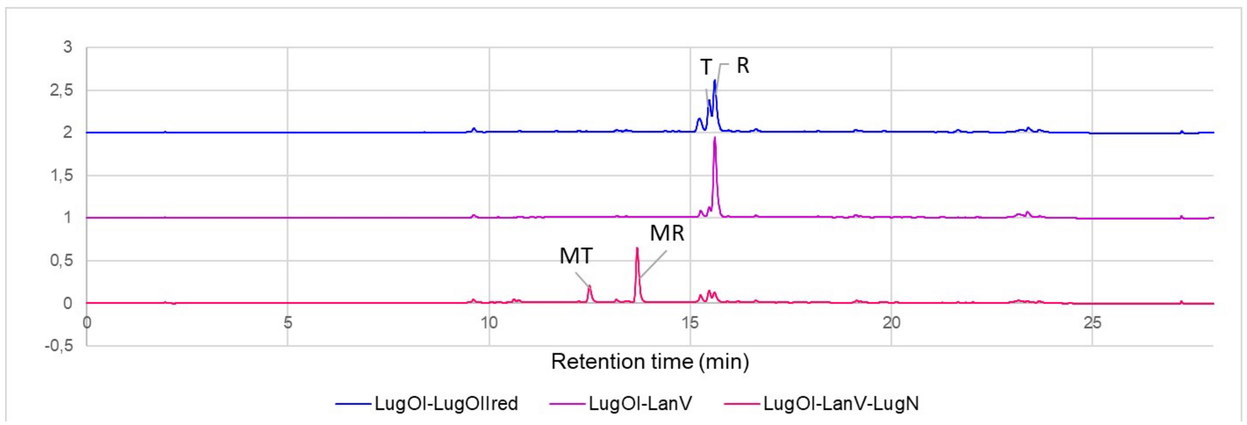

Figure 5. Produced intermediates. Tetrangomycin (T) and rabelomycin (R) were produced with *lug*-genes *lugOI*-l*ugOII*$_{red}$ and l*ugOI*-l*anV*. *LanV* is a *lugOII*$_{red}$ homolog from the landomycin strain. Adding l*ugN* led to the methylation of R and T, producing 8-*O*-methyltetrangomycin (MT) and 8-*O*-methylrabelomycin (MR), respectively.

Some pathway intermediates were successfully produced, as shown in figure 5. However, the gene expression levels need to be optimized with further constructs in order to produce other pathway intermediates. An important intermediate is 8-*O*-methyltetrangomycin, which is necessary in studying the nature of the chemically challenging C-C bond cleavage leading to the unique structure of the C-ring cleaved lugdunomycin. 8-*O*-methyltetrangomycin cannot be formed through 8-*O*-methylrabelomycin, which was the main product in most first-round strains. Therefore, new synthetic promoters with varying activities are used for the second-round strains to lower the activity of *lugOI* leading to a lowered formation of the shunt product rabelomycin. Used promoters are shown in figure 6, which presents the relative strengths of the synthetic promoters with a widely used native *kasOp** promoter as a reference.[3]

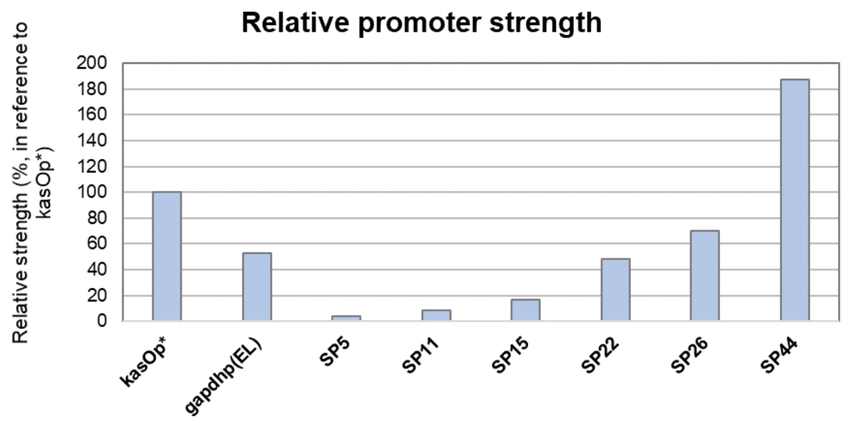

Figure 6. Relative promoter strengths. The relative strengths of native and synthetic promoters used in this study are shown in reference to *kasOp**.[3] The *gapdhp(EL)* promoter was used in all first-round strains, whereas the synthetic promoters *SP5-SP44* are used for the optimized second-round strains.

CONCLUSIONS

- A genetic library of lugdunomycin pathway genes was succesfully constructed with BioBricks
- Some pathway intermediates, such as 8-*O*-methylrabelomycin, were produced and purified from the first-round strains
- Genetic optimization through, for example, varying promoter activities, is needed for the second-round strains to produce other compounds, such as 8-*O*-methyltetrangomycin, which is a key intermediate in the chemically challenging C-ring cleavage
- Enzymatic assays are performed in order to determine the role of the produced intermediates in the lugdunomycin pathway
- Future objective is to add a third plasmid to further guide the pathway towards lugdunomycin

REFERENCES

1. Palmu, K., Ishida, K., Mäntsälä, P., Hertweck, C. & Metsä-Ketelä, M. (2007) Artificial Reconstruction of Two Cryptic Angucycline Antibiotic Biosynthetic Pathways. *ChemBioChem* **8**:1577–1584.
2. Elsayed, S. S., Van Der Heul, H. U., Xiao, X., Nuutila, A., Baars, L. R., Wu, C., … Van Wezel, G. P. (2023) Unravelling key enzymatic steps in C-ring cleavage during angucycline biosynthesis. *Commun Chem* **6**:281.
3. Bai, C., Zhang, Y., Zhao, X., Hu, Y., Xiang, S., Miao, J., … Zhang, L. (2015) Exploiting a precise design of universal synthetic modular regulatory elements to unlock the microbial natural products in *Streptomyces*. *Proc Natl Acad Sci USA* **112**:12181–12186.