Discovery of novel antibiotics by genetic engineering of *Streptomyces* soil bacteria

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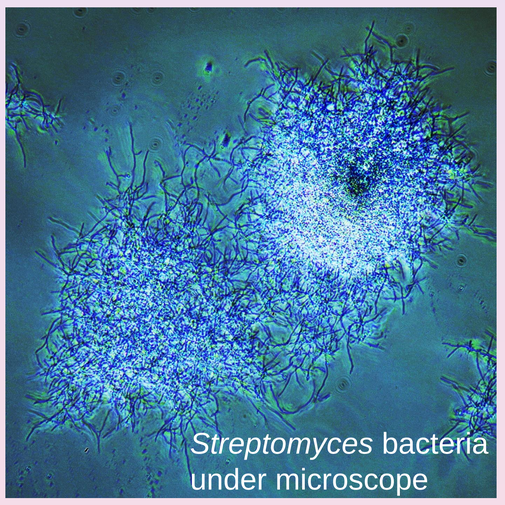


Introduction

Streptomyces are soil dwelling bacteria that produce a vast variety of natural products used as antibiotics and anticancer agents. Modified versions of the natural compounds with higher solubility and potency can be produced by genetically engineering their production pathways in the host organism.

Tetracenomycins are antibiotics produced by *Streptomyces*, and it was recently discovered that they bind to a novel region in the ribosome and thus inhibit protein synthesis in the pathogenic bacteria. Inspired by this discovery, our research group set out to generate modified versions of 8-demethyl-tetracenomycin C (8-DMTC) by attaching different sugar units to it.

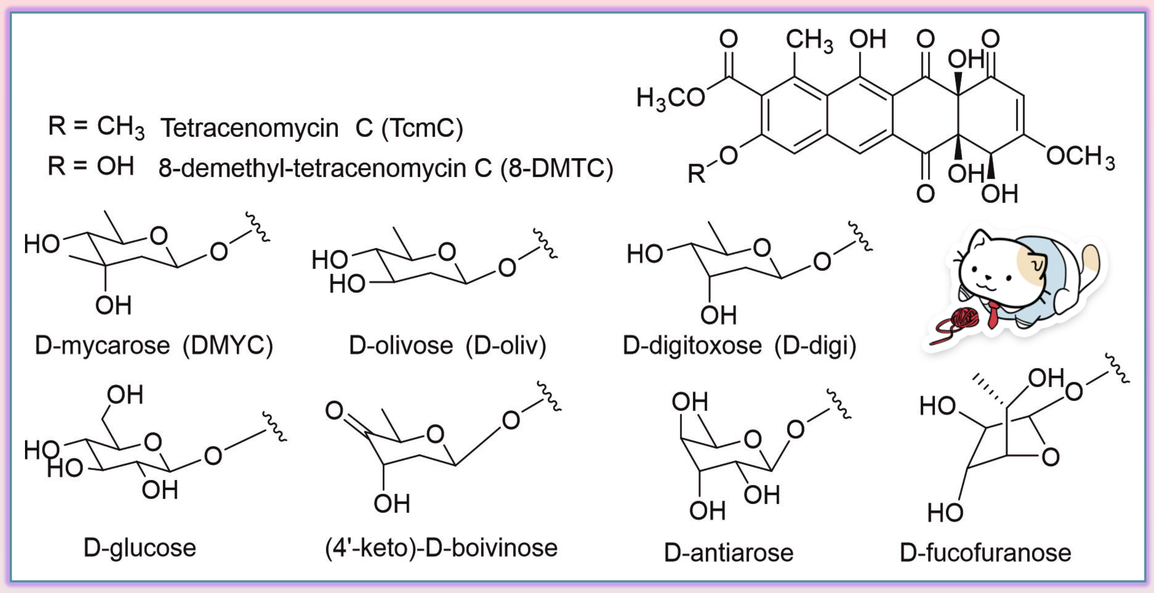


Figure 1. Compounds in this study. A) Tetracenomycin C and 8-demethyl-tetracenomycin C. **B)** Different sugar units that were attached to 8-DMTC to generate new antibiotics. Wavy line indicates the attachment point of the sugars to R in TcmC.

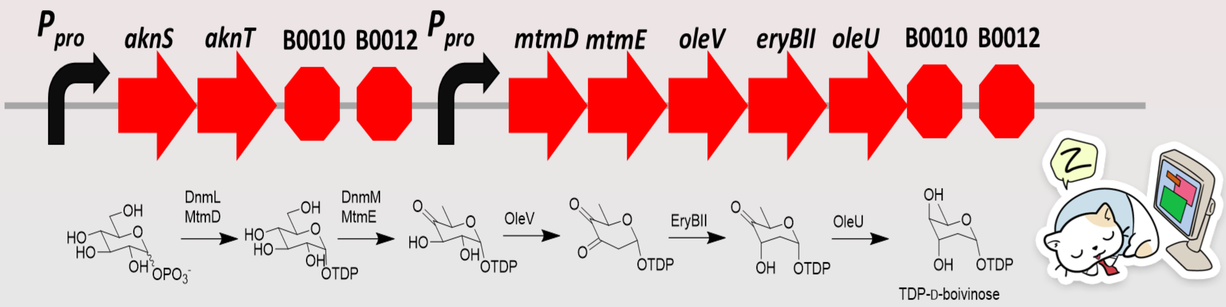


Figure 2. Example of a biosynthetic pathway to generate the sugar unit D-boivinose. The plasmid contains promoters (black arrow), enzymes responsible for modifying glucose (red arrow), and terminators (red octagon). Figure courtesy of collaborator Eric Nybo, Ferris State University, USA

Aims

1. **Produce new antibiotics by genetically engineering the production pathways in *Streptomyces* bacteria**
2. **Screen for increased solubility and potency of the new antibiotics**

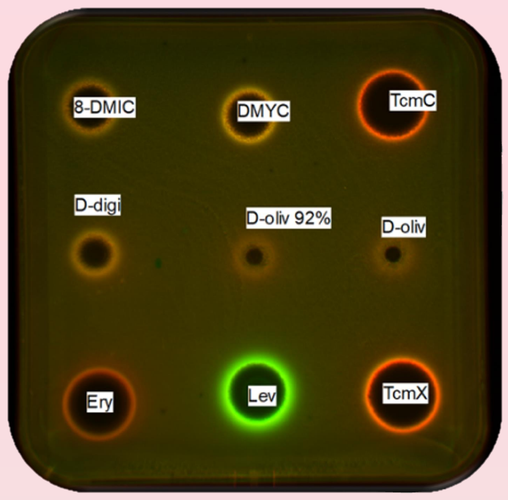
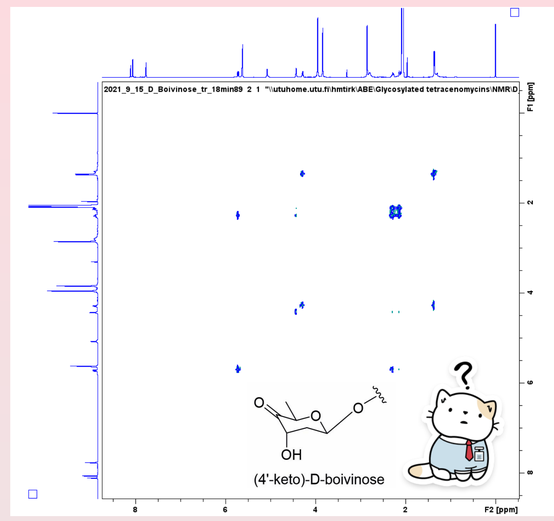


Figure 3. Glycosylated 8-DMTC derivatives inhibit protein translation. In-vivo dual-reporter assay shows inhibition of translation in red and induction of DNA damage in green. Figure courtesy of collaborator Ilya Osterman, Moscow State University, Russia.

Figure 4. COSY measurement of (4'-keto)-D-boivinose. Blue peaks on the axis depict the protons (H) in the compound. It's possible to decipher the structure of the sugar unit by following which peaks are connected (blue dots in the middle).

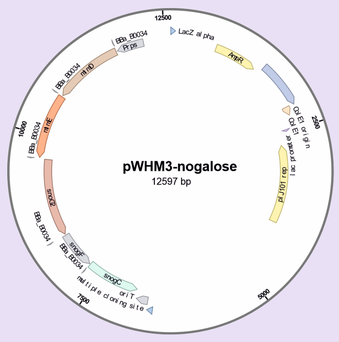


Results and Conclusions

Seven different sugar gene pathways (**Fig. 1**) were generated as plasmids utilizing BioBricks cloning (**Fig. 2**). Sugar units were attached to 8-DMTC utilizing a flexible glycosyltransferase ElmGT expressed by host strain. Most of the pathways in this study were functional, however in the pathway for D-boivinose (**Fig. 2**) the intermediate before the final product was produced.

Preliminary results show that glycosylation of 8-DMTC effects the inhibition of translation (**Fig 3**). Branched chain sugars (D-mycarose) were found out to be the most potent of the glycoside compounds tested so far.

Materials and Methods



1. Generate sugar producing plasmids and conjugate them to *Streptomyces* strain expressing 8-DMTC.

2. Compound production in *Streptomyces coelicolor* M1146

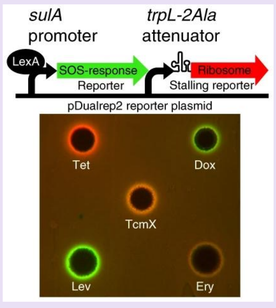
3. Two-phase extraction of compounds from culture using ethyl acetate

4. Chromatographic separation of the compounds based on either size or hydrophobicity

5. High-performance liquid chromatography separation of the compounds based on hydrophobicity

6. Pure compound

7. NMR and high-resolution mass spectrometry to solve the structure of the compound (**Fig. 4**).



8. Bioactivity measurements in vivo: inhibition of translation and DNA damage induction