Transfer-to-Excellence (TTE) Program

Investigating Enzymes used in Biosynthesis of a Potential Anticancer Drug

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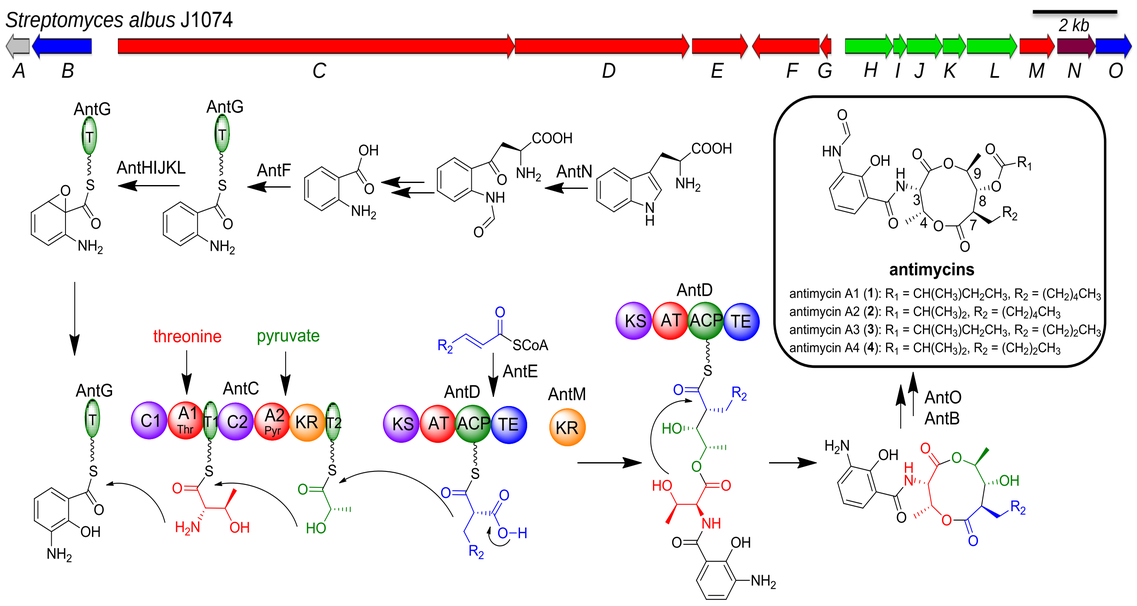
Introduction/Background

Antimycins are inhibitors of the electron transport chain. They are cytotoxic because they interrupt the transmission of the electrons between cytochromes b and c_1 by binding to cytochrome c_1 oxidoreductase. Recent cancer research further confirms that antimycin analogues have potential for being anticancer agents because they selectively inhibit Bcl_2/$BclX_L$ related antiapoptic proteins. In order to produce antimycin analogues exhibiting advanced pharmaceutical effect, the enzymatic mechanism responsible for the antimycin scaffold assembly needs to be clearly elucidated. A putative biosynthetic cluster for the antimycin biosynthesis was reported in 2011 (ref 5). Based on this information, Zhang lab proposed a pathway for antimycin biosynthesis in *Streptomyces albus* (Map 1). The goal of this project is to determine the functions of the enzymes, AntB and AntO, whose functions have not yet been elucidated, in antimycin biosynthesis. Our hypothesis is that AntO is responsible for initial removal and/or re-insertion of the N-formyl group on the aromatic moiety of the antimycins while AntB is responsible for addition of R1 group on the antimycins.

Proposed Antimycin Biosynthetic Pathway



Method

Mutation Preparation/Identification

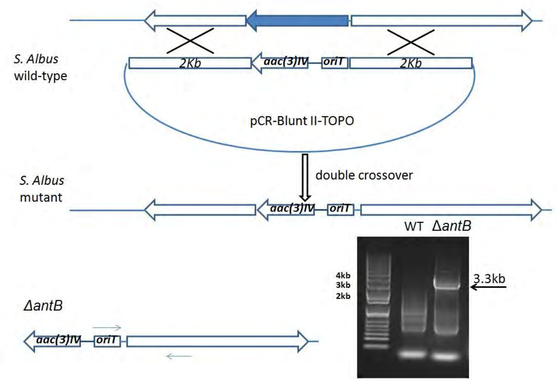
- Potential Mutant constructs were prepared.
- Colonies were screened for double crossover mutants (Fig 1).
- Once a double crossover mutant was identified, the mutant strain was screened for antimycin production.

Screening Mutant Strains for Antimycin Production

- Mutant strains were grown in liquid cultures.
- Supernatant extracts were analyzed by HPLC and LCMS.

Results/Data Analysis

Fig 1. Double crossover mutant and its gel photo



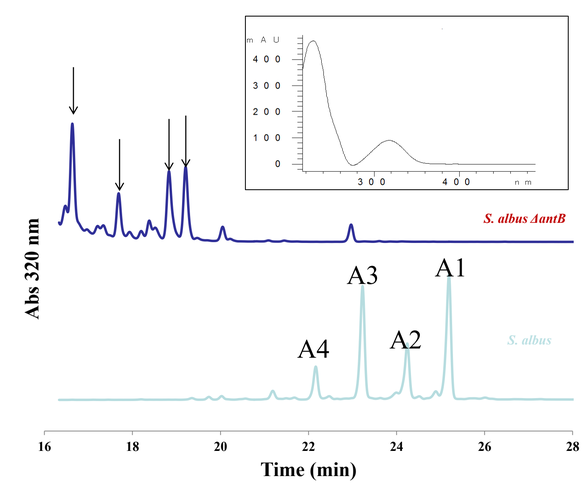
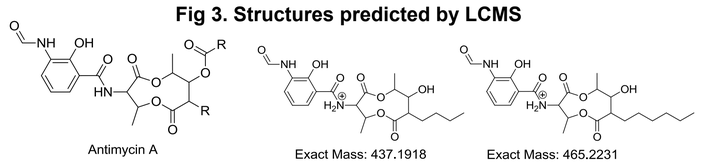


Fig 2. HPLC trace of the products produced by wildtype, compared with those of Δ *antB*. UV spectrum of antimycin shown in top right.

Fig 3. Structures predicted by LCMS



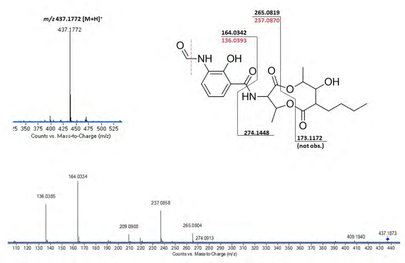


Fig 4. ESI-MS and ESI-MSMS spectra of *ΔantB m/z* 437.1772 $[M+H]^+$

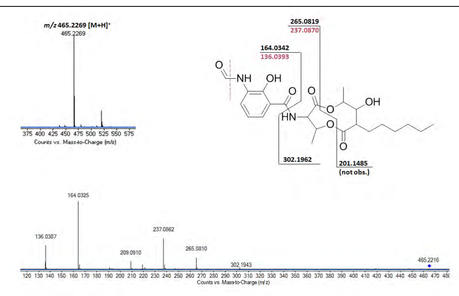


Fig 5. ESI-MS and ESI-MSMS spectra of *ΔantB m/z* 465.2269 $[M+H]^+$

Discussion/Conclusion

- We were successfully able to knockout *antB* in *S. albus.*
- RP-HPLC analysis of S. albus Δ*antB* supernatant extracts revealed several compounds with similar UV spectra as the antimycin standards (A1-A4) (Fig 2).
- Then, we further checked the molecular mass of each compound with LCMS (Fig 3).
- ESI-MS and ESI-MSMS analysis confirmed that the antimycin-like compounds produced by *S. albus* Δ *antB* lack the R1 group confirming that AntB is involved in the addition of the R1 group in antimycin biosynthesis (Fig 4 &5).

Future Directions

- Do exactly the same steps to confirm the function of AntO once we get the Δ *antO* mutant.
- Purify both AntB and AntO to do in-vitro analysis.

References

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