Anthracycline biosynthesis in Streptomyces: engineering, resistance and antimicrobial activity

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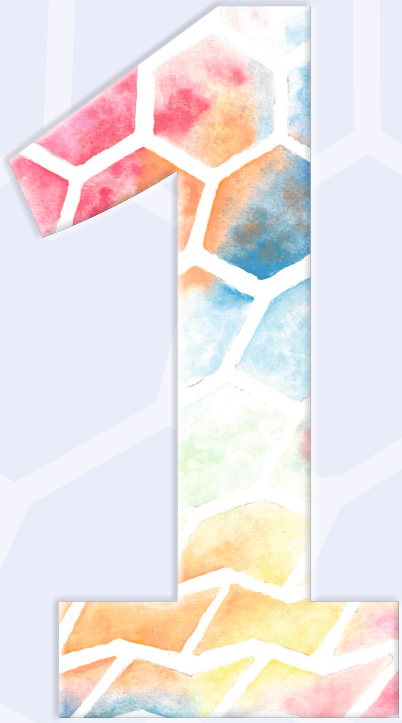

Thesis outline

Actinobacteria are a diverse phylum of Gram-positive bacteria with a multicellular lifestyle[1]. These bacteria are well known for the production of bioactive natural products, many of which have applications in the fields of human, animal and plant health[2–4]. The genes involved in the production of these bioactive metabolites are typically clustered on the genome in so-called biosynthetic gene clusters (BGCs). The production of bioactive metabolites is strictly regulated by a complex network of cluster-situated and global regulators[5,6]. Additionally, BGCs often harbour resistance genes to counteract the toxicity of their cognate products[7].

Subject of this thesis are the anthracyclines, glycosylated aromatic polyketides produced by Actinobacteria. The most renowned anthracyclines are daunorubicin and doxorubicin, natural products that are produced by *Streptomyces peucetius*[8–10]. Although these compounds were initially discovered by their antibiotic activity, their potent anticancer activity led to their development as anticancer drugs. Despite their remarkable efficacy against acute leukaemia and various solid tumours[11,12], their application is limited by severe side effects, such as cardiotoxicity, therapy-related tumours and infertility[13]. Notably, the toxicity of anthracyclines also prevented their application as antibiotics. **Chapter 2** provides a review of recent developments in anthracycline research. In this review, we discuss: (1) the biological role of anthracyclines in producer strains; (2) clinical applications and limitations of anthracyclines as anticancer drugs; (3) new insights into anthracycline biosynthesis; (4) newly discovered anthracyclines via genome mining, metabolic engineering, and combinatorial biosynthesis; and (5) strategies for improved production levels of anthracyclines.

The mode-of-action of anthracyclines involves interaction with DNA, resulting in DNA double-strand breaks via the trapping and poisoning of topoisomerase II onto the DNA, and chromatin damage via the eviction of histones at defined sites in the genome[14,15]. Anthracycline-induced cardiotoxicity results from the combination of both activities, while compounds inducing only chromatin damage maintain anticancer efficacy with limited side effects[14,16]. Importantly, *N,N*-dimethylation of the amino sugar moiety of doxorubicin resulted the absence of DNA damaging activity, while showing increased anticancer activity[16]. Consequently, *N,N*-dimethyldoxorubicin (DMdoxo) was proposed as a promising alternative to doxorubicin with potentially prolonged treatment options. Subsequently, a comprehensive evaluation of a structurally diverse library of anthracyclines revealed that the presence of a tertiary amine on the first sugar moiety generally results in the loss of DNA-damaging activity while exhibiting improved cytotoxicity[17–19].

For the evaluation of anticancer properties, DMdoxo was synthesised through organic chemistry[16]. Although naturally occurring DMdoxo has never been reported, the *N,N*-dimethylated amino sugar is found in other anthracycline biosynthetic pathways. In **Chapter 3**, we describe the engineering of the doxorubicin biosynthetic pathway in *S. peucetius* for the biosynthesis of *N,N*-dimethylated anthracyclines. Through the introduction of enzymes from the *Streptomyces galilaeus* aclarubicin pathway and the *Streptomyces purpurascens* rhodomycin pathway, we achieved the biosynthesis of *N,N*-dimethyldaunorubicin. However, two factors limited the product yield of the engineered strain: (1) our results suggest that the P450 monooxygenase DoxA is

inhibited by the *N,N*-dimethyl moiety of the unnatural substrates; and (2) *N,N*-dimethylated anthracyclines proved to exert a stronger cytotoxic effect on *S. peucetius* compared to the natural variants, resulting in product toxicity.

The susceptibility of *S. peucetius* to *N,N*-dimethylated anthracyclines evoked our interest in exploring anthracycline resistance mechanisms in actinomycetes. In **Chapter 4**, we investigated anthracycline resistance within our laboratory's actinomycete collection. Additionally, we performed adaptive laboratory evolution experiments of selected streptomycetes with the toxic compound DMdoxo. The results indicate that DMdoxo exerts higher antibacterial activity than doxorubicin, and that challenge with DMdoxo induces the activation of cryptic resistance export systems in strains that do not produce anthracyclines themselves.

The severe side effects associated with anthracyclines prevented their development as antibiotics. Nevertheless, detoxified anthracyclines with limited side effects may offer a potential therapeutic window for their use as antibiotics. In **Chapter 5**, we evaluated the antimicrobial activity of a library of 35 structurally diverse anthracyclines[17–19] against ESKAPE pathogens. Compounds with potent cytotoxic activity against human cancer cell lines also exhibited strong antimicrobial activity, particularly against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Generally, *N,N*-dimethylated anthracyclines showed increased antimicrobial activity.

Actinobacteria continue to be an important source of natural products with medicinal properties[2–4]. For the discovery of new compounds and the development of efficient producer strains, high-throughput multi-omics analysis in a useful tool. In **Chapter 6**, we developed an integrated quantitative proteomics and metabolomics workflow for small-scale *Streptomyces* cultures. The workflow provided good quality data from a technical perspective, although biological reproducibility should be optimised in future work.

Finally, the main findings of this work are summarised and discussed in **Chapter 7**.