Antibiotic discovery by induction of inactive antibiotic gene clusters

The World Health Organisation (WHO) has declared antimicrobial resistance (AMR) as one of the top 10 global public health threats facing humanity (WHO, 2017). In 2019 there was an estimated 5 million deaths associated with bacterial AMR (Murray et al., 2022) . On the other hand our supply of new compounds has dwindled drastically leaving our pipeline in a precarious state.

This was attributed to several effects. One of which was the onset of combinatorial synthesis which was very successful in tackling numerous diseases but only had limited success when tackling pathogenic bacteria due to decreased drug penetration and high levels of resistance frequency. Another reason was that 50 years ago antibiotics were readily available, cheap and effective. The pharmaceutical industry saw therefore little benefit in developing further drugs. Another reason was the high level of rediscovery (Katz & Baltz, 2016). It was assumed that the majority of compounds from a natural source has been discovered and there is little benefit in searching further.

Antibiotics are traditionally sources from filamentous soil bacteria called actinomycetes which produced 2/3 of all natural products. The mindset that no new antibiotics can be sourced from this group of bacteria changed radically in 2002 when the first full genome sequence of a model actionomycete (*Streptomyces coelicolor*) was determined. While it was known that *Streptomyces coelicolor* was able to produce 4 secondary metabolites (actinorhodin, prodiginine, CDA and the grey spore pigment) the genome sequence revealed a further 18 gene clusters characteristic for secondary metabolites (Bentley et al., 2002). This led to a renaissance of natural product discovery. However, one fundamental question remained: **How to access antibiotics which are inactive under laboratory conditions?**

While several methods such a coculturing with other organisms, changing culture conditions and genetic engineering of the gene cluster have been applied successfully no gold standard method has emerged.

I hypothesize that antibiotic production can be induced via transposon mutagenesis. Transposons are small mobile elements which integrate themself on the chromosome in a random manner (Cain et al., 2020). I created a transposon with a strong activator on each side of the mobile element. The transposon jumps in the vicinity of the antibiotic biosynthesis cluster and activate its genes.

This project aims to develop this method further and create a robust method for the discovery of new antibiotics to tackle the current antibiotic supply crisis.

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