Bacterial warfare: Identifying new antibiotics made by bacteria

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What is the problem?

Antimicrobial resistance (AMR) is of critical concern in modern medicine, with an estimated 10 million deaths every year by 2050 if we continue on our present course (UK Government AMR report, 2014). The health professions are finding that resistance to new classes of antibiotic often appears in disease causing bacteria only a few years after they start to be used and some particularly successful harmful bacteria have developed resistance to all available antibiotics, highlighting the need for new antimicrobial compounds to be discovered and developed.

What are we interested in?

Most classes of antibiotics come from molecules made by bacteria and fungi which the bacteria or fungi use in the environment to compete with each other to obtain resources and expand their own communities (see Figure 1). If we can identify these compounds we can hopefully discover new antimicrobial agents.

In previous research in my supervisor's lab, we found that a particular bacterium called *Serratia marcescens* could produce a new antibiotic called althiomycin. In this project we wanted to see if different strains of *Serratia marcescens* might produce other new antimicrobials that could be used as antibiotics.

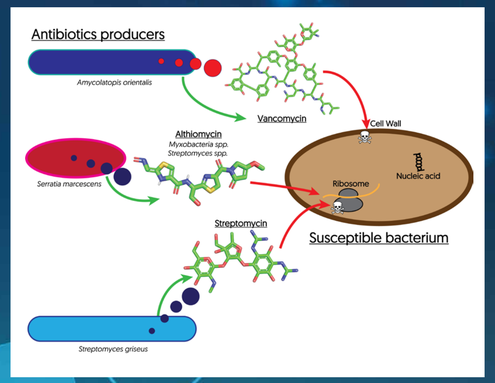

Figure 1 Antibiotics produced by bacteria.

Three different antibiotics produced by bacteria are shown. The green arrows show the bacterial species of origin and the red arrows point to their site of action in other bacteria. Vancomycin targets the bacterial cell wall and is a last resort drug used against a number of bacterial infections. Streptomycin and althiomycin inhibit protein synthesis by targetting bacterial ribosomes (the site where proteins are made).

What did we do?

We developed a visual way to identify if our *S. marcescens* strains of interest could inhibit the growth of a target bacterium using an antibiosis halo assay (see Figure 2).
We tested a collection of seventeen *S. marcescens* strains (inhibitor strains) against a selection of other bacteria (reporter strains) to see which *S. marcescens* strains produced large 'halos', indicating they were producing high amounts of a diffusible antimicrobial molecule(s). Using this information, we then selected a strain and randomly introduced mutations (changes in the DNA sequence) in to the bacteria and studied the effect of the changes. This technique is known as a random mutagenesis screen. We used this to look for variants (mutants) of *S. marcescens* which showed 'reduced killing' or 'loss of killing' in our antibiosis halo assay with the intention of identifying the gene whose loss resulted in this 'reduced/loss of killing'ability. This would hopefully tell us which gene is needed for *S. marcescens* to make the new antimicrobial molecule.

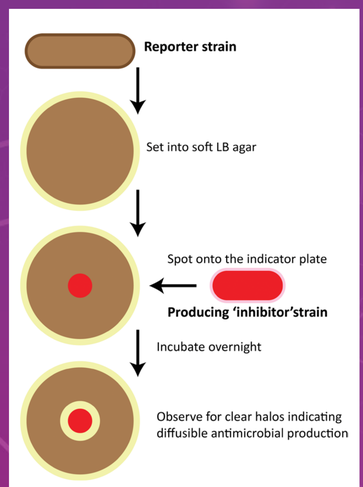

Figure 2 A schematic of the antibiosis halo assay. Both reporter strain (the target) and producing 'inhibitor' strains of interest were grown overnight. The target strain was introduced into the LB agar growth medium (a jelly like material) before a spot of the producing 'inhibitor' strain of interest was added to the plate. The plate was then incubated overnight and clearances were observed the next day, which indicated the presence of a diffusible antibiotic that had killed the reporter strain.

What did we find?

We found that our collection of *S. marcescens* strains had a wide range of antibacterial activity (see Figure 3). The majority of our strains were able to inhibit growth of target strains at 30°C, however, only a small selection of strains could inhibit bacterial competitor growth at 37°C. Interestingly, we discovered that closely genetically related strains can exhibit vastly different antibacterial activity.

We selected strain SJC1005 for our random mutagenesis screen and we found six mutants (out of a 600 mutant screen) that had a decreased 'halo', compared to the non-mutated SJC1005 strain, against a group of bacterial target strains known as Gram-positive bacteria, however, they had minimal change in halo against another group of bacteria, known as Gram-negative bacteria. Gram positive and Gram negative bacteria have differences in the structure of their cell wall.

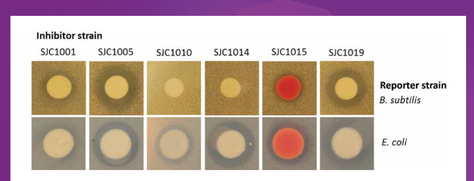

Figure 3 An example of the diverse antimicrobial activity we observed in our *Serratia marcescens* strains. Inhibitor strains are labelled on the top, while the reporter (indicator) strains are labelled on the right. Clearances around the spot indicates the production of inhibitory or bacteria-killing diffusible products.

What does this mean?

We have shown that this antibiosis halo assay is a viable method to look for diffusible antimicrobial products produced by *S. marcescens*. We observed even closely related strains give different results in these assays, suggesting that different strains make different antimicrobial molecules. These strains do not contain genes for making well-known antibiotics so these antimicrobial molecules may be candidates for new antibiotics. Our genetic mutants likely disrupted a gene involved in making an antimicrobial molecule that was effective against Gram-positive bacteria, as inhibition of Gram-negative bacteria was still retained. The next step will be to try to identify what this gene is.

Overall, we have shown that many possible antimicrobials produced by bacteria remain to be discovered and hopefully they may be used as effective antibiotics in time to come.

Who am I?

I am a 4th year BSc (Hons) Microbiology student at the University of Dundee. I undertook my Vacation Scholarship in summer 2017, which sparked my interest in research. Following on from this project, I conducted my final year project working on the how *Serratia marcescens* is able to kill competing bacteria using a bacterial 'spear gun' called the Type VI Secretion system. Following this exposure to academic research, I have been inspired to continue my scientific training and will progress onto a PhD program at Imperial College London in October 2018 to study the molecular and cellular basis of infection. I am interested in microbial interactions, how microbes cause disease and protein secretion in bacteria.

Brian was supervised during his Vacation Scholarship project, *"Identifying new antimicrobial agents produced by Serratia marcescens"* by Dr Sarah Coulthurst.