School of Physics and Astronomy

Modelling the growth of microbial biofilms in heterogeneous antimicrobial concentrations First Year Summary

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May 2018

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1 Project Outline

Antimicrobial resistance is one of, if not the, most important issue in modern medicine. Despite the massive prevalence of antibiotic usage, with over 260,000,000 prescriptions being issued in the US alone each year, surprisingly little is known about the underlying pharmacodynamics, and what factors influence the emergence of resistance. This lack of understanding is becoming increasingly critical as our supply of viable antibiotics dwindles in comparison to the increasing number of resistant strains of bacteria. In 2014 there were over 480,000 cases of drug-resistant tuberculosis reported, with only half of those cases able to be successfully treated. Experts estimate that if current trends continue, the number of deaths caused by drug-resistant bacteria will number in the millions, making further research in the field vital.

Due to the enormity of this issue, it would be foolish to leave the responsibility of progress to just the biologists and chemists of the world. This project therefore aims to use stochastic computational modelling techniques to glean insight on how the presence of antimicrobials control microbial colonies from a more physics based perspective. In particular, this project aims to investigate the underlying dynamics which influences how microbial populations, e.g. biofilms, form and proliferate when exposed to spatially heterogeneous drug concentrations.

While copious research has been undertaken on the mechanisms of antibiotics, the majority of these have been performed under very idealised conditions; with uniform antibiotic and nutrient concentration, and monospecies populations. However, recent developments

suggest that real-world factors which are ignored in these scenarios, actually have serious influence over the development of these microbial populations. In particular, how drug gradients allow microbes to expand spatially, the effects of differing nutrient-dependent growth rates on antimicrobial susceptibility, and competition between species with differing physiological attributes.

This project aims to utilise stochastic modelling techniques to investigate how these parameters affect the growth and development of microbial biofilms, and their expansion along microbial gradients. While these findings will obviously have important medical applications, they could also be of benefit in more industrial applications. In particular, impeding, or even halting the formation of biofilm formation on industrial shipping vessels. The shipping industry currently consumes an exorbitant amount of fuel. If it was a sovereign nation, it would be the 7^{th} largest producer of CO_2 on the planet and it's estimated that up to 45% of this fuel consumption in some cases is spent overcoming the additional drag caused by the formation of marine biofilms. Therefore any progress in our ability to reduce the impact of this biofouling would be of great economic and environmental significance.

Meetings with our industrial partner AkzoNobel has promoted discussions into the effects, and optimal design for antimicrobial paints. These coatings are currently the industry standard for controlling marine biofouling, so research into how factors such as combinations of anti-microbials, and the release rates thereof affect biofilm development is key.

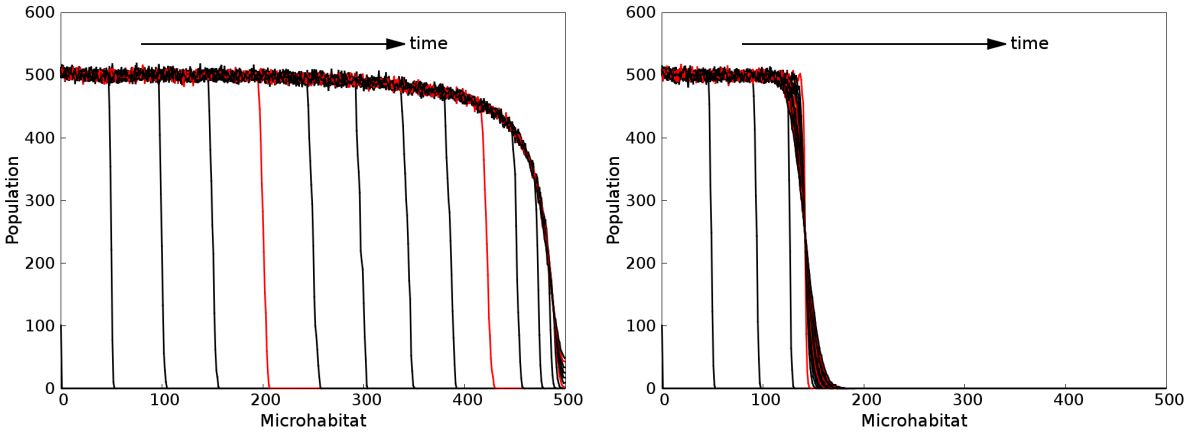
Additionally, this poses an interesting investigation into symmetry. In clinical situations, antibiotics are applied to the external surface of the biofilm, but in the industrial scenario the antimicrobial paints leach antimicrobials from the same surface as the biofilm is attached to. Therefore raising the question as to whether the direction of the gradient also has a role in how microbial populations proliferate through space.

Progress so far

So far several investigations have been performed into the effects of antibiotic gradients on the evolution and proliferation of bacterial colonies. The first project undertaken was to replicate the results of a PRL paper co-authored by my supervisor, which modelled how the steepness of antibiotic gradients in conjunction with differing mutational pathways affected the time taken for resistance to emerge. This was primarily intended as a "warm-up" project, in order to gain familiarity with modelling these sorts of systems, and to build a foundation upon which further projects could be built. These simulations consisted of a simple 1D system comprising several interconnected microhabitats each of which had a carrying capacity and concentration of antibiotic, which both affected the growth rate of the bacteria within.

Following on from this, work on the next project began swiftly. Here, research was performed on the differing effects of growth-rate dependent antibiotics, and their comparative abilities on impeding the spatial expansion of an initial inoculation of bacteria. Two systems were constructed, one with antibiotics which were more effective against fast-growing bacteria, the other with slow-growing bacteria targeting antibiotics. In this model, the carrying capacity factor was replaced with a parameter which represented the concentration of nutrients remaining in the microhabitat, each replication which oc-

curred resulted in nutrients being consumed. This corresponded to a reduction in the overall growth rate, which in turn altered the susceptibility of the bacteria to the applied antibiotic.



(a) SGBTA (b) FGBTA

Figure 1: Spatial distributions of bacterial populations exposed to SGBTA and FGBTA respectively. The steepness of the antibiotic gradient has been chosen here such that the concentration does not reach the MIC until the last few microhabitats. As can be seen, the SGBTA is inferior at inhibiting bacterial growth.

By sampling the spatial distribution of the bacteria over regular intervals, a comparison could be drawn to the differing efficacies of the two sets of antibiotics in curtailing population expansion. As can be seen in Figure 1, the antibiotics which target the fast-growing tip of the advancing population is more effective at inhibiting the spatial expansion of the population than the drugs which target the slow growing bulk towards the rear of the colony. These experiments were repeated for a variety of gradients, uniform concentrations and with more realistic growth curves taken from in vitro experiments performed in laboratory conditions. The corresponding results have now been collated and are in the process of being written up into a paper intended for publication.

Outlook

Following a meeting with our industrial partners AkzoNobel, it was decided that work would continue for the meanwhile on the nascent multispecies model. They mentioned how designing the right combination and release schedule of antimicrobials in order to promote the emergence of favourable biofilm compositions was of particular interest to them, so they intend to send us data on various bacterial species and their corresponding growth rates/susceptibilities to several biocides such that they could be incorporated into the multispecies model in order to deliver more quantitative, rather than simple proof-of-concept results.

For future projects, it's planned that we will move away from the discrete 1D microhabitat model and begin work on a more intricate continuum model. This would allow for the incorporation of more realistic dynamics such as flow, surface roughness and diffusion of the antibiotics, rather than the current fixed gradients.

2 Critical Dependencies

As this is a predominantly computational project, there aren't a large number of critical dependencies to consider. At the moment, the work being undertaken is reasonably broad, so there is the slight risk of duplicate research being published elsewhere before mine is complete, but as the research plan becomes more defined and focused, this should reduce the risk of that. Computation time has yet to become too much of an issue, but as the complexity of the model increases, it may do.

From the regular discussions I have had with my supervisor, I feel my progress has been reasonably satisfactory for my first year. There were times that I felt myself spread quite thinly due to my SUPA and tutoring commitments, but now that I have some experience in how much time these consume, this should be less of a concern going forward.

3 Training and Courses

Courses Attended in First Year

Name	Origin	Assessed	Hours
Intro to Soft Condensed Matter	SUPA	Yes	20
Physics of Biological Evolution	SUPA	Yes	10
Maths Primer	SUPA	Yes	6
Python	SUPA	Yes	8
Preparing for the first year review	Transkills	No	3

Courses to be Attended in 2018-2019

Name	Origin	Assessed
Computational principles to organize complexity	Summer school - INPHYNI	No
Advanced Data Analysis	SUPA	Yes
Introducing Biology to Physicists	SUPA	Yes
Hands-on Writing	Transkills	No

4 Teaching

Name	Semester	Hours
MfP1 Tutorials	1	40
Practical Physics (SciProg)	1	15
Physics 1B Labs	2	30

I intend to undertake a similar amount of teaching in my second year, but most likely in different courses.