Ichip Modification to Detect Growth Inducing Interactions Amongst Unculturable Soil Microbes in Coculture

Antibiotic Discovery: Interactions between Government and Pharmaceutical Companies in The Context of Large Technological Systems

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By

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

By 2050, it is estimated that the number of people worldwide who will die from drug-resistant disease will increase from 700,000 to 10,000,000 at the current rate (Plackett, 2020). Given this expected increase, one would expect pharmaceutical companies to be actively invested in making new antibiotics. However, over the last 20 years, 15 of the 18 largest pharmaceutical companies have left antimicrobial research (Finlay et al., 2019). This could be the reason why there were only 6 new antimicrobial drugs approved from 2010-2014, which compared to the 19 approvals made from 1980-1984, seems small (Ventola, 2015). My STS topic will investigate why these large companies are abandoning antibiotic development, and what is or can be done to bring them back. The framework from which I will look at this topic will be based on the ideas presented in "The political economy of technoscience: An emerging research agenda" by Kean Birch (Birch, 2013).

One reason why pharmaceutical companies might be stepping away from antibiotics is that it has become more difficult to discover them. Antibiotics by definition come from microbes, so in order to look for potential antibiotics, it would be of great help to know how to grow them. However, of the estimated 1 trillion microbial species (Locey & Lennon, 2016), we have only described roughly 12,000 as of 2017 (Overmann et al., 2017). This means that to discover new antibiotics, we most likely have to first learn how to culture currently unculturable microbes. The focus of my capstone project is to grow previously uncultured microbes in the hopes that this method could one day be used to screen for novel antibiotics. This will be done by modifying an existing technology called the

ichip to include the ability to grow microbes in coculture (which is when two microbial species are grown together).

Technical Topic

The reason why so many microbes remain unculturable is likely because the conditions we grow them in don't match the conditions of their natural habitats. Researchers cannot test all possible combinations of these conditions simply because there are too many of them, like which nutrients are present, the pH, and temperature (Stewart, 2012). However, they must be growing somewhere. The isolation chip (ichip) was developed in 2010 to address this lack of culturable microbes. Researchers placed a single microbial cell into one of many small diffusion chambers they made on a piece of Delrin plastic, which they called the ichip (Nichols et al., 2010). These diffusion chambers were then covered with a membrane that had 0.03 μm sized pores. This allows for small molecules to enter into the ichip, but not other cells.

The ichip was then returned to the original environment the cells were taken from for two weeks to allow for growth. After this period, they sequenced the 16S rRNA gene of the microbes, which is a common way to identify microorganisms. When comparing these results to sequences of known microbes, they were able to identify new species (Nichols et al., 2010). The ichip was later used to discover a new antibiotic called teixobactin, which is currently owned by NovoBiotic Pharmaceuticals (Piddock, 2015). According to their website, as of 2021 teixobactin is in late stage preclinical development, which highlights the importance of the ichip (*Press Releases*, n.d.).

My technical capstone project involves modifying the ichip to incorporate coculture with the objective to grow microbes the original ichip couldn't grow. This will be done by making half of the diffusion chambers twice as large as all the other diffusion chambers (Figure 1). Since the ichip is loaded by dilution, this doubling in volume will allow two cells to be placed into those diffusion chambers, on average. After the modified ichip is placed back into the environment the soil sample came from, we can sequence each diffusion chamber's 16S rRNA. The small diffusion chambers require no extra hassle to sequence their 16S rRNA, but we will have to use something like shotgun Illumina sequencing (Ong et al., 2013) to sequence the large diffusion chambers 16S rRNA, since they have 2 or more unique sequences in them. Since many microbes must be screened to increase the likelihood of finding a new species, my partner and I will separately carry out the same experiment from start to finish on samples taken from the same environment in order to increase the number of 16S rRNA sequences for the project.

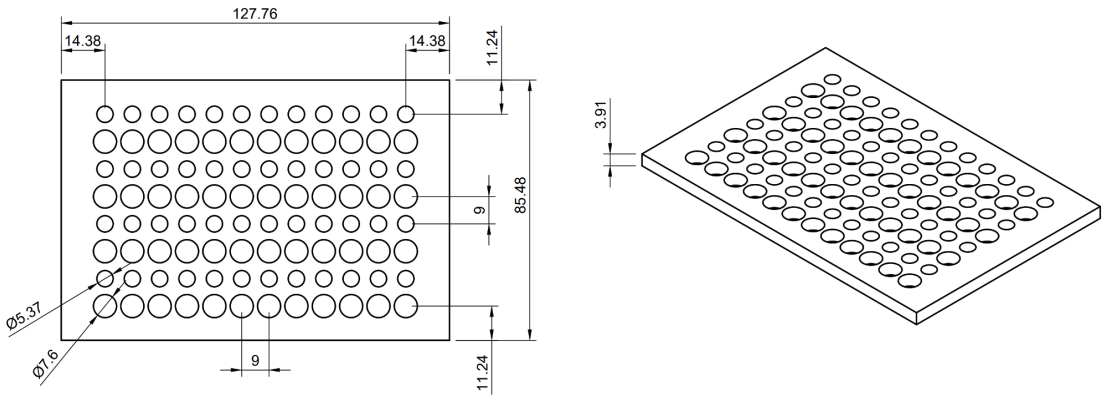

Figure 1: Engineering drawing of the modified ichip. Each cylindrical hole in the material (acrylic) is a diffusion chamber. All units are in millimeters.

Coculturing should provide an advantage over the ichip because it will allow for the better replication of the natural environment unculturable bacteria are currently living in. Other microbes are an important part of a given microbe's environment because different microbial species can communicate and share nutrients with each other (D'Onofrio et al., 2010), and there can be thousands of different species in a gram of soil (Torsvik & Øvreås, 2002). This modification to the ichip will hopefully allow for the growth of new microbial species, which will not only further our knowledge, but these microbes could be producing useful secondary metabolites. Secondary metabolites from microbes constitute half of the world's pharmaceuticals (as of 2009), and have been used as antibacterials, immunosuppressants, and hypocholesterolemic drugs (Demain & Sanchez, 2009).

STS Topic

There are not only technical problems that can describe why large pharmaceutical companies are leaving antibiotic research, such as the lack of cultivable microbes described above, but there are also economic problems. "The political economy of technoscience: An emerging research agenda" by Kean Birch delves into this idea. In this research paper he gives the example that pharmaceutical research is not only driven by the moral imperative to help others, but also by how much money can be made with that research. He also argues that technoscience (technology and science) is typically thought of as influencing the economy, and that we should also look at how the economy impacts technoscience (Birch, 2013). This thinking is very applicable to the decline in antibiotic research, because it costs an estimated $1.5 billion to develop an antibiotic, but the average antibiotic only brings in

$46 million a year (Plackett, 2020). In addition to this, the fact that patents last 20 years since they were filed (Petrova, 2014) makes it easy to see how the economy can influence the creation of technoscience.

So why doesn't the need for new antibiotics translate into profits for pharmaceutical companies? An article by Benjamin Plackett titled "No money for new drugs" offers up reasons as to why (Plackett, 2020). An antibiotic's revenue is determined by its price and how much is sold, and both of these are low for antibiotics. For the price, many governments, such as the United Kingdom, Australia, and Canada, have a say in what the drug price is (Plackett, 2020). This means that pharmaceutical companies will have a harder time adjusting the price to make sure they can at least break even. In effect, these policies are choosing no drugs over expensive ones. As for how much is sold, the nature of antibiotics themselves means that patients aren't on them for long (Plackett, 2020). The duration of treatment varies by infection, but examples of durations for common bacterial infections are 5 to 7 days for community acquired pneumonia, and 3-7 days for uncomplicated urinary tract infections (Wilson et al., 2019). While short treatment durations are better for the patient, they aren't good for pharmaceutical companies.

Kalydeco, which is a treatment for cystic fibrosis, is an example of a drug that has a high price and a high amount sold: two pills a day for a year costs $294,000, and people are on it for life (Kaiser, 2012). Now if you were a pharmaceutical company, which type of drug would you focus on? Antibiotics and a drug like Kalydeco both have the capacity to help

people, but it is way easier to bring in a profit with a drug like Kalydeco than it is with an antibiotic.

In addition to all these challenges for antibiotic discovery, another reason Plackett considers is that doctors don't want to prescribe new antibiotics because it is in the public's best interest to keep resistance to this new treatment as low as possible (Plackett, 2020). While this might be best for public health, it hurts pharmaceutical companies. A pharmaceutical company that discovers an antibiotic is normally protected from competition for between five to ten years, so when the time comes that a new antibiotic is widely needed, it's not hard to see how some other company can just start making that drug without having the cost of discovering it. This lowers the price of the antibiotic, which means the company that discovered it gets even less money if it were used right away (Plackett, 2020).

In order to help out the antibiotic industry, The GAIN (Generating Antibiotic Incentives Now) act was passed in 2011. One thing it does is give 5 extra years of protection against competition to companies who discover an antibiotic (Ambrose, 2011). While this is a step in the right direction, when looking at the cost of antibiotic development, this additional five years does very little. Five extra years at $46 million a year in revenue, on average, still looks small when compared to the $1.5 billion development cost (Plackett, 2020). Given that antimicrobial resistance costs the U.S. $55 billion annually (Dadgostar, 2019), my solution to bring more pharmaceutical companies back into antibiotic research is for the government to subsidize most of the development costs for each new antibiotic

produced. While this will cost money up front, it has the potential to save even more down the road. In addition to this, the government could reward subsidies for each stage an antibiotic reaches in the stages of drug development.

Next Steps

1. Find more detail describing the GAIN act, as well as what other professionals have to say about its effects.
2. Find more current or previous policies directed at antibiotic discovery and briefly discuss what they do. For these policies, find what experts say the implications of these policies could be. Is there anything they would improve or change?
3. Incorporate how long antibiotics are typically in high demand into a specific amount the government will give out in incentives.
4. Break down the amount governments will give out into the phase of drug development. This way, antibiotic companies are not taking an all or nothing risk when they start out discovering a new antibiotic. Briefly touch on each phase a new drug has to go through in order to be approved by the FDA.
5. What examples, if any, are there for government subsidies in the pharmaceutical industry? Would the amount given out to incentivize antibiotic discovery be too much when compared to these existing subsidies, or would it be feasible for the government to subsidize antibiotic development?

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