The Quest for Novel Antimicrobials from Bacteria

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> If the world fails to mount a more serious effort to fight infectious diseases, antimicrobial resistance will increasingly threaten to send the world back to a pre-antibiotic age (Gro Harlem Brundtland)

Bad Bugs Fight Back

Imagine a world without antibiotics. A world where diseases such as syphilis, tuberculosis, meningitis were still commonplace, where a scrape on a leg could develop into life-threatening illness and the plague was still to be feared. Such was life before 1928, the year Alexander Fleming revolutionised the field of medicine by accidentally discovering penicillin, a mould which could kill bacteria. This drug became the world's first antibiotic and it spurred scientists to discover other classes of antimicrobial compounds, which together dramatically reduced the spread of disease.

However, this golden era of medicine will not last forever. After only a few years of use, disease-causing bacteria had emerged which were resistant to antibiotics. The most common of these so-called "superbugs", methicillin-resistant *Staphylococcus aureus* (MRSA), is now responsible for an increasing number of deaths, and is practically untreatable. Similarly, incidents of multi- drug-resistant tuberculosis are on the rise. Other drug-resistant bacteria are causing difficult-to-treat illnesses like pneumonia, urinary tract and bloodstream infections that lead to over 25,000 fatalities a year in the EU.

As time goes by, it is becoming increasingly likely that the discovery of the miracle-cure penicillin was a lucky break. Over the last 80 years, very few antimicrobials have been found that can rival its power. And for those that do, their shelf-life is limited due to bacterial resistance, a reality which has discouraged investment from the pharmaceutical giants.

New approaches and new sources are required to uncover novel antimicrobial agents. The good news is that there *are* other antimicrobial agents, albeit many which possess a smaller range of bacterial targets, and lack the mass destructive power of the classic penicillin-type antibiotics. One such type of antimicrobial agents are the bacteriocins.

https://doi.org/10.33178/boolean.2012.14

Bacteriocins: Good Bugs Provide an Answer

Conceptually, the idea is simple. Bacteria are miniature, thriving machines with one aim: to survive. These little organisms occupy niche environments across the globe, to which they have adapted to survive. One key component in this strategy is their need to outcompete their rivals — especially other species of bacteria, to emerge as the dominant population in their environment. An essential element of this survival strategy lies in their ability to produce compounds that can destroy their enemies. These antibacterial agents, termed bacteriocins, are potent toxins, which penetrate or prevent the formation of the cell membrane, or skin, of other bacteria, causing them to bleed out their internal organs, culminating in cell death. These antimicrobial compounds are made of protein (and therefore, are referred to as peptides), which means they can be easily digested by humans and are thus considered harmless. What's more, bacteriocins are thought to be tougher for bacteria to develop resistance to.

Any environment where there are several communities of bacteria struggling for dominance are good, potential sources for bacteriocin discovery, for example, fermented food (cheese, milks, sausage... etc). Bacteriocins are unique because they are produced by bacteria, and their range of targets can be quite specific, which is useful when one only wants to kill certain types of bacteria. The aim of my research it to use the latest advancements in technology to expedite the screening of bacteria, and to find novel antibacterial agents, specifically, bacteriocins.

Advanced Culture Screening

For years, the standard method to discover new bacteriocins was to use an "overlay" approach. Here, bacteria from any source are grown on a nutritious medium called agar. An indicator bacterium, i.e. one which we would like to kill, is poured over the other bacteria. Any resulting area of clearing in the indicator overlay results in the detection of a possible bacteriocin. This technique, while still useful, is laborious and can be slow to yield results.

My current research uses a modernised approach to screen samples for antimicrobial activity. The most recent samples come from fermented food products, such as kefir, a yoghurt-style milk beverage. Here, the milk is fermented by a complex, unidentified group of bacteria to form an acidic beverage. The resulting product is diluted and spread onto different types of nutritious agars, which are incubated at different temperatures (typically 30° or 37°C) until colonies are grown. The QPIX2, a specially designed machine, can scan the surface of the agar, and using needles, picks each of the colonies and stores them in a liquid medium. These are frozen at -80°C. This process is repeated until a frozen library consisting of tens of thousands of colonies is created. Up to 2,300 of these colonies are then stamped onto large agar plates and allowed to grow. What follows is the actual

experiment for antimicrobial activity. The plates are overlaid with another layer of agar which is mixed with an "indicator" bacterium, (the microbe one wants to show activity against), such as *Staphylococcus aureus*, responsible for MRSA, or *Listeria*, a common food pathogen. The indicator will grow as a cloudy lawn over the existing colonies, and any resulting areas of clearing, or zones, indicate the presence of an antibacterial peptide. The colony number is recorded and cultured once again from the original frozen library for further testing. The producing colony is identified using specialised techniques, the full antimicrobial spectrum is determined and the peptide is eventually isolated and purified. Peptides discovered during the high-throughput screen are currently undergoing laboratory investigation.

Genetic Screening

All living life contains some form of DNA, be they human, plant or bacteria. DNA is, simply put, 4 repeating letters, A, T G and C, which, depending on their arrangement, form a sequence. These sequences encode the instructions for every action an organism takes, from making new proteins to cell reproduction. Technological advancements have made the sequencing of DNA common practice, wherein small sequences of DNA, like genes, or large scale sequences, such as an organism's total DNA, can be determined, or "mapped". These sequences are publicly available in enormous online databases, such as Genbank, for the benefit of other scientists.

Another aspect of my research was to screen all the bacterial DNA in these databases for the presence of new bacteriocins. This was achieved by taking the sequence of a well known bacteriocin, called Nisin, and entering it into an online search engine called BLAST, to screen against all the fully sequenced bacteria in the database, which, at the time of the study was 1178. This generated large volumes of genetic information, which were analysed in-depth for the presence of characteristic bacteriocin-associated genetic traits. Certain species of bacteria were shown to contain stretches of DNA similar, but not identical, to Nisin. This was considered to be a positive indication of a peptide that is biologically functional.

Forty nine "clusters" were shown to contain the group of genes necessary to produce an antimicrobial peptide. The bacteria which possess the codes for bacteriocin production are considered potential producers of antimicrobials. These species of bacteria were shown to come from a wide variety of unrelated habitats, such as mammalian intestines, soil, skin, dairy produce, and even deep-sea hydrothermal vents, demonstrating just how universally important bacteriocin production is for bacterial survival.

Moving Forward

It remains to be proven via laboratory experiments if these new peptides are indeed functional, and if so, how potent and wide-ranging these bacteriocins are. Thanks to the volume of genetic information we have acquired, we now have clues regarding the nature of the peptide. Previously published research has established that certain codes are common to groups of bacteriocins, and have been shown to play an important role in the biological activity of the peptide. With this pre-established information, we can begin research on how our 49 potential peptides might function, and determine just how novel these bacteriocins are.

Considering that it takes at least 10 years of trials before a new drug can reach the market, the time to make novel drug discoveries is now. Here, I have described our attempts to discover next-generation antibiotics, novel antimicrobials produced by bacteria, using high-throughput robotic screening and genetics. Bacteriocin-producers discovered by our high-throughput and genetic screening are currently in the next phase of analysis. The age of the superbug is fast-approaching and our arsenal of drugs is diminishing. The race is on.

Alan Marsh is a third year PhD student under the supervision of Dr Paul Cotter and Prof Paul Ross, whom he would like to acknowledge for their support. Alan is funded by the Alimentary Pharmabiotic Centre, a research division of the Science Foundation Ireland (SFI).