# Hypothesis driven drug discovery

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## Introduction

Antibiotics were discovered over 50 years ago, providing a long sought after treatment for infectious diseases. These drugs effectively suppressed diseases such as tuberculosis and infections such as those caused by *Staphylococcus aureus*.

Many thought that struggle for control over bacterial disease was over. However, the ability of bacteria to rapidly acquire new genes by lateral gene transfer, coupled with the often inappropriate use of antibiotics, meant that resistance to antibiotics began to emerge in many pathogens. Infectious diseases are still one of the world's leading cause of death, killing at least 17 million people annually [1].

The majority of currently prescribed antibiotics were originally derived from bacteria and fungi. Microbes produce antibiotics to inhibit other microorganisms that might be competitors. If a cell produces antibiotics it must also be insensitive to those compounds, or must carry genes that provide resistance to the antibiotics. Consequently, it seems likely that for every antibiotic produced there is at least one natural mechanism of resistance [2].

The ability of bacteria to capture foreign genes meant that as antibiotic usage increased, there were enormous selective advantages for bacteria that accomplished these rare transfer events. Although individual lateral transfer events may be rare, the huge populations of bacteria worldwide, coupled with the power of natural selection, meant that the appearance of resistant strains of bacteria was inevitable.

## Prospecting strategies

To ensure that effective antibiotics will be available for future use, it is important to devise new strategies for discovering such compounds. There are two main approaches that can be used to discover antimicrobials. The first is development of next-generation chemotherapeutic agents using combinatorial chemistry. This method has an element of trial and error, as there are an enormous number of compounds that could potentially be synthesized from the existing structures, yet only a small number that retain drug-like characteristics [3]. This means that unless the unsuitable compounds can be eliminated prior to synthesis, the process may not be cost effective and can take a long time to develop an end product.

The second approach in the discovery of antimicrobials uses natural products to prospect for new compounds. This relies on nature doing the combinatorial chemistry for us, since different species have been producing antimicrobials for millions of years. Approximately 25% of currently prescribed drugs have been directly derived from natural products and it is still a relatively neglected avenue of drug prospecting [4].

One major advantage of targeting unexplored natural products in drug discovery is the increased chance of finding groups of compounds that are entirely novel. Antibiotics that do not belong to any of the known classes, or that are derived from unusual organisms, might have longer useful lives before resistance appears.

In screening natural products for new compounds, the general method has been to randomly screen a large number of samples for a compound with the desired mode of action [5]. This approach is time consuming and expensive, as the majority of samples tested do not exhibit the appropriate characteristics. Furthermore, there is a large degree of replication involved, since the same compounds can be discovered many times. A more cost effective approach might be to carry out directed searches, only screening samples that are likely to have the desired activity.

## Directed screening

This directed approach has already been successful in a number of areas in biology. For example, the Polymerase Chain Reaction (PCR) is a rapid method used to amplify large amounts of DNA from a small sample. It requires multiple cycles of heating and cooling that would normally inactivate enzymes. A major breakthrough in this technique was the use of *Taq* polymerase isolated from *Thermus aquaticus* which lives in hot springs. The *T. aquaticus* enzyme is heat resistant, and is therefore not degraded by the high temperatures required in PCR [6].

Bioremediation is another field where a directed approach has been used to prospect for new enzymes that are capable of breaking down pollutants. The idea behind such research was that bacteria and fungi that grow in polluted areas must be able to tolerate (and potentially break down) the toxic compounds in these areas. Consequently a number of bacteria and fungi from these areas were examined and found to contain enzymes that could break down wastes such as polychlorinated biphenyl [7].

Marine microbiologists have also been successful in approaching the problem of biofouling from an evolutionary perspective. In this case the hypothesis is that where the colonisation of bacteria will be deleterious to the host, it is likely that the host has evolved mechanisms to prevent such colonisation. The red marine

alga *Delisea pulchra* has been the focus of numerous studies and it has been found that the signaling system that allows bacteria to colonise surfaces is inhibited by furanones produced by the alga [8].

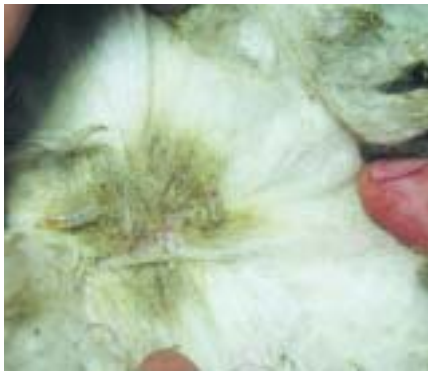
The common theme amongst these examples is the use of biological, natural history and evolutionary knowledge to inform the search for genes, enzymes or compounds with the desired activities. A similar approach can be taken when prospecting for antimicrobials. The directed approach has already been successful in isolating an antimicrobial from the Australian bull ant *Myrmecia gulosa*. It was predicted that ants should produce antimicrobials because they live in high density colonies where the genetic relatedness between individuals is high, and consequently the potential for disease spread is also high [9]. A broad-spectrum antimicrobial is excreted from the metapleural glands in these ants [10].

## Insects and antimicrobials

Insects are highly suitable target organisms in the search for new antimicrobials since they continually come in contact with potentially pathogenic microorganisms, they often live at high densities, and yet very few become infected. This resistance to disease, coupled with low extinction rates, has helped their evolutionary success. They are a prime example of a relatively unexplored resource.

The size of this resource is also attractive. Insects are found in almost all niches and there are more than one million described species within the class Insecta, with possibly seven million yet to be described [11].

**Figure 1. Sheep blowfly larvae.**



The majority of antibiotics used today were originally isolated from bacteria. Insects are known to produce antibiotics and these have different structures from those currently in use. Because such compounds are not from bacteria, any genes conferring resistance to them may not be as easily transferred into pathogens, and hence their effective therapeutic life may be longer. Our research at Macquarie University has focussed on using hypothesis driven drug discovery to explore the insect world.

## Current research

The aim of our current research is to develop and test evolutionary hypotheses that might inform the search for antimicrobials, thus reducing the time and expense spent in this activity. The first hypothesis tested was that the production of antimicrobials would vary within the life history stages of a species, particularly when the life history stages occupy different environments with respect to microbial challenge.

Four species of Diptera (flies) were selected based on the environments in which they live. The first species tested was the sheep blowfly *(Lucilia cuprina)*. This fly causes fly strike in sheep by laying its eggs in moist wool soiled by faecal matter. Once the eggs hatch, the larvae feed on the wool and flesh of the sheep. When the larvae are ready to pupate, they drop off the sheep and pupate in the ground. The adults then

**Figure 2. Head of a fly belonging to the Family Calliphoridae – the 'blowflies'.**

emerge and the cycle continues (Figures 1 & 2).

Due to the different environments encountered by each life stage, it was predicted that the larval and adult stages would produce antimicrobials constitutively since they are mobile feeding phases that encounter substrates containing a variety of pathogens. In contrast, pupae are sedentary and enclosed in a protective case and would therefore be unlikely to come in contact with many pathogens.

The same predictions were made for the house fly *(Musca domestica)* and the vinegar fly *(Drosophila melanogaster)*. The house fly feeds on feces and garbage in both the larval and adult stages whilst the vinegar fly feeds on rotting fruit as a larvae and an adult.

The fourth species tested was the Queensland fruit fly *(Bactrocera tryoni)*. This fly lays its eggs in fresh fruit and when the larvae hatch they feed on the fruit. It was predicted that the larval and pupal stages would not produce antimicrobials constitutively as the environments in which these life stages live have a relatively low number of microbial pathogens. The adults were predicted to produce antimicrobials as they are sexually active and will come in contact with a number of environments and other individuals.

Our results generally support the prediction that the production of antimicrobials varies with the life history stages of a species when the life history stages occupy different environments with respect to microbial challenge. Antimicrobials were found in most larvae, few pupae and all adults. Research is currently underway to identify whether the compounds produced by the adults of all four fly species are closely related.

Hypothesis driven drug discovery appears to be a successful way to prospect for new compounds, as it reduces the need to test organisms that are unlikely to produce antimicrobials in the first place. This method of drug discovery can be applied to a number of areas when searching for novel pharmaceutical products. For example, when prospecting for new sunscreens, it would make sense to target organisms that are exposed to UV radiation, while anti-inflammatory agents and immuno-suppressants are likely to be found in parasites that invade host tissues [12].

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