SLIME OF THE TIMES

Sarah J Pitt and **Alan Gunn** write about their search for new antibiotics, which has taken them out of the laboratory and into the vegetable patch.

The quest for antimicrobial agents has taken researchers to some interesting places, both geographically and conceptually. The first synthetic antibiotics used in human medicine were the sulphonamides. These were developed by chemists in Germany in the 1930s who had been thinking about the observation that certain dyes were used in staining bacteria and parasites for diagnostic identification. They wondered whether specific binding of dyes could be used to prevent the growth of microorganisms inside the human body. Through trial and error and gradual alterations to chemical structure of the original compound, an effective antibacterial agent called Prontosil was produced. It was used successfully to treat streptococcal and staphylococcal infections in humans before the outbreak of the Second World War. The discovery that a common soil fungus, *Penicillium chrysogenum*, produces a substance which also has an inhibitory effect on *Staphylococcus* and *Streptococcus* spp. was made a few years earlier. However, Fleming's discovery remained an interesting laboratory finding with potential. It was not until the 1940s that Florey and Chain developed the technique for producing penicillin in large quantities which then made it available for use in patients.

Natural sources of antibiotics

Scientists often follow up reports of "natural" antibiotics to establish whether

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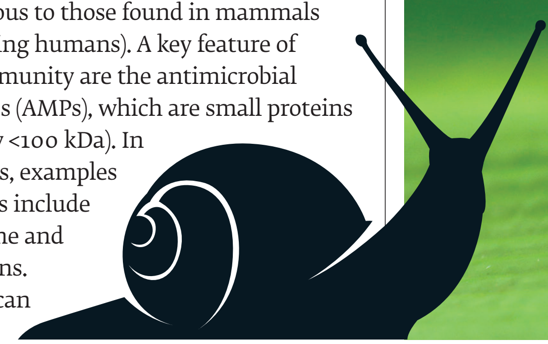
they really work (and if so, what is the mechanism of action) and some traditional remedies have turned into commercial products. For example, Manuka honey has been long recognised as having skin healing properties and clinical trials have confirmed this. Since it is feasible to produce honey in large quantities, this is now available as a "medical grade" format, used to treat chronic wound infections. Another strategy is to identify animals, fungi, and plants that produce antimicrobials. For example, marine sponges are a rich source of bioactive chemicals, including antibiotics. This is not particularly surprising because sponges must defend themselves chemically since they cannot move away from or actively defend themselves against attack. However, as is the case with many natural substances, translating laboratory findings into clinical treatments is proving difficult, because isolating chemicals and scaling up production is not easy.

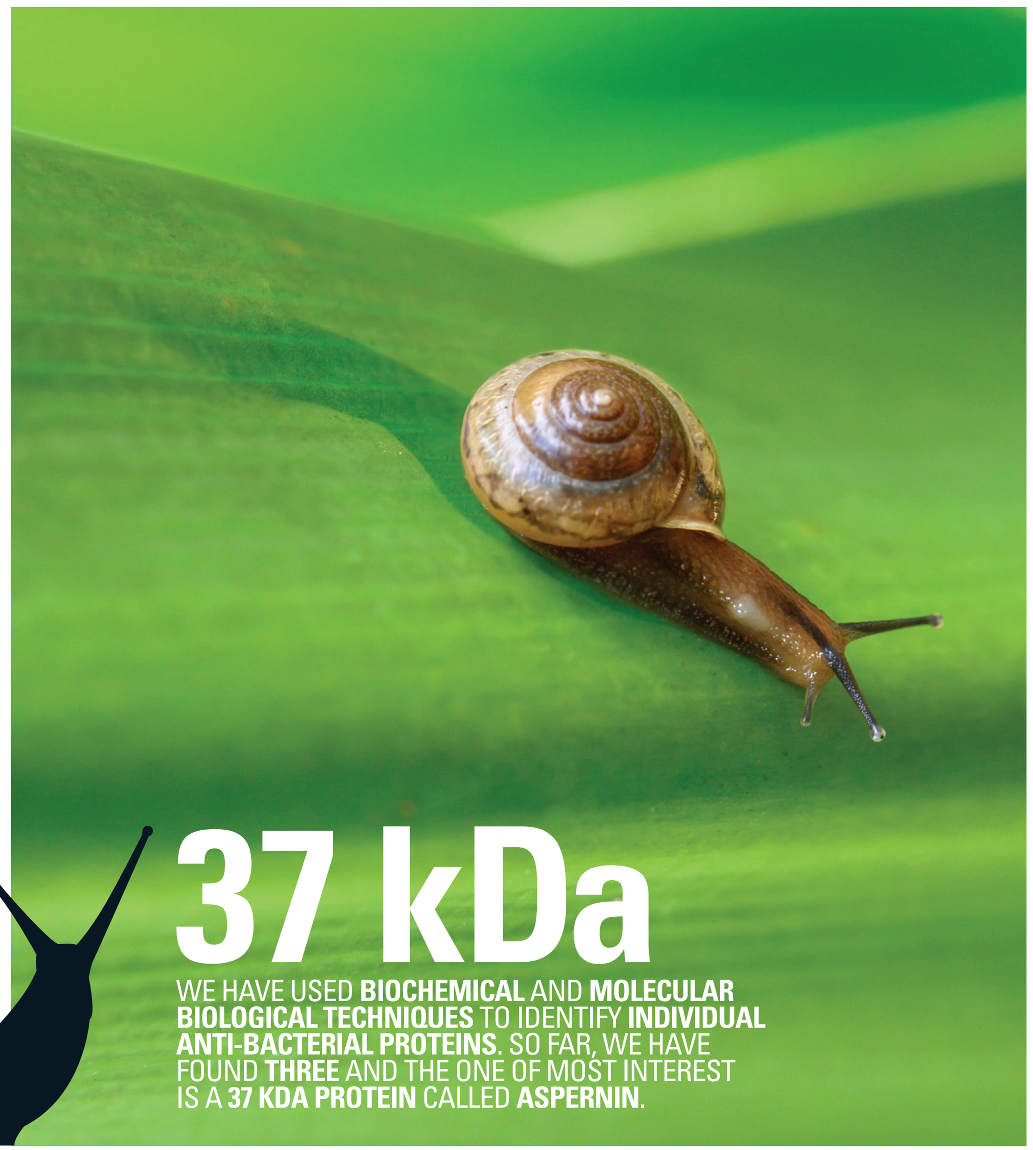
Antimicrobial protection in soil invertebrates

The soil microbiome is enormously diverse and contains many organisms that could be pathogenic to animals living within it. Thus, invertebrates living in or on soil, such as worms, slugs and snails are likely to have strategies to protect themselves against infection. Could these be exploited to find new antimicrobials? Advances in immunology have demonstrated that invertebrates have a well-developed innate immune system, with some components analogous to those found in mammals (including humans). A key feature of this immunity are the antimicrobial peptides (AMPs), which are small proteins (usually <100 kDa). In humans, examples of AMPs include lysozyme and defensins. Molluscan AMPs, mostly occur within the haemolymph or in association with the haemocytes. Laboratory experiments have demonstrated that some mollusc AMPs can inhibit the growth of common bacteria and yeasts. However, to date, research is mainly focussed on their role in mollusc biology (particularly commercial marine species, such as mussels and oysters). Translation of this information into producing new types of antimicrobial agents with widespread applications is rather limited.



Mucus as protection against the soil environment

As well as internal protection, slugs and snails also produce mucus both to help them move across surfaces and to defend themselves against predators. Mucus production helps to prevent bacteria from establishing biofilms on the mollusc skin and then entering the animal's body. Since mucus is made continually, this also helps to ensure that any potential pathogenic organisms are sloughed off as the slug or snail moves through the soil.



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However, the constant production of mucus is clearly not enough on its own and so the possibility that it might have actively anti-microbial constituents led a research team in Japan to investigate this in the 1980s and 1990s. They collected and analysed mucus from the Giant African Land Snail, *Achatina fulica*. They reported finding a relatively large (160 kDa) protein, called Achacin, which inhibited the growth of *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Using these organisms as examples of Gram-positive and Gram-negative bacteria, they suggested that Achacin had broad spectrum activity. A Chinese team subsequently isolated and characterised a 9 kDa protein from *Achatina fulica* mucus which they showed had antibacterial activity and was also effective against the yeast *Candida albicans*. There is virtually no information on the presence of antiviral substances in mollusc mucus but molluscs, like all other organisms, suffer from viral diseases so they are likely to exist. For example, researchers in Brazil identified a fatty acid-based substance in the mucus of the slug *Phyllocaulis boraceiensis* which appeared to prevent the replication of measles virus in monkey kidney cells.

Antibacterial proteins in mucus from the garden snail

In our work, we have shown that the mucus from the brown garden snail, *Cornu aspersum* (also called *Helix aspersa*) has antibacterial properties. In preparations of whole mucus we found a repeatable, specific activity against *Pseudomonas aeruginosa*. It does not seem to have the broad spectrum effect which was reported for the Giant African Land Snail mucus. However, the mucus preparations have activity against all the type culture collection strains and all the clinical isolates of this bacterium that we have tested it against. It is not clear why this might be the case. *Ps. aeruginosa* is a key soil organism as well as an important opportunistic pathogen. Guides to keeping snails as pets list pseudomonal infection as a particular hazard, while experiments on *C. aspersum* in the 1980s showed that challenge with *Ps. aeruginosa* elicited a strong immune response and high doses were potentially fatal. This suggests that the very specific antibacterial effect that we observed is due to a natural requirement for the snail to protect against this bacterium. We have used biochemical and molecular biological techniques to identify individual anti-bacterial proteins. So far, we have found three and the one of most interest is a 37 kDa protein called Aspernin. Since *Ps. aeruginosa* is found in chronic wound infections and is an important cause of respiratory infections in patients with cystic fibrosis, it is hoped that Aspernin could be incorporated into a topical cream or an aerosol preparation. Cosmetic preparations (anti-wrinkle creams, face masks) containing *C. aspersum*/*H. aspersa* mucus are already widely available. It is claimed that they reduce the appearance of wrinkles, blemishes and scars. Although they contain extracts of whole mucus, rather than identifiable individual components, this means that it is possible to scale up the production of the mucus from snails and incorporate it safely into marketable products. It should therefore be feasible to produce clinically useful quantities of an antibacterial formulation.

CORNU ASPERSUM

Disseminated into many parts of the world intentionally as a food delicacy, and accidentally by the movement of plants.

Distribution
This snail is found in the UK, western Europe and along borders of the Mediterranean and Black Seas. It has also been introduced into the Atlantic Islands, South Africa, Haiti, New Zealand, Australia, Mexico, Chile and Argentina.

Description
The shell is large, globose, moderately glossy and sculptured with fine wrinkles. Adult shells (four to five whorls) measure 28 to 32mm in diameter

Reproduction
Mating requires four to 12 hours. Oviposition occurs three to six days after fertilisation The number of eggs deposited at one time varies from 30 to 120.

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Conclusion

Antimicrobial resistance is a major public health problem. It is important to ensure that the drugs which are clinically useful at the moment are used wisely and sparingly. However, there are already strains of microorganisms that are resistant to most, if not all, of the antibacterial agents available to treat them. Thus, there is a pressing need for new antimicrobial agents, but these may come from surprising places. So next time you see a slug or snail in the garden – even if it is munching on your prize cabbages – you might see it in a different light. BMS

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Sarah features on the latest IBMS podcast, which can be streamed at ibms.org/resources/podcasts