10.1071_ISSN0311-4546EC107p8

Blowfly inspires novel cleansing

Organophosphate-degrading enzymes have been trialled successfully.

During the late 1980s, CSIRO entomologists Dr John Oakeshott and Dr Robyn Russell were immersed in studies of the sheep blowfly and associated mysteries of insecticide resistance. Little did they guess their work would lead to the use of enzymes to combat toxic pesticide residues.

Oakeshott, Russell and their colleagues at CSIRO Entomology discovered that the sheep blowfly had evolved resistance to the insecticide used against it via a mutation that enabled the fly maggots to degrade the insecticide to non-toxic products.

The resistant flies had mutated the active site of a particular esterase enzyme, which meant that their metabolism could now break down the normally highly toxic insecticide (an organophosphate) and render it harmless. This work led the scientists to the idea of using a similar technique to clean up pesticide residues in the environment.

For the past few years, the CSIRO group, in partnership with Dr Hung Nguyen and his colleagues at Orica Australia, have been trying to mimic the success of the sheep blowfly by developing commercial enzyme technologies for bioremediation.

There is mounting pressure to reduce agricultural pesticide residues, as they can be a threat to both the environment and to the marketing of many of Australia's commodities. Grains, cotton, wool, meat, fruit and vegetables, nursery plants and cut flowers are all at risk.

Contaminated waters, such as irrigation wastes on rice and cotton farms, or spent animal dip liquors, are also environmental hazards and ones that the scientists believe are amenable to enzyme treatment.

The CSIRO Entomology team mostly looks for suitable enzymes in bacteria, rather than insects. This is because bacteria can degrade larger amounts of residue. For example, soil in cotton fields is full of bacteria that either use pesticide residues as a source of nutrients, or detoxify the residues to survive and multiply.

Backyard serendipity

'What's amazing is that when you look in the right place, you find enzymes,' Russell says. 'It depends where you go for your bit of dirt. Our best microbial enzyme came from a back yard in Brisbane. A lady regularly washed her dog there with an insecticide formulation and we found resistant bacteria in the soil.'

To obtain an enzyme from such a source, an enrichment culture of the bacteria is set up in the laboratory. Insecticide is added and left for a while, so selecting for still more resistant microbes. The culture is then shaken and divided into sub-cultures, which are tested for their ability to degrade pesticide. If such activity occurs, the culture is progressively diluted to isolate the organism responsible.

Once a promising strain of bacteria has been isolated, the gene responsible for producing the enzyme – or several genes and enzymes – has to be identified and cloned. An 'expression system' in which to multiply the gene for commercial use must also be developed. This involves inserting the gene into a, usually circular, unit of bacterial DNA known as a plasmid, and allowing the bacteria to multiply.

A single bacterial cell can hold many of these plasmids, so the copy number of the gene is greatly increased, thereby increasing its expression. The scientists are at this stage with the enzyme for degrading organophosphate pesticide, and are aiming for expression levels at least 10 times that of the wild-type bacteria.

The enzyme is obtained by breaking open the bacterial cells (cell lysis) to release the enzyme and other cell contents. This means the enzyme preparation, or 'cell lysate', contains no living cells or organisms. The preparation then has to be refined and formulated until it is stable, yet effective. Orica Australia is now working with CSIRO Molecular Science to develop this process and, as the preparation, or end-product, is an enzyme, it would need to be registered as an agrochemical.

In the meantime, Orica Australia has tested the organophosphate degrading enzyme produced from the wild type, or natural, bacterial strain to see how good it is in cleansing irrigation tail water. A trial on a cotton farm in New South Wales in March was spectacularly successful, reducing residue levels in more than 80 000 litres of contaminated water by 90% in 10 minutes.

Horticulture Australia Limited also supports of research into commodity clean-up. Fruits and vegetables such as lettuce and broccoli, that are normally washed, are especially suitable as the appropriate enzyme could be added to the washing liquid. The team has already trialled its organophosphate degrading enzyme for these purposes in the laboratory and achieved a greater than 90% reduction in residue levels.

More enzyme will be required when the exposure time is short, and less if it can act on the residue for several hours. Another possibility is to retail the formulation for use on fruit and vegetables in the home.

Enzymes also have potential in the decontamination of polluted soils. Soluble formulations could be added directly to damp soils or, more likely, genetically engineered into a microbe or plant that will absorb the pesticide over time and degrade it to harmless products.

'Early analysis indicates that enzyme preparations should be cost-effective in many applications,' Russell says. 'But we still have quite a long way to go. We have patents on three enzymes so far and we are also looking for enzymes to degrade pyrethroid and carbamate insecticides, a herbicide group and endosulfan, which lurk in the soil in toxic forms.

'It is an exciting area of research and, given the growing interest in clean and green produce, we believe we are on the right track in pursuing enzyme bioremediation solutions to residue problems. Our sheep blowfly research really started something.'

More about cleaning enzymes

Russell R Harcourt R Sutherland Nguyen H and Oakeshott J (1999) Can microbes help to clean water? Microbiology Australia, 20:14–17.

Sreve Davidson