

PESTICIDE DETOXIFICATION USING ENZYMATIC BIOREMEDIATION

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Pesticides provide essential protection in the production of many agricultural commodities. However, increasing pesticide use as a result of increased production has led to community concern about the social and environmental impacts of pesticide residues. Of particular concern is the contamination of irrigation run-off and drainage water, agricultural soils and horticultural products.

Detoxification of pesticide residues in contaminated soil has been achieved by introducing and/or encouraging the growth of microorganisms capable of detoxifying the residues on site – a technology known as bioremediation (pesticide detoxification using biological material). A well known example of this, frequently cited in the media, is the clean up of the contaminated Olympic sites in Sydney. This method of bioremediation is based on traditional composting techniques and relies on microbial growth to metabolise the toxicants. The detoxification process is generally slow, taking weeks to months to accomplish. Furthermore, the methodology is not suited to the generally low aeration, low nutrient state of contaminated water. However, the microorganisms which break down toxicants in contaminated soil can be sources of enzymes that will detoxify pesticide residues in such situations. The application of these enzymes is particularly suited to pesticide-contaminated water as they can achieve rapid remediation without the addition of nutrients or aeration.

The problem of pesticide contamination of irrigation water must be resolved before it can be released into the waterways. CSIRO Entomology, in conjunction with members of the Advanced Water Technologies business of Orica Australia Pty Ltd. and CSIRO Molecular Sciences, has successfully developed enzyme-based bioremediation technologies for detoxifying pesticides in contaminated water prior to its release from the farm. This technology is being extended to include the clean up of pesticide spills, the clean up of rinsings from spray equipment washdown, and for treatment of horticultural produce. This paper describes the progress of this joint initiative.

The search for such enzymes can be divided into four stages. Initially, a source of enzyme is identified in bacteria or from other natural sources. Next, the gene encoding the enzyme is then identified and isolated. If necessary, protein-engineering technologies are used to design a

stable, easily manufactured product. Finally, the enzyme is produced by industrial-scale fermentation and bioprocessing for introduction to the contaminated water.

Current research focuses on several major insecticide classes including organophosphates, carbamates, synthetic pyrethroids and the organochlorine, endosulfan. Below we describe progress we have made with each of these classes of compounds.

ORGANOPHOSPHATES

We have isolated a soil microorganism that can detoxify the organophosphate class of compounds. This particular microorganism had adapted to utilise the pesticide as a nutrient source. The organophosphate-degrading enzyme isolated from this bacterium attacks the phosphoester bond of aromatic oxon and thion organophosphates, but does not have activity against the aliphatic versions of these compounds. However, protein engineering technologies have been employed to create a synthetic variant of this enzyme that degrades the aliphatic compounds. Collectively, the original enzyme and variants are predicted to degrade 90% of registered organophosphate compounds.

The performance of the original enzyme for decontaminating organophosphates has been evaluated in several field trials. In the first field trial, methyl parathion levels in 80,000 L of fast flowing run-off water in cotton farm drainage channels were reduced by 90% in less than ten minutes. This is a low concentration/high volume source of pesticide-contaminated water, which also contains high levels of silt and other particulate matter. In a second field trial, enzyme treatment of rinsate from the washdown of pesticide spray equipment achieved a reduction in methyl parathion concentration of 90% in 10 minutes, and 99% after 1 hour. In contrast to the run-off water in the first trial, this rinsate is a high concentration/low volume source, which also contains organic solvents.

Thus, the technology has proved to be an effective and powerful tool for the rapid degradation of pesticide residues. The results from these field trials indicate a range of conditions in which the organophosphate-degrading enzyme can perform. The application range of the technology has been broadened further to include diazinon detoxification in spent sheep-dip liquor, and the treatment of methyl parathion residues on the surface of leafy green vegetables. In a recent laboratory trial, the concentration of diazinon was reduced from 4.7 parts per million to below 1 part per billion (99.98% reduction), within 1 hour. In the trial involving leafy green vegetables, residues on the surface of baby bok choy were reduced by up to 95%. Given the complex nature of the surface of bok choy, this trial further demonstrated the utility of the enzyme technology.

CARBAMATE COMPOUNDS

A bacterium that degrades the carbamate insecticide, carbaryl, has also been isolated in our laboratory from carbamate-contaminated soil. In contrast to the organophosphate degrading bacterium described above, which has the ability to degrade many different organophosphates, this bacterium has a narrow substrate range and does not detoxify other carbamate pesticides. However, the enzyme that is responsible for this activity is stable under a variety of conditions, does not require any co-factors for activity and results in substantial detoxification

of carbaryl, a pesticide used worldwide on horticultural produce. CSIRO Entomology will use protein engineering technologies in an attempt to increase the substrate range of this enzyme so that it can be used to detoxify other carbamate compounds.

PYRETHROID COMPOUNDS

Another hydrolase enzyme with potential for use in bioremediation has been isolated from insects that are resistant to certain pesticides. Protein technologies have again been employed to design variants of this enzyme that can degrade pyrethroid insecticides. These enzymes are stable against a range of biotic and abiotic challenges, with half-lives in some agricultural waste streams of a number of days. These enzymes can be produced on a large scale in bacteria using fermentation techniques, and they are being trialed for commercial use in the detoxification of pyrethroid residues.

ENDOSULFAN

CSIRO Entomology has also isolated bacteria that degrade the two isomers of endosulfan and the toxic metabolite of endosulfan, endosulfan sulfate. These bacteria were isolated by providing endosulfan as the only source of sulfur to a soil microbial population. Sulfur is an essential component of living matter. Therefore only the bacteria that could release the sulfur from endosulfan could survive. Removal of sulfur from either endosulfan or endosulfan sulfate results in substantial detoxification of these compounds. The enzymes responsible for this activity belong to a class of enzymes termed monooxygenases, which differ from the hydrolase enzymes described above in that they require co-factors for activity. CSIRO and Orica Australia are currently investigating the use of these systems for bioremediation.

COMMERCIALISATION

Commercialisation of the enzymatic bioremediation technology is likely to involve three types of products. A freeze-dried enzyme powder will be available for treatment of residues in contaminated water. This powder will have a shelf life of several months and will be reconstituted prior to application to the contaminated water. Another potential product form is a polyurethane pad containing immobilised enzymes. Prototypes of this product have proven effective for soaking up and decontaminating pesticide spills. Finally, a self-contained user-friendly device containing immobilised enzyme that will allow the detection of pesticide residues at concentrations as low as 20 parts per billion is being developed.

Chemical pesticide usage is unlikely to decline substantially in the near future, despite the development of transgenic crops and other alternative biological controls. Environmental and safety concerns are leading to increasingly stringent residue requirements by regulatory authorities. Whilst these concerns are being addressed through better pesticide and water management practices, there is an increasing need for rapid and effective remediation technologies in many industrial and agricultural processes. The CSIRO-Orica Australia joint initiative described in this paper is developing biotechnologies to address this need.

The first step is to identify the problem. In this case, the problem is that the company is not meeting its sales targets. This is a common problem for many companies, and it can be caused by a variety of factors. Some of the most common causes are:

- 1. Poor product quality
- 2. Inadequate marketing
- 3. High prices
- 4. Poor customer service
- 5. Lack of innovation

Causes of the Problem

The first cause of the problem is poor product quality. If the product is not of high quality, customers will not buy it. This can be caused by a variety of factors, such as using low-quality materials or not testing the product thoroughly before it is released.

Causes of the Problem

The second cause of the problem is inadequate marketing. If the company is not reaching its target audience, it will not be able to sell its product. This can be caused by a variety of factors, such as not having a clear marketing strategy or not using the right channels to reach the target audience.

Causes of the Problem

The third cause of the problem is high prices. If the product is priced too high, customers will not buy it. This can be caused by a variety of factors, such as not having a clear understanding of the market or not being able to compete on price.

The fourth cause of the problem is poor customer service. If customers are not satisfied with the company's customer service, they will not buy its product. This can be caused by a variety of factors, such as not having enough customer service staff or not training the staff properly.

The fifth cause of the problem is lack of innovation. If the company is not offering new and improved products, it will not be able to attract new customers. This can be caused by a variety of factors, such as not having enough resources to invest in research and development or not having a clear vision for the future.