Enzymatic bioremediation: From enzyme discovery to field applications

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Abstract

Chemical pesticides will continue to be a major part of agricultural systems throughout the world in the foreseeable future. However, a growing concern about the non-target toxicity of these chemicals has led to requirements for effective pesticide remediation technologies in a range of industries and applications. Many of these applications require rapid action and therefore traditional remediation strategies are inadequate. This has led to an interest in the use of formulated enzymes rather than live bacteria as bioremediation agents. This process known as Enzymatic bioremediation is particularly suited to situations where rapid remediation is needed, ranging from minutes to a few hours. CSIRO Entomology is involved in an initiative with Orica Australia Ltd and Horticulture Australia Limited to isolate and develop enzymes for treatment of pesticide-contaminated waste waters from agricultural production and processing industries. Water quality regulations, particularly in Australia, are becoming increasingly stringent necessitating efficient and affordable treatment strategies. This paper describes progress by CSIRO on the isolation and development of enzymes that detoxify pesticides containing ester bonds, including organophosphates, carbamates and synthetic pyrethroids. These compounds comprise the major insecticides that are most problematic with respect to residue issues and in fact account for the majority of insecticide sales both in Australia and world-wide.

Media summary

Enzymes isolated from microorganisms and insects for the treatment of agricultural residues, developed jointly by CSIRO, Entomology, Orica Australia Ltd and Horticulture Australia Limited.

Key Words

Enzymatic bioremediation, organophosphates, carbamates, pyrethroids, waste water

Introduction

Pesticides provide essential protection in the production of many agricultural commodities. 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, pesticide spills, contaminated rinsings from spray equipment and animal dips, and used pesticide containers. This paper describes the application of modern biology to develop enzymatic bioremediation technologies to mitigate the effects of pesticide residues in a joint CSIRO-Orica Australia Ltd initiative.

Previous bioremediation technologies have centered around growth of microorganisms capable of detoxifying residues, a method based on traditional composting techniques. This method is slow, taking weeks to months to accomplish and is not suited to the generally low aeration, low nutrient state of contaminated water. The technology developed by CSIRO involves isolating and optimizing enzymes, producing them on a large scale and applying them to contaminated environments, where they function to rapidly degrade pesticides and then are themselves readily degraded. This technology is particularly suited to the treatment of waste waters from agricultural production and processing industries and in fact any aqueous environment. Development efforts are currently focused on enzymes for the bioremediation of a variety of insecticide (organophosphates, carbamates, pyrethroids, endosulfan, neonicotinoids, benzoyl ureas), herbicide (glyphosate, paraquat, triazines, phenyl ureas), and fungicide (carbamate) targets. This paper briefly describes CSIRO's progress in developing enzymes for some of the ester containing insecticidal compounds.

Methods

The prerequisites for a bioremediation enzyme to function effectively *ex vivo* in waste water are demanding (Table 1). The enzyme must be able to degrade the pesticide to products that are substantially less toxic and more readily biodegradeable and must function independently of cofactors, which would be prohibitively expensive. Furthermore, the enzyme must function in waste water having low and high pesticide concentrations such as agricultural run-off and equipment rinsate, respectively.. The enzyme also needs to be stable under a variety of conditions including situations that involve high organic matter (eg animal dips), high particulate matter (eg muddy agricultural water) or high solvent concentrations (eg used pesticide containers). Finally, the enzyme must degrade as broad a range of target pesticides as possible, and be cost-effective to produce.

Table 1. Properties required by enzymes for effective water bioremediation

Property	Issues
Degradation of pesticide at such bonds as to render the product non-toxic	
Do not require cofactors	Co-factors are prohibitively expensive
Rapid performance at a range of concentrations	Low K_m (high substrate affinity) and high k_{cat} (turnover). Performance criteria are more relaxed for longer treatments
Environmental stability over a range of conditions ie pH and temperature	Conditions vary in different applications, a product is required to function under all conditions
Few or no environmental inhibitors	Reliable, reproducible application conditions
Detoxifies many if not all pesticides in a class	Multiple enzymes are costly
Cost effective production and formulation	Required to provide affordable product

Development of enzymatic bioremediation products involves four stages: bioprospecting to identify enzyme templates, isolation of the gene/enzyme system, adaptation of the enzyme to fulfil performance criteria, and downstream development to produce a stable, reliable product. Gene/enzyme bioprospecting at CSIRO Entomology involves both microbial and entomological fauna. However, as the most problematic pesticides are those that are not readily degraded in the environment, the enzymes that we isolate are often sub-optimal and do not fulfil our selection criteria. The requirements for substantial detoxification without co-factors are absolute and templates that fulfil these requirements must be identified through bioprospecting. All other requirements can be addressed using modern molecular biological techniques.

Below we describe our progress in relation to several major insecticides that are most problematic in respect of residue issues: the organophosphates, carbamates and synthetic pyrethroids. These compounds in fact account for a majority of insecticide sales worldwide.

Results and Discussion

Organophosphates

The organophosphates contain ester bonds, hydrolysis of which leads to substantial detoxification. Aa hydrolytic enzyme has been isolated from an organophosphate tolerant soil microorganism that had adapted to grow in organophosphate contaminated soils, where the pesticides had become a worthwhile nutrient (phosphorous) source. The enzyme attacked the phosphoester bond of aromatic oxon and thion organophosphates which account for around 50% of available organophosphates. The enzyme performs very well under field conditions including in fast flowing run-off water in cotton farm drainage channels (low concentration/high volume source of pesticide-contaminated water, which also contains high levels of silt and other particulate matter) and in rinsate from the washdown of pesticide spray equipment (high concentration/low volume source, which also contains organic solvents).

Carbamates

A carbamate contains both ester and amide bonds, and hydrolysis of either detoxifies the compound. A variety of carbamate degrading enzymes, both esterases and amidases, have been sourced by CSIRO Entomology. All these enzymes have been isolated from soil bacteria found at carbamate-contaminated sites. The enzymes are all stable under a variety of conditions, do not require co-factors for activity and result in substantial detoxification of their substrates. Enzyme evaluation is underway and further development will involve protein engineering technologies to increase the substrate range of this enzyme so that it can be used to detoxify carbamate compounds.

Pyrethroids

Pyrethroids also contain an ester bond, hydrolysis of which leads to substantial detoxification. An esterase enzyme isolated from insects that are resistant to certain pesticides is capable of this detoxification.One enzyme isolatedfrom the house fly is stable against a range of biotic and abiotic challenges and is effective against pyrethroids with half-lives in some agricultural waste streams of several days. The enzymes can be produced on a large scale in bacteria using fermentation techniques, and is being trialled for commercial use in the detoxification of pyrethroid residues.

Other targets

CSIRO's discovery program is most advanced for the ester containing compounds described above. Whilst CSIRO have isolated and characterised several enzymes that detoxify the sulfite di-ester insecticide, endosulfan, these enzymes require co-factors and as such are not suitable for enzymatic bioremediation. The search for hydrolytic enzymes that can catalyse a similar reaction continues. Enzymes that degrade the triazine herbicides have been sourced and CSIRO are currently improving their activity, particularly towards atrazine. CSIRO also have recently commenced bioprospecting to find enzymes that degrade the neonicotinoids and phenyl/benzoyl urea compounds.

Commercialisation

Orica Australia Ltd has undertaken large-scale production of CSIRO enzymes using industrial fermentation techniques. Field trials for various applications have demonstrated the utility of these enzymes in all situations tested to date. These trials have included the treatment large volumes of fast flowing run-off water in drainage channels on a cotton farm, and treatment of spent dip liquors. In the laboratory similar levels of reduction in organophosphate residues were achieved on the surface of commodities such as leafy vegetables, whose complex surface structure might be expected to be problematic for effective remediation.

Conclusions

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.

Acknowledgements

We are grateful for the financial support of Orica Australia Ltd, Horticulture Australia Limited, the Cotton Research and Development Corporation, the Australian Cotton CRC, the CRC for Sustainable Rice Production, CRC for Sugar Industry Innovation through Biotechnology and Australian Centre for International Agricultural Research.