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with planetary health"

Molecular cloning and recombinant protein expression of 9-lox gene from solanum tuberosum in pichia pastoris yeast cells

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Abstract

Synthetic fungicides have effectively controlled crop pathogenic fungi, but their continued use has been detrimental to natural biological systems, and sometimes resulted in development of fungal resistance. They also have undesirable effects on non-target organisms and foster environmental and human health concerns, thus, new biodegradable natural alternatives have to be investigated. Lipoxygenases (LOX) are ubiquitous non-heme iron containing dioxygenases that catalyze the addition of molecular oxygen to polyunsaturated fatty acids (PUFAs) such as linoleic acid to form oxylipins that possess anti-microbial activity. The aim of this study was to generate a recombinant 9-Lipoxygenase protein for the chemo-enzymatic synthesis of oxylipin-based biodegradable fungicides. Golden gate assembly, a molecular cloning method that allows assembly of various DNA fragments into a complete piece using Type IIs restriction enzymes, and T4 DNA ligase was used to clone the complete 9-LOX gene into the expression plasmid vector pPICZalphaB. Protein expression in Pichia pastoris yeast cells was induced by addition of absolute methanol after every 24h for up to four days. Analysis of protein expression from cell lysates was achieved by SDS-PAGE and Western blotting probed with anti-histidine antibodies, which showed putative protein bands of 97kDa representing recombinant 9-LOX protein. In the future, optimisation studies on the yeast kex2 convertase and the alpha- secretion factor need to be performed in order to enable the secretion of recombinant Solanum tuberosum 9-LOX

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protein, since the protein in this study was recovered from cell lysates. Currently, the experiments underway involve testing the recombinant enzyme in the first catalytic step of generating oxylifungi/biodegradable fungicide as the overall project goal.

Keywords: 9-Lipoxygenase, Biodegradable fungicides, golden gate cloning, Pichia pastoris, Solanum tuberosum