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Motif search-aided screening of the *Pseudomonas syringae* pv. maculicola genome for genes encoding tertiary alcohol ester hydrolases

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Tertiary alcohol ester (TAE) hydrolases are a group of esterases (EC 3.1.1.-) that catalyze the kinetic resolution of TAEs and as a result, they are sought-after for the production of optically pure tertiary alcohols (TAs) which are useful as building blocks for number biologically active compounds. What sets these enzymes apart is, the presence of a GGG(A)X-motif in the active site which appears to be the main reason behind their activity towards the sterically demanding TAEs. The genome of *Pseudomonas syringae* pv. maculicola (Psm) comprises a multitude of genes that encode esterases. We therefore, hypothesize that some of these genes encode TAE hydrolases. In this study, Psm was screened for TAE hydrolase activity using the linalyl acetate (LA) plate assay and a positive reaction was observed. As a result, the genome of Psm was screened for esterases with a GGG(A)X-motif using the motif search tool and two potential TAE hydrolase genes (PsmEST1 and 2, 1100 and 1000 bp, respectively) were identified, PsmEST1 was amplified by PCR and the gene sequenced for confirmation. Analysis of the sequence data with the SingnalP 4.1 server revealed that the protein comprises a signal peptide (22 amino acid residues) on the N-terminus. Primers specific for the gene encoding the mature protein (without the signal peptide) were designed such that they contain NdeI and XhoI restriction sites for directional cloning of the PCR products into pET28a. The gene was expressed in E. coli JM109 (DE3) and the clones screened for TAE hydrolase activity using the LA plate assay. A positive clone was selected, overexpressed and the protein purified using nickel affinity chromatography. The activity of the esterase towards LA was confirmed using thin layer chromatography.

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