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ABSTRACT BOOK

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Medium chain length polyhyoxyalkanoates (mcl-PHA) model compounds for the discovery of novel PHA depolymerases

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PHAs are naturally made microbial polyesters that have been commercialized as biodegradable plastics. However, it has been shown that these materials are not so easily biodegraded in natural environments [1]. PHA depolymerases are key PHA degrading enzymes and their identification and characterization is of great interest and importance. Currently, screening is done on polymeric substrates using techniques such as clear zone assays on agar or weight loss measurements. Results obtained using these different methods cannot be directly compared, since they depend highly on the polymer used, PHA granules preparation and assay conditions [2].

In order to design a more specific test for the determination of PHA depolymerase activity, we synthesized 3-hyoxyalkanoate monomers (3-HA monomer) and 3-hyoxyalkanoic acid dimers (3-HA dimer) and their respective p-nitrophenyl esters, allowing for spectrophotometric determination of their activity [3]. Compounds were characterized using N and FTIR. Para-nitrophenyl labeled substrates were then used in the enzymatic activity assay with the benchmark polyhyoxyoctanoate (PHO) depolymerase from Pseudomonas fluorescens GK13 expressed in Escherichia coli CodonPlus-RIPL hosts. This activity was compared to recombinantly expressed leaf-branch compost cutinase (LCC cutinase) and polyethyleneterephtalate (PET) hyolyzing esterase from Ideonella sakaiensis (IsPETase). Our initial results revealed increased specificity of PHO depolymerase towards newly synthetized substrates, suggesting their suitability for specific screens and isolation of new mcl-PHA depolymerases, as well as in high throughput screening assays designed for guiding their directed evolution.

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