Optimization of a high-throughput impedance spectroscopy-based screening platform for the engineering of PET hydrolases

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Plastic has definitely revolutionized modern society. However, its extended lifespan and fuel origins poses environmental challenges. It is of utmost importance to not only accelerate plastic degradation but also ensure circular economy and retrieve plastic building blocks for new plastic synthesis. Enzymatic degradation has emerged as a promising solution, with thermophilic[1] and mesophilic[2] lipases and cutinases being discovered and engineered for polyethylene terephthalate (PET) hydrolysis. One of these enzymes is PHL7 (Polyester Hydrolase Leipzig 7)[3], a thermophilic hydrolase with high thermostability and degradation activity, making it a promising candidate for protein engineering through mutagenesis strategies. To test the effect of individual and combination of specific mutations, a combinatory mutant library was developed, using degenerated primers and restriction-free cloning method. To screen the vast array of engineered PHL7 mutants, the enGenes eX-press V2 cell system, which decouples biomass with mutated protein production, was employed. This system ensures a high protein yield, allowing to directly use the supernatant for enzymatic activity assays, bypassing the time-consuming purification steps. For the assessment of enzymatic activity, electrochemical impedance spectroscopy was applied and compared to the typically used gravimetric assay. The workflow was assessed on two types of designed PHL7 mutant library: in one, six residues distanced from each other by at least three amino acid residues were tested; in another, six consecutive amino acid residues belonging to the α6/β8 loop region of PHL7 were mutated. The first library was obtained and the workflow proved successful. However, the second library was not obtained suggesting that refinements in the cloning methods to fit the consecutive mutations need to be performed. Furthermore, a normalization strategy of the expression of the protein using split-GFP technology will be tested and implemented.

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