SYNTHETIC BIOCATALYTIC MODULES FOR ENHANCED TRANSFORMATION OF BIOLOGICAL WASTE PRODUCTS

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Many insoluble materials can be used as carriers for the immobilisation of enzymes. Solid-binding peptides (SBPs) are short amino acid sequences that can act as molecular linkers to direct the orientated immobilisation of proteins onto solid materials without impeding their biological activity [1]. Silica-based materials like silica and zeolite have been found to be suitable matrices for enzyme immobilisation in industrial processes. They are inexpensive, offer high mechanical strength and stability, are chemically inert and can be deployed over a wide range of operating conditions. We have constructed biocatalytic modules that are based on the incorporation of a silica-binding SBP ('linker') sequence into several genes for thermostable enzymes to facilitate the immobilisation of the proteins onto silica-based matrices, enabling the hydrolysis of both simple and complex polysaccharides. We have shown also that the procedure is suitable for the construction of complex enzymological pathways.

In proof of concept experiments, the linker (L) sequence was attached to the N- or C-terminus of three thermostable hemicellulases isolated from thermophilic bacteria using genetic engineering techniques [2]. The resulting L-enzymes remained active after fusion and displayed the same pH and temperature optima but differing thermostabilities in comparison to their corresponding enzymes without linker. The linker facilitated the rapid and simple immobilisation of each L-enzyme onto zeolite, resulting in the construction of 'single enzyme biocatalytic modules'. All three L-enzymes co-immobilised onto the same zeolite matrix resulted in the formation of 'multiple enzyme biocatalytic modules', which were shown to degrade various hemicellulosic substrates effectively in a 'one-pot' reaction.

Cell-free synthetic biology circumvents many of the limitations encountered by *in vivo* synthetic biology by operating without the constraints of a cell. It offers higher substrate and enzyme loading and the facile optimisation of enzyme ratios. Some of the challenges of this approach include costly enzyme preparation, biocatalyst stability, and the need for constant supplementation with co-factors. To overcome these challenges, we have developed a molecular toolbox that facilitates the construction of biocatalytic modules with predefined functions and catalytic properties. It consists of three interchangeable building blocks: (a) low-cost inorganic matrices (e.g., silica, zeolite), (b) matrix-specific SBPs and (c) thermostable enzymes. The rational combination of these building blocks allows for flexibility and a 'pick, mix' and re-use' approach with multiple biocatalytic modules available for the assembly of natural and non-natural pathways. Individual immobilised enzymes can be combined rationally to assemble recyclable and product-specific reactions.

We present preliminary results relating to the construction of two synthetic pathways for the conversion of organic wastes such as coffee and plant biomass. The pathway assembly process allows for rapid evaluation for proof of concept and for assessing the parameters for a synthetic pathway, which are very labour- and time-intensive by the *in vivo* approach.

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[2] Care, A, Petroll, K, Gibson, ESY, Bergquist, PL, Sunna, A. (2017) Biotech. Biofuels. 10: 29