RATIONAL DESIGN, CLONING AND EXPRESSION OF TWO BIFUNCTIONAL CHIMERIC ENZYMES TO DEGRADE ARABINOXYLAN

Luana de Fátima Alves¹; Letícia Magalhães Arruda¹; Richard John Ward²
¹Departamento de Bioquímica, Faculdade de Medicina de Ribeirão Preto – USP, São Paulo, Brazil
²Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto – USP, São Paulo, Brazil

Lignocellulose consists primarily of cellulose, hemicellulose and lignin, and engineering enzymes for saccharification of this material is a key technology for development of biomass-based biorefineries. Rational design of hybrid enzymes is a proven strategy for creation of fusions that preserve 3D-structures of the chimeric protein. With the aim of degrading arabinoxylan, two molecular models were created by rational design using a 3D-structure guided strategy. The models were derived from four parental enzymes: a CE4 acetyl xylan esterase (EC 3.1.1.73) and a feruloyl esterase (EC3.1.1.72) both from Clostridium thermocellum, generated a bifunctional chimeric enzyme named FaeAxe; and a arabinofuranohydrolase (EC 3.2.1.55) and a GH11 xylanase (EC 3.2.1.8) both from Bacillus subtilis, generated a bifunctional chimeric enzyme named AraXyl. The fusions were created using overlap PCR, and the resulting products were cloned into the pETSUMO vector. The chimeric proteins were expressed and purified. The catalytic properties of the FaeAxe and AraXyl chimeric enzymes will be compared with the respective parental enzymes in search of an improved synergy between the two parental enzymes. These constructs present the potential for application in bioethanol and cellulose pulp bleaching industries and demonstrate the promise of improving biocatalysts by protein engineering.

Key words: Chimeric enzymes, lignocellulose, rational design
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