IDENTIFICATION OF AMINO ACIDS RESPONSIBLE FOR ACTIVITY OF THE HYDROLASE MO13 SELECTED FROM METAGENOME

Matas Tiškus[1], Nina Urbelienė[1], Audrius Laurynėnas[2], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius Univesity

[2]Department of Bioanalysis, Institute of Biochemistry, Life Sciences Center, Vilnius University

matas.tiskus@gf.stud.vu.lt

Unculturable microbiomes lead to the discovery of new genes, biocatalysts, natural compounds and bioproducts. Screening for novel enzymes that are capable of catalyzing new reactions is constantly needed because the discovery of new enzymes might provide new approaches for designing enzymatic processes.

During this study it was aimed to apply a site-directed mutagenesis for the hydrolase MO13, that was selected from metagenomics library on mineral agar plate with N^4-benzoyl-2'-deoxycytidine as the sole source of uridine. Bioinformatics analysis showed that the selected MO13 clone contained ORF sharing 56% sequence identity with the hypothetical alpha/beta hydrolase (WP_119118064.1) from *Bacillus asahii* belonging to the tannase/feruloyl esterase family. The sequence analysis together with the proteins tertiary structure prediction revealed three amino acids –aspartate 429, histidine 467 and Serine 195 as a potential catalytic triad. Further investigation using a site-directed mutagenesis confirmed that these amino acids were crucial for the enzymatic activity of MO13 hydrolase. This was proved *in vivo* using uridine auxotrophic *Escherichia coli* strain DH10B *ΔpyrFEC* and N^4-benzoyl-2'-deoxycytidine (the sole source of uridine) and *in vitro* with various substrates. The further site-directed mutagenesis targeting two amino acids (Ala146, Arg111) surrounding the proposed enzyme active center showed that the changes at these positions had effect on a substrate specificity of the hydrolase MO13. Changing of Ala146 amino acid to Trp (A146W) and Arg111 to Ala (R111A) strongly inhibited the enzymes ability to hydrolyze terephthalic acid derivatives such as *bis*(2-hydroxyethyl) terephthalate and dimethyl terephthalate. A146W mutant also lost its ability to hydrolyze capecitabine whereas R111A mutant still retained a weak activity towards this substrate.