University of Belgrade
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Book of Abstracts

Editors:

Aleksandar Djordjević, PhD
Danica Šantić, PhD
Marija Jeftić, PhD
Velimir Šećerov, PhD
Zora Živanović, PhD

International Scientific Conference

GREEN AGENDA FOR WESTERN BALKANS

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SOLUBILITY PREDICTION OF THE PET HYDROLYZING ENZYME'S DOUBLE MUTANTS FOR PRODUCTION IN *ESCHERICHIA COLI*

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Abstract: Polyethylene terephthalate (PET) is a widely used plastic material. Due to its convenient physicochemical properties, it has become irreplaceable in many scientific, industrial, medical and everyday uses, leading to an accumulation of this material in the environment and initiating many ecological problems, especially in marine ecosystems. One of the solutions for overcoming this ecological threat may be found in recombinantly produced PET degrading enzymes.

The genes encoding proteins with prominent PET hydrolyzing activity (PETases) that have been successfully produced in *Escherichia coli* are commercially available (Addgene #112203 and #162667). These genes encode *Ideonella sakaiensis* PETase mutant W159H/S238F, and the fusion of the wild-type enzyme to MHETase (*I. sakaiensis* mono-(2-hydroxyethyl) terephthalic acid hydrolyzing enzyme).

Initially, we have done sequence alignment by ClustalW of the sequences corresponding to the entries available in the PAZy database (pazy.eu/doku.php) that contains information on many PET-degrading enzymes. We have identified amino acid substitutions that might be of interest for mutation towards improving the PET hydrolytic activity of *Is*PETase: at position W159 substitutions into H, I and L and at position S238 substitutions into F, T, Y, W, L and G. Since we are aiming to produce all of the abovementioned (double) mutants, we used different bioinformatic tools to predict the expression solubility of the mutated enzymes. To evaluate the accuracy of the available tools we have tested the expression levels and solubility of *Is*PETase W159H/S238F and *Is*PETase-MHETase fusion in *E. coli*. The *Is*PETase W159H/S238F protein was expressed fully soluble only at 20 °C, whereas the larger (~92 kDa) *Is*PETase-MHETase fusion protein was insoluble in all tested conditions. NetSolP () gave the most accurate solubility predictions for the tested proteins and we used it for prediction of the solubility of the aimed mutants.

Keywords: solubility, protein expression, *Escherichia coli,* PETase, bioinformatics

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