# Serbian Biochemical Society
# Seventh Conference

***"Biochemistry of Control in Life and Technology"***

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## Proceedings

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# Directed evolution of cellulase from *Trichoderma reesei* for higher activity and development of microtiter plate assay based on cellobiose dehydrogenase

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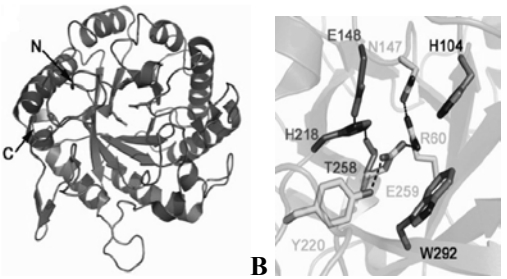
Cellulase (EC 3.2.1.4) are important enzymes in food, paper, textile, detergent and biofuel industries. Most cellulases have low activity and stability. Improving these properties would have substantial impact on numerous industrial processes. Enzymatic properties can be improved by directed evolution, but the screening process is the limiting step. Coupled cellulase assay has been developed in order to improve the screening process. This method does not require boiling samples and allows rapid screening of mutants in a microtiter plate. The aim of this study was to establish enzyme coupled assay where cellulase first hydrolyzes carboxymethylcellulose (CMC), and cellobioses dehydrogenase (CBDH) and dichlorophenolindophenol (DCPIP) is used subsequently for detection of reducing ends[1,2]. Cellulase gene (wt) derived from *Trichoderma reesei* was cloned in the pESC-TRP vector, and expressed in the yeast *S. cerevisiae*. Obtained heterologous protein is used to optimize enzymatic assay conditions, including pH optimum, CMC concentration, and CBDH amount. Libraries were obtained using semi-rational design and mutations were introduced in catalytic site of cellulase[3] as can be observed in the Figure 1.




**A** **B**

**Figure 1.** A: 3D structure of cellulase; B: Catalytic site of cellulase.

Libraries were screened for mutants by previously optimized assay. Selected mutants showed increased cellulase activity as can be observed in the Figure 2.

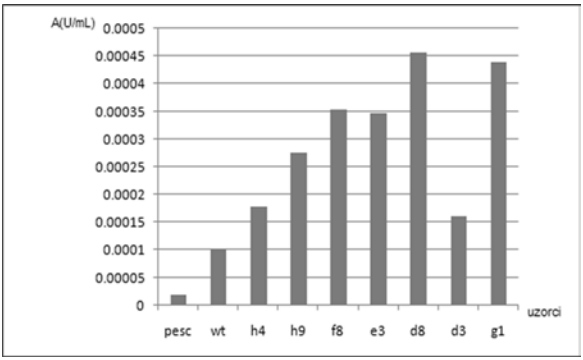



**Figure 2.** The activity of wild-type cellulase and selected mutants.

Cellulase gene (wt) has been recloned in pCTcon2 vector because it allowes for expression on the yeast surface. Also, the library was created in this vector by introducing random mutations using error prone PCR. The gene library was screened with the aforementioned assay and mutants with higher cellulase activity were selected. The aim of this study was to obtaine cellulase expressed on the yeast surface in order to develop fluorescent assay applied in flow cytometry.

Cellulase was successfully produced in *S. cerevisiae*, and libraries yielded mutants with increased cellulase activity. Developed assay allowed us a quick and efficient way of scanning aforementioned gene libraries.

## Acknowledgements

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