



**Serbian Biochemical Society
Ninth Conference**

"Diversity in Biochemistry"

Proceedings

*Faculty of Chemistry – Kolarac Endowment
Belgrade 2019*

Serbian Biochemical Society

President: Marija Gavrović-Jankulović

Vice-president: Suzana Jovanović-Šanta

General Secretary: Milan Nikolić

Treasurer: Milica Popović

Organizing committee

Natalija Polović

Milan Nikolić

Milica Popović

Karla Ilić Đurđić

Dragana Robajac

Romana Masnikosa

Nataša Simin

Aleksandra Stefanović

Jelena Brkljačić

Isidora Protić-Rosić

Ana Simović

Snežana Spasić

Vladimir Mihailović

Ana Miltojević

Srđan Miletić

Scientific committee

Marija Gavrović-Jankulović

Mihajlo B. Spasić

Vesna Niketić

Ivan Spasojević

Dejana Mokranjac

Neda Mimica-Dukić

Snežana Đorđević

Suzana Jovanović-Šanta

Melita Vidaković

Snežana Marković

Olgica Nedić

Ivanka Karadžić

Vesna Spasojević-Kalimanovska

Tanja Ćirković Veličković

Ivan Gržetić

Goran Brajušković

Vesna Vučić

Niko Radulović

Proceedings

Editor: Ivan Spasojević

Cover design: Zoran Beloševac

Publisher: Faculty of Chemistry, Serbian Biochemical Society

Printed by: Colorgrafx, Belgrade

Serbian Biochemical Society
Ninth Conference
with international participation

University of Belgrade – Kolarac Endowment
14-16.11.2019. Belgrade, Serbia

“Diversity in Biochemistry”

Characterization of recombinant *Phanerochaete chrysosporium* cellobiose dehydrogenase mutants with increased oxidative stability from *Pichia pastoris* KM71H strain

Ana Marija Balaz^{1*}, Neda Popov², Olivera Prodanović³, Raluca Ostafe⁴, Rainer Fischer⁵, Radivoje Prodanović²

¹*Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia*

²*Faculty of Chemistry, University of Belgrade*

³*Institute for Multidisciplinary Studies, University of Belgrade*

⁴*Molecular Evolution Protein Engineering and Production facility (MEPEP), Purdue University, West Lafayette, USA*

⁵*Indiana Bioscience Research Institute, Single Cell Analytics Center, Indianapolis, USA*

* e-mail: anam@chem.bg.ac.rs

Phanerochaete chrysosporium is a white rot fungi and it has been known to secrete flavocytochrome enzyme cellobiose dehydrogenase (CDH, EC 1.1.99.18) which contains two domains, a flavine domain and cytochrome domain. Flavine domain contains FAD as prosthetic group and its catalytically active domain, whereas cytochrome domain serves as electron acceptor. Cellobiose and lactose, as well as other β – 1,4 – linked disaccharides and oligosaccharides, have been oxidized by the cellobiose dehydrogenase to their corresponding lactones¹⁻³. CDH can be used for constructing biosensors and therefore directed evolution has been used to produce more active and stable variants of the enzyme. Wild type CDH enzyme was expressed in *S.cerevisiae* INVSc1 cells and used for creation of saturation mutagenesis libraries at M65, M685 and M738 and screening for increased oxidative stability. More stable mutants that were found were recloned into *Pichia pastoris* KM71H strain for higher expression yield. They were afterwards, expressed in *Pichia*, purified and kinetically characterized.

Acknowledgements

This study was supported by Ministry of Education, Science and Technological Development of Republic of Serbia, grant number ON172049 and III46010.

References

1. Harreither W, Sygmond C, Augustin M, Narciso M, Rabinovich ML, Gorton L, Haltrich D, Ludwig R. Catalytic properties and classification of cellobiose dehydrogenases from ascomycetes. *Appl Environ Microbiol* 2011;77:1804-15.
2. Laurent CVFP, Breslmayr E, Tunega D, Ludwig R, Oostenbrink C. Interaction between cellobiose dehydrogenase and lytic polysaccharide monooxygenase. *Biochemistry* 2019;58:1226-35.
3. Hallberg BM, Henriksson G, Pettersson G, Divne C. Crystal structure of the flavoprotein domain of the extracellular flavocytochrome cellobiose dehydrogenase. *J Mol Biol* 2002;315: 421-34.