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#### **Proceedings**

Editor: Ivan Spasojević

Technical secretary: Jelena Nestorov

Cover design: Zoran Beloševac

Publisher: Faculty of Chemistry, Serbian Biochemical Society

Printed by: Colorgrafx, Belgrade

CIP			

# Serbian Biochemical Society Sixth Conference

with international participation

Faculty of Chemistry, University of Belgrade, 18.11.2016. Belgrade, Serbia

"Biochemistry and Interdisciplinarity: Transcending the Limits of Field"

#### **Foreword**

#### Dear Colleagues

It is my distinct pleasure to welcome you to the 6<sup>th</sup> Conference of the Serbian Biochemical Society, entitled "Biochemistry and Interdisciplinarity: Transcending the Limits of Field". It is an honor for me to be selected as the Editor of Proceedings of the Conference. I am grateful to the Steering Committee of Serbian Biochemical Society for giving me this opportunity to shape the premiere forum in biochemistry in the region. We have been tremendously fortunate to have Mihajlo B. Spasić as the first Editor. He nurtured this Conference (and Society) through its re-starting years as it grew in quality and relevance. Clearly, following in his footsteps is a challenge.

We have invited Djuro Josić from the University of Rijeka and eight experts from four major universities in Serbia to give lectures at the  $6^{th}$  Conference. The visit of our dear colleague from Croatia is a part of an initiative for closer collaboration within FEBS3+ (Croatia, Hungary, Slovenia, and Serbia) Meeting Programme that was established by FEBS in 2010. We have also invited students at the final years of PhD studies to present their work in our Proceedings as Abstracts. Official languages at the Conference will be Serbian, Croatian, and English.

I would like to express my gratitude to the members of the Scientific Board who suggested lecturers and to all respected colleagues who accepted the invitation.

Editor of the Proceedings Ivan Spasojević

## Rational design of raw starch degradingα-amylasefrom *Bacillus licheniformis* 9945a for possible surface binding sites identification

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Raw starch degrading enzymes often possess extra substrate binding regions that enhance their activity to starch granule via physical adsorption. These can be found either on separate domains termed starch binding domains (SBDs) or in the form of surface binding sites (SBSs) situated on the surface of enzymes. Conservation of SBS is not known nor expected amongst amylase families. However, within same subfamily it can be expected to have occurrence of same or similar residues being involved in starch binding. Confirmed adsorption ofα-amylase from Bacillus licheniformis ATCC 9945a (BliAmy), a potent enzyme for raw starch hydrolysison raw starch granules is indicative for presence of SBS as enzyme is lacking SBD. Suspected sites responsible in BliAmy were identified by homology modeling and in silico analysis. Site-directed mutagenesis of target amino acid residues was performed. Wild typeenzymeand mutantswere produced using an optimized fed-batch approach in a defined media with significant overexpression of 1.2 g L<sup>-1</sup>. A mixed mode Nuvia<sup>TM</sup>cPrime<sup>TM</sup> resin was used for downstream processing with yields of 96% directly from the fermentation broth. Role of enzyme domain C in raw starch adsorption has been elucidated by deletion analysis. The Langmuir model was applied for adsorption kinetics study of mutants to starch granules, while kinetics of hydrolysis was followed by Michaelis-Menten equation.

#### Acknowledgement

This work was supported by the Serbian Ministry of Education, Science and Technological development, project grant number 172048 and International Centre for Genetic Engineering and Biotechnology (ICGEB) research project grant number CRP/YUG11-02.

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