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## ENZYMOMOLOGY AND MOLECULAR BIOLOGY

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**Enzymatic synthesis of prebiotic galacto-oligosaccharide: Application of nanobiocatalysts and structural characterization of product****Dejan Bezbradica**

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**Statement of Problem:** Galacto-oligosaccharides (GOS) are group of  $\beta$ -galactoside compounds with significant market value due to their prebiotic properties utilized in infant nutrition products. Physiological activity is based on their short chain carbohydrate structure which makes them non-digestible by digestive enzymes, but digestible by beneficial probiotic bacteria with consequential property of selective promotion of their growth and improvement of overall health status. State of the art in current industrial GOS production based on transgalactosylation activity of  $\beta$ -galactosidases implies that attempts for further advance could be focused on: Fine-tuning of physiological properties by targeted control of enzymatic process toward obtaining GOS of desired structure and developing novel immobilized  $\beta$ -galactosidase preparations with improved affinity towards GOS synthesis.

**Methodology & Theoretical Orientation:** For evaluation of the effect of enzyme origin on degree of polymerization and type of  $\beta$ -linkages within obtained GOS compounds, transgalactosylation was performed with different  $\beta$ -galactosidases: from *Aspergillus oryzae* and *Lactobacillus acidophilus*. Elucidation of chemical structures in obtained GOS mixtures was performed using ion-mobility spectrometry–tandem mass spectrometry (IMS-MS/MS) one-step approach. Improvement in the field of  $\beta$ -galactosidase immobilization was attempted by producing novel nanobiocatalyst with functionalized nonporous fumed nano-silica (FNS) particles as immobilization support.

**Conclusion & Significance:** IMS-MS/MS analysis has shown that structure of obtained GOS is influenced by origin of  $\beta$ -galactosidase, since one from *A. oryzae* produced GOSs with  $\beta(1\rightarrow6)$  and  $\beta(1\rightarrow3)$  linkages, while enzyme from *L. acidophilus* produces GOSs with  $\beta(1\rightarrow6)$  and  $\beta(1\rightarrow4)$  linkages. Type of glycosidic linkages influences prebiotic properties of GOS, hence determination of linkage type will have great significance in enabling adequate selection of  $\beta$ -galactosidase for targeted prebiotic application. The immobilization on nano-supports indicated that the most adequate support is one functionalized with amino groups, which enabled several times higher transgalactosylation activities than conventionally immobilized  $\beta$ -galactosidase.

**Biography**

Dejan Bezbradica obtained his PhD degree in Biochemical Engineering and Biotechnology from the Faculty of Technology and Metallurgy in Belgrade in 2007. Since 2013, he is an Associate Professor in the Department of Biochemical Engineering and Biotechnology. During 2009, he was on sabbatical working in the Laboratory of Enzyme Engineering at Institute of Catalysis in Madrid. His scientific work covers following areas: Cell and enzyme immobilization, enzymatic synthesis in microaqueous media, application of membrane reactors in biocatalytic processes; microbial production and purification of industrial enzymes, kinetic modeling of bisubstrate enzymatic reactions, application of enzymes with transglycosylative activity in synthesis of bioactive compounds, chemical modification of enzymes and immobilization supports, and nanobiocatalysis. His recent research activities are focused on the development of food and feed products containing bioactive galactosides with prebiotic activities targeted for specific probiotic species.

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