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11th International Conference
on Fundamental and Applied Aspects of
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Professor Ivan Draganić

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J-09-P

CHARACTERISATION OF EXOPOLYSACCHARIDE PRODUCED BY BACILLUS SP. NS032

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Abstract
In this study, the main structural monomer units and configuration of their glycosidic linkages of a exopolysaccharide obtained from a strain of *Bacillus licheniformis* was investigated. The chemical structure of this polymer, after purification of crude material, was analyzed by chemical methods, planar chromatography, elemental analysis and FTIR.

Introduction
Microbial exopolysaccharides are the group of biopolymers whose structural chemistry is very complex. Usually some types of microorganisms can be characterized by the presence of several carbohydrate polymers. The economic significance of these compounds is related to the fact that some of these polymers is nowadays widely accepted products of biotechnology, while others are in various stages of research. The use of microbial polymers varies widely due to good mechanical properties for application as fiber, films, adhesives, rheology modifiers, hydrogels, emulsifiers, and drug delivery agents. Biopolymers with industrial application are often bacterial and fungal products, like levan, dextran, β-glucan, pullulan. In this work we reported a route to produce of purified exopolysaccharide produced by strain of *Bacillus licheniformis,* and determination of main structural characteristics of this polymer.

Experimental
Exopolysaccharide was produced by strain of *Bacillus licheniformis* which was isolated from petroleum sludge sample taken from Oil Refinery Novi Sad. The strain was identified using 16S rRNA gene sequence analysis [1]. The organism was cultivated in sucrose broth for 10 days at 28 oC [2]. After removal of biomass the crude polysaccharide was isolated from fermentation culture by precipitation with two volumes of ethanol.
Polymer was analyzed for its carbon, hydrogen and nitrogen content using the Vario EL III device (GmbH Hanau Instruments, Germany). Nitrogen content usually was indication for the presence of protein as impurities. After treatments with Dnase, Rnase and Pronase and dialysis, purified polysaccharide was precipitated by acetone and lyophilized. In this way was obtained purified polysaccharide, without nitrogen, with content of C and H that corresponded to polysaccharide molecules (Table 1.). Monomer components was determined by planar chromatography of the acid hydrolizate of the glycan. The ATR-IR spectrum of pure polysaccharide was obtained

using Thermo Nicolet 6700 FT-IR Spectrophotometer with Smart Orbit Diamond ATR (attenuated total reflectance) accessory.

Results and Discussion

Planar chromatography of the hydrolyzed crude polysaccharide, comparing with standards of authentic monosaccharides, showed two main components, fructose and glucose, in about equal proportions. After further treatments, in hydrolysate of purified polymer was detected only one component, D-Fructose, which indicated that investigated polymer is fructan. Additional evidence that purification of polysaccharide was successful was found in the results of elemental analysis of crude and purified polymer (Table 1). The FT-IR spectrum of the purified polysaccharide showed the spectral pattern typical for polysaccharides. As shown in Fig.1, absorption bands between 1128 cm^{-1} and 1014 cm^{-1} corresponded to C-O-C and C-O-H stretching vibrations [3], the bands in the range of 1200-1500 cm^{-1} ascribed to C-H deformation vibration, the band at 1645 cm^{-1} was due to bound water. Strong, wide band at 3000–3500 cm^{-1} and band at 2936 cm^{-1} was assigned to the hydroxyl groups stretching vibration of polysaccharide and C-H stretching [4]. Absorption band in the anomeric region (950-700 cm^{-1}) at 891 cm^{-1} is specific for the β-configuration of the glycosidic linkages. Based on the FT-IR spectra it was concluded that investigated polymer had β-configuration of glycosidic linkages.

Table 1. Elemental chemical composition.

	% N	% C	% H
Crude sample	7.44	30.58	5.13
Purified sample	/	44.42	6.20

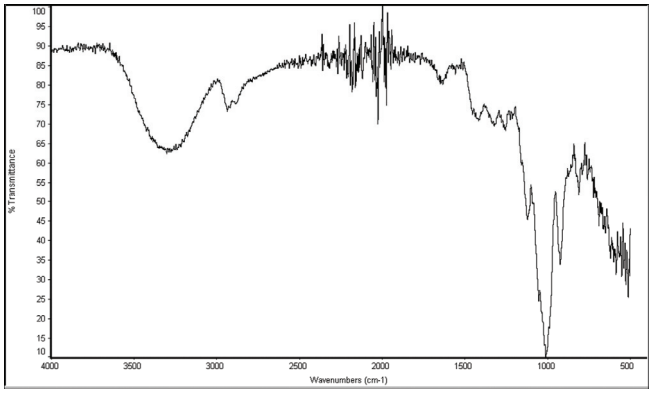

Figure 1. FTIR spectrum of purified exopolysaccharide produced by *B. licheniformis.*

Conclusion

The present study showed, on the basis of chemical methods, planar chromatography, elemental analysis and FTIR, that investigated exopolysaccharide is homopolymer,

composed of a D-fructose, as the O-specific monomer, with β-configuration of the glycosidic linkages.

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