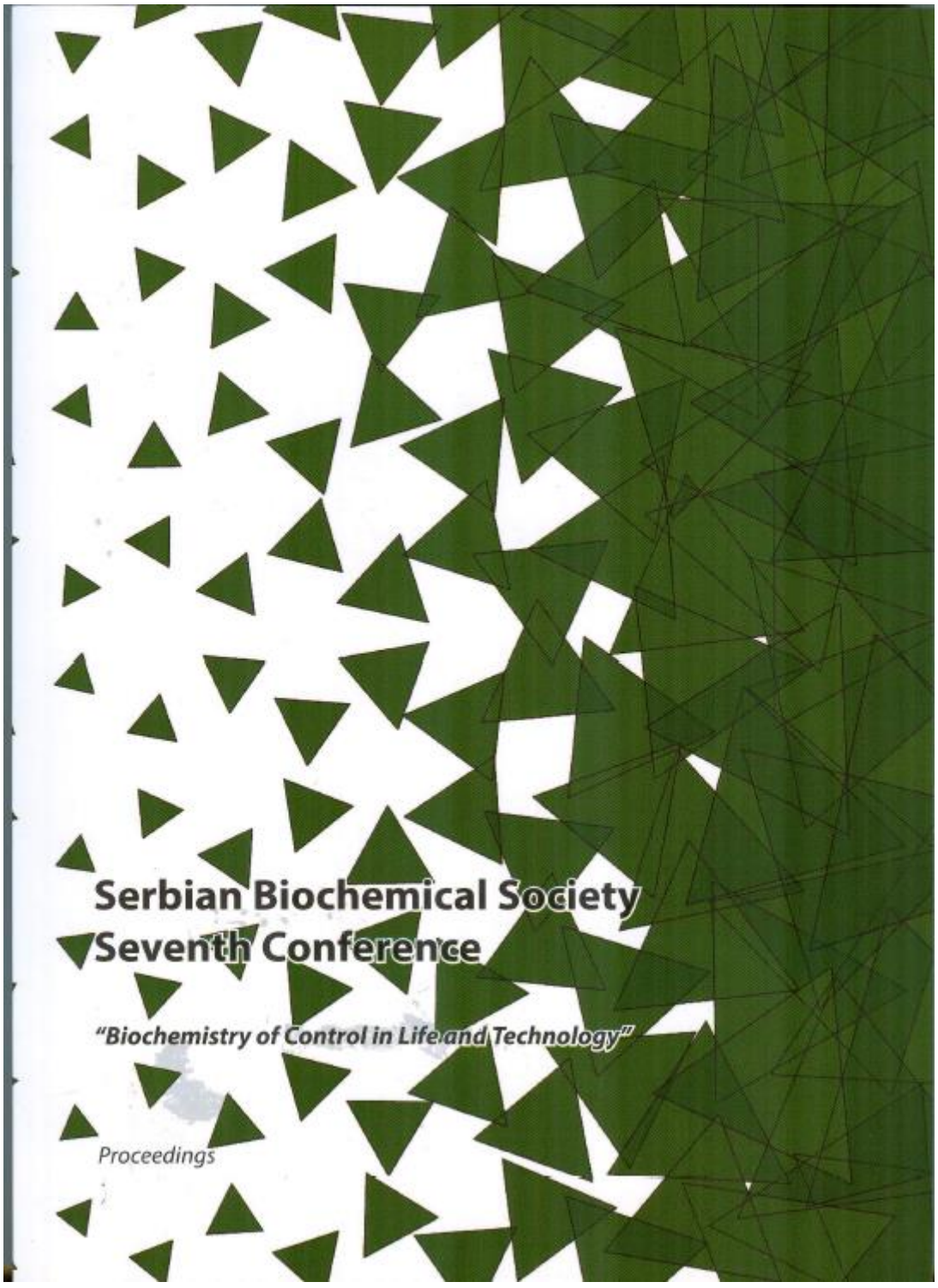
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Structural characterization of EPS produced by *Brachybacterium paraconglomeratum* sp. CH-KOV3

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Microorganisms isolated from polluted environments can be used for bioremediation [1]. However, some of microbial isolates can synthesize various exopolysaccharides (EPSs) [2,3]. This non-toxic, natural, and biodegradable polymers can be used in different industries such as food and cosmetic as water-binding and gelling agents, as probiotics, sweeteners, thickeners, stabilizers. In waste water treatment today they are used as heavy metal removal agents [4]. In medicine EPS have a potential antiviral, immunostimulatory and antitumor activities [5,6].

The aim of this work was structural characterization of EPS produced by *Brachybacterium paraconglomeratum* sp. CH-KOV3. For the structural instrumental analysis of EPS, the following methods were applied: GC-MS (gas chromatography mass spectrometry) and correlated two-dimensional NMR (nuclear magnetic resonance) techniques - DEPT 135 (distortionless enhancement by polarization transfer), COSY (correlation spectroscopy), and HSQC (heteronuclear single quantum coherence). Methylation was performed by the method which described earlier [7]. The permethylated EPS was subjected to reductive cleavage as described by Rolf and Gray [8]. Cleaved monomer units were acetylated. Obtained acetylated, methylated products were analyzed by GC-MS. These analyses were performed on a GCxGC-MS (Shimadzu, Kyoto, Japan). NMR spectra of the isolated EPS were measured on a Bruker AVANCE III 500 spectrometer.

GC-MS analysis - three sets of two peaks were identified [8,9]. First set represented fructofuranoses with (2,6)-linkages and referred to the main chain. Second set of peaks corresponded to the nonreducing terminal units of the glycan molecules. Third set also had two retention times. The identified peaks corresponded to the fructosyl residues that indicate the (2,1) branching of the polysaccharide chain. GC-MS analysis of methylation products suggest that the units in the main chain are (2,6)-linked, the main chain was substituted with single d-fructofuranoses at position O-1. Polysaccharide was of moderate branching.

DEPT 135 spectrum which was used to determine the degree of hydrogenation of each carbon showed intense signals corresponded to CH protons of C-5, C-3 and C-4; CH2

protons of C-6 and C-1. The part of the COSY spectrum of isolated EPS, showed cross peaks H6a/H6b, H5/H6b, H4/H5 and H3/H4 and the absence of any correlation peaks in the region 3.6-3.8 ppm [10]. HSQC spectrum indicates direct correlations between carbons of the sugar units and skeleton protons. Diagnostic cross peaks H5/C5, and H6a, H6b/C6 were detected, and their values are similar to the values of another levan-type fructan [11].

In conclusion, EPS produced by *Brachybacterium paraconglomeratum* sp. CH-KOV3 is a levan-type polysaccharide.

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