Fructan from *Bacillus sp.* NS032 - preparation, characterization and antioxidant activities *in vitro*

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Introduction

Microbial polysaccharides (MPS) are characterized by high structural diversity leading to their numerous applications. Many of these polymers are now widely accepted products of biotechnology with applications in various fields: food industry, cosmetics, agriculture, pharmacy and medicine. In recent years, much attention was given to bacterial exopolysaccharide levan, due to specific physical and chemical properties and non-toxicity, for which it could be applied as a stabilizer, emulsifier, flavor and fragrance carrier, prebiotic, antioxidant and antitumor agent, for encapsulation, etc.

The aim of this work was to investigate the structural characteristics of a exopolysaccharide produced by *Bacillus sp*. NS032. The synthesized MPS was characterized by chemical methods, planar chromatography, elemental analysis, FTIR and NMR spectroscopy. Apart from that, the antioxidant activities in vitro of this polysaccharide were investigated.

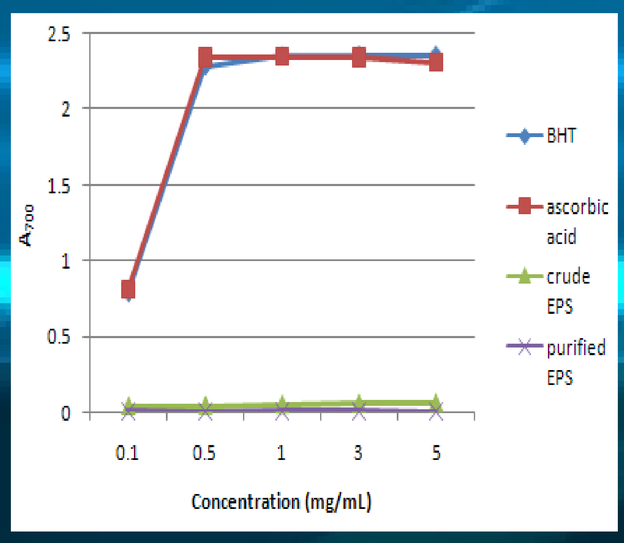

Fig. 3. Reducing power assay of exopolysaccharides

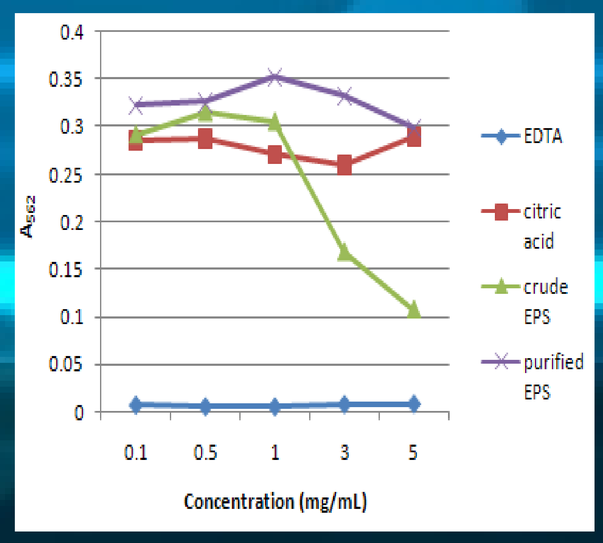

Fig. 4. Chelating activities of exopolysaccharides

Experimental

- Exopolysaccharide was produced by *Bacillus sp.* NS032 [1]. The microorganism was cultivated in sucrose broth for 10 days at 28 °C. After removal of biomass the crude polysaccharide was isolated by precipitation with ethanol. After treatments with Dnase, Rnase and Pronase and dialysis, purified polysaccharide was precipitated by acetone and liophylised.
- The elemental analysis data were obtained using the Vario EL III device (GmbH Hanau Instruments, Germany).
- Monomer components were determined by planar chromatography of the acid hydrolizate of the glycan.
- FT-IR spectrum of pure polysaccharide was obtained using Thermo Nicolet 6700 FT-IR Spectrophotometer in ATR mode.
- NMR spectra of purified MPS were recorded on a Varian Gemini 2000 (200 MHz (^{1}H), and 50,3 MHz (^{13}C).
- Antioxidant activities of crude and purified polysaccharide were evaluated using two different *in vitro* assays systems.

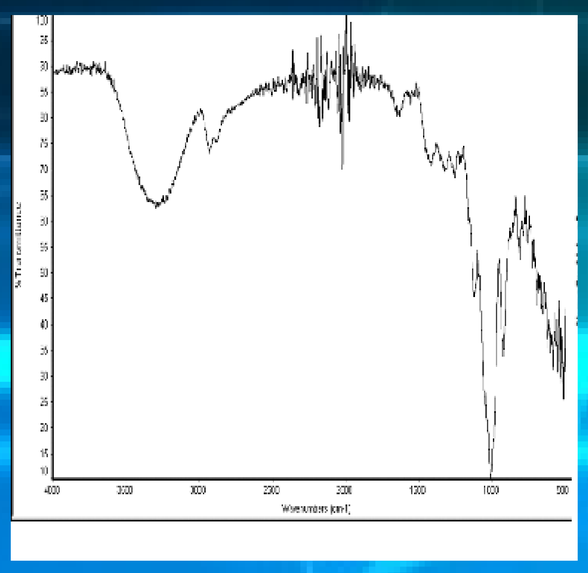

Fig. 1. FT-IR spectrum of purified exopolysaccharide produced by *Bacillus sp.* NS032

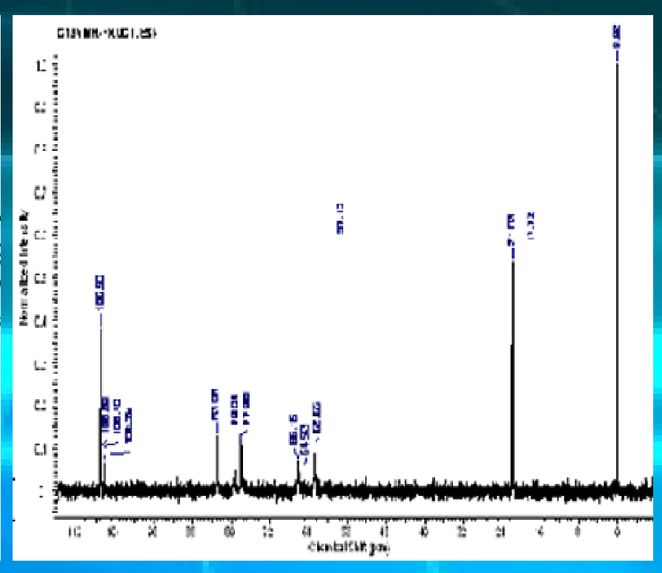

Fig. 2. ^{13}C NMR spectrum of purified exopolysaccharide produced by *Bacillus sp.* NS032

Results

- Planar chromatography of the hydrolyzed crude polysaccharide showed two main components, fructose and glucose, while in hydrolysate of purified polymer was detected only D-fructose, which indicated that investigated polymer was fructan.
- Results of elemental analysis showed that C and H content corresponded to polysaccharide molecules.
- The FT-IR spectrum of the purified polysaccharide (Fig.1) showed absorption peaks between 1128 cm^{-1} and 1014 cm^{-1} attributed to ν_{str} (C-O-C) and ν_{str} (C-O-H), the bands in the range of 1200-1500 cm^{-1} assigned to δ (C-H), the peak 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 ν_{str} (OH) and ν_{str} (C-H), respectively. Absorption band at 891 cm^{-1} is specific for the β-configuration of the glycosidic linkages.
- The ^{13}C NMR spectrum (Fig.2) of purified fructan showed signals at 106.98 ppm, 106.393 ppm and 106.284 ppm which corresponded to anomeric β-C-2 atoms of D-fructofuranosil residues. These signals were from C-2 atoms of linear (2→6)-linked fructofuranose, and from C-6 atoms of branched (2→6)-linked fructofuranose. Chemical shift at 83.08 ppm was from C-5 atoms of β-D-frukfuranosil residues.
- The antioxidant activities of crude and purified polysaccharide were tested using *in vitro* assay systems: ferric-reducing power and chelating activities. In the reducing power assay results showed (Fig.3) that crude and purified polysaccharide did not have antioxidant activity, as was expected based on literary data [3,4]. In the Fe^{2+} chelating assay (Fig. 4), the Fe^{2+} chelating effect the of crude and purified MPS were concentration related.

Conclusion

- The polysaccharide produced by the *Bacillus sp.* NS032 is homopolymer, fructan, with the backbone chain consisting of (2,6) linked β-D-fructofuranose units with side groups linked to the main chain likely through β-(2,1)-glycosidic linkages.
- Investigated MPS belongs to levan-type polysaccharide.
- The antioxidant activities (chelating ability, reducing power) *in vitro* showed moderate or low values of the crude and purified polysaccharides, compared to the commercial antioxidants.

References

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Acknowledgement

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