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СПИСАНИЕ НА МАКЕДОНСКОТО ЛЕКАРСКО ДРУШТВО



5-ти КОНГРЕС НА МИКРОБИОЛОЗИТЕ НА МАКЕДОНИЈА
СО МЕЃУНАРОДНО УЧЕСТВО
5-th CONGRESS OF MACEDONIAN MICROBIOLOGISTS
WITH INTERNATIONAL PARTICIPATION



ЗБОРНИК НА РЕЗИМЕА
и програма

BOOK OF ABSTRACTS
and Programme



28-31 Мај 2014
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B.5 In vitro antioxidant activity of levan produced by *Brachybacterium* sp. CH-KOV3

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Introduction: In this work levan isolated from microorganism *Brachybacterium paraconglomeratum* and fragments obtained by partial hydrolysis of purified polysaccharide were evaluated by antioxidant activity. Levans (fructans) are generally linear polysaccharides containing fructose residues linked

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predominantly by β -(2,6)-glycosidic bonds and one α -glucose at the end of the chain, with branching through the position of C-1.

Aim: The aim of the presented work was investigation and comparison of antioxidant potential of fructan synthesized by the non-pathogenic, rod shaped, Gram-positive *Brachybacterium* sp. CH-KOV3, and fragments obtained by its controlled acid hydrolysis.

Material and methods: The microorganism was isolated from the hydrocarbon polluted sediment of the waste water canal of the industrial zone of Pancevo, Serbia. The bacterial strain identified as *B. paraconglomeratum* using 16S rDNA analysis, was incubated on sucrose-based substrate at 28°C for 7 days. Supernatant obtained after centrifugation was precipitated using cold ethanol. After dialysis and lyophilization polysaccharide was investigated by total hydrolysis with 2M trifluoroacetic acid (3 h, 120°C) and planar chromatography. Part of purified glycan was subjected to controlled acid hydrolysis with 0.125 % oxalic acid (12 min., 80°C). The obtained fragments and purified glycan were subjected to measurement of antioxidant activity.

Results: Exopolysaccharide levan was isolated from fermentation broth of microorganism *B. paraconglomeratum*, after procedure of purification described above. Results of acid hydrolysis and planar chromatography confirmed fructose as dominant component in this polymer. Fragments obtained by partial hydrolysis of levan and purified native fructan were evaluated by antioxidant activity. This examination was performed by DPPH free radical scavenging activity assay, reducing power, and chelating ability on ferrous ions.

Conclusion: Results are pointed out promising potential of levan as antioxidant agent, especially the fragments obtained by its partial hydrolysis. In comparison to other antioxidant tests the chelating activity showed the highest values for both samples, which can be attributed to the conformation of polysaccharide chain in solution.

Keywords: levan, hydrolysis, chelating



IN VITRO ANTIOXIDANT ACTIVITY OF LEVAN PRODUCED BY *Brachybacterium* sp. CH-KOV3



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Introduction

Microbial exopolysaccharides (EPSs) 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.

Levans (fructans) are generally linear polysaccharides containing fructose residues linked predominantly by β -(2,6)-glycosidic bonds and one α -glucose at the end of the chain, with branching through the position of C-1 [1, 2].

Aim

The aim of the presented work was investigation and comparison of antioxidant potential of fructan synthesized by the non-pathogenic, rod shaped, Gram-positive *Brachybacterium* sp. CH-KOV3, and fragments obtained by its controlled acid hydrolysis.

Material and methods

The microorganism was isolated from the hydrocarbon polluted sediment of the waste water canal of the industrial zone of Pancevo, Serbia. The bacterial strain, identified as *B. paraconglomeratum* using 16S rDNA analysis, was incubated on sucrose-based substrate at 28°C for 7 days. Supernatant obtained after centrifugation was precipitated using cold ethanol. After dialysis and lyophilization polysaccharide was investigated by total hydrolysis with 2M trifluoroacetic acid (3 h, 120°C) and planar chromatography. Part of purified fructan was subjected to controlled acid hydrolysis with 0.125% oxalic acid (12 min, 80 °C). The obtained fragments and purified fructan were evaluated by antioxidant activity. This examination was performed by DPPH free radical scavenging activity assay, reducing power and chelating ability on ferrous ions [3, 4].

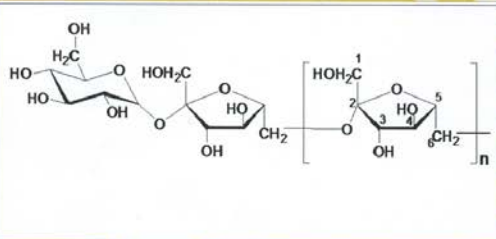


Figure 1. Structure of levan

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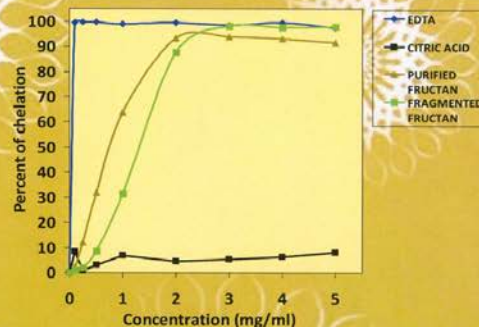


Figure 2. Chelating activities of exopolysaccharides

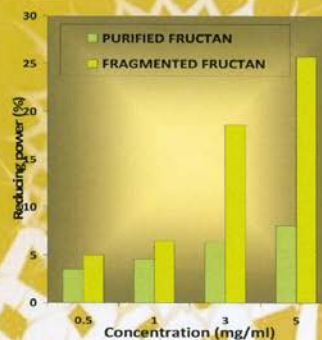


Figure 3. Reducing power assay of exopolysaccharides

Results

- ✓ Exopolysaccharide levan was isolated from fermentation broth of microorganism *B. paraconglomeratum*, after procedure of purification described above.
- ✓ Results of acid hydrolysis and planar chromatography confirmed fructose as dominant component in this polymer.
- ✓ Fragments obtained by partial hydrolysis of levan and purified native fructan were evaluated by antioxidant activity. In DPPH free radical scavenging activity assay results for both samples are similar, about twenty percent.
- ✓ The reducing power (Figure 3) of levan fragments was more pronounced than that of native levan, indicating that the antioxidant activity had been greatly enhanced after acid hydrolysis.
- ✓ In comparison to other antioxidant tests the chelating activity showed the highest values for both. As shown in Figure 2, the chelating activities of purified and fragmented fructans increased with the increase of concentrations.

Conclusion

Results are pointed out promising potential of levan as antioxidant agent, especially the fragments obtained by its partial hydrolysis. In comparison to other antioxidant tests the chelating activity showed the highest values for both samples, which can be attributed to the conformation of polysaccharide chain in solution.