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OXIDATIVE TRANSFORMATION OF LEVAN PRODUCED BY BACILLUS LICHENIFORMIS STRAIN

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ABSTRACT
In this work, microbial polysaccharide levan produced by *Bacillus licheniformis* NS032 was functionalized by introducing aldehyde groups into glycan chain. Resulting polyaldehyde polymer was characterized by FT-IR spectral analyses and content of aldehyde groups was determined titrimetrically.

INTRODUCTION
Levan is microbial polysaccharide produced by broad range of microorganisms [1]. This glycan consisting of β-(2,6)-fructose units with a terminal D-glucose residue (Fig.1). Branching that occurs through β-(2,1)-bonds are often short and sometimes consisting of a single fructose residue. Bacterial levans have the broad spectrum of applications in many fields like food, health care, pharmacy, medical, cosmetic and others [1]. Levan is also successfully subjected to many reactions including grafting reaction with polystyrene [2].

This paper describes the formation of polyaldehyde polymers of partially oxidized levan with the aim of obtaining a suitable reactive derivative enbling covalent coupling with different amino compounds. The presence of aldehyde groups was evidenced by FT-IR spectroscopy data of polyaldehyde polymers.

EXPERIMENTAL
Levan used in this work was produced by the *B. licheniformis* NS032 [3]. Samples of aldehyde-functionalized levans (1.0 g) were obtained with periodate salts (0.5 g-2.0 g) in aqueous solution at room temperature for 24 h. The aldehyde content in polymers was varied over a broad range by changing the reaction conditions. Excess periodate was removed by ethylene glycol and

resulting polyaldehyde glycan was purified by dialysis (MWCO 14,000). Obtained products were lyophilized. The aldehyde content was determined titrimetrically by hydroxylamine hydrochloride method. Characterization of resulting polyaldehyde polymers was performed by FT-IR data (Thermo Nicolet 6700 FT-IR Spectrophotometer in ATR mode).

RESULTS AND DISCUSSION

Polyaldehyde derivatives of levan are obtained by reaction with periodate salts in aqueous solution. The aldehyde content in obtained levan samples was in range 24.4%-69.5%. Levan contains two monomer units in the polysaccharide chain, depending of their linkages in the molecule. However, it contains same types of vicinal diol groups and periodate oxidation results in the same types of dialdehyde structures (Fig. 1). In the molecule of this glycan predominate fructofuranosyl residues, because the glucopyranose unit is located only at the nonreducing end of the polymer chain.

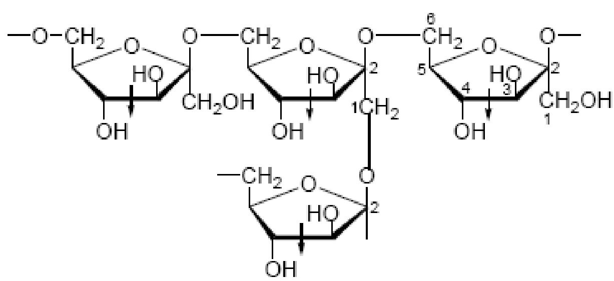

Fig. 1. Sites of oxidation in a periodate-oxidized levan (denoted by arrows)

The aldehyde-functionalized levan can react with various amine-containing compounds, e.g. some drugs or polymers to form Schiff base structures (Fig. 2) or alkylamines if coupling occurs in the presence of a reducing agent. Some periodate oxidized polysaccharides were successfully coupled with various amino compounds that can potentially have many of the uses in many fields, especially in medicine and pharmacy [4]. Polyaldehyde levan, because of its nontoxicity and solubility in water also can have great potential applications.

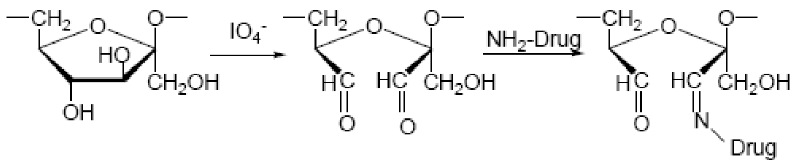

Fig. 2. Schematic representation of coupling reaction of aldehyde-functionalized levan with amine-containing drug

FTIR spectra were used to determine structural changes in the native and the oxidized levan. The FT-IR spectrum of native levan (Fig.3.) revealed characteristic absorption bands for polysaccharide, namely a strong broad band at 3000-3400 cm^{-1} as well as band at 2936 cm^{-1}; they were assigned to the OH stretching vibration and CH stretching, respectively [5]. Strong narrow bands between 1128 cm^{-1} and 1014 cm^{-1} corresponded to C-O-C and C-O-H stretching. The absorption bands arising from C-H deformation vibration were observed in the wavenumber region 1200-1500 cm^{-1}, while absorption band at 1645 cm^{-1} (H-O-H bending) was arising due to bound water. The vibration of C1-H bond in the IR anomeric region (950-700 cm^{-1}) near 891 cm^{-1} is specific for the β-configuration of the glycosidic linkages that are typical for levan.

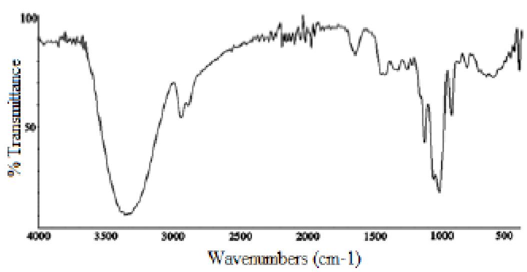

Fig. 3. FTIR spectrum of native levan produced by *B. licheniformis*

After oxidation, the FTIR spectrum (Fig. 4.) showed change in the carbonyl stretching region, while maintaining the general appearance. The new peak of low intensity for C=O groups at 1730 cm^{-1} appeared, which has been attributed to carbonyls in periodate-oxidized polysaccharides in different glycans [6].

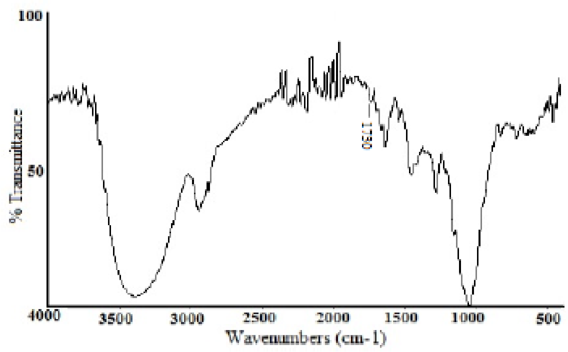

Figure 4. FT-IR spectrum of aldehyde-functionalized levan

CONCLUSION

Polyaldehyde polymers with different carbonyl contents were prepared by the oxidation of native levan using periodate ions in aqueous solution. The presence of aldehyde groups was confirmed by the FT-IR data. Aldehyde-functionalized levan can have potential application in many areas, due to enabling covalent coupling with different amino compounds.

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