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STRUCTURAL CHARACTERIZATION OF MICROBIAL LEVAN BY SMITH DEGRADATION

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ABSTRACT
In this work, microbial polysaccharide levan from *Bacillus licheniformis* strain was firstly oxidized to polyaldehyde, then reduced to polyalcohol, completely hydrolyzed, and finally acetylated. Resulting peracetylated alcohols were characterized by GC-MS and compared to the results from corresponded acetylated standards.

INTRODUCTION
Microbial polysaccharides have a great potential as functional biopolymers for food, industrial, cosmetic or medical applications. Depending on type of monosaccharide units, they could be homopolymers or heteropolymers. Fructans are fructose based homopolymers, with dominant glycosidic linkages: (2,1)- inulin, (2,6)- levan, or mixed type [1].

Levan is composed of β-(2→6) linked β-D-fructofuranose units with occasional β-(2→1) branching and carry D-glucosyl residue at the end of a chain. Microbial levan could be competitive replacement to commercial synthetic polymers due to its biocompatibility, biodegradability and renewability. It also shows a significant antioxidant, antitumor and anti-inflammatory effects [2].

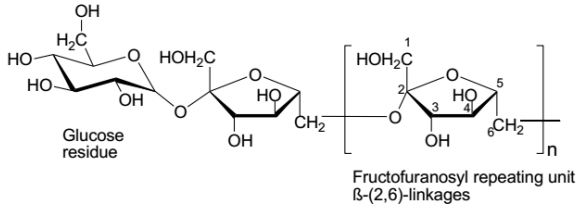

Figure 1. Structure of levan

The aim of this work was structural characterization of levan from *Bacillus licheniformis* strain by periodate oxidation and Smith degradation.

EXPERIMENTAL

Levan used in this work was produced by the *B. licheniformis* strain [3]. Other reagents and solvents were purchased from commercial sources and used as supplied. The dried polysaccharide (100 mg), which was dissolved in 50 mL distilled water, was oxidized with 0.1 M $NaIO_4$ (50 mL) at 20 ^{0}C in the dark. Aliquots were removed at suitable intervals for estimation of periodate and formic acid by the arsenite method and iodometric titration, respectively [4]. The oxidation was completed after 18 h, and the periodate consumption and releasing of formic acid were calculated from analytical data by extrapolation to zero time. The oxidized fructan was then degraded by the Smith procedure [4]. The solution of polyaldehyde was reduced with $NaBH_4$, neutralized and dialyzed. The obtained polyalcohol was hydrolyzed by oxalic acid [5]. The hydrolysate and standards (reduced fructose and glycerol) were acetylated [6] and then analyzed by GS-MS. GS-MS experiments were performed on Agilent 7890A GC system equipped with a HP-5MS column (30 m x 250 μm x 0,25 μm). The oven was programmed to 40 °C for 1 min, and then heated by 10 °C/min to 315 °C (held for 16.5 min). The run time was 45 min. Mass spectra were acquired in the electron ionization mode (EI) with ion energy of 70 eV.

RESULTS AND DISCUSSION

Levan isolated from *B. licheniformis* strain was characterized by elemental analysis, NMR and FTIR spectroscopy [3]. The structure of this polysaccharide was additionally investigated by the periodate oxidation followed by Smith degradation. The extent of periodate oxidation of fructan was monitored at intervals of 6 h [4]. The oxidation became constant after 18 h and corresponded to 0.89 mol of periodate and 0.020 mol of formic acid, respectively, per hexose residue. Oxidized fructan was subjected to Smith degradation (reduction of oxidized polysaccharide with $NaBH_4$ and subsequent complete hydrolysis with acid). After esterification of obtained products, resulting mixture of alditol acetates was subjected to GC-MS analysis (Figure 2). The components were identified by their retention times and typical breakdown patterns obtained on EI-MS. The total ion chromatogram (Fig. 2.) showed dominant peak at 12.98 min retention time, which corresponded to referent standard glycerol triacetate. In the mass spectrum (Fig. 2.) the obtained fragments m/z 43, 103, 116 and 145 are characteristic for fragmentation pattern of glycerol triacetate.

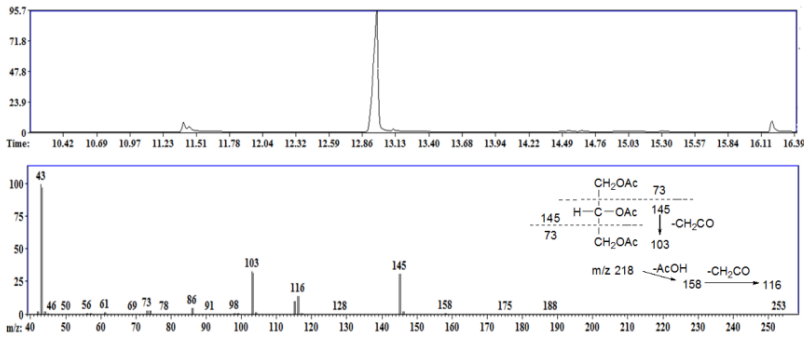

Figure 2. GC-MS of levan after periodate oxidation and Smith degradation

It was found that the periodate oxidation and Smith degradation of investigated fructan were resulted one component, glycerol, i.e. loss of fructose. After acetylation it is detected as glycerol peracetate. Scheme of these reactions is shown in Fig. 3.

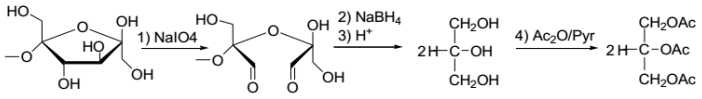

Figure 3. Reaction scheme of the periodate oxidation, Smith degradation and acetylation of 2,6-O-substitued fructofuranose, of which consisting the main chain of levan

These results indicated the presence of (2,6)-linkages in the main chain of the investigated fructan. Branching through the position of O-2 and terminal nonreducing glucose unit also give only glycerol, i.e. loss of fructose, as a result of these oxidative transformations. Products of Smith degradation indicated that the investigated glycan does not contain (2,3)- or (2,4)- linkages, considering that these types of bonds do not give glycerol as the resulting product. Obtained results are in accordance with the data for levans from other microbial sources [7].

CONCLUSION

Structure of levan isolated from *B. licheniformis* strain was investigated by periodate oxidation followed by Smith degradation. On the basis of the GC-MS analysis of degraded products, it has been found only one component, glycerol. The obtained results are in consistency with those related to the structures of levan from other microbial sources. The differences can exist

in the mode and frequency of branching, as well as in the length of the side chains, which will be the subject of further investigations.

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