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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.22 Synthesis of pullulan-based silver nanoparticles and their anti-microbial activity for prevention of food spoilage

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**Introduction:** Microbial polysaccharides have been used as stabilization agent for the synthesis of silver nanoparticles. A new class of materials based on such nanoparticles may found a wide range of potential applications including prevention of food spoilage.

**Aim:** The aim of the presented work was the synthesis of silver nanoparticles (Ag-NPs) based on periodate oxidized pullulan and investigation thus obtained material as an antibacterial agent.

**Material and methods:** Pullulan used in this work is produced by the yeast-like fungus *A. pullulans*, strain CH-1. Ag-NPs were synthesized by heating solutions of silver nitrate and oxidized pullulan at temperature of 121°C. Formation of nanoparticles was visually monitored by changing the color of the solution from colorless to yellow brown. The characterization of Ag-NPs was performed by UV-Vis spectroscopy, scanning electron microscopy (SEM), and electron diffraction spectroscopy (EDX). The antibacterial activity of produced Ag-NPs was tested using the strain of *Micrococcus lysodeikticus* and it was shown significant activity against them.

**Results:** Pullulan CH-1 is a linear  $\alpha$ -D-glucan having glucose residues connected by (1,4)- and (1,6)-glucosidic linkages, with some (7%) maltotetraosyl units. The results present in this work showed improved procedure for production of Ag-NPs based on polyaldehydo polymer obtained by treatment

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pullulan with periodate ions. Ag-NPs produced were characterized by UV-VIS data. Ultraviolet visible absorption spectra of these solutions showed sharp absorption at 400-440 nm, relating to the plasmon resonance of silver ions. SEM data indicated that nanoparticles formed were polydispersed. EDX spectrum showed presence of silver, by sharp signal in the silver region at 3 keV. Antibacterial activity of Ag-NPs was proven, too.

**Conclusion:** Obtained results showed that synthesized antibacterial material, based on oxidized pullulan Ag-NPs is promising candidate for a wide range applications, among them is very important food industry, as food packaging products, as barrier materials and antimicrobials for prevention of food spoilage.

Keywords: pullulan, nanoparticles, antibacterial activity





# SYNTHESIS OF PULLULAN-BASED SILVER NANOPARTICLES AND THEIR ANTIMICROBIAL ACTIVITY FOR PREVENTION OF FOOD SPOILAGE



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## Abstract

In this work we investigated the synthesis and antimicrobial properties of a pullulan based silver nanoparticles. Pullulan is nontoxic and biodegradable polysaccharide, therefore has wide applications, from food, as additive, to environmental remediation agents [1].

## Introduction

Nanoparticles can be usually synthesized in different ways: by physical, chemical and biological methods. The aim of the presented work was the synthesis of silver nanoparticles by periodate oxidized polysaccharide, pullulan, that produced by the yeast-like fungus *Aureobasidium pullulans*, strain CH-1 (IChTM, Collection of Microorganisms). It is a linear  $\alpha$ -D-glucan having D-glucopyranose units connected by (1,4)- and (1,6)-glucosidic linkages. The maltotriose repeating units, connected by (1-6)- $\alpha$ -D-linkages (Fig. 1), maltotetraosyl units (about 7%) being randomly inserted in the polymer chain are the main structural characteristics of this polysaccharide [2,3]. Potential application of pullulan through various chemical modifications obtaining new biologically active derivatives is very relevant.

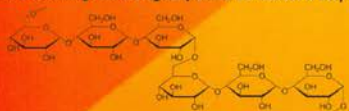


Fig. 1 Pullulan

Polyaldehyde derivatives of pullulan were obtained by reaction with periodate salts in aqueous solution. In the polyaldehydes thus prepared the aldehyde content can be varied in a wide range, depending on the reaction conditions. Pullulan contains three different anhydroglucoside moieties in the repeating unit and it contains different types of vicinal diol groups, therefore, periodate oxidation of this glucan results in different types of dialdehyde structure (Fig. 2).

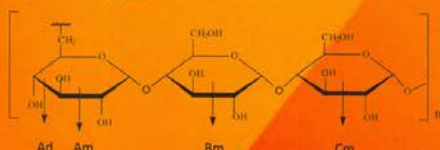


Fig. 2 Different places of oxidation in a periodate-oxidized pullulan. Am, Bm and Cm are singly oxidized units, Ad is a doubly oxidized unit.

## Aim

The aim of the present work was test synthesis of pullulan-based silver nanoparticles of periodate oxidized pullulan and silver ions with the aim of broadening knowledge in this area. Thus obtained silver nanoparticles can be used in many applications, including the food industry.

## Material and methods

Pullulan-based silver nanoparticles of oxidized pullulan and silver ions were synthesized by reducing silver nitrate salts with 1% solution of periodate oxidized pullulan by heating at temperature of 121 °C.

After cooling at room temperature the solution was used for the characterization of obtained nanoparticles.

## References

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## Results and Discussion

The aqueous silver ions were reduced to metallic silver by reaction with periodate oxidized pullulan. Formation of nanoparticles was visually monitored by changing the color of the solution from colorless to yellow brown. Ultraviolet visible absorption spectra (UV-Vis) of these solutions (Fig. 3) showed sharp absorption maxima at range 400-440 nm, relating to the plasmon resonance of silver ions.

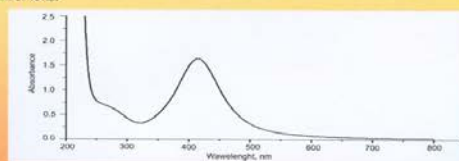


Fig. 3. UV-Vis spectrum of the solution of pullulan-based silver nanoparticles

The characterization of obtained products was performed by scanning electron microscopy (SEM) and energy dispersive spectrometry (EDX), too (Fig. 4. a, b).

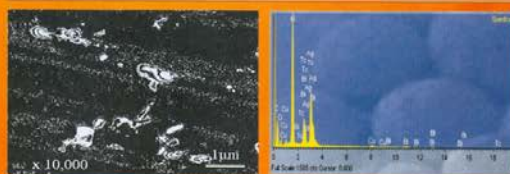


Fig. 4. SEM micrograph (a) and EDX spectrum (b) of dried sample nanoparticles obtained by reducing silver nitrate with periodate oxidized pullulan

## Antimicrobial activity of silver nanoparticles

The antibacterial activity of silver nanoparticles was tested using the strain of *Micrococcus lysodeikticus*, and it was shown significant activity against them (Fig. 5).

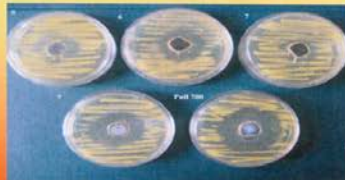


Fig. 5. Results of antibiogram test of pullulan-based silver nanoparticles using the strain *Micrococcus lysodeikticus*

\* Diameter of inhibition zone increases with the greater degree of oxidation of the pullulan chain (range 10% - 70%). I.e. higher number of the -CHO groups affects on an increased formation of nanoparticles by reduction of silver salt to nano Ag<sup>0</sup>.

## Conclusion

The synthesized pullulan-based silver nanoparticles is promising candidate for a wide range applications, among them is very important food industry, as food packaging products, as barrier materials and antimicrobials for prevention of food spoilage.

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