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Biosynthesis of dextran by *Weissella confusa* and its *In vitro* functional characteristics

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ABSTRACT

The aim of this study was to monitor the influence of the fermentation conditions on the exopolysaccharides (EPS) biosynthesis. For this, different culture media compositions were tested on an isolated lactic acid bacteria (LAB) strain, identified by 16S rDNA sequence as being *Weissella confusa*. It was proved that this bacterial strain culture in MRS medium supplemented with 80 g/L sucrose and dissolved in UHT milk produced up to 25.2 g/L of freeze-dried EPS, in static conditions, after 48 h of fermentative process. Using FTIR and NMR analysis, it was demonstrated that the obtained EPS is a dextran. The thermal analysis revealed a dextran structure with high purity while GPC analysis depicted more fractions, which is normal for a biological obtained polymer. A concentration up to 3 mg/mL of dextran proved to have no cytotoxic effect on normal human dermal fibroblasts (NHDF). Moreover, at this concentration, dextran breaks up to 70% of the biofilms formed by the *Candida albicans* SC5314 strain, and has no antimicrobial activity against standard bacterial strains. Due to their characteristics, these EPS are suitable as hydrophilic matrix for controlled release of drugs in pharmaceutical industry.

1. Introduction

Exopolysaccharides (EPS) are extracellular biomacromolecules, who have received special attention in the last decade due to numerous applications, such as in the pharmaceutical, medical and food industries. These biopolymer are generally recognized as safe (GRAS) for human health and are ideal candidate for food industry, being used as gelling, emulsifying, stabilizing and thickening agents [1]. EPS produced by lactic acid bacteria (LAB) have immune-modulatory, antitumor, anti-inflammatory and immune-stimulator effects, and act as oxidizing agents. Their structural properties determine the biological activity and technological applications [2], therefore more knowledge about EPS isolation techniques, chemical composition and structure are of interest for the potential applications.

Dextran is an EPS biosynthesized by several types of lactic acid bacteria, such as *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Streptococcus mutants* and *Weissella confusa*. Depending on the strain and the composition of the culture medium, it can be obtained with a low or high molecular weight (10–150 kDa) [3].

This biopolymer is a very complex glycan composed of units of α-D-glucose with α-(1 → 6) linear bonds and different percentages of α-(1 → 4) α-(1 → 3) and α-(1 → 2) branches. The branching degree depends on the nature of dextransucrase biosynthesized by the microbial strain. This enzyme hydrolyses the glycosidic bond in sucrose, releasing glucose which is further use in the biosynthesis of dextran and fructose. Both of them are involved in different metabolic processes [4]. EPS molecules are associated with one another, but can also interact with other molecules situated in their proximity, such as proteins, lipids, inorganic ions or other macromolecules found on the cell membrane surface [5].

The study of dextran obtained from the fermentation of *Weissella* spp. strains, especially *Weissella confusa (W. confusa)*, has recently entered into the attention of the scientific community. It was only in 2012 when the gene encoding (LBAE K39) of *W. confusa* biosynthesizes dextransucrase, which has a size of 180 kDa, was completely sequenced, thus becoming available for various appli-

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cations [6]. *W. confusa* is known to biosynthesis high amounts of non-digestible oligosaccharides and mainly dextran as extracellular polysaccharides [7–9]. These polymers are receiving increased attention because of both potential application as probiotics and wide range of industrial uses, especially for bakeries [10–12] and for the production of cereal-based fermented functional beverages [13].

Moreover, dextran has different applications in pharmaceutical and light industries [14]. It is used in medicine as antithrombotic agents, reducing blood viscosity and increasing its volume [15]. Dextran-based nanoparticles have applications in targeted drug therapy, where dextran is used as coating, or it can be functionalized with other compounds in order to obtain specific properties [16]. Dextran is also used as coating for protection against oxidation of metal nanoparticles [17].

The aim of this study was to find the optimum conditions for obtaining high EPS amounts by fermentative methods. For this purpose, the compositions of the culture medium were varied, and the obtained polymers were extracted, purified and characterized. The used bacterial strain was isolated from commercial yoghurt and identified by molecular biology techniques as *Weissella confusa*. In order to select the potential medical applications of the biopolymer we conducted a series of biological assays: determination of cytotoxicity on fibroblasts, antibacterial testing and the antifungal susceptibility test against one of the most known pathogen nowadays, *Candida albicans*.

2. Materials and methods

2.1. Microorganisms

The lactic acid bacteria strain coded PP29 was isolated from Romanian commercial yoghurt in the laboratories of Centre of Advanced Research in Bionanoconjugates and Biopolymers (InteliCentru) of the "Petru Poni" Institute of Macromolecular Chemistry, Iasi, and kept at −80 °C in Man Rogosa Sharpe medium (MRS) supplemented with 20% glycerol.

2.2. Molecular identification of the bacterial strain

The PP29 LAB strain was identifying by 16S rRNA gene sequence analysis. The bacterial DNA was extracted from 24 h cultures grown on MRS agar plates at 30 °C. DNA purification was made in duplicate with the Genomic DNA Purification Kit (Thermo Scientific) and the elution was made in 100 μL nuclease free water. The spectrophotometric quantification was made in NanoDrop. A 10 ng/μL dilution was made for both samples and 5 μL were used for the PCR reactions. The primer pair: 27F 5′ AGAGTTTGATCMTGGCTCAG 3′ and 1492R 5′ TACGGYTACCTTGTTACGACTT 3′ were used for 16 S rDNA gene amplification of [18]. The PCR reactions were performed in a total volume of 25 μL (12.5 μL GoTaq® Master Mix, 2.5 μL forward primer 10 mM, 2.5 μL reverse primer 10 mM, 2.5 μL nuclease free water and 5 μL DNA). The reaction mixture was first incubated for 10 min at 95 °C and then cycled for 35 times 30 s at 95 °C followed by one cycle of 4 min at 60 °C. The electrophoresis migration of the products was conducted in 2% agarose gel electrophoresis in order to verify the reaction and the possible contaminations. The PCR products were purified with Wizard SV Gel and PCR Clean-Up Kit (Promega) and a new electrophoresis migration was made with the final products in order to verify the purification and to quantify the quantity to be sequenced. Sequencing reactions were prepared using primers 27F/1492R. DNA sequencing was carried out by using GenomeLab™ Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter). The sequencing products were purified with glycogen, sodium acetate and Na_2-EDTA, as indicated by the sequencing kit and were migrated in the sequencer GenomeLab™ GeXP Genetic Analysis System with the migration program LFR-a. The sequences were interpreted, exported in Chromas Lite program (version 2.01) and examined with deposited sequences by using nucleotide BLAST program (NCBI, http://www.ncbi. nlm.nih.gov) and BLAST search tools [19].

2.3. Fermentations conditions

The strain isolation and purification was made in Petri dishes using MRS agar supplemented with 1% $CaCO_3$, incubated at 30 °C for 48 h [20].

For experimental fermentations were used three culture media denoted MDI, MDII and MDIII. The culture medium compositions were the following: **MDI**: MRS (55.3 g/L), fructose (40 g/L), glucose (40 g/L), dissolve in distillate water; **MDII**: MRS (55.3 g/L) and sucrose (80 g/L) dissolved in distillate water; **MDIII**: MRS (55.3 g/L) and sucrose (80 g/L) dissolved in UHT milk (with the following nutritional information/100 mL: energy value – 44 kcal, proteins – 3 g, lipids – 1.5 g (saturated fatty acids – 0.9 g), sugar – 4.5 g, calcium ions – 120 mg). All the fermentations were made in static (S) and dynamic conditions (D).

The culture medium was sterilized at 110 °C for 30 min and inoculated with 30% of fresh inoculum (24 h) with A_{600nm} of 0.5 [21]. The samples were incubated at 33 °C for 48 h without pH correction during fermentation, under static and dynamic conditions (at 100 rpm in an orbital incubator) [22]. Before performing the EPS extraction and purification, the culture was heated at 100 °C for 15 min in order to inactivate the enzymatic equipment capable of degrading the biopolymer [23].

2.4. EPS isolation and purification

The cells and proteins were removed by precipitation with 20% trichloroacetic acid (TCA) followed by centrifugation at 10,000 rpm for 10 min at 4 °C. The EPS were separated by precipitation with three volumes of cold ethanol for 24 h at 4 °C [22]. The EPS were collected by centrifugation at 12,000 rpm for 15 min at 4 °C, washed with ethanol three times, resuspended in double distilled water (DDW) and subjected to dialysis through a membrane with a porosity of 14,000 Da against DDW for three days at room temperature. For analysis, the EPS samples were coded as it follows: I-PP-29 – the PP29 strain fermented in MDI in dynamic conditions, I-PP-29-S – the PP29 strain fermented in MDI in static conditions, II-PP-29 – the PP29 strain fermented in MDII in dynamic conditions, II-PP-29-S – the PP29 strain fermented in MDII in static conditions, III-PP-29 – the PP29 strain fermented in MDIII in dynamic conditions, III-PP-29-S – the PP29 strain fermented in MDIII in static conditions and subjected to freeze drying process. The amount of the polymer was expressed in g of dry biopolymer per liter culture medium [24].

2.5. Gel permeation chromatography analysis

To estimate the distribution of the EPS molar masses, gel permeation chromatography (GPC) was used. Measurements (weight average of molecular weight number (Mw), the average molecular number (Mn) and the polydispersity index (PDI)) were recorded on a Polymer Laboratories System (PL-GPC 120, Varian) equipped with refractive index detector and three PL-aquagel packed columns filled with beads of porous gel composed of vinyl copolymers (cross-linked) with polymeric hydroxyl functionality (8 μm particle size and 20, 40 and 60 Å pore type), connected in series and placed in the column oven at 30 °C. The samples concentration was 0.8 mg/mL in H_2O (filtered through a cellulose filter with 0.45 μm pore size) and 0.02 M $NaNO_3$ solution was used as mobile phase with a flow rate of 1.0 mL/min. The calibration curve was made

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