EFFECTS OF CULTURE MEDIUM COMPOSITION ON BIOSYNTHESIS OF EXOPOLYSACCHARIDES

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In memory of Acad. Cristofor I. Simionescu,
for his contributions to the chemistry of cellulose
and the creation of this journal

The study investigated the influence of culture medium composition on the amount and structure of exopolysaccharides produced by the fermentation of lactic acid bacteria. It was established that the most favorable culture medium composition was the standard MRS prepared in milk supplemented with sucrose. Dynamic conditions were found more suitable for the biosynthesis of exopolysaccharides. An amount of 9 g dry polymer/L of culture medium was obtained. Gel permeation chromatography highlighted the presence of multiple exopolysaccharide fractions, mainly with a low degree of heterogeneity. FTIR, ^{1}H and ^{13}C-NMR analyses showed that dextran was the main biosynthesis product. The thermal stability of the obtained biopolymers was determined by TGA and DSC analyses. Both methods indicated a pure and stable polymer, comparable with dextran.

Keywords: lactic acid bacteria, exopolysaccharide, GPC, TGA/DSC, FTIR, NMR

INTRODUCTION

In recent years, a gradually increasing demand in the use of natural polymers for various industrial applications has led to boosting development of microbial polysaccharide production processes. Various types of microorganisms can synthesize and excrete extracellular polysaccharides. These biopolymers generally include capsular and slime exopolysaccharides (EPSs).[1] The microorganisms responsible for the biosynthesis of EPSs are lactic acid bacteria (LAB). On the other hand, LAB are considered as GRAS ("generally recognized as safe") and can inhibit the growth of different bacteria, yeasts and fungi through the production of organic acids, hydrogen peroxide, enzymes, defective phages, lytic agents and antimicrobial peptides, or bacteriocins.[2] The EPSs excreted by LAB can be regarded as a safe biological polymer and offer an alternative source of microbial polysaccharides in order to be used in the food and medical industries and others.[3]

EPSs accumulate on the microbial cell surface, providing protection of the LAB cells, stabilizing the membrane structure against external environmental factors and serving as a carbon and energy reserve. These are renewable resources, of great biotechnological importance. Microbial polymers have various industrial applications, being used in the food industry as thickeners and gelling agents,[3] in the pharmaceutical industry as hydrophilic matrix for controlled release of drugs,[4] for the development of bacterial vaccines and to enhance non-specific immunity. Also, EPSs are used to improve the water-holding capacity of soil and for its detoxification of heavy metals.[5]

The main commercial EPSs, including dextran, a biopolymer synthetized by LAB, are used as blood plasma substitutes in clinical applications, as standards for size-exclusion chromatography, and as ingredients in cosmetics, bakery and industrial frozen products.[6] LAB can also biosynthesize extracellular xanthan, gellan, glucans, pullulan, *etc*.[7]

The high demand for natural polymers has led to the development of a new research area, that of EPSs, which can be obtained from various microbial fermentations, using different compositions of the culture media.

The aim of this study has been to establish the effect of different compositions of the culture media and different fermentation conditions on the molecular weight, structure and amount of biosynthesized EPSs.

EXPERIMENTAL

Materials

The lactic acid bacterial strain (denoted as PP15) was isolated from Romanian commercial UHT milk according to a protocol published by Hwanhlem *et al.* in 2014,[8] in the laboratories of the Centre of Advanced Research in Bionanoconjugates and Biopolymers (IntelCentre), "Petru Poni" Institute of Macromolecular Chemistry, Romanian Academy. After incubation for 48 h at 30 °C, colonies showing different morphologies were selected and plated on MRS agar for strain purification processes. The pure strain was kept at -80 °C in Man Rogosa Sharpe (MRS) broth supplemented with 20% glycerol.[8]

The MRS broth, trichloroacetic acid, ethanol, calcium carbonate, agar and potassium bromide were purchased from Sigma-Aldrich. The yeast extract was purchased from Bio Springer and the sucrose, fructose and glucose were commercial ones. The UHT milk was purchased from a local market. The pullulan standard, Type P-82, Lot 01101, was purchased from Shodex Denko KK, Japan. D_2O (99.9 atom% D, density of 1.107 g/mL) and 3-(Trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP, 98 atom% D), were purchased from Euriso-top, France.

Fermentation conditions

The LAB strain was isolated from Romanian commercial UHT milk, using MRS agar supplemented with 2% $CaCO_3$ on Petri dishes, incubated at 30 °C for 48 hours. After obtaining a pure culture, the LAB strain was transferred on MRS broth supplemented with 20% glycerol and kept at -80 ºC.[8]

For EPS biosynthesis, three different culture media (MDI, MDII and MDIII) were used. The composition for MDI was the following: 55.3 g/L MRS broth, 40 g/L fructose, 40 g/L glucose, dissolved in double distilled water (DDW). The composition of MDII was: 55.3 g/L MRS broth, 80 g/L sucrose dissolved in DDW, while MDIII consisted of: 55.3 g/L MRS broth, 80 g/L sucrose, dissolved in UHT milk (characteristics of UHT milk: 44 kcal/100 mL energy value, 3 g/100 mL proteins, 1.5 g/100 mL lipids (0.9 g/100 mL saturated fat acids), 4.5 g/100 mL sugars, 120 mg/100 mL calcium ions).

The culture medium was sterilized at 110 °C for 30 minutes and inoculated with 30% of 24 h fresh inoculums, with absorbance of 0.5 at 600 nm.[9] The samples were incubated at 33 °C for 48 hours, without pH control, under static and dynamic conditions (in an orbital incubator shaker at a slow rotational speed, of 100 rpm).[10] In order to inactivate the enzymes capable of polymer degradation, the cultures were heated at 100 °C for 15 min.[11-13] Then, the samples were subjected to EPS extraction and purification.

Isolation and purification of EPSs

The first step consisted in the removal of proteins and cells by precipitation with 20% trichloroacetic acid (TCA), followed by centrifuging at 10.000 rpm for 10 min at 4 °C. The resulting supernatants were precipitated overnight at 4 °C with three volumes of cooled ethanol to perform EPS precipitation.[10] The precipitate was collected by centrifugation at 12.000 rpm for 15 min at 4 °C. The EPS were washed 3 times with chilled ethanol, re-dissolved in DDW and dialyzed (14.000 Da cut-off) against distilled water for 3 days at room temperature. The compounds were subjected to a Martin Christ dry-freezing process, in an ALPHA 2-4 LD Plus Freeze-Dryer, and the results were expressed as polymer dry mass in one litter of fermented culture medium.[14]

The names of the samples were denoted as follows: xPP15y, where: x represents the culture medium (MD) denoted I (MDI), II (MDII) and III (MDIII); y corresponds to the fermentation conditions, namely S (for static conditions) and blank (for dynamic conditions).

Gel permeation chromatography (GPC)

The number average molecular weight (Mn) and the weight average molecular weight (Mw) of the EPS samples were determined by gel permeation chromatography (GPC). 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 ºC, was used. The samples were dissolved in the mobile phase (0.02M $NaNO_3$, 0.001M NaH_2PO_4, pH 7) at 1 mg/mL, filtered through a 0.45 µm cellulose filter and injected through a manual injection valve equipped with a 100 µL loop at 1.0 mL/min. The standards used to calibrate the system were P-82 Shodex Denko pullulan standards of defined Mw, ranging from 6 to 805 kDa. All the data provided by the GPC system were collected and analyzed using Cirrus GPC software.

Nuclear Magnetic Resonance spectroscopy (NMR)

NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer equipped with a 5 mm inverse detection z-gradient probe, operating at 400.1 and 100.6 MHz for ^{1}H and ^{13}C nuclei. The ^{1}H NMR experiments were recorded with suppression of the water signal, using the standard NOESY-presaturation pulse sequence. The experiments were recorded with TopSpin 2.1 PL6 spectrometer control and processing software. For NMR analysis, the compounds were dissolved in deuterated water with TSP used as internal standard. The spectra were recorded at room temperature. Chemical shifts are reported in ppm and referred to TSP (ref. ^{1}H 0.00 ppm and ^{13}C 0.00 ppm).

Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for KBr pellets, in the range of 4000-400 cm^{-1} at a resolution of 2 cm^{-1}, on a Bruker Vertex 70 spectrometer (Bruker Optics, Germany). The pellets were prepared with a certain EPS sample/KBr ratio. The data processing was performed by means of OPUS 6.5 software (Bruker Optics, Germany). The spectral regions of interest were baseline-corrected by using the interactive concave rubber band method. The position and the estimated number of the sub-bands were determined by using the second derivative spectrum and the Savitzky-Golay algorithm with a nine-point smoothing factor. The curve fitting analysis used the mixed Gauss-Lorentz functions, where the peak position was maintained fixed and the intensity, shape and width were considered as variable parameters.

Thermal analysis – thermogravimetry (TGA) and differential scanning calorimetry (DSC)

The TGA and DTG analyses of EPS samples were performed on STA 449F1 Jupiter NETZSCH equipment. DSC measurements were performed on a Maia F3 200 DSC device (Netzsch, Germany). Approximately 10 mg of freeze-dried sample were used for the analysis. Measurements were carried out in the temperature range of 30-700 °C, applying a heating rate of 10 °C/min. Nitrogen purge gas was used as inert atmosphere at a flow rate of 50 mL/min. Samples were heated in open Al_2O_3 crucibles. The device was calibrated for temperature and sensitivity with indium, according to standard procedure.

RESULTS AND DISCUSSION

Composition of culture media and fermentation conditions

Many researchers showed that EPS amounts and properties are greatly dependent on the microorganism's type, the fermentation conditions and medium composition.[1] Also, the EPS biosynthesis depends on the type of the carbon sources, nitrogen sources, fermentation time, agitation rate and incubation temperature.

In order to monitor the effect of culture medium composition on the development of a lactic acid bacterial strain and the biosynthesis of exopolysaccharides, MRS broth – a standard culture medium for lactic acid bacteria was chosen as a growth base. In the compositions of the MRS broth, besides growth factors, 20% glucose was added as a carbon source for bacterial growth. Taking into account the literature data, according to which a concentration of 100 g/L sugars is the most favorable for EPS biosynthesis,[15] an amount of 80 g/L sugars was added to the final medium composition. On the other hand, although many sugars are used for fermentations, such as glucose, fructose, mannose, sucrose, lactose, maltose, only sucrose is able to induce dextransucrase production. The dextransucrase system can produce dextran using sucrose as carbon substrate. In certain cases, a homologous series of isomalto-oligosaccharides is produced.[16]

For the MDI, fructose and glucose were chosen in the same amount, because these two sugars are most easily degraded by LAB strains. The final composition was the following: 55.3 g/L MRS broth, 40 g/L fructose and 40 g/L glucose dissolved in DDW.

For the MDII, sucrose was chosen as carbon source, because it is a disaccharide with one molecule of glucose and one of fructose. What is notable about sucrose is that, unlike most disaccharides, it is more easily degraded by microorganisms through the hydrolytic pathway. The hydrolysis breaks off the glycosidic bond and converts sucrose into glucose and fructose. If the enzyme sucrase is added, the reaction will proceed rapidly. LAB strains biosynthesize sucrase very fast in order to use this sugar as a carbon source. The final MDII composition comprised: 55.3 g/L MRS broth, 80 g/L sucrose dissolved in DDW.

The composition of MDIII consisted of 55.3 g/L MRS broth and 80 g/L sucrose dissolved in commercial UHT milk. It was decided to add a supplementary nitrogen source to facilitate rapid LAB growth and enzyme biosynthesis. In the milk composition, proteins, lipids and 120 mg calcium ions/100 mL of milk were found. The presence of calcium is important because a dependence of

dextransucrase activity on the presence of calcium has been observed. By including 2% calcium ions in the culture media, some researchers reported that the viscosity of dextran was improved.[16]

The fermentation time is a very important factor for EPS biosynthesis, because the maximum EPS biosynthesis is recorded when LAB are in the exponential growing stage, while, during the plate stage and the decrease stage, the EPSs are used in LAB metabolism as a carbon source.[10] The literature data specify that the fermentation time also plays a role in EPS viscosity, thus, over 50 hours of fermentation led to a significant decrease in the medium viscosity.[16] Following this, the fermentation time was fixed at 48 h.

The agitation rate influences the oxygenation of LAB strains and, in this context, it is very important to know the type of LAB, if they are aerobic or anaerobic microorganisms.[10] To this end, the fermentations were carried out under static and dynamic conditions, at 100 rpm.

The incubation temperature of the LAB strains depends on LAB species and on their utilization. For EPS biosynthesis, the optimum growth temperature was fixed at 33 °C,[10] taking into account that a temperature over 30 °C increases the biosynthesis of the high molecular weight fraction.[16]

Gravimetric evaluation of EPSs

The amounts of EPS biosynthesis were initially evaluated gravimetrically by measuring the polymer dry mass (PDM) after 48 h of drying at 42 °C,[17] and the results are presented in Table 1.

Natural polymers are favored over synthetic ones due to their biodegradability, low cost and non-toxicity, but they also acquire certain limitations like microbial contamination,[18] this being the reason for the culture being inactivated before EPS extraction and purification, by heating at 100 °C for 15 min.[13]

From the strain fermentations under static conditions in MDI and MDII, no EPSs were obtained at the end of the extraction and purification processes. The EPSs were present in such low amounts that they were co-precipitated along with proteins in the phase of precipitation with TCA. From Table 1, it can be noticed that, from MDI and MDII, a small quantity of EPSs was obtained only under dynamic conditions, a higher amount being achieved for the fermentation conducted in MDII (5.05 g/L). The composition of these two culture media was dissolved in DDW.

A difference was made by MDIII, where the composition of the MDII was maintained, but the components were dissolved in UHT milk. Under static conditions, 8.0 g/L EPSs were obtained by biosynthesis, which is the highest amount recorded for these experiments. Anyway, the best results were achieved for the fermentation performed in MDIII under dynamic conditions (9.0 g/L), which are the most suitable conditions for EPS biosynthesis by the strain denoted PP15. UHT milk has a positive influence on the EPS biosynthesis; the effect is due to the protein from the milk (mainly casein), which is present under the form of spherical casein aggregates (submicelles), held together by calcium phosphate linkages. Once the casein micelles are degraded by the LAB enzymatic system, the calcium is released into the culture media and used as enzyme cofactor or in the LAB metabolism in order to influence the EPS biosynthesis.

Table 1
Amount of EPS extracted from fermented culture media

Fermentation conditions	EPS from MDI, g/L	EPS from MDII, g/L	EPS from MDIII, g/L
Static fermentations	-	-	8.0
Dynamic fermentations	2.48	5.05	9.0

Table 2
Relevant molecular parameters of EPS samples

Samples	Fraction	Mn, kDa	Mw, kDa	PDI
IPP15	Fr. 1	342,656.37	662,565.00	1.93
	Fr. 2	5,099.47	6,081.70	1.19
	Fr. 3	7.11	10.25	1.44
IIPP15	Fr. 1	1,044,796.83	1,953,770.01	1.87
	Fr. 2	21,015.18	26,461.23	1.26
	Fr. 3	107.83	141.91	1.31
	Fr. 4	6.88	8.79	1.28
IIIPP15	Fr. 1	560,236.00	1,264,950.80	2.25
	Fr. 2	566.02	1,176.15	2.07
	Fr. 3	6.30	9.63	1.52
	Fr. 4	0.12	0.29	2.00
IIIPP15S	Fr. 1	86,727.40	198,756.20	2.30
	Fr. 2	210.78	412.36	1.95
	Fr. 3	7.12	8.15	1.14
	Fr. 4	0.25	0.28	1.13

Mn – molecular number; Mw – molecular weight; PDI – polydispersity index

Molecular weight determination of EPSs

The average molecular weight of EPS depends on the monosaccharide composition and polymer conformation, along with the sucrose concentration and the other nutrients present in the culture medium. According to J. Han *et al.*,[19] the glucansucrase is the key enzyme responsible for the conversion of sucrose in the predominant dextran EPS biosynthesis, along with Leuconostoc species. The obtained data confirmed that the sucrose amount added to the culture medium determines an increase in the molecular weight of EPSs; therefore at 80 g/L of added sucrose, the estimated average molecular exceeded 2000 kDa.[19]

The weight-average molecular weight (Mw), the number-average molecular weight (Mn) and the polydispersity index (PDI) of the IPP15, IIPP15, IIIPP15 and IIIPP15S samples are presented in Table 2. The IPP15S and IIPP15S samples were not investigated because of some impediments, as explained above. It can be noticed that sample IPP15 presented three characteristic fractions, while the other samples (IIPP15, IIIPP15 and IIIPP15S) exhibited four fractions with different molecular weights. The first fraction (Fr. 1) of all EPS samples has the highest Mn, Mw, and a higher level of PDI, indicating a polydisperse biopolymer. The ratio of these two values (Mw/Mn), namely, PDI, is a measure of the width of molecular mass distribution, and is important because it influences the functional EPS properties.[20] According to the research group of Y. Yang,[20] a value of 1.05 for the PDI of the obtained EPS indicates a low degree of heterogeneity of the polymer chain length. Moreover, low values of the polydispersity index for EPSs demonstrate that these EPS molecules exit much less dispersed in aqueous solution without forming large aggregates. Taking this into account, it can be assumed that the obtained EPS formed complex aggregates.[21]

The next fraction (Fr. 2) with various molar masses had different PDI for all samples, showing monodisperse structures for samples IPP15 and IIPP15, and a polydisperse configuration for IIIPP15 and IIIPP15S. Fr. 3 had a similar GPC profile for all the studied EPSs with monodisperse molecules, with an exception, sample IIPP15, which had a higher molar mass. The polydispersity of IPP15, IIPP15 and IIIPP15 samples showed a medium molecular weight distribution, probably due to culture medium composition or the filtration process, which retained the unsolved products from the samples. The same trend was observed for fraction 4 (Fr. 4), with PDI values around 1, which indicated the presence of monodisperse polymers, where all the chain lengths were equal. Overall, the sample IIPP15 obtained under dynamic conditions displayed the highest molecular weight with an increased viscosity both in aqueous and mobile phase solutions, with a medium molecular weight distribution. The diversity in molecular weight of the EPS samples could be explained by the diversity of LAB strains, culture medium compositions and/or the EPS structures.[21] In our specific case, the diversity in molecular weight of the EPS samples is due to the presence of three different culture media (MDI, MDII and MDIII) used for the fermentation processes of one LAB strain (PP15).

Nuclear Magnetic Resonance spectroscopy (NMR) analyses

The obtained ^{1}H and ^{13}C NMR spectra showed the characteristic signals of a complex mixture of EPSs obtained from different culture media. Based on previously reported data,[6,22] and the NMR analysis of the commercially available standard, it was deduced that one of the EPSs present in the mixture was dextran.

The ^{1}H NMR spectrum corresponding to dextran is presented in Figure 1 (top spectrum). Several signals belonging to the glucose units can be observed in the region between 3.5-4.1 ppm, while the protons from position 1 of the α-(1→6)-linked glucose units appear as a doublet at 4.99 ppm. The same signal, from 4.99 ppm, could be identified in all the ^{1}H NMR spectra of the samples I to IIIPP15, as exemplified in Figure 2.

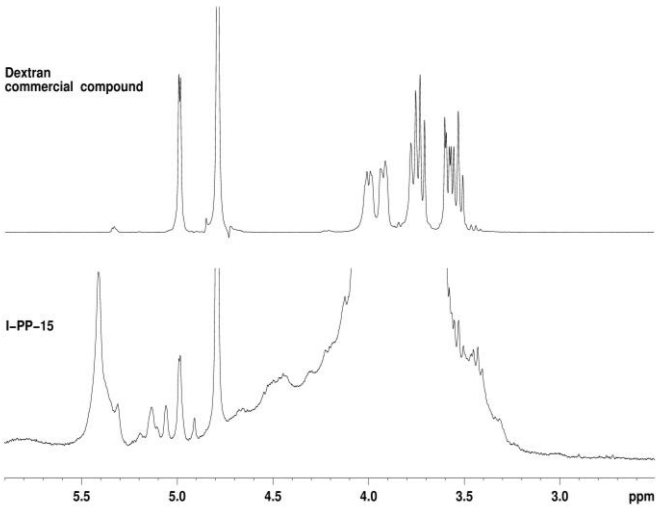

Figure 1: ^{1}H NMR spectra of a commercially available dextran sample (top) and of sample IPP15 (bottom), recorded in D_2O

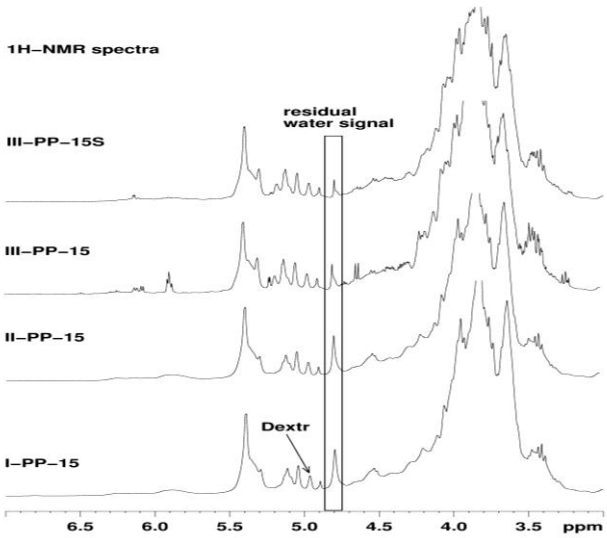

Figure 2: ^{1}H NMR spectra of samples IPP15 to IIIPP15, recorded in D_2O, with suppression of the water signal (abbreviation: dextr – dextran)

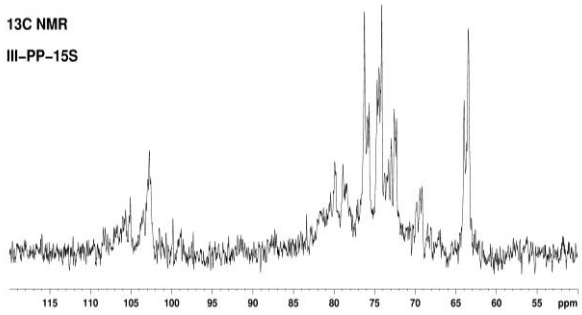

Figure 3: ^{13}C NMR spectrum corresponding to sample IIIPP15S, recorded in D_2O

In the ^{1}H NMR spectra of samples IPP15 to IIIPP15, several signals can be observed in the region between 4.86-5.50 ppm, characteristic of different types of anomeric protons. This can suggest the presence of more than one EPS structure. The number of anomeric signals is the same in all the analyzed samples, as exemplified in Figure 2, indicating the biosynthesis of similar EPSs in all the studied culture media.

The presence of the EPS mixture was supported by the ^{13}C NMR experiment as well. In the ^{13}C NMR spectrum, recorded for sample IIIPP15S, there are several signals in the region characteristic of C_1 carbons (100-110 ppm) and C_6 carbons (60-66 ppm), as presented in Figure 3. This additional information supports the initial assumption regarding the presence of the EPS in the mixture.

Fourier-transform infrared spectroscopy (FTIR) analyses

From the spectral point of view, IPP15 and IIPP15, as well as IIIPP15 and IIIPP15S samples are similar. The FTIR spectra of the two classes have similar features, such as the broad stretching vibration of the hydroxyl groups, centered at 3412 cm^{-1}, the amide I (1670 cm^{-1}) and amide II bands (1545 cm^{-1}), the succession of CH and CH_2 bending vibrations observed at 1455, 1419 and 1370 cm^{-1}, and the amide III band around 1234 cm^{-1}. Figure 4 highlights the common absorptions for the representative sample of each species, *i.e.* IPP15 (blue trace) and IIIPP15 (red trace).

The intense and broad band in the IPP15 sample with maxima around 1030 cm^{-1} is characteristic of glucopyranose residues, being a superposition of C-O-C stretching, C-OH stretching and bending vibrations. With the help of the second derivative spectra, the sub-bands characteristic of glycosidic bridges and vibrations of free hydroxyl groups can be distinguished (Fig. 5). Thus, some of the free and hydrogen-bonded primary alcoholic groups (C_6-O_6H) are identified by the $\nu(C_6\text{-}O_6H)$ vibrations at 996 and 1022 cm^{-1}, respectively. The intense peak at 1078 cm^{-1} is also connected with $\nu(C_6\text{-}O_6H)$ and $\delta(C_4\text{-}C_5)$,[23] but have some contribution from $\delta(C_1\text{-}H)$.[24] Two weak sub-bands observed at 1106 and 1127 cm^{-1} are indicative of $\nu(C_3\text{-}O_3H)$ and $\nu(C_4\text{-}O_4H)$ stretching vibrations, respectively.[25]

The peak appearing at 1151 cm^{-1}, the shoulder around 965 cm^{-1} and a distinctive band around 835 cm^{-1} are characteristic of the stretching vibrations of the glycosidic linkages in α-D-glucopyranoses.[26] In the anomeric region, the weak band around 835 cm^{-1} is a combination from the stretching of the C_1-O_5-C_5 bond and the deformation of the C_1-H, C_5-H and C_6-H bonds.[26] The second derivative of this band separates two sub-bands, located at 832 and 845 cm^{-1}. The first one would indicate the $\alpha(1\rightarrow3)$ anomeric configuration.[27] The sub-band at 845 cm^{-1} and the weak band around 930 cm^{-1} (combined ν(C-O) and ν(C-C-O) in the glycosidic link) are specific to $\alpha(1\rightarrow6)$ glycosidic linkages,[28] so the dominant structure seems to be a dextran type.

The sub-band at 1055 cm^{-1} has contributions from $\nu(C_2\text{-}O_2H)$,[25] and is also specific to α-D-glucoses.[24] The 1300-1200 cm^{-1} region is dominated by the amide III band, peaking near 1234 cm^{-1}. The underlying components are the bending vibrations of the C_6-O_6-H and O_6-C_6-H bonds, located around 1274 cm^{-1}, and the bending vibrations (δ(O-CH) and δ(CCH)) of the ring – hydrogen atoms that contribute mainly to the sub-band at 1263 cm^{-1}.[26]

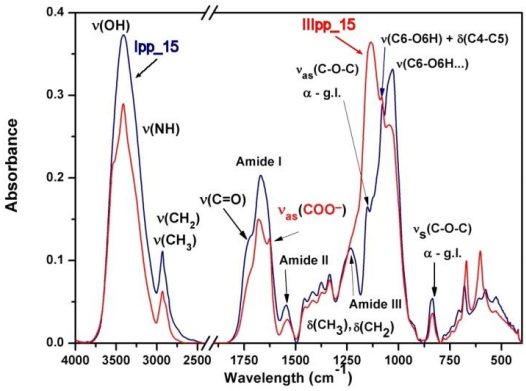

Figure 4: Infrared spectra of IPP15 (blue trace) and IIIPP15 (red trace)

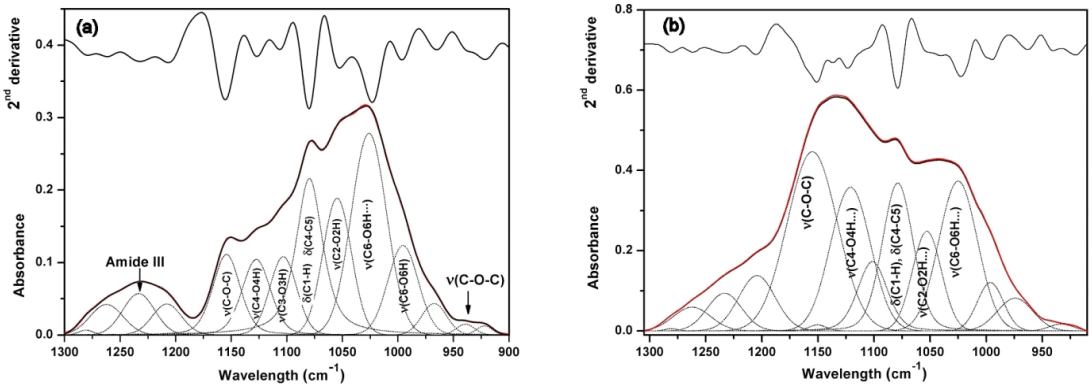

Figure 5: Curve fitting analysis of FTIR spectra of IPP15 (a) and IIIPP15 (b) in the region of characteristic vibrations of glucopyranose residues. The red line represents the overall fit curve. The second derivative spectrum is added at the top of each figure

In the spectrum of IIIPP15, the "sugar" band has a completely different profile, with the main maxima centered at 1135 cm^{-1}. The second derivative spectrum is more complex than that of IPP15 and indicates some new structures. The decrease of the sub-band assigned to the bending vibration at the C_3 position (1104 cm^{-1}) and broadening of ν(C-O-C) of the glycosidic linkage (1155 cm^{-1}) are accompanied by the red-shift with +4 cm^{-1} of the bending vibration at the C_4 position (1127 cm^{-1}). The appearance of the new sub-band at 1135 cm^{-1} might arise from the formation of new inter-unit links of the C_1-O-C'$_4$ types. Two new peaks have emerged at 1681 and 1626 cm^{-1}, and may be connected to ν_{as}(C=O) in an amine unit, and to ν(C=O) in a carboxylate group, respectively. The sharp peaks observed around 3550 and 3413 cm^{-1} would correspond to the ν(NH_2) vibration. The above observations suggest that the third culture medium led to the development of another exopolysaccharide bearing α(1→4) glycosidic linkages, in addition to the dextran structural type.

Thermal analyses (TGA and DSC)

TGA is a versatile analytical tool that measures weight loss of a material as a function of temperature.[5] Figure 6 presents the TGA curves of IPP15, IIPP15, IIIPP15 and IIIPP15S samples. Analyzing the curves from Figure 6, it can be noticed that all the samples exhibit a similar thermal degradation pattern, like that of natural dextran, which degrades in two stages.[29]

In our case, the first stage of EPS thermal degradation (Fig. 6) ranges between 60-126 °C, with a mass loss from 12.65 to 14.23%, which is attributed to physical and chemical loss of moisture from the analyzed materials.[30] The second major stage, from 197 to 304 °C (28.00-55.50% mass loss), corresponds to random polymer backbone scissions. Figure 7 presents the second heating curves of the studied samples. It may be noticed that the studied structures exhibit a glass transition temperature domain (T_g) in the range of 116-220 °C. This is in good agreement with the findings by Scandola M. *et al.*,[31] who reported a T_g value of 223 °C for natural dextran.

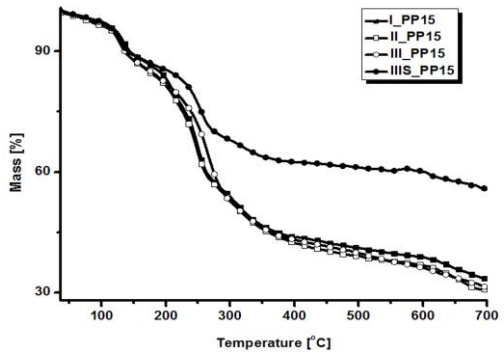

Figure 6: TG and DTG curves of EPSs

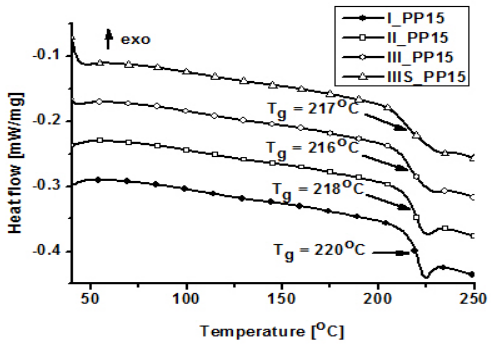

Figure 7: Second heating curves of studied EPSs

CONCLUSION

The isolated LAB strain produced the highest amount of EPS under aerobic conditions in the MDIII culture medium (9.0 g dry polymer/L culture medium).

The IIPP15, IIIPP15 and IIIPP15S samples exhibited four fractions with different molecular weights, while the IPP15 sample presented three characteristic fractions. The first fraction of each EPS sample was characterized by high Mn, Mw and PDI characteristics. The second and third fractions showed a low PDI, indicating low polydispersity of the sample, even if the fractions possessed different Mn.

In the ^{1}H NMR spectra of the EPSs produced by the LAB strain in different culture media, several signals in the region of 4.86-5.50 ppm can be observed. These signals are characteristic of different types of anomeric protons, suggesting the presence of more than one EPS structure. The ^{1}H NMR analysis, in correlation with FTIR analysis, demonstrated that the dominant structure in the EPS biopolymers is dextran.

It should be mentioned that the IIIPP15 sample had a completely different FTIR profile, suggesting that the MDIII culture medium led to the appearance of $\alpha(1\rightarrow4)$ glycosidic linkages, in addition to the dextran structural type.

Thermal analyses (TG, DTG and DSC) revealed that all the samples exhibited a similar thermal degradation pattern to that of natural dextran.

Due to their characteristics, the obtained EPSs are a suitable candidate for the development of different hydrophilic matrices for controlled drug delivery.

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