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Research article

Chemical and Thermal Characterization of an exopolysaccharide from *Lactiplantibacillus plantarum* BAL-29-ITTG

Caracterización Química y Térmica de un exopolisacárido-proveniente de Lactiplantibacillus plantarum BAL-29-ITTG

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Abstract. - *Exopolysaccarides (EPS) are biopolymers, which can be produced by lactic acid bacteria. In this work an EPS from Lactiplantibacillus plantarum BAL-29-ITTG was characterized by ^{1}H, ^{13}C, COSY, TOCSY and HSQC nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and viscometry. Thermal analysis and viscometry results suggested that ESP had a high molecular weight with a branched structure; to determine its main monosaccharides, the experimental chemical shifts of hydrogens and carbons obtained by NMR were loaded and compared with the database in the online software CASPER: http://www.casper.organ.su.se./casper/ Results showed that at least eight monosaccharides are present as components of this EPS, the most likely monosaccharides identified were: β-D-glucopyranose 1-4 and 1-6 linked: →4)-β-D-Glc-(1→; →6)-β-D-Glc-(1→ and α-D -manose 1-3, 1-4 and 1-6 linked: →3)-α-D-Man-(1→; →4)-α-D-Man-(1→; →6)-α-D-Man-(1→, although, data from FTIR and NMR also suggest N-acetylated residues.*

Keywords: Exopolysaccharides; Monosaccharides determination; Nuclear magnetic resonance; Polysaccharides composition; Polysaccharides characterization.

Resumen. – *Los exopolisacáridos (EPS) son biopolímeros que pueden ser producidos por bacterias ácido lácticas, En este trabajo, un EPS proveniente de Lactiplantibacillus plantarum BAL-29-ITTG fue caracterizado mediante resonancia magnética nuclear (RMN) de ^{1}H, ^{13}C, COSY, TOCSY y HSQC, espectroscopía de infrarrojos (FTIR) calorimetría diferencial de barrido (DSC), análisis termogravimétrico (TGA) y viscosimetría. Los resultados de análisis térmicos y viscosimetría indican que este EPS tiene una estructura ramificada y una masa molar alta; para determinar los monosacáridos principales, los desplazamientos químicos de carbono e hidrógeno obtenidos mediante RMN fueron cargados y comparados con la base de datos del software en línea CASPER: http://www.casper.organ.su.se/casper/ Los resultados mostraron que, al menos, ocho monosacáridos diferentes componen este EPS, los más probables identificados fueron: β-D-glucosa con uniones 1-4 y 1-6: →4)-β-D-Glc-(1→; →6)-β-D-Glc-(1→ y α-D-manosa con uniones 1-3, 1-4 y 1-6: →3)-α-D-Man-(1→; →4)-α-D-Man-(1→ y →6)-α-D-Man-(1→, aunque los datos de FTIR y RMN sugieren también la presencia de residuos N-acetilados.*

Palabras clave: Exopolisacáridos; Determinación de monosacáridos; Resonancia magnética nuclear; Composición de polisacáridos; Caracterización de polisacáridos.

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1. Introduction

Carbohydrates are some of the main components in living organisms. Monosaccharides can be in lineal or in cyclic form, for the last one, anomers α or β are possible. Polysaccharides are formed through glycosidic linkage between many monosaccharides, for this reason they also are named glycans, because they have many hydroxyl groups in their composition, the linkage can be at different positions, more detailed information on this can be found in the work of Flitsch [1]. On the other hand, they generally are attached to other biomolecules forming glycoconjugates, for example, with proteins or lipids form glycoproteins or glycolipids respectively. For the mentioned above, their complete chemical structure characterizations can be highly complex.

Exopolysaccharides (EPS) are extracellular biopolymers that can be produced by plants, fungi or bacteria [2, 3]. Bacterial polysaccharides also included lipopolysaccharides (LPS), peptidoglycans, teichoic acids (TA), capsular polysaccharides (CPS) [4]. Although EPS can also be extracted from plants, bacterial EPS have more diverse structures, therefore more diverse properties and bioactivities, hence, they have recently received significant attention in different fields of science and technology for different applications, for example in medicine or food package [2, 5]. These biopolymers are mainly composed of repetitive sugar units and, depending on their location, could be found in capsular form, they are closely associated with the cell surface and in free form, weakly bound or even totally secreted into the extracellular environment. According to their chemical composition, EPS are classified into homopolysaccharides, which are made up of units of a single type of monosaccharide such as glucose or fructose and heteropolysaccharides, which are made of two or more different monosaccharide units (glucose, xylose, fructose, mannose, galactose, rhamnose, *N*-acetylglucosamine, gluronic acids, etc.) having different chemical structures and linkages, [2, 5, 6].

The number of investigations dedicated to the applications of bacterial EPS has increased in recent decades. Due to bacterial EPS possess various unique beneficial properties such as, high adhesive capacity, biocompatibility, biodegradability, gelation ability, non-toxicity, pseudoplasticity, viscoelasticity and thixotropic nature [2, 7]. EPS have also been reported to withstand various environmental stresses, such as high temperature, high pH, freezing, thawing, or high salt concentrations [8], therefore, they have wide commercial applications, for example, in the food packages, pharmaceutical and cosmetic industries, among others [2]. Furthermore, some bacterial EPS also possess antitumor, antioxidant, antibiofilm, anti-inflammatory, antibacterial, antiviral, cholesterol-lowering, prebiotic, wound healing or immunomodulatory activities [9, 10, 11].

As mentioned before, EPS can be extracted from different sources but, particularly lactic acid bacteria (LAB) are microorganisms present in food production and in turn these are beneficial to human health. [12, 13]. In general, LAB are widely recognized as safe, due to this they are mainly used in fermented foods [14]. LAB exhibits a variety of probiotic effects, which are closely related to their metabolites, including organic acids, bacteriocin, exopolysaccharides (EPS) and others [15, 16, 17]. However, the development and application of EPS from BAL are relatively limited compared to polysaccharides derived from animals and plants [18].

The bioactivities presented by the EPS produced by the BAL are related to their structure. Mainly, the monomeric composition, molecular weight

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and charge of EPS depend on the bacteria, likewise, the biological functions such as antioxidant, antitumor and antibiofilm activity depend on the composition and structure of EPS [19, 20]. Certain structural features may be associated with specific bioactivities. For example, the presence of α-(1 → 3) and α-(1 → 6) linkages in the main chain and α-(1 → 3) linkages in the branched chains of the EPS produced by L. plantarum (LAB) has been associated with immunomodulatory activity [19].

Jiang and Yang, reported that, although EPS produced by lactic acid bacteria (LAB) contain similar monosaccharide components (galactose, glucose, rhamnose, etc.), these had different properties owing their structural difference [21]. They also reported that, these properties were affected by their molecular weight distribution, the type of glycosidic bond, their charge, side chains, the rigidity of the molecules that form the EPS, among others; regarding this, it was mentioned that the structural characteristics, uses and bioactivities of EPS depend on the type of microorganism and the medium in which they were grown up [2, 5]. Because bacterial EPS have many functional groups, for example, hydroxyl, carboxyl, carbonyl or acetyl, which allow their modification to obtain new properties [8].

Ramírez-Pérez et al. reported that LAB isolated from a fermented beverage, known as "taberna" (*Lactiplantibacillus plantarum BAL-03-ITTG*, *Lactiplantibacillus plantarum BAL-05-ITTG*, *Limosilactobacillus fermentum* BAL-21-ITTG, *Lactiplantibacillus pentosus* BAL-22-ITTG, *Lactiplantibacillus fabifermentans* BAL-27-ITTG, *Lactiplantibacillus paraplantarum* BAL-28-ITTG, *Lactiplantibacillus plantarum* BAL-29-ITTG), can use different carbon sources and, when they are cultured in MRS broth (de Man, Rogosa y Sharpe), these can be produced as free and capsular EPS. The highest production of ERPS from *Lactiplantibacillus plantarum* BAL-29-ITTG, was 478.0 ± 16.97 mg L^{-1}) [22]. On the other hand, the EPS production from *Lactiplantibacillus plantarum* BAL-29-ITTG was also evaluated using modified MRS broth at different conditions such as carbon source (sucrose and lactose), concentration (10 and 30 g/L), nitrogen source and concentration (yeast extract and ammonium sulphate, 5 and 15 g L^{-1}), temperatures (20 and 40 °C) and agitation (0 and 150 rpm) [23], an experimental design of Plackett Burman was used, some exopolysaccharides obtained exhibited higher antioxidant and antibiofilm activity against *E. coli, S. aureus and P. aeruginosa* with values between 23 and 77%. Under optimal conditions the production of *Lactiplantibacillus plantarum* BAL-29-ITTG, was 619.66 ± 83.21 mg L^{-1}. In this work, the culture medium corresponding to the optimal conditions was selected and used [23], *Lactiplantibacillus plantarum* BAL-29-ITTG was grown in a stirred tank fermenter and the extracellular EPS was characterized by viscometry to obtain its molecular weight, by thermal analysis to determine its thermal transition such as glass transition temperatures (T_g) or melting point (T_m) and by FTIR-ATR and NMR to obtain its main monosaccharides composition.

2. Methodology

2.1 Biologic material and strain recollection

L. plantarum BAL-29-ITTG was obtained from the Research Laboratory, Instituto Tecnológico de Tuxtla Gutiérrez, Chiapas, México. The strain was maintained in glycerol (30% v/v) at -18°C, it was reactivated by two successive soup MRS (De Man, Rogosa y Sharpe), using a (10 % v/v) of inoculum. The vessels or tubes were incubated for 12 h at 37 °C and stirred at 110 rpm using a LumistellMR IRO 65 stirrer with controlled temperature. The Applikon® Biotechnology

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model ez2-control bioreactor containing MRS broth was then inoculated and the effect of aeration (0 vvm and 1 vvm) and agitation (150 rpm and 250 rpm) was evaluated. After 24 h of incubation. The cultures were centrifuged at 4500 rpm for 30 minutes at 4 °C, two volumes of cold absolute ethanol were added to the obtained free cells soup, these were shaken vigorously and then incubated for 12 h at 4 °C. The precipitated EPS were dissolved using ultrapure water and dialyzed for two days using Spectra/Por 1, MWCO 6-8 kD membranes, water was changed each 8 h, EPS were freeze-dried at 0.860 bar a -40 °C using a LABCONCO FreeZone4.5 freeze dryer.

2.2 EPS characterization

Molecular weights were calculated by viscometry using a manual glass Ubbelohde viscometer, the temperature was maintained at 20 °C using an oil bath. EPS solutions were prepared at five different concentrations, from 1 to 5 mg mL^{-1}, using distilled water as solvent, once the temperature was constant, the flow times were recorded and the relative viscosity (η_{rel}) was calculated using the equation 1, specific viscosity (η_{sp}) was calculated using the equation 2, reduced viscosity (η_{red}) was calculated using the equation 3, inherent viscosity (η_{inh}) was calculated using the equation 4 and, the Mark-Houwink Sakurada (equation 5) was used to calculate the viscosity average molecular weight (M_v)

$$\eta_{rel} = t/t_o \quad (1)$$

$$\eta_{sp} = \eta_{rel} - 1 \quad (2)$$

$$\eta_{red} = \eta_{sp}/C \quad (3)$$

$$\eta_{inh} = Ln(\eta_{rel})/C \quad (4)$$

$$\eta = K \bullet M^{\alpha} \quad (5)$$

where t is the time of flow of each sample, t_0 is the time of flow of the solvent, C is the concentration (g mL^{-1}), η is the intrinsic viscosity at zero concentration, α and K are empirically determined constants.

Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (FTIR-ATR) was recorded from 650 to 4000 cm^{-1} with 16 scans using a Fourier Transform Perkin Elmer (Spectrum 400, Walthem, MA, USA) in Attenuated Total Reflection mode (ATR) using a diamond/ZnSe crystal with single reflection.

Differential Scanning Calorimetry (DSC) was carried out using a Modilated DSC, TA Instruments model Q2000. Samples were cooled to -30 °C, an isothermal was maintained for 5 min; afterward the temperature was modulated to +/−1 °C enery 60 s; and then, a heat ramp of 10 °C min^{-1} up to 180 °C under nitrogen atmosphere was applied. Two cycles were recorded, the results showthe second one. Glass transition temperatures (*Tg*) were calculated by using the Universal Analysis 2000 software from TA Instruments. Decomposition temperatures (*Td*) were measured by thermogravimetric analysis (TGA) using a TA Instrument, Discovery model, Mew Castle. DE, USA. A heat ramp of 20 °C mil-1 was used from 20 up to 600 °C with a nitrogen flow of 50 mL min^{-1}.

Nuclear magnetic resonance (NMR) data were obtained at 25 or 70 °C using a Bruker AVANCE III HD NMR spectrometer operating at 400.13 or 100.62 MHz for ^{1}H and ^{13}C respectively. 20 mg of each sample were dissolved using 0.5 mL of deuterium oxide (D_2O) 99% from Merk, Toluca

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México, after that, samples were lyophilized and dissolved again using 0.5 mL of D_2O as solvent.

The main monosaccharides of this EPS was selected by comparison between experimental data from NMR and the database from the software online CASPER [24], for this, the main chemical shifts obtained by ^{1}H, ^{13}C, COSY, TOCSY and HSQC NMR spectroscopy were loaded and, once the software CASPER generated the most probable monosaccharides moieties, the ones with the lower error and agreement with the data from FTIR were selected.

3. Results and discussion

3.1 Molecular weight

Five different concentrations of EPS were prepared from 1 to 5 mg mL^{-1} using distillated water as solvent, the times of flow were measured at 20 °C using a Cannon-Ubbelohde viscometer. Once the intrinsic viscosity was determined by Huggins and Kramer equations (**Figure 1**), the viscosity average molecular weight was calculated using the Mark-Houwink equation, constant from dextran, K = 0.0443 mL g-1 and α = 0.043, were used. In **Table 1** the times of flow and different viscosities are shown. The intrinsic viscosity calculated was 12.19, therefore the M_n calculated was 471.8 kDa, this value was in the same order of magnitude as an EPS isolated from *Limosilactobacillus reuteri* C66 (370 kDa) [25]. The low intrinsic viscosity and high molecular weight suggest a compact structure in solution.

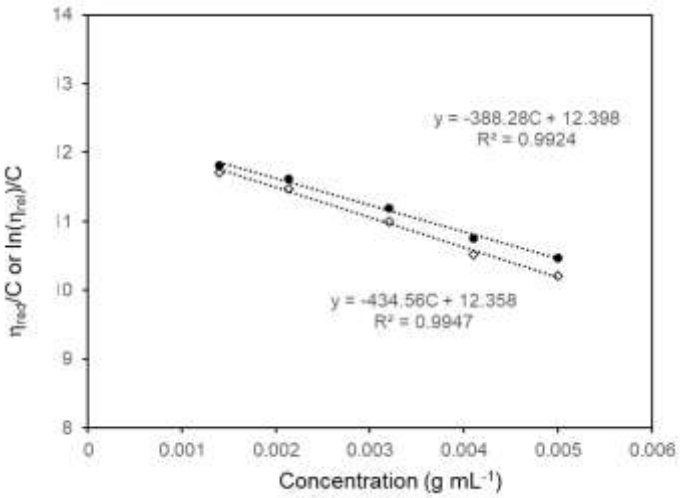

Figure 1. Plot of: (•) reduced viscosity (Huggins) and (◊) inherent viscosity (Kramer).

Table 1. Data to obtain the intrinsic viscosity of the EPS

Concentration (g/mL)	Time of flow* (s)	η_{rel}	η_{sp}	η_{red} (mL/g)	η_{inh}
0.00000	121.0	-	-	-	-
0.00142	123.0	1.0165	0.0165	11.6401	11.5449
0.00213	124.0	1.0248	0.0248	11.6237	11.4820
0.00320	125.3	1.0358	0.0358	11.1915	10.9957
0.00400	126.3	1.0441	0.0441	11.0193	10.7833
0.00500	127.3	1.0523	0.0528	10.4683	10.2036

*Average of three measurements

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3.2 Composition

3.2.1 FTIR-ATR analysis

FTIR-ATR spectrum of EPS (**Figure 2**) showed de characteristic absorption bands, the corresponding to the OH was centered around 3266 cm^{-1}, the stretching vibration of the methylene groups ($-CH_2$) was observed at 2925 cm^{-1}, the vibration of carboxylic groups (C=O) at 1644 cm^{-1} characteristic of acetyl group *N*-linked or stretching vibration of mannose [26] which corresponds with the observed by NMR and suggested by CARPER, a band with low intensity at 1531 cm^{-1}, suggest *N*-acetylated residues [27], the N-H vibration above 3200 cm^{-1} was not observed, however, a strong band at 1219 cm^{-1} was attributed to acetyl groups [28] and, pyranose ring [29]. The ether groups (C-O-C) were assigned at 1030 and 1049 cm^{-1}. In the anomeric region, the band at 879 cm^{-1} could be due to β-glycosidic linkage between monomers [30], the band at 860 cm^{-1} could be attributed to β-D-glucopyranose, the corresponding to α-D-glucopyranose was not observed [31], the three bands at 913, 879 and 778 cm^{-1} corresponding to β-D-glucose [28], the two bands at 913 and 778 cm^{-1} could be due to α-polyglucosanes with (1:6-linkages) [31]. The band at 811 cm^{-1} could be due to manose [28]. These bands indicate that the EPS contained mainly β glycosides linkages in its structure, this data also is agreement with data obtained from NMR (**Table 2**).

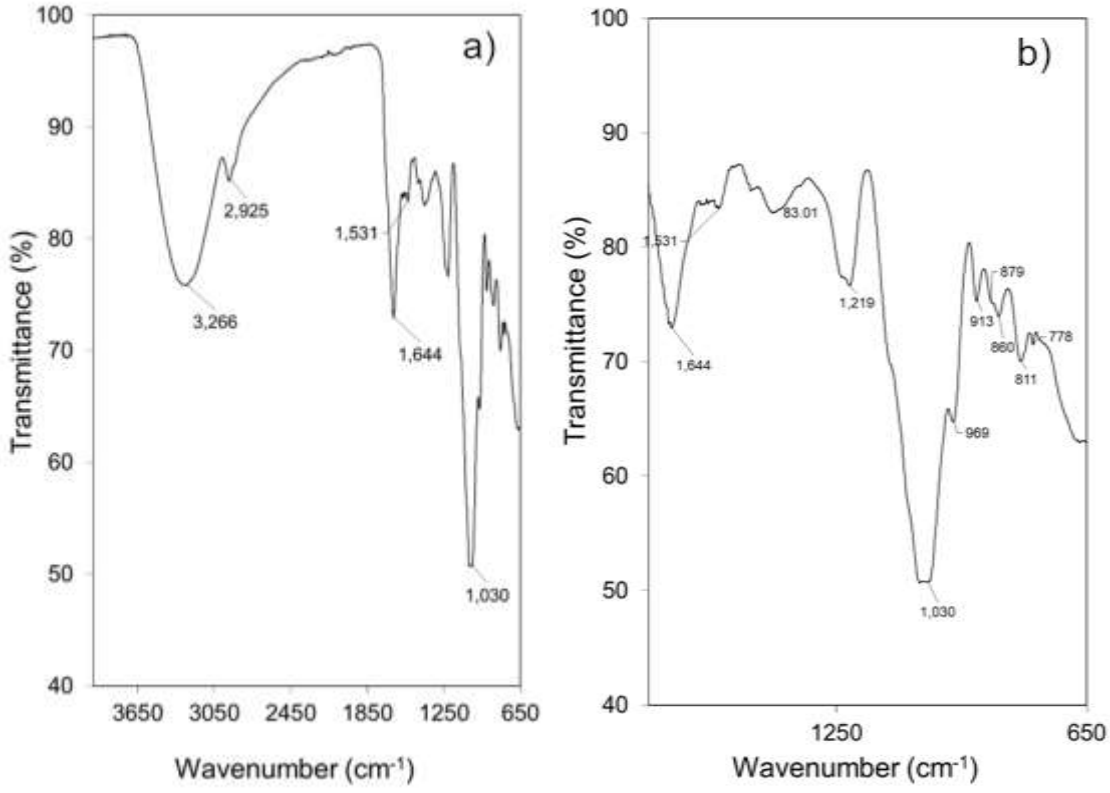

Figure 2. FTIR-ATR spectrum of the EPS and amplification of the anomeric region.

3.2.2 Thermal characterization

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Thermo-gravimetric analysis showed four main steps of weight loss (**Figure 3**), the first one at temperature below 150 °C indicated moisture [32], but at lower temperature, around 43 °C, loss weight could be attributed to residual ethanol from the extraction method [33]. A rapid degradation started at 220 °C, this showed a maximum around 265 °C, followed by another with a maximum at 340 °C, after that, the degradation continued gradually, the final residue at 600 °C was 35.8%, it is known that EPS can have ionizable functional groups [2] such as phosphate or sulphate [4] then, this high percentage can be due to inorganic material. Similar values have been observed, for example, in EPS from L. Plantarum CNPC003 [34]. On the other hand, by modulated differential scanning calorimetry, two glass transition temperatures were observed (**Figure 4**) the first one, at low temperature (~7-8 °C), could be due to branching, whereas the second one, at high temperature (~157 °C) was attributed to the mail chain [33], these branched structure could explain its high molecular weight calculated by viscometry.

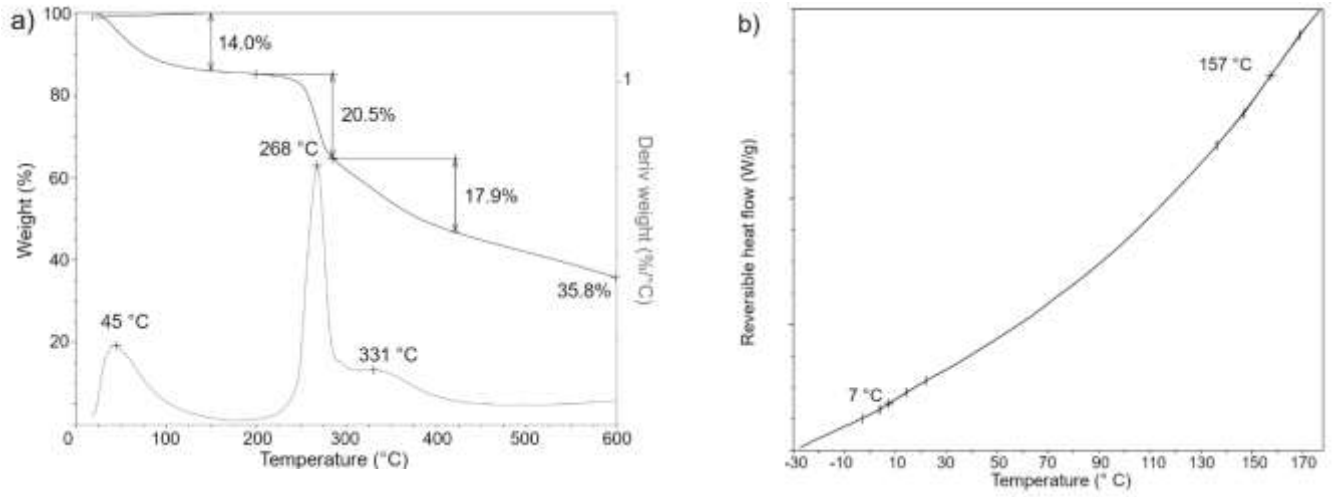

Figure 3. Thermo-Gravimetric Analysis and Differential Thermal Analysis (a) and, Differential Scanning Calorimetry of the obtained EPS (b), lower value corresponding to the *Tg* of branched structure and higher one corresponding to *Tg* of the main chain.

3.2.3 Nuclear Magnetic Resonance

To decrease the signals from hydroxyl groups, sample was first dissolved using D_2O to exchange hydrogens atoms bonded to heteroatoms and then. It was lyophilized, the sample was dissolved again using the same solvent and NMR spectra were recorded. For ^1H NMR the spectra were recorded at 25 or 70 °C, the last one because many NMR spectra available have been recorded at this temperature and, the chemical shift from DHO moves to higher field [35] allowing a better observation of the anomeric region, reported chemical shifts are relative to the signal of HDO which appeared at 4.71 ppm at 25 °C but, it appeared at 4.30 ppm at 70 °C, although, the values of chemical shifts from EPS were similar at both temperatures. Water suppression was applied to observe the anomeric region between 4.4 and 5.6 ppm, the hydrogens H2-H6 were observed between 3.2 and 4.4 ppm (**Figure 4**), similar patterns have been observed for other EPS [17], finally weak signals were observed between 0.8 and 2.4 ppm which can be assigned to aliphatic moieties.

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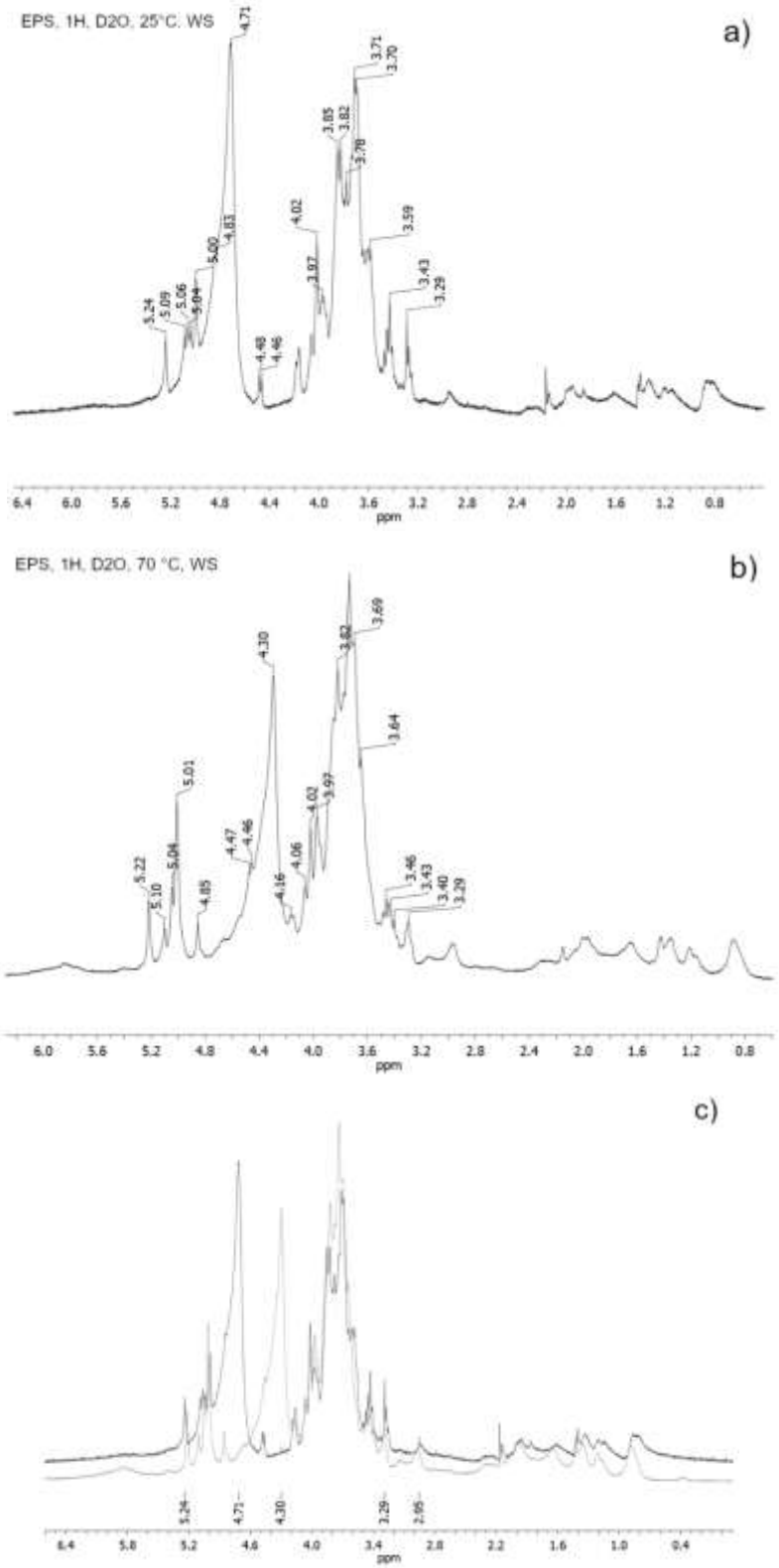

Figure 4 ^{1}H NMR spectra with water suppression at a) 25 °C, b) 70 °C and c) overlapping spectra at 25 °C (dark line) and 70 °C (light line)

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^{13}C NMR spectrum was recorded (**Figure 5**), in the anomeric region of the ^{13}C spectrum, between 98 and 106 ppm, eight signals were observed, this result indicated that, this EPS is a heteropolysaccharide with a complex structure. For an EPS from Limosilactobacillus fermentum D12, seven anomeric carbons were observed, these were assigned to galactofuranose and glucopyranose residues [36]. To the correct assignation, 2D ^{13}C-^{1}H HSQC NMR spectrum was recorded at 70 °C (**Figure 6**), in this spectrum, eight correlations in the anomeric region were also observed (**Figure 6a**).

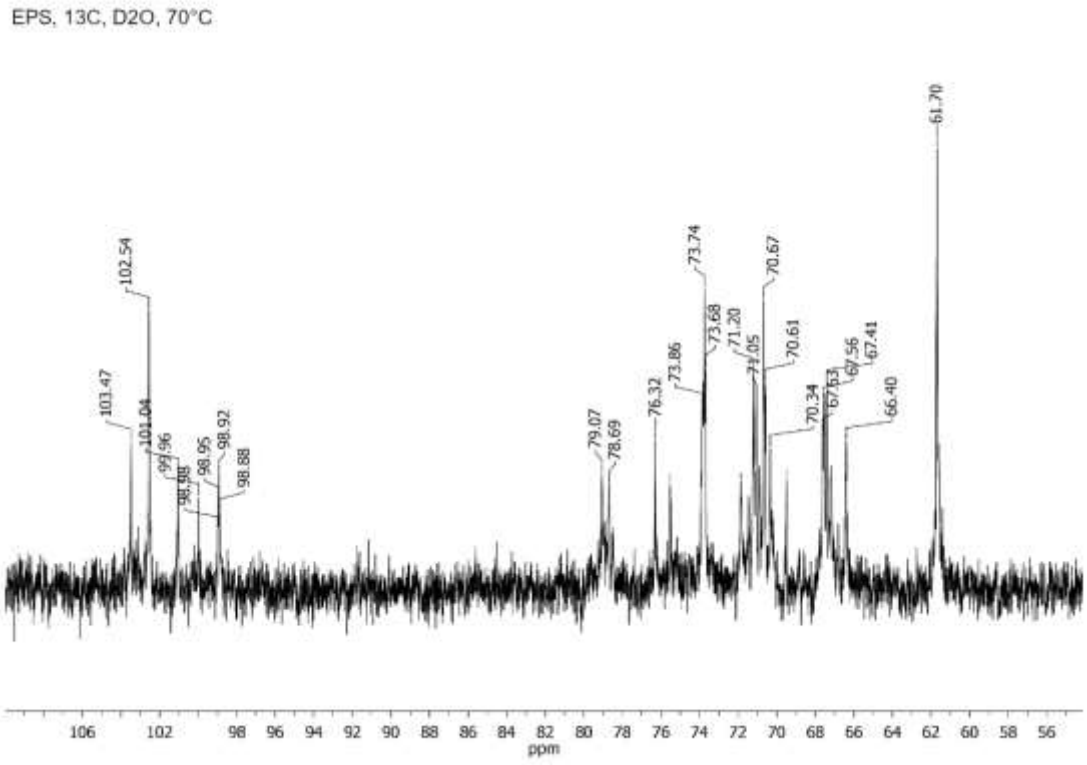

Figure 5. ^{13}C NMR spectrum of EPS.

Correlation spectroscopy H-H (COSY) was recorded at 70 °C (**Figure 7a**). No correlation was observed between the region of sugar rings and the signals below 2.5 ppm, taking into consideration the observed in the FTIR spectrum, these last signals could be due to methyl from *N*-acetyl groups. On the other hand, in the anomeric region, only seven correlations from H1-H2 could be observed (**Figure 7b**), the hydrogen of the anomeric carbon at 4.49 ppm showed the highest intensity, therefore, the complete spin system, H2-H3, H3-H4, H4-H5, H5-H6 and H5-H6' could be assigned by COSY. Chemical shifts are shown in **Table 2**.

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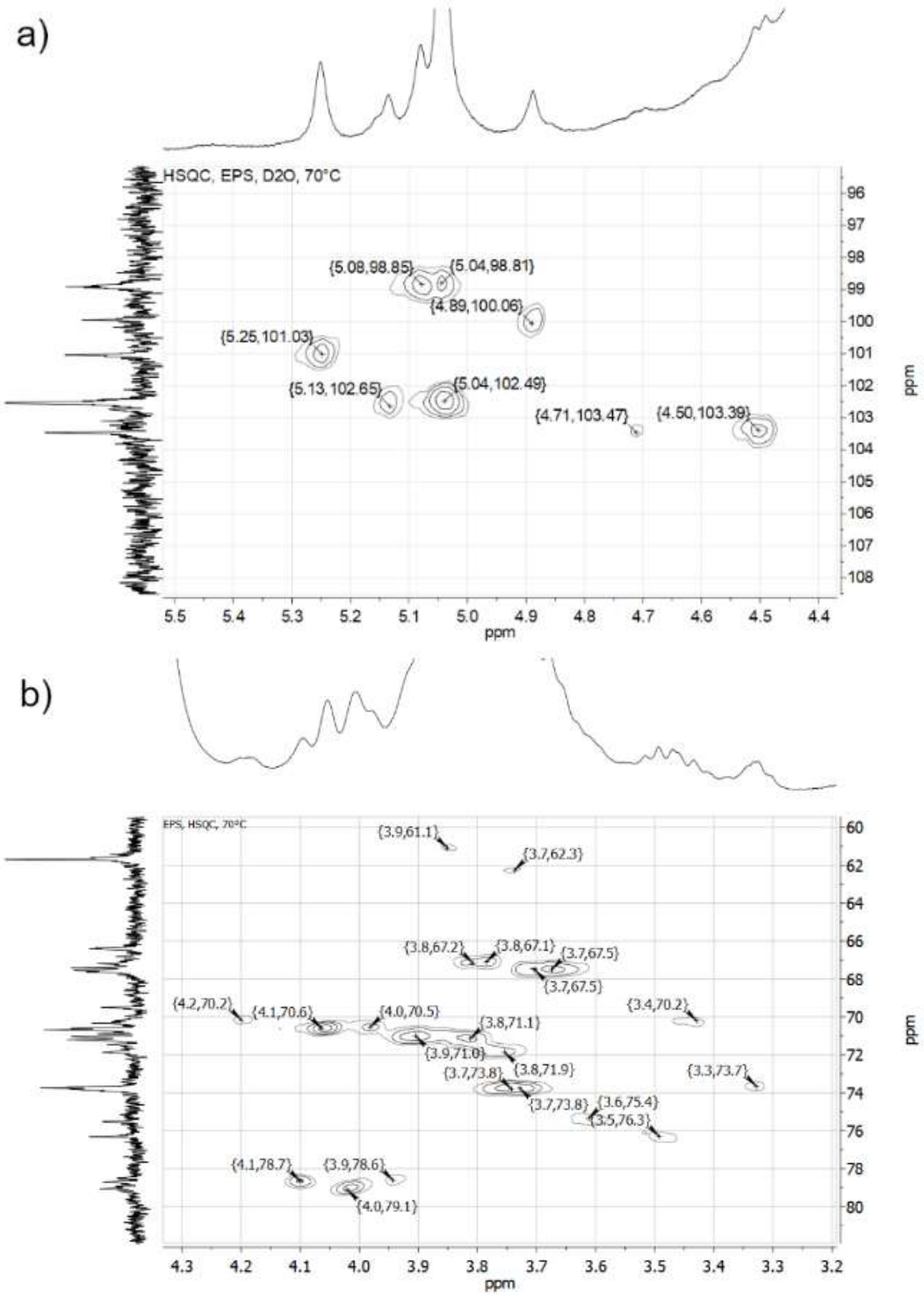

Figure 6. ^{1}H-^{13}C HSQC spectroscopy a) anomeric region and b) saccharide ring region.

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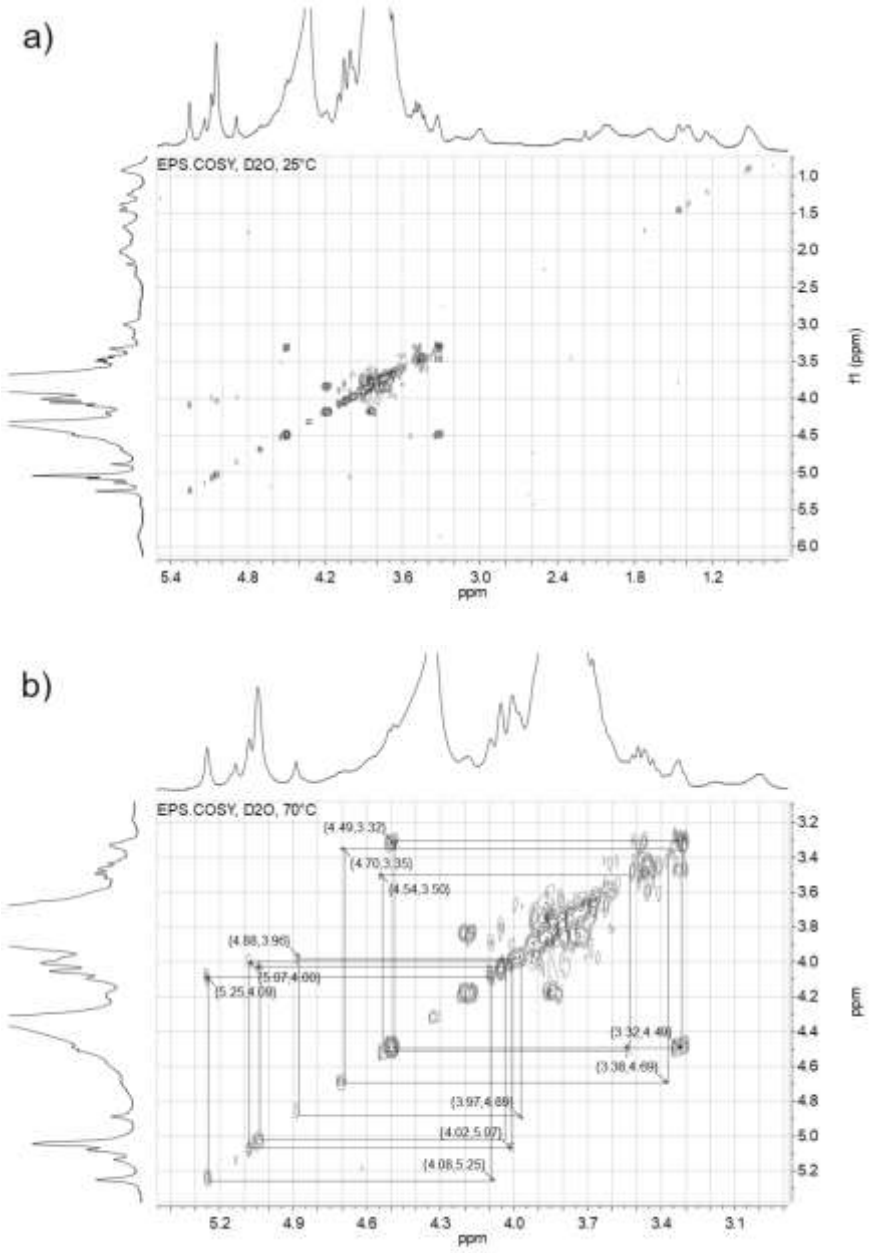

Figure 7. Correlation spectroscopy ^{1}H-^{1}H (COSY) of the EPS and b) H1-H2 correlation observed.

To observe the total spin systems in the saccharide rings, total correlation spectroscopy (TOCSY) was carried out but, due to the higher number of signals, only two spin systems, from two sugar residues could be totally assigned and, for one more, only five correlations could be observed (**Table 2**), after this assignation, the ^{13}C chemical shifts were obtained by the HSQC NMR spectrum and the data were compared with the database from the online software CASPER (http://www.casper.organ.su.se/casper/determine.php) For each experimental data set, different saccharides were suggested but, only those with similar ^{1}H and ^{13}C chemical shifts values and lower error were selected.

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Table 2. Selection of monosaccharides based on comparison between the experimental and database NMR chemical shifts $^{1}H(^{13}C)$.

1	2	3	4	5	6	Residue*	Error
4.49 (103.4)	3.30 (73.6)	3.45 (70.3)	3.63 (75.5)	3.82 (67.2)	3.94, 4.18 (78.7)	**Exp****	
4.53 (103.7)	3.34 (74.0)	3.51 (76.7)	3.47 (70.6)	3.62 (75.8)	3.88, 4.20 (69.5)	→6)-β-D-Glc-(1→	0.33
4.54 (103.2)	3.39 (73.9)	3.66 (75.1)	3.64 (79.7)	3.65 (75.8)	3.82, 3.99 (61.1)	→4)-β-D-Glc-(1→	0.33
5.24 (101.0)	4.09 (78.7)	3.90 (71.0)	3.78 (73.4)	3.50 (76.3)	3.62 (75.4)	**Exp****	
5.24 (101.9)	4.02(71.7)	3.95(71.7)	3.85 (75.0)	3.75 (73.2)	3.80, 3.88 (62.0)	→4)-α-D-Man-(1→	0.33
5.10 (98.9)	4.04 (70.7)	3.86 (78.5)	3.64 (67.5)	3.76 (73.4)	-	**Exp****	
4.90 (100.6)	3.99 (70.8)	3.83 (71.7)	3.74 (67.6)	3.82 (72.0)	3.77, 3,85 (61.8)	→6)-α-D-Man-(1→	0.00
5.07 (102.9)	4.18 (70.6)	3.95 (79.0)	3.77 (67.1)	3.78 (74.3)	3.77, 3,85 (61.8)	→3)-α-D-Man-(1→	1.00

**Saccharide residue suggested by comparison between experimental data and database from the CASPER sofware, the selected ones were chosen based on the lower error.

*The experimental assignation was based on ^{1}H-^{13}C (HSQC), ^{1}H-^{1}H (COSY) and ^{1}H-^{1}H (TOCSY) NMR

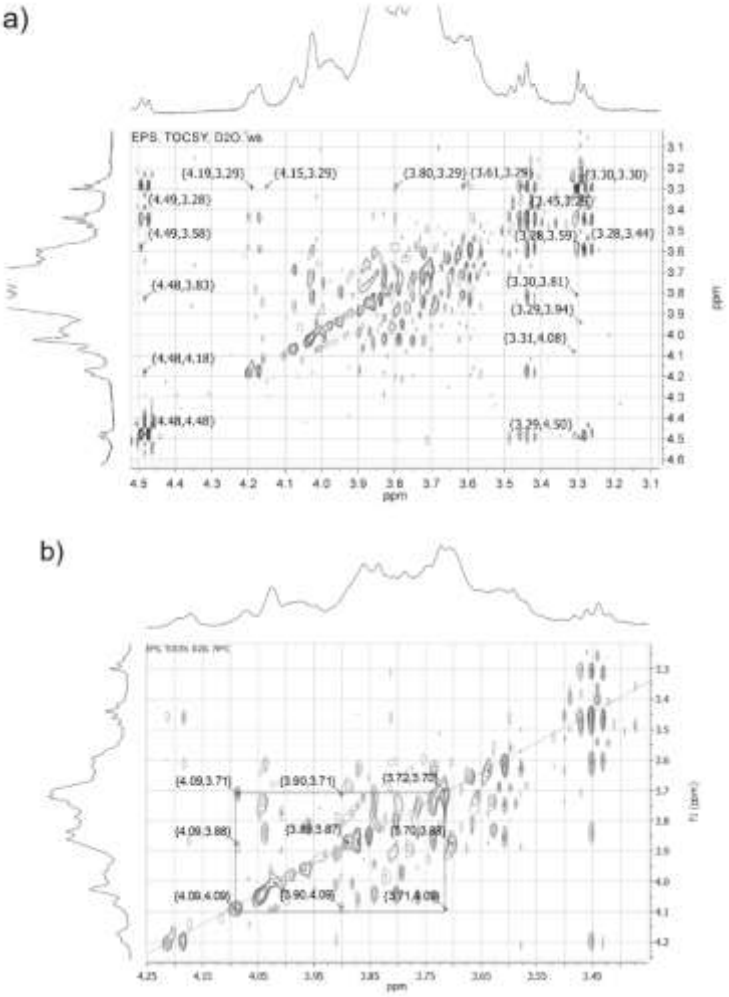

Figure 8. TOCSY from ESP: a) total correlation of anomeric hydrogen at 4.50 ppm and b) correlation H2 up to H4 of residue with anomeric hydrogen at 5.25 ppm.

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For the hydrogen of the anomeric carbon at 4.49 ppm, the total correlation could be assigned (**Figure 8a**), this corresponds to the observed by COSY. According to the predicted chemical shifts from CAPER, the experimental signals could correspond to →6)-β-D-Glc-(1→ or →4)-β-D-Glc-(1→ . It is important to note that these results are consistent with those obtained by FTIR where stretching vibration from mannose was observed as well as vibrations from pyranose ring, β-glycosidic linkages and β-D-glucopyranose.

For anomeric hydrogens at 5.24, H1-H2 correlation was determined by COSY, the correlation of H2 with H3 up to H6 could be assigned (**Table 2**), the anomeric carbon at 101.0 ppm and its hydrogen at 5.24, could be assigned to →4)-α-D-Man-(1→ residue. This corresponds again with what was observed in the FTIR-ATR spectrum.

For the anomeric hydrogen at 5.10 ppm, only the correlation of H2 up to H5 could be assigned (**Table 2**). In this case, the structures suggested using CASPER correspond to 1-3 and 1-6 linked α-D-Mannose. These main components are like EPS produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* although with different linkage [26].

For hydrogens of the other anomeric carbons, because of the high number of signals, only some correlations could be done, results are shown in **Table 3**. The components were selected only by comparison between C-H chemical shifts of the anomeric carbon, the H1-H2 correlation from sugar rings and the values obtained by CASPER. In this case, NAc residues were suggested, these results are according to the observed by COSY and FTIR. For some experimental data, different sugars were equally likely.

Table 3. Suggested monosaccharides residues by CASPER based on the chemical shifts of the anomeric carbon, C1-H1 and H1-H2 correlation.

^{13}C	HSQC ^{1}H	COSY H1-H2	Residue*
103.5	4.50	4.50-3.31	→6)-β-Glu-(1→
	4.53	4.53-3.50	→4)-β-Gal-(1→
			→6)-β-Gal-(1→
	4.71	4.71-3.35	→6)-β-Glu
102.5	5.04	5.04-4.04	→6) α-D-Man^{i} (1→
	5.13	5.13-NObs	
101.0	5.25	5.25-4.09	→4)-α-D-Man-(1→
			→4)-α-D-GlcNAc-(1→
100.0	4.89	4.88-3.98	→6)-α-D-Man-(1→
			→6)-α-D-GlcNAc-(1→
			→6)-α-D-Gal-(1→
			→4)-α-D-Gal^{i}-(1→
98.98	5.04	5.04-4.04	→6)-α-D-GlcNAc-(1→
	5.08	5.08-4.01	→3)-α-D-Gal (1→
98.95	5.04	5.04-4.04	6)-α-D-GlcNAc-(1→
	5.08	5.08-4.01	→3)-α-D-Gal (1→
98.92	5.04	5.04-4.04	6)-α-D-GlcNAc-(1→
	5.08	5.08-4.01	→3)-α-D-Gal (1→
98.88	5.04	5.04-4.20	6)-α-D-GalNAc-(1→
			4)-α-D-GalNAc-(1→

*Residue was selected by minor error between the experimental chemical schiffs and the calculated ones using CASPER, anomeric carbon and H1 and H2 were the main criteria.

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4. Conclusions

Because the EPS produced by *Lactiplantibacillus plantarum* BAL-29-ITTG under the optimal reported culture conditions have shown antioxidant and antibiofilm activity against *E. coli*, *S. Aureus* and *P. aeruginosa*, the need for its chemical and thermal characterization arises; although a compete characterization is very difficult due to its complex structure, according to the data obtained in this work, the studied EPS is a branched heteropolysaccharides with a higher molecular weight and, compacted in aqueous solutions; It had a degradation temperature like others produced EPS but, it had a higher content of inorganic material such as mineral salts. According to the comparative between database from the software CASPER and the experimental NMR and FTIR data, this EPS from BAL-29-ITTG, is essentially composed of glucopyranose 1-4 and 1-6 linked, mannopyranose, 1-3, 1-4 y 1-6 linked and some N-acetylated moieties. The sugars that make up these EPS are linked mainly by β and α-glycosidic bonds. These same sugars linked by these bonds coincide with those found in the EPS produced by other strains of *Lactiplantibacillus plantarum* isolated from other sources. All the above indicates that BAL-29-ITTG produces extracellular EPS with promising physicochemical and biotechnological characteristics.

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Rony Obed Suchiapa Diaz: Methodology, formal analysis, research, *Lucia María Cristina Ventura Carrasco:* Validation, resources, writing: proofreading, supervision, project administration and acquisition of funds; *Alejandro Ramírez-Jiménez:* Validation, formal analysis, research, writing-original draft, supervision.

References

[1] S. L. Flitsch, "Enzymatic Carbohydrate Synthesis", en *Comprehensive Chirality*, Elsevier, 2012, pp. 454-464. doi: 10.1016/B978-0-08-095167-6.00727-8.

[2] A. I. Netrusov, E. V. Liyaskina, I. V. Kurgaeva, A. U. Liyaskina, G. Yang, y V. V. Revin, "Exopolysaccharides Producing Bacteria: A Review", *Microorganisms*, vol. 11, n.º 6, p. 1541, jun. 2023, doi: 10.3390/microorganisms11061541.

[3] S. A. M. Moghannem, M. M. S. Farag, A. M. Shehab, y M. S. Azab, "Exopolysaccharide production from Bacillus velezensis KY471306 using statistical experimental design", *Brazilian Journal of Microbiology*, vol. 49, n.º 3, pp. 452-462, jul. 2018, doi: 10.1016/j.bjm.2017.05.012.

[4] S. R. Dave, K. H. Upadhyay, A. M. Vaishnav, y D. R. Tipre, "Exopolysaccharides from marine bacteria: production, recovery and applications", *Environmental Sustainability*, vol. 3, n.º 2, pp. 139-154, jun. 2020, doi: 10.1007/s42398-020-00101-5.

[5] L. A. Silva, J. H. P. Lopes Neto, y H. R. Cardarelli, "Exopolysaccharides produced by Lactobacillus plantarum: technological properties, biological activity, and potential application in the food industry", *Ann Microbiol*, vol. 69, n.º 4, pp. 321-328, abr. 2019, doi: 10.1007/s13213-019-01456-9.

[6] E. Korcz y L. Varga, "Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry", *Trends in Food*

ISSN: 2594-1925

Science & Technology, vol. 110, pp. 375-384, abr. 2021, doi: 10.1016/j.tifs.2021.02.014.
[7] A. T. Adesulu-Dahunsi, A. I. Sanni, K. Jeyaram, J. O. Ojediran, A. O. Ogunsakin, y K. Banwo, "Extracellular polysaccharide from Weissella confusa OF126: Production, optimization, and characterization", *International Journal of Biological Macromolecules*, vol. 111, pp. 514-525, may 2018, doi: 10.1016/j.ijbiomac.2018.01.060.
[8] Nguyen, Phu-Tho, T.-T. Nguyen, D.-C. Bui, P.-T. Hong, Q.-K. Hoang, y H.-T. Nguyen, "Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications", *AIMS Microbiology*, vol. 6, n.º 4, pp. 451-469, 2020, doi: 10.3934/microbiol.2020027.
[9] A. K. Abdalla *et al.*, "Exopolysaccharides as Antimicrobial Agents: Mechanism and Spectrum of Activity", *Front. Microbiol.*, vol. 12, p. 664395, may 2021, doi: 10.3389/fmicb.2021.664395.
[10] T. Lin, C. Chen, B. Chen, J. Shaw, y Y. Chen, "Optimal economic productivity of exopolysaccharides from lactic acid bacteria with production possibility curves", *Food Science & Nutrition*, vol. 7, n.º 7, pp. 2336-2344, jul. 2019, doi: 10.1002/fsn3.1079.
[11] Z. Liu *et al.*, "Characterization and bioactivities of the exopolysaccharide from a probiotic strain of Lactobacillus plantarum WLPL04", *Journal of Dairy Science*, vol. 100, n.º 9, pp. 6895-6905, sep. 2017, doi: 10.3168/jds.2016-11944.
[12] R. D. Ayivi *et al.*, "Lactic Acid Bacteria: Food Safety and Human Health Applications", *Dairy*, vol. 1, n.º 3, pp. 202-232, oct. 2020, doi: 10.3390/dairy1030015.
[13] L. Yu *et al.*, "Purification, characterization and probiotic proliferation effect of exopolysaccharides produced by *Lactiplantibacillus plantarum* HDC-01 isolated from sauerkraut", *Front. Microbiol.*, vol. 14, p. 1210302, jun. 2023, doi: 10.3389/fmicb.2023.1210302.
[14] J. Wang *et al.*, "Optimization of Exopolysaccharide Produced by Lactobacillus plantarum R301 and Its Antioxidant and Anti-Inflammatory Activities", *Foods*, vol. 12, n.º 13, p. 2481, jun. 2023, doi: 10.3390/foods12132481.
[15] J. Xiong, D. Liu, y Y. Huang, "Exopolysaccharides from Lactiplantibacillus plantarum: isolation, purification, structure–function relationship, and application", *Eur Food Res Technol*, vol. 249, n.º 6, pp. 1431-1448, jun. 2023, doi: 10.1007/s00217-023-04237-6.
[16] D. Meghwal, K. K. Meena, R. Singhal, L. Gupta, y N. L. Panwar, "Exopolysaccharides producing lactic acid bacteria from goat milk: Probiotic potential, challenges, and opportunities for the food industry", *ap*, vol. 12, n.º 2, dic. 2023, doi: 10.54085/ap.2023.12.2.22.
[17] M. Ayyash *et al.*, "Exopolysaccharide produced by the potential probiotic Lactococcus garvieae C47: Structural characteristics, rheological properties, bioactivities and impact on fermented camel milk", *Food Chemistry*, vol. 333, p. 127418, dic. 2020, doi: 10.1016/j.foodchem.2020.127418.
[18] Q. Xu, M.-M. Wang, X. Li, Y.-R. Ding, X.-Y. Wei, y T. Zhou, "Antioxidant and anti-inflammatory activities and action mechanisms of exopolysaccharides from Lactiplantibacillus plantarum Z-1", *Food Bioscience*, vol. 62, p. 105247, dic. 2024, doi: 10.1016/j.fbio.2024.105247.
[19] T. Bouzaiene *et al.*, "Exopolysaccharides from *Lactiplantibacillus plantarum* C7 Exhibited Antibacterial, Antioxidant, Anti-Enzymatic, and Prebiotic Activities", *Fermentation*, vol. 10, n.º 7, p. 339, jun. 2024, doi: 10.3390/fermentation10070339.
[20] M. Kowsalya *et al.*, "Extraction and characterization of exopolysaccharides from *Lactiplantibacillus plantarum* strain PRK7 and PRK 11, and evaluation of their antioxidant, emulsion, and antibiofilm activities", *International Journal of Biological Macromolecules*, vol. 242, p. 124842, jul. 2023, doi: 10.1016/j.ijbiomac.2023.124842.

ISSN: 2594-1925

[21] Y. Jiang y Z. Yang, "A functional and genetic overview of exopolysaccharides produced by Lactobacillus plantarum", *Journal of Functional Foods*, vol. 47, pp. 229-240, ago. 2018, doi: 10.1016/j.jff.2018.05.060.

[22] J. I. Ramírez-Pérez *et al.*, "Effect of linear and branched fructans on growth and probiotic characteristics of seven Lactobacillus spp. isolated from an autochthonous beverage from Chiapas, Mexico", *Arch Microbiol*, vol. 204, n.º 7, p. 364, jul. 2022, doi: 10.1007/s00203-022-02984-w.

[23] L. M. C. Ventura Canseco, R. O. Suchiapa Díaz, M. C. Luján Hidalgo, y M. Abud Archila, "Producción y bioactividades de los exopolisacáridos de *Lactiplantibacillus plantarum* BAL-29-ITTG utilizando un diseño experimental Plackett-Burman", *Rev. Mesoamericana de Investigación*, vol. 3, n.º 3, 2023, doi: 10.31644/RMI.V3N3.2023.A03.

[24] K. M. Dorst y G. Widmalm, "NMR chemical shift prediction and structural elucidation of linker-containing oligo- and polysaccharides using the computer program CASPER", *Carbohydrate Research*, vol. 533, p. 108937, nov. 2023, doi: 10.1016/j.carres.2023.108937.

[25] A. K. M. H. Kober *et al.*, "Exopolysaccharides from camel milk-derived Limosilactobacillus reuteri C66: Structural characterization, bioactive and rheological properties for food applications", *Food Chemistry: X*, vol. 25, p. 102164, ene. 2025, doi: 10.1016/j.fochx.2025.102164.

[26] A. A. Al-Nabulsi *et al.*, "Characterization and bioactive properties of exopolysaccharides produced by Streptococcus thermophilus and Lactobacillus bulgaricus isolated from labaneh", *LWT*, vol. 167, p. 113817, sep. 2022, doi: 10.1016/j.lwt.2022.113817.

[27] T. Hong, J.-Y. Yin, S.-P. Nie, y M.-Y. Xie, "Applications of infrared spectroscopy in polysaccharide structural analysis: Progress, challenge and perspective", *Food Chemistry: X*, vol. 12, p. 100168, dic. 2021, doi: 10.1016/j.fochx.2021.100168.

[28] M. Mathlouthi y J. L. Koenig, "Vibrational Spectra of Carbohydrates", en *Advances in Carbohydrate Chemistry and Biochemistry*, vol. 44, Elsevier, 1987, pp. 7-89. doi: 10.1016/S0065-2318(08)60077-3.

[29] X. Wang, C. Shao, L. Liu, X. Guo, Y. Xu, y X. Lü, "Optimization, partial characterization and antioxidant activity of an exopolysaccharide from Lactobacillus plantarum KX041", *International Journal of Biological Macromolecules*, vol. 103, pp. 1173-1184, oct. 2017, doi: 10.1016/j.ijbiomac.2017.05.118.

[30] O. Braissant *et al.*, "Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat: EPS turnover in a hypersaline microbial mat", *FEMS Microbiology Ecology*, vol. 67, n.º 2, pp. 293-307, feb. 2009, doi: 10.1111/j.1574-6941.2008.00614.x.

[31] S. A. Barker, E. J. Bourne, M. Stacey, y D. H. Whiffen, "Infra-red spectra of carbohydrates. Part I. Some derivatives of D-glucopyranose", *J. Chem. Soc.*, p. 171, 1954, doi: 10.1039/jr9540000171.

[32] J. Wang, X. Zhao, Y. Yang, A. Zhao, y Z. Yang, "Characterization and bioactivities of an exopolysaccharide produced by Lactobacillus plantarum YW32", *International Journal of Biological Macromolecules*, vol. 74, pp. 119-126, mar. 2015, doi: 10.1016/j.ijbiomac.2014.12.006.

[33] F. Zamora, M. C. González, M. T. Dueñas, A. Irastorza, S. Velasco, y I. Ibarburu, "Thermodegradation and thermal transitions of an exopolysaccharide produced by *Pediococcus damnosus* 2.6", *Journal of Macromolecular Science, Part B*, vol. 41, n.º 3, pp. 473-486, jun. 2002, doi: 10.1081/MB-120004348.

[34] V. B. Bomfim *et al.*, "Partial characterization and antioxidant activity of exopolysaccharides produced by Lactobacillus plantarum CNPC003", *LWT*, vol. 127, p. 109349, jun. 2020, doi: 10.1016/j.lwt.2020.109349.

[35] H. E. Gottlieb, V. Kotlyar, y A. Nudelman, "NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities", *J. Org. Chem.*,

ISSN: 2594-1925

vol. 62, n.º 21, pp. 7512-7515, oct. 1997, doi: 10.1021/jo971176v.
[36] N. Čuljak *et al.*, "Limosilactobacillus fermentum strains MC1 and D12: Functional properties and exopolysaccharides characterization", *International Journal of Biological Macromolecules*, vol. 273, p. 133215, jul. 2024, doi: 10.1016/j.ijbiomac.2024.133215.

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