Physicochemical and rheological properties of some exopolysaccharides produced by lactic acid bacteria isolated from plant origin materials

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*SILVIA-SIMONA GROSU-TUDOR1, MIHAELA-MARILENA STANCU1, IULIA-ROXANA ȘTEFAN2,1, CĂLINA-PETRUȚA CORNEA2, MEDANA ZAMFIR1

1Institute of Biology Bucharest of the Romanian Academy, Splaiul Independentei No. 296, Bucharest 060031, Romania
2University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd, 011464 Bucharest, Romania

*Correspondent footnote
Phone: +40 21 221 92 02; Fax: +40 21 221 90 71; Email: silvia.grosu@ibiol.ro

Abstract

Lactic acid bacteria (LAB) isolated from different plant-origin materials were screened for their ability to produce exopolysaccharides (EPS). The taxonomic affiliation of the EPS-producing strains was determined on the basis of their 16S rRNA sequences. Nine of the 146 tested strains have been shown to produce EPS in MRS medium with sucrose, all belonging to Leuconostoc mesenteroides species. One strain, namely L. mesenteroides 109, has been shown to produce large amounts of EPS, of about 19 g/L. All isolated EPS have a high molecular mass, of above 1400 kDa, and a monomer composition dominated by the presence of glucose. The rheological properties and the EPS production in different growth media were studied for four LAB strains producing high amounts of EPS, L. mesenteroides 109, 112, 124, 127, and one strain, namely Weissella cibaria 120, which was not able to produce EPS when grown in MRS with sucrose. Among the EPS producing strains, the most promising one regarding the potential application in the food industry is L. mesenteroides 109, as it produces considerable amounts of EPS (over 25 g/l), together with a high viscosity (over 2400 mPa s) in soy milk supplemented with sucrose.

Key words: lactic acid bacteria, exopolysaccharides, physicochemical characterisation, rheological properties

1. Introduction

Exopolysaccharides (EPS) produced by lactic acid bacteria (LAB) have gained considerable attention in the fermented dairy industry because of their potential applications as viscosifiers, texturizers, and emulsifying agents (GROBBEN & al. [1]). They also possess antitumoral (EBINA & al. [2]), immunostimulatory (HOSONO & al. [3]), macrophage (NISHIMURA-UEMURA & al. [4]), and lymphocyte activating (KITAZAWA & al. [5]) activities. Furthermore, they enhance the colonization of the gastrointestinal tract by probiotic bacteria and act as antioxidants (POLAK-BERECKA & al. [6]). Regarding their physiological role, EPS from LAB have been claimed to protect cells from detrimental environmental conditions, such as dehydration, macrophages, antibiotics, and bacteriophages, to sequester essential cations, and to be involved in adhesion and biofilm formation (LOOIJESTEIJN & al. [7]). EPS produced by the food-grade microorganisms with GRAS (Generally Recognised As Safe) status are important sources of natural alternatives to commercial additives of plant or animal origin. Most of these additives used to improve the rheological properties of the product are chemically modified (ROLLER & DEA [8]) and...
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hence are not allowed in many European Union countries (GIBSON & ROBERFROID [9]). Since the popularity of natural food products without any additives has increased, the use of EPS-producing LAB could result in safe, natural, and healthy end-products, with enhanced texture and improved stability, which may have an important impact on the development of novel products (SCHELLHAASS & MORRIS [10]).

The mechanism of EPS synthesis has been extensively studied for years (WELMAN & MADDOX [11]). The amounts of EPS produced by LAB is strongly dependent on the type of LAB strains, the growth medium composition (carbohydrate and nitrogen source, C/N ratio, vitamins, salts and other supplements) (SEESURIYACHAN [12]). Moreover, fermentation conditions such as temperature, environmental pH, and the presence of oxygen also have a significant impact on EPS synthesis (SVENSSON & al. [13]). The relationship among the amounts of the EPS, composition/structure, and functionality is not yet completely elucidated. A rational screening for novel EPS from particular LAB strains that are characterized by a unique structure or molecular mass is of outmost importance regarding possible application in food industry (GROSU-TUDOR & ZAMFIR [14]).

Fermented foods (dairy, cereals, fruits and vegetables) constitute a very important part of the daily diet in Romania and they are still produced, at a large extend, in a traditional way. Our previous studies have shown that spontaneously fermented dairy products are important sources of LAB strains with functional properties (ZAMFIR & al. [15]; VAN DER MEULEN & al. [16]; GROSU-TUDOR & al. [17]), including EPS-producing strains.

In the present study, strains isolated from fresh or fermented vegetables, but also from other plant-origin materials were screened for EPS production. One of the main plant-origin source was 

borş, an easily produced sour liquid that results from a fermentation process of wheat bran and maize flour, used to sour the typical Romanian soups named „ciorbe”. The article describes our results concerning the selection of some EPS-producing LAB strains and the detailed characterization of these metabolites produced in different growth conditions.

2. Materials and methods

Bacterial strains and growth conditions

The 146 LAB used throughout this study were isolated from: fermented vegetables (cabbage, cucumbers, tomatoes, paprika, etc.: 22 strains), fresh vegetables (carrots, paprika, tomatoes, cucumbers, red and white cabbage, etc.: 62 strains), apple juice (6 strains), flowers (3 strains), and 

borş (53 strains). Strains were isolated by plating on MRS agar (DE MAN & al. [18]), modified MRS agar with 50 g/l of sucrose instead of glucose (MRS-s) and modified MRS agar with 20 g/l of fructose instead of glucose (MRS-f). All strains were stored at -85°C in their corresponding isolation medium, containing 25% (v/v) of glycerol as a cryoprotectant. To obtain fresh cultures from the frozen stock, strains were propagated twice in fresh liquid medium, before the experiments. When screened for EPS production, LAB strains were grown in MRS-s. Glucomannans from the growth medium that could interfere with the EPS screening were removed through ultrafiltration according to the method described by VAN DER MEULEN & al. [16].

Screening for EPS production

The newly isolated LAB strains were screened for EPS production through Gel Permeation Chromatography (GPC), using a Jasco HPLC System (Jasco Europe, Cremella, Italy), equipped with an UltrahydrogelTM Linear column (Waters Corp., Milford, Mass., USA), kept at 35°C, and coupled to RI-2031 refractive index detector (Jasco). Samples were prepared according to the method described by VAN DER MEULEN & al. [26] prior to the injection on the GPC column. The EPS were eluted with 0.1M NaNO₃ at a flow rate of 0.6
Dextran standards with molecular masses ranging from 80 kDa to 1.4 MDa (Sigma-Aldrich, Switzerland) were used to estimate the molecular mass of the purified EPS.

**Identification of EPS-producing LAB strains**

The taxonomic affiliation of the EPS-producing LAB strains was determined on the basis of their 16S rRNA sequence. PCR amplification of 16S rRNA gene and purification were performed as previously described by STANCU [19]. Sequencing of amplification products was performed by Macrogen Europe (Amsterdam, The Netherlands). DNA sequencing runs were assembled using the BioEdit software. The sequences were compared to those from databases using the BLAST search program.

**EPS isolation and quantification**

From the GPC-positive strains, EPS were isolated according to a two-step precipitation protocol described by DE VUYST & al. [20]. The LAB strains were cultivated in filtered MRS-s for 12 h, with no pH control or agitation. Total EPS yields were determined gravimetrically by measuring the polymer dry mass (PDM) after 48 h of drying at 42°C. Further purification of the EPS was done by ultrafiltration using a Vivaspin 6 ultrafiltration module with a 10-kDa MM cut-off (Sartorius Stedim Biotech GmbH, Goettingen, Germany). The retentate obtained after two centrifugation steps was adjusted to 2 mL with ultrapure water and used for further analysis.

**Monomer analysis**

The purified EPS were hydrolyzed for 6 h at 100°C with 8N HCl, evaporated in an Eppendorf AG centrifugal concentrator (Eppendorf, Hamburg, Germany) and resuspended in ultrapure water. Monosaccharide composition of EPS was determined by automated Thin-Layer Chromatography (TLC) (CAMAG, Muttenz, Germany) using the ascending technique with silica gel 60 F254 precoated glass sheets (Merck, Damstadt, Germany). The sugars were eluted with a mixture of 1-butanol/acetic acid/water, 6/1/2 (v/v) and the bands were visualized by spraying with p-aminobenzoic acid (WALL [21]). Glucose, galactose, rhamnose, manose, ribose, xylose (all from Fluka, Sigma-Aldrich, Switzerland), fructose (Merck KGaA, Darmstadt, Germany), arabinose (Veb Berlin Chemie, Germany), glucosamine and galactosamine (both from Calbiochem, Inc. San Diego, Calif., USA) were used as standards. Alternatively, HPLC was used to determine the sugar composition of the hydrolyzed EPS. A Jasco HPLC system (Jasco Europe, Cremella, Italy), equipped with a Carbo Sep Coregel 87P column (Teknokroma, Spain), kept at 85 °C, and coupled with RI-2031 refractive index detector (Jasco) was used for separation. Elution was performed with MilliQ water, at a flow rate of 1 ml/min. Arabinose, fructose, galactose, glucose, maltose, mannose, rhamnose, ribose, sorbose, sucrose, and xylose at a concentration of 0.1 mg/ml were used as standards.

**Effect of growth medium on EPS production**

Four LAB strains that were shown to produce high amounts of EPS in MRS-s and one strain that does not produce EPS when grown in MRS-s were selected further to investigate the effect of growth medium on EPS biosynthesis.

Cow’s milk and soy milk, both supplemented with 50 g/l of sucrose or with no addition of sucrose were inoculated with 2% of fresh LAB cultures obtained in MRS. Uninoculated milks were used as controls. LAB strains were cultivated overnight at 28°C. The pH was measured at the end of the fermentation and the EPS were isolated and quantified as previously mentioned. The monomer analysis of the purified EPS was performed, too.

**Rheological measurements**

The apparent viscosity of the cultures obtained with the selected strains grown in cow’s or soy milk (with and without added sucrose) was measured at 25°C using a DV-II Pro Brookfield digital viscometer (Lorch, Germany) with a cylindrical spindle (no. S 63).
cylindrical spindle no. S 61 was used to measure the viscosity of the controls (uninoculated milks). Spindle speeds of 10, 20, 50, and 100 rpm were applied. According to the manual instructions, the torque to rotate the spindle in the samples had to be in the range of 10% to 95%. Values out of this range were not valid. Therefore, a spindle speed of 50 rpm was chosen for the measurements in case of all cultures and 100 rpm for uninoculated milks. Apparent viscosity measurements were recorded in mPa s.

3. Results and discussions

Besides dairy products, plant origin foods (fresh or fermented fruits, vegetables or cereals) play an important role in the culinary habits of the people in Romania and they are still unexplored food ecological niches. Some of these products are known for their positive effects in humans, especially due to the high vitamin and mineral content. The literature data available concerning the microbial contents and functionality of these food products is scarce (GROSU-TUDOR & ZAMFIR [22]; WOUTERS & al. [23]).

A laboratory culture collection was recently build up with LAB strains isolated from various fresh or fermented vegetables and other plant-origin material collected from farmhouses or small local factories (results not published). In the present study, these newly isolated strains were screened for EPS production. EPS were isolated, purified and characterized.

Screening for EPS production

The GPC-based screening revealed nine EPS-producing LAB strains. An individual peak, eluted at around 10 min, could be detected for all these strains, as shown in Fig. 1A for the strain *Leuconostoc mesenteroides* 127. For the EPS-negative strains no peaks could be detected, thereby proving that contaminants were removed from the medium through ultrafiltration (Fig. 1B). The low frequency of the EPS-producing strains compared with the total number of the strains tested is in agreement with other studies (GRSOU-TUDOR & ZAMDIR [14]; VAN DER MEULEN & al. [16]).

![Figure 1. Gel Permeation Chromatography analysis of an EPS positive strain *Leuc. mesenteroides* 127 (A) and an EPS negative strain *Lactobacillus fermentum* 8 (B).](image-url)
Identification of the EPS-producing LAB strains

Based on the analysis of the 16S rRNA gene sequencing, all nine EPS-producing LAB strains have been identified as *Leuconostoc mesenteroides*. Table 1 shows the identity of these strains and the similarity to other bacterial strains from the public database.

A high incidence of EPS production among *Leuconostoc* strains was previously observed in a screening of LAB strains isolated from fermented dairy products (GROSU-TUDOR & al. [17]) and fermented vegetables (GROSU-TUDOR & ZAMFIR [14]).

Table 1. Taxonomic affiliation of the EPS-producing LAB strains

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Closest bacterial strain name (accession number)</th>
<th>Percentage of nucleotide identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leuc. mesenteroides</em> 93</td>
<td><em>Leuc. mesenteroides</em> PON89</td>
<td>90</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 109</td>
<td><em>Leuc. mesenteroides</em> PON89</td>
<td>90</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 112</td>
<td><em>Leuc. mesenteroides</em> ER8</td>
<td>99</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 113</td>
<td><em>Leuc. mesenteroides</em> PON89</td>
<td>90</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 116</td>
<td><em>Leuc. mesenteroides</em> PON89</td>
<td>90</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 124</td>
<td><em>Leuc. mesenteroides</em> ER8</td>
<td>99</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 127</td>
<td><em>Leuc. mesenteroides</em> ER8</td>
<td>99</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 133</td>
<td><em>Leuc. mesenteroides</em> ER8</td>
<td>99</td>
</tr>
<tr>
<td><em>Leuc. mesenteroides</em> 138</td>
<td><em>Leuc. mesenteroides</em> ER8</td>
<td>99</td>
</tr>
<tr>
<td><em>Weissella cibaria</em> 120*</td>
<td><em>Weissella cibaria</em> gz-106</td>
<td>99</td>
</tr>
</tbody>
</table>

*This strain does not produce EPS in MRS-s, but was chosen for further experiments and, therefore, identified at species level*

EPS isolation and characterization

From all GPC positive strains, EPS could be isolated in various amounts from cultures obtained in filtered MRS-s, by acetone precipitation. One strain, namely *L. mesenteroides* 109 has been shown to produce large amounts of EPS, of about 19 g/l and three other *L. mesenteroides* strains, namely 112, 124, and 127 were able to produce around 10 g/l of EPS (Table 2). The other strains produced lower amounts of EPS, of about 5 g/l in the case of *L. mesenteroides* 93 and about 3 g/l in the case of strains 113, 116, 133 and 138 (Table 2).

Although unusual for LAB strains, the production of high amounts (upto 50 g/l) of EPS by *Leuconostoc* species has been described by other authors (ULLRICH [24]). Similar high EPS yields, of over 10 g/l, were obtained in our previous studies on LAB isolated from fermented dairy products (GROSU-TUDOR & ZAMFIR [14]; GROSU-TUDOR & al. [17]).

The GPC chromatograms revealed that all EPS eluted before the elution of the largest dextran standard available, with a molecular mass of 1.4 MDa, indicating that the molecular mass of all EPS exceeded this value (Table 2). High molecular mass EPS (both homopolysaccharides and heteropolysaccharides) produced by LAB strains isolated from fermented dairy products or fermented vegetables have been previously reported (GROSU-TUDOR & ZAMFIR [4]; GROSU-TUDOR & al. [17]). An estimation of the molecular mass of a certain EPS can be essential for its characterization, taking into account that the molecular mass is an important factor in determining the intrinsic viscosity and functional properties of EPS (RUAS-MADIEDO & al. [25]).
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Table 2. EPS-producing strains and characterization of the EPS isolated from MRS-s cultures

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>EPS yield* (g/l)</th>
<th>Molecular mass</th>
<th>Monomer composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. mesenteroides 93</td>
<td>Cucumber</td>
<td>5.7 ± 0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 109</td>
<td>Bell pepper</td>
<td>19.1 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 112</td>
<td>Carrot 1</td>
<td>10.8 ± 0.1</td>
<td>&gt; 1.4 MDa</td>
<td>Glucose</td>
</tr>
<tr>
<td>L. mesenteroides 113</td>
<td>Carrot 2</td>
<td>3.0 ± 0.5</td>
<td>&gt; 1.4 MDa</td>
<td>Glucose</td>
</tr>
<tr>
<td>L. mesenteroides 116</td>
<td>Green beans</td>
<td>3.6 ± 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 124</td>
<td>Yellow beans</td>
<td>10.1 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 127</td>
<td>White cabbage</td>
<td>10.5 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 133</td>
<td>Orach</td>
<td>3.8 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. mesenteroides 138</td>
<td>Lovage</td>
<td>2.8 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*EPS yield in filtrated MRS-s supplemented with 50 g/l of sucrose.

High molecular mass polymers can be used as viscosifiers, emulsifiers, or stabilizing agents to improve the rheological properties and the texture of food products (PATEL & al. [26]). The monomer composition of the isolated EPS was determined by TLC of the hydrolyzed samples. For all EPS tested, several bands could be observed (Fig. 2). The major band corresponds to glucose (Rf = 0.276), while for the others bands it is difficult to say if they correspond to other monosaccharides from the EPS composition, or to oligosaccharides resulted from an incomplete hydrolysis.

The HPLC analysis confirmed the presence of glucose monomers in the composition of all the hydrolyzed EPS. Figure 3 shows the HPLC chromatogram of the hydrolyzed EPS isolated from strain L. mesenteroides 127. Besides the peak corresponding to glucose, several Romanian Biotechnological Letters, Vol. 22, No. 4, 2017
other small peaks were detected, eluted before glucose, most probably corresponding to some oligosaccharides. A very high peak, eluted at about 11.3 min, corresponds, most probably, to a secondary product of the hydrolysis step, since it is detected in all hydrolyzed EPS samples.

Figure 3. HPLC chromatogram of an EPS positive strain *Leuc. mesenteroides* 127.

**Effect of growth medium on EPS production**

It has been shown that EPS production is strictly correlated with bacterial growth, being in general higher when the producing strain is grown under optimal conditions. Four LAB strains producing high amounts of EPS, namely *L. mesenteroides* 109, 112, 124, 127 and one strain, *Weissella cibaria* 120 (isolated from yellow beans), that was not able to produce EPS when grown in MRS-s, were selected to be further used to evaluate the EPS production in different growth media.

Firstly, cows and soy milk were used for the growth. None of the five selected strains was able to grow in cow’s milk after 48 h of incubation. However, in soy milk they showed a good growth, reaching a final pH between 4.65 ± 0.00 and 4.85 ± 0.14 after 24 h of incubation (Table 3).

**Table 3. EPS production and viscosity of the cultures obtained in soy milk**

<table>
<thead>
<tr>
<th>Strain</th>
<th>pH</th>
<th>EPS yield (g/l)</th>
<th>Viscosity (mPa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. mesenteroides</em> 109</td>
<td>4.85 ± 0.14</td>
<td>10.0 ± 0.1</td>
<td>652.2 ± 34.2</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 112</td>
<td>4.71 ± 0.07</td>
<td>7.9 ± 0.4</td>
<td>804.7 ± 54.5</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 124</td>
<td>4.71 ± 0.08</td>
<td>9.3 ± 0.1</td>
<td>756.5 ± 34.1</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 127</td>
<td>4.65 ± 0.00</td>
<td>8.7 ± 0.1</td>
<td>643.5 ± 13.5</td>
</tr>
<tr>
<td><em>W. cibaria</em> 120</td>
<td>4.79 ± 0.00</td>
<td>8.7 ± 0.8</td>
<td>390.6 ± 39.9</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>8.41 ± 0.17</td>
<td>0.4 ± 0.1</td>
<td>12.3 ± 0.5</td>
</tr>
</tbody>
</table>

The EPS production in soy milk was, in general, lower than in MRS-s. Among the five strains, *L. mesenteroides* 109 reached the maximum EPS yield (10.0 ± 0.1 g/l) followed by *L. mesenteroides* 124 producing 9.3 ± 0.1 g/IPES. Surprisingly, *W. cibaria* 120 produced a high amount of EPS in soy milk (8.7 ± 0.8 g/l), although it was not able to synthesize EPS in filtered MRS-s. It is well known that the EPS yield is generally affected by the composition of the growth medium.
the medium used for microbial cultivation (DE VUYST & al. [27]) and an enhancement of EPS production by modifying the growth medium has been also investigated by other authors (SEESURIYACHAN [12]). Minor amounts of polysaccharides could be isolated from uninoculated soy milk (0.4 ± 0.1 g/l), most probably coming from the soybean, while from uninoculated cow’s milk, as expected, no polysaccharides could be isolated.

Addition of sucrose to soy milk resulted in a significant increase of the EPS yields for most of the tested strains, as compared to the ones in soymilk without sucrose or in MRS-s. For instance, in the case of *L. mesenteroides* 109, the EPS yield was 25.8 ±1.7 g/l, almost three times higher than in soy milk without sucrose (Table 4). All five strains grew very well in soy milk with sucrose, the final pH considerably dropping in time, reaching values between 4.37 ±0.08 and 4.68 ± 0.05 after 24 h of incubation (Table 4).

**Table 4. EPS production and viscosity of the cultures obtained in soy milk and cow’s milk supplemented with sucrose**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Soy milk (50 g/l sucrose)</th>
<th>Cow’s milk (50 g/l sucrose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>EPS yield (g/l)</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 109</td>
<td>4.55 ± 0.10</td>
<td>25.8 ± 1.7</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 112</td>
<td>4.48 ± 0.01</td>
<td>15.6 ± 0.3</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 124</td>
<td>4.50 ± 0.06</td>
<td>15.9 ± 0.1</td>
</tr>
<tr>
<td><em>L. mesenteroides</em> 127</td>
<td>4.37 ± 0.08</td>
<td>14.8 ± 0.9</td>
</tr>
<tr>
<td><em>W. cibaria</em> 120</td>
<td>4.68 ± 0.05</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>8.41 ± 0.17</td>
<td>0.4 ± 0.1</td>
</tr>
</tbody>
</table>

The addition of sucrose to cow’s milk also allowed the growth of the five strains, although slightly slower compared with soy milk. Moreover, the EPS yields obtained in this medium were lower than the ones in soy milk with sucrose, but comparable, for most strains, with the ones obtained in MRS-s (Table 4). No differences in the molecular weight and monomer composition of the EPS isolated from different media were detected (results not shown).

**Rheological properties**

In recent years, whey separation is a common problem in fermented milk products. This problem often appears as a result of shrinkage of a gel causing an expulsion of liquid (MISTRY & HASSAN [28]). Conventionally, different types of thickeners, stabilizers and synthetic chemicals are being used to avoid this problem (RAMANWAMY & BASAK [29]) but these synthetic chemicals are not allowed in some parts of the world (WAND & al. [30]). EPS produced by LAB have gained attention because these polymers are able to improve the rheological properties of fermented milks (FRANCOIS & al. [31]), and they may function as natural alternatives to commercial stabilizers (CERNING [32]). The physical properties of yoghurt can be improved by the use of EPS produced in situ or added as bioingredients (DOLEYRES & al. [33]). Also EPS have been shown to increase the moisture retention in cheese (DABOUR & al. [34]). The viscous behavior of EPS is dependent of its structure and
mass (FREITAS & al. [35]) and it is affected by various factors such as salts, ionic strength, pH and temperature. Information concerning the rheological properties are essential for the selection of the EPS with potential application for a certain type of product. The rheological behavior of the five EPS-producing strains in cow’s milk and soy milk with and without addition of sucrose was investigated during this study. The viscosities recorded at 25°C for the cultures obtained in these media are presented in Tables 3 and 4. The viscosities of fermented soy milks ranged from 390.6 ± 39.9 to 804.7 ± 54.5 mPa s, depending on the LAB strain. Soy milk fermented with *L. mesenteroides* 112 exhibited the highest viscosity among the cultures obtained in soy milk without sucrose, although the EPS production was the lowest. Similar results were obtained by PRADIP & al. [36] on dahi prepared with different EPS producing or nonproducing LAB strains. On the other hand, *W. cibaria* 120 exhibited the lowest viscosity in soy milk without sucrose, although the EPS yield was quite high in these conditions (Table 3). In this case, it seems that higher EPS production made fermented soy milk more adhesive, which would indicate a contribution of EPS to the tendency of the product to adhere to the surface of other materials (PRADIP & al. [36]). This is in accordance with the results obtained by PRADIP & al. [36] from dahi prepared with EPS producing LAB strains. On the contrary, the viscosity of fermented soy milk supplemented with sucrose increased gradually with the EPS production by the selected LAB strains (Table 4). The highest viscosity (2443.4 ± 43.8 mPa s) obtained in soy milk fermented with *L. mesenteroides* 109 corresponded with the highest EPS production (25.8 ± 1.7 g/l). Similar reports that EPS could improve the viscosity of yoghurt were obtained by ZHANG & al. [37]. The high viscosity of soy milk fermented with *L. mesenteroides* 109 suggests that this strain has a good thickening ability and therefore can be used to obtain fermented products based on soy milk, with improved rheological properties. The viscosities recorded in fermented cow’s milk supplemented with sucrose are presented in Table 4. The values ranged from 262.5 ± 6.5 to 486.5 ± 18.5 mPa s, depending on the LAB strain. The highest viscosities were observed for *L. mesenteroides* 124 and 109, followed by *L. mesenteroides* 112. However, the values obtained were five times smaller than in soy milk supplemented with sucrose. The cow’s milk fermented with *L. mesenteroides* 127 and *W. cibaria* 120 had the lowest viscosities although the EPS production for *L. mesenteroides* 127 was amongst the highest (10.7 ± 0.2 g/l). Again, there is no correlation between EPS production and viscosities obtained. In the case of EPS produced by LAB, it has been shown that the factors that are likely to influence the viscosity of dairy fermented products are: EPS concentration, but also EPS composition, charge, spatial arrangements, rigidity, and its ability to interact with proteins in the fermented products (CANQUIL & al. [38]; GANTES & al. [39]). Although the EPS synthesized by LAB selected in our studies, seems to have the same molecular weight and monomer composition, their rheological properties differ considerably. The contribution of the EPS producing strains to the textural properties seems to be a result of the secretion of extracellular polysaccharides and the ability of the polysaccharides to form strands, which connect bacteria to the casein micelles (TAMINE & al. [40]). It is speculated that the increased viscosity of EPS-containing foods may increase the residence time of ingested fermented product in the gastrointestinal tract and therefore be beneficial to a transient colonization by probiotic bacteria (ISMAIL & NAMPOOTHIRI [41]).

### 4. Conclusions

In conclusion, this study provide information about new LAB strains isolated from plant origin materials (fresh/fermented vegetables, fruits and cereals), able to produce large amounts of EPS, with potential application in food biotechnology (i.e. to improve the rheological properties of fermented products). The EPS isolated during this study are homopolysaccharides, composed of glucose, and they have a high molecular mass. The
growth media did not influence the molecular mass and monomer composition of the EPS, but significantly affected the production yield and the rheological properties. Among the EPS producing strains, the most promising one regarding the potential application in the food industry is \textit{L. mesenteroides} 109, as it produces considerable amounts of EPS (over 25 g/l), together with a high viscosity in soy milk supplemented with sucrose (over 2400 mPa s).

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