Culture Conditions Determine the Balance between Two Different Exopolysaccharides Produced by *Lactobacillus pentosus* LPS26

Jorge-Ignacio Sánchez, Beatriz Martínez, Rafael Guillén, Rufino Jiménez-Díaz, and Ana Rodriguez

Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Ctra. Infiesto s/n, 33300 Villaviciosa, Spain, and Instituto de la Grasa (IG-CSIC), Departamento de Biotecnología de Alimentos, Apartado 1078, 41012 Seville, Spain

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*Lactobacillus pentosus* LPS26, isolated from a natural fermentation of green olives, produces a capsular polymer constituted of two exopolysaccharides (EPS): EPS A, a high-molecular-weight (high-*M*<sub>m</sub>) polysaccharide (1.9 × 10<sup>6</sup> Da) composed of glucose and rhamnose (3:1), and EPS B, a low-*M*<sub>m</sub> polysaccharide (3.3 × 10<sup>4</sup> Da) composed of glucose and mannose (3:1). Fermentation experiments in a chemically semidefined medium with different carbon sources (glucose, fructose, mannitol, and lactose) showed that all of them except fructose supported EPS A production rather than EPS B production. The influence of temperature and pH was further analyzed. As the temperature dropped, increased synthesis of both EPS was detected. The control of pH especially enhanced EPS B production. With regard to this, the maximum total EPS production (514 mg liter<sup>−1</sup>) was achieved at a suboptimal growth temperature (20°C) and pH 6.0. Continuous cultures showed that EPS A, synthesized mainly at low dilution rates, is clearly dependent on the growth rate, whereas EPS B synthesis was hardly affected. EPS production was also detected in supplemented skimmed milk, but no increase on the viscosity of the fermented milk was recorded. This could be linked to the high proportion of the low-*M*<sub>m</sub> polysaccharide produced in these conditions in contrast to that observed in culture media. Overall, the present study shows that culture conditions have a clear impact on the type and concentration of EPS produced by strain LPS26, and consequently, these conditions should be carefully selected for optimization and application studies. Finally, it should be noted that this is, to our knowledge, the first report on EPS production by *L. pentosus*.

Microbial exopolysaccharides (EPS) are a wide group of secreted polymers that can be tightly attached to the cell surface (capsular polysaccharides) or released as extracellular slime in the surroundings of the cell (20). In the natural environments, polysaccharides may be associated with virulence and cell protection against desiccation, osmotic stress, antibiotics, toxic compounds, and bacteriophage or protozoa attack (11, 40). Several food applications as emulsifiers, thickeners, viscosifiers, and stabilizers are known for these molecules (40). Among microbial EPS, those produced by lactic acid bacteria (LAB) are receiving increasing attention because these microorganisms have a ‘food-grade’ status. The rheological properties of EPS produced by LAB have found a major application in the manufacture of fermented dairy products such as yogurt, cheese, or fermented milks, although they also play a role in the elaboration of fermented meats and vegetables (2, 33, 42, 45). In addition, they have also been proven to have beneficial effects on human health such as cholesterol-lowering, antitumoral, and immunomodulating activities and prebiotic effects (8, 29, 37). Some studies have also related the production of EPS in LAB with a partial resistance to phage infections, one of the main problems in the dairy industry (16, 24).

EPS from LAB can be composed of a single type of sugar monomer (homopolysaccharides) or several types of monomers (heteropolysaccharides [HePS]). Moreover, EPS produced by different strains vary in sugar composition, chain length, degree of branching, or sugar linkages. All of these factors determine the rheological and health-promoting properties of EPS (36, 37). EPS can be produced either in a bioreactor, followed by the appropriate downstream processing for their further use as food additives, or in situ during the fermentation process. Thus, they have great potential to become alternatives for the currently used stabilizers and thickeners. Thus far, the use of these EPS as bioingredients in the food industry depends on the economic yield of the process which, in many cases, is limited by the low quantities of polymers produced by LAB (40 to 600 mg liter<sup>−1</sup>) compared to other polymers traditionally used such as xanthan or gellan (10 to 25 g liter<sup>−1</sup>) produced by non-food-grade microorganisms (11). It should be also noticed that homopolysaccharides are produced to a much greater extent than HePS. In this regard, the conditions for efficient production of EPS in LAB should be optimized. It is known that the biosynthesis of EPS is strain dependent and influenced by fermentation conditions and medium composition (23). In fact, mesophilic strains seem to produce maximum levels of EPS in suboptimal conditions for the bacterial growth, whereas EPS production appears to be growth associated in thermophilic strains (10).

*Lactobacilli* are often used in mixed starters in the manufacture of dairy products. In addition, secondary microbiota of most ripened cheeses mainly consist of mesophilic lactobacilli that play an important role in flavor and texture development.
Lactobacilli are also the predominant microbiota in “natural fermented” meat and vegetables and used as starters in controlled fermentations of these products (4). Within the lactobacillus group, Lactobacillus pentosus is a facultative heterofermentative microorganism that has been isolated from the adventitious nonstarter LAB in several cheese varieties (27, 39), and it is also involved in the fermentation of meat (13), fish (30), and vegetables (38).

EPS production by lactobacilli has been recently reported (1, 31, 41, 43). However, no EPS produced by L. pentosus has been characterized thus far. With regard to this, we have studied the production of two different EPS by L. pentosus LPS26, a strain isolated from a natural fermentation of olives. The influence of culture conditions, the carbon source, and the growth rate on the yield and the composition of EPS are reported. According to our results, the ratio between these two EPS can be modulated, and the production levels can be optimized by the culture conditions.

MATERIALS AND METHODS

Bacterial strains and culture conditions. L. pentosus LPS26 was isolated from a Spanish-style green olive fermentation by J. L. Ruiz-Barba and R. Jiménez-Díaz at the Instituto de la Grasa-CSIC (Seville, Spain). This strain, formerly classified as L. plantarum according to its carbon fermentation profile (25), has been recently identified as L. pentosus (J. L. Ruiz-Barba, unpublished data) by molecular techniques (44).

Lactoccus lactis subsp. lactis IPLA947 was isolated from an artisan cheese (9). Strains were routinely cultured at 30°C in MRS or Lactococcus lactis molecular techniques (44). Several carbon sources (glucose, lactose, fructose, and mannitol), sugar concentrations (5, 20, 30, and 40 g liter−1), temperatures (20, 25, and 30°C), and pH levels (6.0, 5.0, and not controlled) were used depending on the assay.

Samples from batch and continuous cultures were taken, and the bacterial growth (expressed as CFU ml−1), pH (in uncontrolled-pH cultures), and EPS production were determined. Decimal dilutions of culture samples were made in quarter-strength Ringer solution (Oxoid, Basingstoke, Hampshire, United Kingdom). Appropriate dilutions were plated in duplicate on MRS agar (Scharlab) and incubated for 48 h at 30°C. Sugars and organic acids were simultaneously determined by HPLC (5).

Kinetic parameters. In batch culture assays, the maximum specific growth rate (μmax) was experimentally determined in the exponential growth phase as μmax = ln(X1/X0)/(t1 − t0), where X0 and X1 are the numbers of CFU ml−1 and t0 and t1 are the times along the exponential phase, respectively. The EPS yield (YEPS) was expressed as milligrams of EPS produced per gram of sugar consumed, and the volumetric productivity (Pv) was expressed as milligrams of EPS produced per liter per hour. For the calculation of these parameters, maximum bacterial growth (determined at the end of the exponential phase for each experiment) and maximum EPS production (determined at the end of the incubation period) were used. In continuous cultures, kinetic parameters were determined when steady-state conditions were assessed. In this case, Pv was calculated as milligrams of EPS produced per liter at each dilution rate (D).

Milk cultures. Pure and mixed cultures containing either L. pentosus LPS26 alone or both L. pentosus LPS26 and L. lactis subsp. lactis IPLA947 were prepared in UHT commercial skimmed milk supplemented with 2% of powder skimmed milk (Scharlab). Overnight cultures in MRS or M17, respectively, were used as inocula to give an initial population of about 2 × 108 CFU ml−1 for L. pentosus LPS26 and 7.5 × 108 CFU ml−1 for L. lactis subsp. lactis IPLA947, and cultures were incubated at 25°C for 7 h. When indicated, milk was supplemented with glucose (0.5% [wt/vol]) and yeast extract (0.5% [wt/vol]). EPS counts were performed by plating appropriate dilutions on MRS agar (Scharlab) supplemented with vancomycin (30 mg ml−1). IPLA947 counts were performed on M17 agar (Scharlab). Plates were prepared in duplicate and incubated for 48 h at 30°C. Isolation and quantification of EPS was performed as described above. To discard any influence of yeast extract present in supplemented milk on EPS quantification, uninoculated samples were tested to check for the absence of interfering polymers. The viscosity of the fermented milks was determined according to the Posthumus method (46) using a pure culture of L. lactis subsp. lactis IPLA947 as a reference sample.

RESULTS

L. pentosus LPS26 displayed a soft, loose pellet after centrifugation. In MRS-agar, colonies had a rosy phenotype, in-
The influence of carbon source on growth and EPS production was studied in SDM with glucose, fructose, and mannitol (sugars present in olive flesh) and lactose. The concentration of carbon source was 30 g liter\(^{-1}\), and the temperature of incubation was 30°C. Table 2 summarizes the results obtained. Mannitol and fructose provided the highest (2.4 × 10\(^5\) CFU ml\(^{-1}\)) and the lowest (1.5 × 10\(^3\) CFU ml\(^{-1}\)) viable counts, respectively, and no significant differences were detected between mannitol and glucose (\(P > 0.01\)). Fructose and mannitol supported the lower EPS synthesis (\(P < 0.01\)), whereas glucose and lactose were more favorable sugar sources for EPS production. The synthesis of the high-\(M_w\) EPS (EPS A) seemed to be more dependent on the carbon source than the low-\(M_w\) EPS (EPS B) since larger variations were detected in the former (Table 2). A higher ratio of EPS A to EPS B was obtained with mannitol, glucose, and lactose. However, an inverse ratio was detected with fructose (Table 2). Slight variations in the constituent monosaccharides of both EPS were observed within the different carbon sources. Glucose content ranged from 71 to 80%, and the rhamnose content ranged from 20 to 29% in EPS A, whereas the glucose content ranged from 60 to 76%, and the mannose content ranged from 24 to 30% in EPS B (data not shown). For subsequent assays, glucose was selected as the carbon source.

To determine any effect of glucose concentration, batch cultures were performed at 30°C for 72 h, and polymer synthesis

![Graph](image-url)

**FIG. 1.** Size exclusion chromatogram showing the two EPS produced by *L. pentosus* LPS26. Dotted lines flank the fractions collected for sugar composition analysis of the two EPS.

### TABLE 1. Composition of the two EPS produced by *L. pentosus* LPS26

<table>
<thead>
<tr>
<th>EPS</th>
<th>Glucose (%)</th>
<th>Rhamnose (%)</th>
<th>Mannose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>73</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>76</td>
<td>0</td>
<td>24</td>
</tr>
</tbody>
</table>

* Incubation was performed in batch cultures at 30°C without pH control in SDM supplemented with glucose (30 g liter\(^{-1}\)) as the carbon source.

### TABLE 2. Effect of carbon source on growth and EPS production by *L. pentosus* LPS26

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>Amt of EPS (mg liter(^{-1}))</th>
<th>Maximum growth (CFU ml(^{-1}))</th>
<th>EPS A/EPS B ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>26.3(^a) 55.6(^b) 81.9(^c)</td>
<td>1.5 × 10(^8) 3:7</td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>91.2(^b) 34.6(^a) 125.8(^c)</td>
<td>2.4 × 10(^7) 7:3</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>134.0(^b) 32.0(^a) 166.0(^c)</td>
<td>1.3 × 10(^6) 8:2</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>116.6(^a) 48.3(^b) 164.9(^c)</td>
<td>5.8 × 10(^5) 7:3</td>
<td></td>
</tr>
</tbody>
</table>

* Incubation was performed in batch cultures at 30°C without pH control for 72 h in SDM supplemented with 30 g of each carbon source liter\(^{-1}\).

* Data are mean values (n = 3). Within the column, means with the same superscript letter are not significantly different (\(P > 0.01\)).
was tested at different concentrations of glucose (Table 3). Higher viable counts and an extended exponential phase (12 to 24 h) were observed as the sugar concentration increased. No significant differences were observed between glucose at 20 and 30 g liter⁻¹ (P > 0.01). Regarding the total EPS production, it was first detected after 8 h of incubation, and a clear inverse relationship between temperature and EPS production was observed (P < 0.01). Consequently, total polymer synthesis determined after 72 h of incubation was about 1.2- and 1.6-fold higher at 20°C than at 25 and 30°C, respectively. The EPS yield was also higher at 20°C (23.1 mg g of glucose⁻¹) than at 25°C (17.6 mg g of glucose⁻¹) and 30°C (12.4 mg g of glucose⁻¹) (P < 0.01). In contrast, the lower temperature resulted in lower viable counts, with the differences being significant between 20 and 25°C.

The influence of pH (pH 6.0, 5.0, and no pH control) on bacterial growth and EPS synthesis was determined in batch cultures performed with glucose (30 g liter⁻¹) as the carbon source at 20°C for 72 h. A clear influence of pH EPS production was observed (Fig. 3). The maximum total EPS production reached under uncontrolled pH conditions (pH dropped to 3.88) was 265 mg liter⁻¹ compared to 352 mg liter⁻¹ at pH 5.0 and 511 mg liter⁻¹ at pH 6.0. The control of pH favored the rise of both EPS (P < 0.01), but the increase of EPS B was more pronounced. Higher cell viability was also detected at pH 5.0 (P < 0.01). Regarding the EPS yield, it was notably higher at pH 5.0 (38.7 mg g of glucose⁻¹) than at pH 6.0 (22.4 mg g of glucose⁻¹) or at a uncontrolled pH (23.1 mg g of glucose⁻¹) (P < 0.01). It should be also mentioned that fermentation under controlled pH conditions showed a shift to a mixed-acid metabolism, resulting in a clear increase in formate production (108 mM) compared to the low level detected under an uncontrolled pH (0.55 mM) (data not shown).

We have, therefore, defined the growth conditions that could be picked from batch cultures with the purpose of EPS production by L. pentosus LPS26. These conditions consisted

![FIG. 2. Effect of temperature on growth and EPS production by L. pentosus LPS26. Incubation was performed in batch cultures without pH control in SDM supplemented with glucose (30 g liter⁻¹). The data are mean values (n = 3). Within the same variables (i.e., EPS production, cell growth, and EPS yield), means with the same lowercase letter are not significantly different (P > 0.01).](image-url)
of SDM with glucose at 30 g liter$^{-1}$ (pH 6.0) and an incubation temperature of 20°C. In this way, we can ensure a total EPS production of 511 mg liter$^{-1}$.

**EPS production in continuous culture.** Continuous cultures were used to accurately study the influence of the growth rate on the synthesis of both EPS. Taking into account the growth conditions that provided the highest levels of EPS in batch cultures, a range of dilution rates (D) (0.02 to 0.11 h$^{-1}$) was assayed. As expected, steady-state growth, as assessed by the CFU ml$^{-1}$, was maintained at the different dilution rates since the critical growth rate ($0.12$ h$^{-1}$) corresponding to the $\mu_{max}$ calculated from batch cultures was not exceeded. The highest EPS A production (336.3 mg liter$^{-1}$) was noted at $D = 0.02$ h$^{-1}$ (14% of $\mu_{max}$), and higher dilution rates resulted in a considerable decrease (Fig. 4). On the other hand, the production of EPS B did not seem to be affected by the growth rate since a low amount (<50 mg liter$^{-1}$) was produced at dilution rates ranging from 0.02 to 0.07 h$^{-1}$. At the highest $D$ value (92% of $\mu_{max}$), EPS B was hardly detected (2.5 mg liter$^{-1}$). Consequently, the total EPS, mainly composed of EPS A,
showed a maximum (354.3 mg liter\(^{-1}\)) at the lowest D value (0.02 h\(^{-1}\)).

Residual glucose was detected throughout the range of dilution rates. Similar sugar consumption (ca. 67% of initial concentration) and lactate production (150 mM) were observed at low D (0.02 to 0.04 h\(^{-1}\)), increasing residual glucose and decreasing lactate concentrations were detected. Organic acids other than lactate, such as acetate (16.5 mM) and formate (4.1 mM), were also detected over the range of dilution rates, with no substantial variation on their concentrations (data not shown).

It should be noticed that bacterial growth and, in particular, EPS production were notably higher at D = 0.02 h\(^{-1}\). This D value provided also the maximum EPS yield (18.9 mg of EPS g of glucose\(^{-1}\)) and a volumetric productivity of 7.08 mg liter\(^{-1}\) h\(^{-1}\).

Production of EPS in milk. To evaluate the potential of the EPS-producing strain \textit{L. pentosus} LPS26 as an adjunct culture for the elaboration of fermented dairy products and to measure the amount and EPS ratio produced in milk, fermentations were carried out in milk. \textit{L. pentosus} LPS26 alone was not able to grow in milk, most likely due to a low proteolytic activity on milk casein (data not shown). Therefore, supplementation of milk with glucose and yeast extract was required. In single cultures performed in supplemented milk, LPS26 reached 10^9 CFU ml\(^{-1}\) and produced 140.1 mg of total EPS liter\(^{-1}\). Cocultures of LPS26 and the acidifying strain \textit{L. lactis} IPLA947 were also performed in both milk and supplemented milk. In milk, EPS production was rather low (12.8 mg liter\(^{-1}\)), with EPS B being predominantly produced. LPS26 reached levels of 6.3 \times 10^7 CFU ml\(^{-1}\), whereas viable counts of 6.2 \times 10^6 CFU ml\(^{-1}\) were detected for IPLA947. Supplementation of milk resulted in both a clear rise of the microbial counts (2.5 \times 10^8 CFU of LPS26 ml\(^{-1}\) and 1.8 \times 10^9 CFU of IPLA947 ml\(^{-1}\)) and a notable increase in the total EPS production (162.9 mg liter\(^{-1}\)), mainly due to the great increase showed by EPS B (Fig. 5). As a whole, supplementation of milk significantly affected both EPS production and viable counts (P < 0.01). Unfortunately, no differences in the viscosity of fermented milks were observed in the presence of the EPS-producing strain LPS26.

FIG. 5. Growth and EPS production of EPS by \textit{L. pentosus} LPS26 in pure and mixed dairy cultures. Cultures were incubated at 25°C for 72 h. "(sp)" indicates milk supplemented with glucose (0.5% [wt/vol]) and yeast extract (0.5% [wt/vol]). The data are mean values (n = 3). Within the same variables (i.e., EPS production, cell growth, and EPS yield), means with the same lowercase letter are not significantly different (P > 0.01).

DISCUSSION

This is, to our knowledge, the first report on EPS produced by a strain of \textit{L. pentosus}. The strain \textit{L. pentosus} LPS26, involved in green-olive fermentation, produces a capsular polymer consisting of two EPS that differ in size and sugar composition. The synthesis of more than one type of EPS by LAB has been previously reported (11, 18, 26). For instance, the production of two polymers of 8.5 \times 10^5 and 4 \times 10^4 Da and different sugar compositions was observed in \textit{L. plantarum} EP56 (41).

Glucose and rhamnose present in EPS A are usual components of many EPS characterized to date (22, 34), whose mechanisms for the synthesis of their sugar-nucleotide precursors and incorporation to the polysaccharide chain are well known (12). In contrast, there are no data in the literature regarding metabolic routes that could explain the incorporation of mannose units in HePS (as is the case for EPS B) produced by LAB, although the presence of this sugar has been already described (21). Some authors suggest that the detection of sugars such as mannose, arabinose, or xylose is due to contamination from material coming from cell wall components present in the medium composition, such as glucomannans from yeast extract or peptone, that interfere with the isolation, purification, and structural characterization of EPS (6). However, the use of a medium such as the SDM minimizes the presence of these interfering components (19). Furthermore, analysis performed in uninoculated medium processed in the same way as the cultures did not show the presence of any polymer (data not shown), confirming that mannose was a genuine component of EPS B.

Our results revealed that culture conditions have a clear
impact on growth and EPS production by *L. pentosus* LPS26. We were able to improve EPS production by up to 511 mg liter$^{-1}$ in batch cultures, a relatively high value compared to those generally described for HePS produced by LAB, for which EPS production ranges between 150 and 600 mg liter$^{-1}$ (7). Nevertheless, it is far from the EPS amount produced by *L. rhamnous* RW-9595M (1,275 mg liter$^{-1}$), described as the highest EPS-producing strain among LAB (14). Remarkably, the tested conditions differently affected the production of the two EPS in our strain, and thus the ratio between both EPS can be modulated.

The carbon source has a marked influence on growth and EPS production by *L. pentosus* LPS26 and also on the ratio of both EPS. Glucose provided higher EPS production and clearly supported EPS A synthesis over EPS B. In contrast, fructose favored the synthesis of EPS B. Similar observations were reported for *L. delbrueckii* subsp. *bulgaricus* NCFB 2772, which produced a high-$M_w$ EPS and a low-$M_w$ EPS with a molar ratio of 1:1 in glucose, and the low $M_w$ EPS was nearly the only one in fructose (18). In our case, certain uncoupling between growth and EPS production occurred in *L. pentosus* LPS26, since the maximum viable counts (detected after 12 to 24 h of incubation) did not correspond with the maximum EPS production (detected after 72 h of incubation). This has been also described in other *Lactobacillus* (15) and *Lactococcus* (23) species. We have only observed slight differences in EPS production using different glucose concentrations. Interestingly, the EPS yield, i.e., the amount of EPS produced per glucose consumed, was highest at the lowest sugar concentration, suggesting that an excess of the sugar source is not needed to stimulate EPS synthesis, but it may even decrease the efficiency of its synthesis.

The increase of EPS production at low temperatures, as happened with EPS A and EPS B, seems to be a common feature of mesophilic EPS-producing LAB, in which suboptimal growth conditions result in improved EPS production (10). To explain this, it has been suggested that slowly growing cells exhibit much slower cell wall polymer biosynthesis and that more lipid carrier precursor molecules are available for EPS biosynthesis (40). Control of the pH also enhanced the EPS production by *L. pentosus* LPS26. Control of the pH fostered mainly the synthesis of EPS B, although EPS A production was also favored. Again, certain uncoupling between growth and EPS production was observed, since the maximum viable counts were reached at pH 5.0, and pH 6.0 resulted in the maximum EPS production, in contrast to previous reports (17, 28).

The results obtained with continuous cultures indicate that EPS A and EPS B have different production kinetics. EPS A is mainly synthesized at a very low D value (0.02 h$^{-1}$); hence, its production would be considered non-growth associated. This finding is in agreement with the results obtained in batch cultures where the production of EPS A was mainly detected at the end of the exponential phase and maintained for at least 72 h (data not shown). In contrast, the production of EPS B did not seem to be affected by the growth rate. Similar sugar consumption and lactate production was observed at a lower D (0.02 to 0.04 h$^{-1}$), although great differences in EPS production were observed. This finding suggests that glucose consumed at the lowest dilution rate is more efficiently rerouted toward EPS synthesis. Thus, after sugar uptake an important proportion of carbon source is metabolized toward the formation of the sugar nucleotides precursors involved in EPS biosynthesis (3).

Continuous culture could outperform batch culture for the production of a potential bioingredient such as EPS produced by LAB by eliminating the inherent idle time for cleaning and sterilization and the long lags before the organisms enter a period of high productivity. Moreover, continuous culture allows a higher automation of processes and lowers labor costs. In this regard, the greatest volumetric productivity (7.08 mg liter$^{-1}$ h$^{-1}$) obtained with a D of 0.02 h$^{-1}$ was similar to that calculated for batch culture in the same conditions (7.13 mg liter$^{-1}$ h$^{-1}$). However, the time required for cleaning and sterilization in batch cultures was not taken into consideration for this calculation, and thus the volumetric productivity obtained with continuous culture would be actually higher.

In the present study, we have also evaluated the potential application of *L. pentosus* LPS26 for the manufacture of dairy fermented products as an in situ EPS producer. The results showed that EPS B was the main polymer produced in milk cultures, in contrast to what we observed in SDM, where EPS A was the most abundant EPS type. This is another example of the effect that medium composition has on the ratio of different EPS produced by a particular strain. On the other hand, no effect of the EPS on the viscosity of the fermented milks was observed. This could be explained by an insufficient EPS production. However, similar or even lower EPS concentrations have been shown to be enough to have a significant effect on viscosity (36). Therefore, this lack of thickening effect may be related to the structural characteristics of EPS B. In this regard, molecular size, branching degree, or type of linkages are determining factors for the rheological properties of EPS (36). Mainly, molecular size seems to be a critical parameter for determining the viscosity-intensifying properties of EPS since high-$M_w$ polymers possess a higher intrinsic viscosity (32, 35). In this assay, therefore, the absence of higher viscosity could be explained by the synthesis, almost exclusively, of EPS B. Culture conditions that enhance production of the high-$M_w$ polysaccharide (EPS A) in milk should therefore be determined.

Further research is being performed to determine the structures of both EPS to get a deeper insight into the structure-function relationships regarding their applications as texturing agents for both dairy and nondairy products and their potential prebiotic effect.

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