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EXOPOLYSACCHARIDE PRODUCTION BY SELECTED LACTIC ACID BACTERIA ISOLATED FROM FERMENTED VEGETABLES

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Abstract

Lactic acid bacteria (LAB) play a key role in the food fermentation process since they contribute to the texture, flavor, quality and conservation of the fermented products. Several LAB strains have been shown to produce exopolysaccharides (EPS), with potential applications in food industry, since they can act as natural thickeners that improve the texture of the final product, decrease syneresis and reduce the fat levels in fermented foods. In situ production of EPS by LAB to get a desired texture and mouthfeel of some fermented products is being explored, in order to replace polysaccharides from plants or animals, currently in use. Moreover, it has been suggested that some EPS produced by LAB have prebiotic activity, contributing to the promotion of human gastrointestinal health. During this study, five new EPS-producing LAB strains have been selected from 21 strains isolated from fermented vegetables. The mucoidness/ropiness of the colonies developed on MRS agar media containing different carbon sources (glucose, sucrose, fructose, galactose, lactose, or xylose) was firstly observed. EPS presence in the culture supernatant was detected through gel permeation chromatography (GPC). The EPS material was isolated from these strains by acetone precipitation, then dialysed, dried and weighted. The molecular mass was estimated by the same GPC method, while the monomer composition was determined by automated thin layer chromatography (TLC), after hydrolysis with 8N HCl. One of the positive strain, Leuconostoc mesenteroides/pseudomesenteroides 406 has been shown to produce large amounts of EPS, of about 15 g/L and two strains, Leuconostoc citreum/lactis/garlicum 167 and Leuconostoc sp. 208 were able to produce around 6 g/L of EPS. All isolated EPS have a high molecular mass, of above 1400 KDa, and a monomer composition dominated by the presence of glucose. The influence of the growth medium composition and incubation temperature on the EPS biosynthesis was also investigated. Three LAB strains, that were shown to produce high amounts of EPS, have been selected to be used in this study. It was shown that some mild stress conditions might stimulate, in some cases, the EPS-production.

Keywords: *exopolysaccharides, fermented vegetables, lactic acid bacteria, salinity stress.*

INTRODUCTION

Lactic acid bacteria (LAB) have been used around the world to improve the preservation, sensorial characteristics and nutritional value of a large variety of products, such as milk, meat and vegetables (Doyle and Beuchat, 2007; Wood and Holzapfel, 1995; Wood, 1997). Several LAB strains can also contribute to the improvement of the texture and viscosity of fermented products by means of the synthesis of exopolysaccharydes (EPS). EPS can be classified into two groups: homopolysaccharides (HoPS) and hetero-polysaccharides (HePS) (De Vuyst and Vaningelgem, 2003; Hassan, 2008). HoPS are composed of one type of monosaccharide subunits, while HePS are formed from a backbone of repeated subunits of different monosaccharides, e.g. D-galactose, D-glucose or L-rhamnose, in different ratios. Well-known examples of HoPS include the dextrans and fructans produced by *Leuconostoc mesenteroides* and *Streptococcus salivarius*, respectively (De Vuyst and Degeest, 1999), while HePS are synthetized by many LAB including strains of *Streptococcus thermophilus, Lactococcus lactis* and a number of lactobacilli (De Vuyst el at. 2001).

Although their function and relevance for the bacteria are not completely understood, it has been suggested that EPS may play a significant role in the protection of cells against dehydration, phagocytosis, phage attacks or toxic compounds (De Vuyst et al., 2001). EPS may also contribute to the adhesion of microorganisms onto solid surfaces and to intercellular communications (De Vuyst and Degeest, 1999). EPS may also alter the techno functional properties (e.g. viscosity or water binding capacity) of fermented foods such as

yogurt, cheese or sourdough (Hassan and Awad, 2005; Costa et al., 2010) and, consequently, the sensory properties of these products (Mozzi et al., 2006). Therefore, LAB that show a capacity to excrete EPS can be used to replace thickeners and stabilizers such as polysaccharides of animal (gelatin), plant (starch, pectin) or other microbial origin (xanthan). A further advantage is their GRAS (Generally Recognized As Safe) status, which means that LAB and their metabolites are considered safe and no declaration is needed when they are added to food.

The mechanism of EPS synthesis has been extensively studied for years (Joly et al., 2002; Wellman and Maddox, 2003). The amounts of EPS produced by LAB is strongly dependent on the growth medium composition (carbohydrate and nitrogen source, C/N ratio, vitamins, salts and other supplements) (De Vuyst and Degeest, 1999; De Vuyst et al., 2001; Seesuriyachan, 2012). Moreover, fermentation conditions such as temperature, environmental pH, and the presence of oxygen also have a significant impact on EPS synthesis (Boels et al., 2003; Svensson et al., 2005).

The aim of this study was to isolate and characterize some EPS produced by LAB isolated from traditional Romanian fermented vegetables and to study the influence of growth medium composition and the incubation temperature on the biosynthesis of these EPS.

MATERIALS AND METHODS

Bacterial strains and media

LAB used throughout this study were isolated from traditional Romanian fermented vegetables: green tomatoes (1 strain), cauliflower (2 strains), carrots (4 strains) or brine (14 strains). Strains were isolated by plating on MRS agar (de Man et al. 1960), purified, identified to species level by $(GTG)_5$-PCR fingerprinting and 16S rRNA sequencing (Wouters et al., 2013). Strains were stored at -75°C in MRS broth containing 25% (v/v) of glycerol as a cryoprotectant.

When screened for EPS production, LAB strains were grown in modified MRS medium containing 50 g/L sucrose instead of glucose. Glucomannans that could interfere with the EPS screening were removed through ultrafiltration according to the method described by Van der Meulen et al. (2007).

Screening for EPS production

A preliminary method to evaluate the capacity to produce EPS, was by observing the mucoidness/ropiness of the colonies developed on MRS agar media containing different carbon sources: glucose (20 g/L), sucrose (50 g/L), fructose (20 g/L), galactose (20 g/L), lactose (20 g/L), or xylose (20 g/L).

The 21 LAB strains were then screened for EPS production through gel permeation chromatography (GPC), using a Jasco HLPC system (Jasco Europe, Cremella, Italy), equipped with an Ultrahydrogel™ Linear column (Waters Corp., Milford, Mass., USA), kept at 35°C, and coupled to a RI-2031 refractive index detector (Jasco). Samples were prepared according to the method described by Van der Meulen et al. (2007). The EPS were eluted with 0.1 M $NaNO_3$ at a flow rate of 0.6 mL/L. Dextran standards ranging from 80 kDa to 1.4 Mda (Sigma-Aldrich, Switzerland) were used to estimate the molecular mass of the EPS.

EPS Isolation and Quantification

Isolation of EPS was carried out from 24 h cultures obtained in 25 mL of filtered MRS supplemented with 50 g/L of sucrose, without pH control or agitation. EPS was isolated according to De Vuyst et al. (1998). Total EPS yields were determined gravimetrically by measuring the polymer dry mass (PDM). Further purification of the EPS was done by dialysis against distilled water at 4°C for 7 days, with a water replacement twice a day.

Monomer Analysis

The purified EPS were hydrolysed 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, 2005). Glucose, galactose, rhamnose, fructose, mannose, ribose (Fluka, Sigma-Aldrich), arabinose (Veb Berlin Chemie, Germany), glucosamine and galactosamine (Calbiochem, Inc. San Diego, Calif., SUA) were used as standards.

Effect of NaCl and incubation temperature on EPS production

Three high EPS-producing LAB strains belonging to *Leuconostoc* genus have been selected to determine the effect of incubation temperature and addition of NaCl on the biosynthesis of EPS.

Different medium variants were inoculated with 2% of fresh cultures obtained in filtered modified MRS (with 50 g/l of sucrose). Four incubation temperatures were tested: 20°C, 28°C, 37°C, 42°C, and three concentrations of salt: 1%, 3%, and 5%, respectivelly (both at 20°C and 28°C).

Growth parameters (final pH, optical density at 600nm and viable cell counts) were determined after 24 h, except when no visible growth was observed and cultures were left for 24 or 48 h more. EPS were isolated and quantified as previously mentioned.

RESULTS AND DISCUSSIONS

Screening for EPS production

Most of the strains used in this study grew in all media tested, but it was observed a preference for glucose, sucrose and fructose as energy source. In some cases, due to the small size of the colonies, the mucoid or ropy phenotype could not be detected. Eight strains were very mucoid on media containing sucrose (Fig. 1, Table 1). None of the tested strains showed a ropy phenotype.

The nomenclature used to describe the different EPS-producing phenotypes of LAB is confusing, and terms as “ropy”, “mucoid”, and “slime” have been indistinctly used. However, not all the mucoid or slime-producing strains are ropy. The mucoid colonies have a glistening and slimy appearance on agar plates, but are not able to produce strands when extended with a stik, whereas the ropy colonies form a long filament by this method (Knoshaug et al., 2000). Some LAB can express both ropy and mucoid phenotypes depending on the culturing conditions (Dierksen et al. 1997; Cerning et al., 1994).

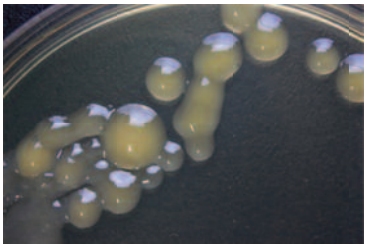


Figure 1. Mucoidness of the colonies developed on agar plates by *Lactobacillus plantarum* 235

EPS characterization

The GPC-based screening revealed five EPS-producing LAB strains. An individual peak, eluted at around 10 minutes, could be detected for all these strains, as shown in Fig. 2 for strain *Leuconostoc mesenteroides/ pseudomesenteroides* 406. For the EPS-negative strains, no peaks could be detected, thereby proving that contaminants were removed from the medium through ultrafiltration.

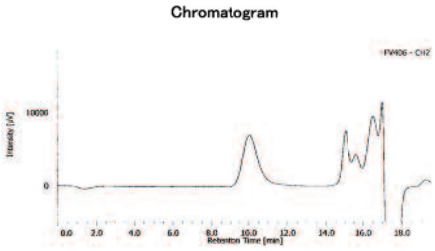


Figure 2. Gel Permeation Chromatography (GPC) of the EPS produced by the positive strain *Leuconostoc mesenteroides/ pseudomesenteroides* 406

All five EPS-producing strains belong to the genus *Leuconostoc*. 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 et al., 2013) and in fermented vegetables (Grosu-Tudor and Zamfir, 2013). The high incidence of the EPS-producing strains compared with the total number of tested strains is in agreement with other studies (Adebayo-tayo and Onilude, 2008). However, the authors admit that mesophilic strains have

the highest potential for EPS production and this concurs with our findings. The EPS production of the five selected strains isolated from Romanian fermented vegetables could be correlated with the mucoidness of the colonies developed on MRS with 50 g/L of sucrose (Table 1). From all GPC-positive strains, EPS could be isolated in various amounts from cultures obtained in filtered modified MRS, by acetone precipitation. *Leuconostoc mesenteroides/pseudomesenteroides* 406 has been shown to produce large amounts of EPS, of about 15 g/L and two strains, *Leuconostoc citreum/lactis/garlicum* 167 and *Leuconostoc* sp. 208 were able to produce around 6 g/L of EPS. For two *Leuc. citreum* strains 96 and 247, EPS yielded 0.21 and 1.02 g/L, respectively.

Table 1. Growth and mucoidness of LAB strains on agar MRS with different carbon sources

Strain	**MRS-glc**	**MRS-suc**	**MRS-fruct**	**MRS-galact**	**MRS-xil**	**MRS-lact**
unidentified 56	+	m	+	+/-	+	+/-
Leuc. mesenteroieds 69	+	+	+	+	+	+
***Leuc. citreum* 96**	**+/-**	**vm**	**+**	**-**	**-**	**-**
Leuc. mesenteroides 97	+	vm	+	+	+	+
***Leuc. citreum/lactis/garlicum*167**	**+**	**vm**	**+**	**-**	**+**	**-**
Lb. parabrevis 196	+	+/-	+	+/-	+	+/-
Leuc. mesenteroides 197	+	+	+/-	+	+	+
Lb. plantarum 198	+	-	+	-	-	-
***Leuc*. sp. 208**	**+**	**vm**	**+**	**-**	**+/-**	**-**
unidentified 234	+	+	+	+	+	+
Lb. plantarum 235	+	vm	+	+	+	+
Leuc. mesenteroides/ pseudomesenteroides 246	+	vm	+	+	+/-	+
***Leuc. citreum* 247**	**+/-**	**vm**	**+/-**	**+/-**	**-**	**+/-**
Lb. pentosus 265	+	+	+	+	+	+
Lb. parabrevis 341	+	+	+	+	+	+/-
Leuc. mesenteroides 355	+	+	+	+	+	+
Lb. brevis 403	+	+	+	+	+	+
***Leuc. mesenteroides/ pseudomesenteroides* 406**	**+**	**vm**	**+**	**-**	**-**	**+/-**
Lb. brevis 530	+	+	+	+	+	+
Lb. plantarum 616	+	+	+	+	+	+
Lb. plantarum 619	+	+	+	+	+	+

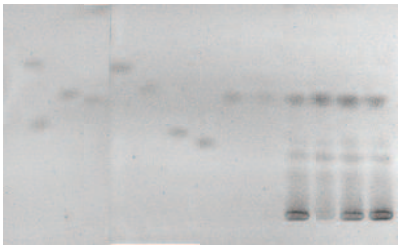
vm = very mucoid; m = mucoid;
+ = good growth; +/- = poor growth; - = no growth

The GPC chromatograms revealed that all EPS eluted before the elution of the largest dextran standard available (molecular mass of 1.4 MDa), indicating that the molecular mass of all EPS exceeded this value. High molecular mass EPS (both HoPS and HePS) produced by LAB strains isolated from fermented dairy products or fermented vegetables have been previously described (Vaningelgem et al., 2004c; Van der Meulen et al., 2007; Grosu-Tudor and Zamfir, 2013). An estimation of the molecular mass of a certain EPS can be important for its characterization, taking into account that the molecular mass is an important factor in determining the intrinsic viscozity and functional properties of EPS (Ruas-Madiedo et al., 2002). High molecular mass polymers can be used as viscosifiers, emulsifiers, gelling, or stabilizing agents to modify the rheological properties and texture of food product (Joly et al., 2002; Patel et al., 2010).

The monomer composition of the isolated EPS was determined by TLC of the hydrolyzed samples. For the strain *Leuc. citreum* 96, a light fading band was detected, corresponding to glucose, while for the other four strains tested, several bands could be observed (Fig. 3).

The major band corresponds to glucose, while the others might be other monosaccharides from the EPS composition, which could not be correlated with any of the sugars used as standards. However, the presence of these additional bands might be also explained by an incomplete hydrolysis of the EPS. Further use of additional standards or the use of complementary analysis, such as HPLC, would

bring more accurate information about the exact monomer composition of the isolated EPS.



1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 3. Monomer composition analysis by TLC: 10 = *Leuc. citreum* 96; 11 = *Leuc. citreum/lactis/garlicum*167; 12 = *Leuc*. sp. 208; 13 = *Leuc. citreum* 247; 14 = *Leuc. mesenteroides/ pseudomesenteroides* 406. Rhamnose (1), galactose (2), mannose (3), fructose (4), ribose (5), arabinose (6), glucosamine (7), galactosamine (8), and glucose (9) were used as standards.

Effect of NaCl and incubation temperature on EPS production

EPS production has been shown to be strictly correlated with bacterial growth, being in general higher when the producing strain is grown under optimal conditions. Therefore, the medium composition, the addition of extranutrients or nutrients for growth enhancement, the carbon to nitrogen ratio, but also the incubation temperature, the oxygen tension, the pH level and the incubation time are important factors affecting both the growth and the EPS production.

Three LAB strains producing high amounts of EPS – *Leuc.* sp. 208 and *Leuc. mesenteroides/ pseudomesenteroides* 406 from the present screening and *Leuc. citreum* 52 from a previous screening (Grosu-Tudor and Zamfir, 2013) - were selected to be used further to evaluate the EPS production in different growth conditions. Firstly, different incubation temperatures (20°C, 28°C, 37°C and 42°C) were tested.

The three strains grew well at all these temperatures (Table 2). The strains *Leuc. citreum* 52 and *Leuc*. sp. 208 showed the largest number of viable cells after 24 h of incubation at 20°C, whereas *Leuc. mesenteroides/ pseudomesenteroides* 406 after 24 h of incubation at 28°C. The poorest growth was shown for *Leuc. mesenteroides/pseudomesen-teroides* 406 after 48 h of incubation at 37°C and 42°C. This was expected, since leuconostocs are known to be mesophilic, with optimal growth temperature between 20°C and 30°C (Wood and Holzapfel, 1995). The pH dropped during the fermentation, reaching final values around 3.5 – 4.0 (Table 2).

The EPS yields varied significantly with the incubation temperature. EPS biosynthesis has been shown to be growth-related, the optimal growth conditions being favourable to the production (Torino et al., 2001, Zhang et al., 2011). However, there are also reports that optimal conditions for EPS production by some LAB strains might be different from those for their oprimal growth (Gamar et al, 2007) and EPS production has been considered by some authors as a mechanism of bacterial self-protection against unfavourable conditions (Ruas-Madiedo et al., 2002; Lin and Chang Chien, 2007).

In our studies, this was the case for two strains, *Leuc. citreum* 52 and *Leuc*. sp. 208, reaching the highest EPS yields of 14.52 g/L and 8.43 g/L, respectively, at incubating temperature of 20°C, although the best growth was obseved at 28°C. *Leuc*. sp 208 doubled the EPS yield at 20°C compared with the optimal growth temperature (Table 2). On the contrary, EPS biosynthesis of *Leuc. mesenteroides/ pseudomesenteroides* 406 reached the highest yield of 16.02 g/L at the optimal growth temperature, 28°C. Zamfir et Grosu-Tudor (2014) reported similar results in case of some EPS-producing LAB strains isolated from Romanian dairy products.

The EPS yield is generally affected by the composition of the medium used for microbial cultivation (De Vuyst et al. 2001), and an enhancement of EPS production by modifying the growth medium has been also investigated (Svensson et al., 2005; Boels et al., 2003; Seesuriyachan, 2012). Sometimes, EPS has been shown to be produced in higher amounts in order to protect microbial cells from stress conditions (Jolly et al., 2002). Therefore, the cultivation of LAB under several stress conditions in order to obtain a higher yield of EPS was investigated. As a stress factor, the presence of different concentrations of NaCl in the growth medium was used.

Table 2. Growth parameters and EPS yields of the tested strains at different temperatures

Strain	Temperature	O.D. 600nm	Final pH	Cell count (CFU/mL)	EPS yield (g/L)
Leuc. citreum 52	20°C	5.753	4.12	3.5×10^{13}	14.52
Leuc. citreum 52	28°C	7.807	3.80	5.5×10^{12}	11.73
Leuc. citreum 52	37°C	6.304	3.83	5.7×10^{11}	5.28
Leuc. citreum 52	42°C	3.108	4.27	3.05×10^{8}	0.31
Leuc. sp. 208	20°C	6.202	4.11	4.05×10^{13}	8.43
Leuc. sp. 208	28°C	8.333	3.72	4.2×10^{13}	4.56
Leuc. sp. 208	37°C	6.323	3.63	9.3×10^{12}	0.12
Leuc. sp. 208	42°C	3.939	3.95	2.2×10^{11}	0.28
Leuc. mesenteroides/pseudomesenteroides 406	20°C	5.215	4.38	2.6×10^{12}	13.47
Leuc. mesenteroides/pseudomesenteroides 406	28°C	6.126	4.33	1.8×10^{13}	16.02
Leuc. mesenteroide/pseudomesenteroidess 406	37°C	0.357	5.47	7.9×10^{8}	0.90
Leuc. mesenteroides/pseudomesenteroides 406	42°C	0.288	5.63	2.5×10^{7}	0.38

All tested strains showed a good growth in the presence of NaCl at 28°C (Table 3). Concerning the EPS production, *Leuc. mesenteroides/pseudomesenteroides* 406 reached the maximum yield in the presence of 5% NaCl, after 24 h of incubation at 28°C (25.83 g/L), higher than the amount produced under optimal conditions (16.02 g/L) and three times the value obtained in the presence of 1% NaCl (8.68 g/L). This is in accordance with the results obtained by Seesuriyachan et al. (2012), who report increased EPS production by *Lactobacillus confusus* under high salinity stress. Although *Leuc. mesenteroides/ pseudomesenteroides* 406 reached the highest EPS yields at 5% NaCl, the biomass production was the lowest, showing that high salinity stress can have a negative impact on microbial growth. On the contrary, *Leuc.* sp 208 and *Leuc. citreum* 52 showed a good growth in the presence of all concentrations of NaCl used, but they lost the ability to synthesise EPS under salinity stress, except for *Leuc. citreum* 52, which still produced 1.68 g/L EPS in the presence of 1% NaCl. Evidence of inhibition of EPS production by NaCl has been also reported in the cultivation of *Lactobacillus helveticus* ATCC 15807 (Torino el al., 2005) and *Pediococcus parvulus* 2.1 (Velasco et al., 2006).

Table 3. Growth parameters and EPS yields under salinity stress at 28°C

Strain	NaCl	O.D. 600nm	Final pH	Cell count (CFU/mL)	EPS yield (g/L)
Leuc. citreum 52	1%	7.461	3.69	5×10^{13}	1.68
Leuc. citreum 52	3%	5.863	3.71	2.7×10^{12}	0
Leuc. citreum 52	5%	5.042	3.52	1.2×10^{13}	0
Leuc. sp. 208	1%	7.759	3.66	1.5×10^{13}	0
Leuc. sp. 208	3%	6.080	3.67	7.6×10^{13}	0
Leuc. sp. 208	5%	5.751	3.40	7.3×10^{13}	0
Leuc. mesenteroides/pseudomesenteroides 406	1%	5.995	4.15	2.6×10^{13}	8.68
Leuc. mesenteroides/pseudomesenteroides 406	3%	5.789	4.09	1.6×10^{13}	16.53
Leuc. mesenteroides/pseudomesenteroides 406	5%	4.748	4.18	8.05×10^{11}	25.83

When incubated at 20°C, *Leuc. mesenteroides/pseudomesenteroides* 406 grew well in the presence of all concentrations of NaCl tested (Table 4). The other two strains showed a good growth in the presence of 1% NaCl (24 h) and a slower growth in the presence of 3% NaCl (48 h), while at higher concentration of NaCl (5%) they needed 72 h for growth. Under these growth conditions, *Leuc*. sp. 208 has lost again the ability to synthetize EPS, while *Leuc. citreum* 52 was still able to produce low amounts in the presence of 1% NaCl, although much lower compared with the yields obtained under

normal conditions. *Leuc. mesenteroides/ pseudomesenteroides* 406, was able to produce very high EPS amounts (over 11 g/L) at 20°C, in the presence of NaCl upto 5%. However, in the presence of 5% NaCl, the production was lower, probably due to the combined effect of two stress factors (lower incubation temperature and high salinity) (Table 4).

Table 4. Growth parameters and EPS yields under salinity stress at 20°C

Strain	NaCl	O.D. 600nm	Final pH	Cell count (CFU/mL)	EPS yield (g/L)
Leuc. citreum 52	1%	2.083	5.04	3.1×10^{10}	0.716
Leuc. citreum 52	3%	5.096	3.98	1.2×10^{13}	0
Leuc. citreum 52	5%	2.274	4.35	2.4×10^{13}	0
Leuc. sp. 208	1%	2.684	4.80	9×10^{10}	0
Leuc. sp. 208	3%	5.728	3.80	1.8×10^{13}	0
Leuc. sp. 208	5%	4.589	3.64	3.1×10^{12}	0
Leuc. mesenteroides/pseudomesenteroides 406	1%	6.405	3.95	1.7×10^{13}	17.42
Leuc. mesenteroides/pseudomesenteroides 406	3%	5.611	4.03	3.2×10^{12}	17.60
Leuc. mesenteroides/pseudomesenteroides 406	5%	2.883	4.66	1.5×10^{10}	11.91

CONCLUSIONS

In conclusion, this study provides information about new LAB strains isolated from fermented vegetables, 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, most probably, HoPS, composed of glucose solely, and they have a high molecular mass. The incubation temperature and the presence of NaCl in the growth medium significantly affected the EPS production. The highest EPS yield, of over 25 g/L, was obtained for *Leuc. mesenteroides/pseudomesenteroides* 406 grown at 28°C, in the presence of 5% NaCl.

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REFERENCES

Adebayo-tayo B., Onilude A.A., 2008. Screening of lactic acid bacteria isolated from nigerian foods for EPS production, Wolrd Applied Sciences Journal, 4, p 741-747.

Boels I.C., van Kranenburg R., Kanning M.W., Chong B.F., de Vos W.M., Kleerebezem M., 2003. Increased exopolysaccharide production in *Lactococcus lactis* due to increased levels of expression of the NIZO B40 eps gene cluster. Applied and Environmental Microbiology, 69, p 5029-5031.

Cerning J., Renard C.M.G.C., Thibault J.F., Bouillanne C., Landon M., Desmazeaud M., Topisirovic L., 1994. Applied and Environmental Microbiology, 60, p 3914-3919.

Costa N.E., Hannon J.A., Guinee T.P., Auty M.A.E, McSweeney P.L.H, Beresford T.P., 2010. Effect of exopolysaccaride produced by isogenic strains of *Lactococcus lactis* on half-fat Cheddar cheese. Jouranl of Dairy Science, 93, p 3469-3486.

De Man J.C., Rogosa M., Sharpe M.E., 1960. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology, 23, p 130-135.

De Vuyst L., De Vin F., Vaningelgem F., Degeest B., 2001. Recent developments in the biosynthesis and applications of heteropolysaccharides from lactic acid bacteria. International Dairy Journal, 11, p 687-707.

De Vuyst L., Degeest B., Heteropolysaccharides from lactic acid bacteria. FEMS Microbiol., 1999, 23, 153-177.

De Vuyst L., Vanderveken F., Van de Ven S., Degeest B., 1998. Production by and isolation of exopolysaccharides from *Strepcocccus thermophilus* grown in a milk medium and evidence for their growth-associated biosynthesis. Journal of Applied Microbiology, 84, p 1059-1068.

De Vuyst L., Vaningelgem F., 2003. Developing new polysaccharides, p. 275–320. In Mc Kenna, B.M. (Ed.), Texture in Food –Volume 2: Semi-solid Foods. Woodhead Publishing Ltd. Cambridge, UK.

Dierksen K.P., W.E. Sandine, J.E. Trempy., 1997. Expression of ropy and mucoid phenopypes in *Lactococcus lactis.* Journal of Dairy Science, 80, p 1528-1536.

Doyle M.P., Beuchat L.R., 2007. Food Microbiology: Fundamentals and Frontieres, 3rd ed. Washington, DC: ASM Press.

Gamar L., Blondeau K., Simonet J.M., 1997. Physiological approach to extracellular polysaccharide

production by Lactobacillus rhamnosus strain C83. Journal of Applied Microbiology, 83, p 281-287.
Grosu-Tudor S., Zamfir M., 2013. Functional properties of lactic acid bacteria isolated from Romanian fermented vegetables, Food Biotechnology, 27 (3), 235-248.
Grosu-Tudor S., Zamfir M., Van der Meulen R., De Vuyst L., 2013. Isolation of novel homopolysaccharide-producing lactic acid bacteria from Romanian raw milk and fermented dairy products, European Food Research and Technology, 237 (4), p 609-615.
Hassan A.N., 2008. ADSA foundation scholar award:possibilities and challenges of exopolysaccharide-producing lactic cultures in dairy foods. Journal of Dairy Science, 91, p 1282-1298.
Hassan A.N., Awad S., 2005. Application of exopolysaccharide-producing cultures in reduced-fat Cheddar cheese: cryo-scanning electron microscopy observations. Journal of Dairy Science, 88, p 4214–4220.
Jolly L., Vincent S.J., Dubic P., Neeser J.R., 2002. Exploiting exopolysaccharides from lactic acid bacteria. Antoine van Leevuwenhoek, 84, p 367-374.
Knoshaug E.P., Ahlgren J.A., Trempy J.E., 2000. Growth associated exopolysaccharide expression în *Lactococcus lactis* subsp. *cremoris* Ropy352, Journal of Dairy Science, 83, p 633-640.
Lin T.Y., Chang Chien M.F., 2007. Exopolysaccharide production as affected by lactic acid bacteria and fermentation time. Food Chemistry, 100, p 1419-1423.
Mozzi F., Vaningelgem F., Hebert E.M., Van der Meulen R., Foulquie Moreno M.R., Font de Valdez G., 2006, Diversity of heteropolysaccharide-producing lactic acid bacteria strains and their biopolymers. Applied and Environmental Microbiology, 72, p 4431–4435.
Patel A.K., Michaud P., Singhania R.R., Soccol C.R., Pandey A., 2010. Polysaccharides from probiotics: new developments as food additives. Food Technology and Biotechnology, 48, p 451-463.
Ruas-Madiedo P., Hugenholtz J., Pieternela Z., 2002. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. International Dairy Journal, 12, p 163-171.
Seesuriyachan P., 2012. Statistical modeling and optimization for exopolysaccharide production by Lactobacillus confusus in submerged fermentation under high salinity stress, Food Science and Technology, 21 (6), p 1647 -1654
Svensson M., Waak E., Svensson U., Radstrom P., 2005. Metabolically improved exopolysaccharide production by *Steptococcus thermoplilus* and its influence on the rheological properties of fermented milk. Applied and Environmental Microbiology, 71, p 6398-6400.
Torino M.I., Hebert E.M., Mozzi F., Font de Valdez G.J., 2005. Applied Microbiology, 99, p 1123-1129.
Torino M.I., Taranto M.P., Sesma F., De Valdez G.F., 2001. Heterofermentative patters and exopolysaccharide production by *Lactobacillus helveticus* ATCC 15807 in responce to envoronmental pH. Journal of Applied Microbiology, 91, p 846-852.
Van der Meulen R., Grosu-Tudor **S.,** Mozzi F., Vaningelgem F., Zamfir M., De Vuyst L., 2007. Screening of lactic acid bacteria isolated from dairy and cereal products for exopolysaccharide production and genes involved, International Journal of Food Microbiology, 118, p 250-258.
Vaningelgem F., Van der Meulen R., Zamfir M., Adriany T., Laws A.P., De Vuyst L., 2004. Streptococcus thermophillus ST 111 produces a stable high-molecular-mass exopolysaccharide in milk-based medium. International Dairy Journal, 14 , p 857-864.
Velasco S., Arskold E., Paese M., Grage H., Irastorza A., Radstrom P., van Niel E.W., 2006. International Journal of Food Microbiology, 111, p 252-258.
Wall P.E., 2005. Thin Layer Chromatography. A modern practical Approach. Cambridge: The Royal Society of Chemistry, Thomas Graham House.
Welman A.D., Maddox I.S., 2003. Exopolysaccharides from lactic acid bacteria:perspectives and challenges. Trends in Biotechnology, 21, p 269-274.
Wood B.J.B. 1997. Microbiology of fermented foods. London: Blackie Academic and Professionals.
Wood B.J.B., Holzapfel W.H., 1995. The Genera of lactic acid bacteria, Vol. 2. Glasgow: Blackie Academic and Professionals.
Wouters D., Grosu-Tudor S., Zamfir M., De Vuyst L., 2013. Applicability of *Lactobacillus plantarum* IMDO 788 as a starter culture to control vegetable fermentations, Journal of the Science of Food and Agriculture, 93, p 3352 -3361.
Zamfir M., Grosu-Tudor S.S., 2014. Stress response of some lactic acid bacteria isolated from Romanian artisan dairy products, World Journal of Microbiology and Biotechnology, 30 (2), 375-384.
Zhang T., Zhang C., Li S., Zhang Y., Yang Z., 2011. Growth and exopolysaccharide production by Streptococcus thermophilus ST1 in skim milk. Brazilian Journal of Microbiology, 42, p 1470-1478.