sciendo

ASN, Vol. 9, No 3, Pages 71–83, 2022

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University of Shumen: Chemistry, Physics, Biology, Geography

Journal homepage: https://content.sciendo.com/view/journals/asn/asn-overview.xml

Structural characterization of an exopolysaccharide produced by *Lactobacillus plantarum* Ts

Stephany Toschkova

[1] *University of Shumen, Faculty of Natural Sciences, Department of Chemistry,*
115 Universitetska Str., 9700 Shumen, Bulgaria

Abstract: *An exopolysaccharide (EPS) was isolated from Lactobacillus plantarum Ts and purified by size exclusion chromatography train. L. plantarum showed the highest production (9.4 ± 0.8 g/l) of EPS. Furthermore, L. plantarum was cultured in medium with volume of 5 L and the EPS was extracted by ethanol precipitation. By HPLS analysis demonstrated, that the EPS has two fractions - polymer and olygomer. The molecular mass of the EPS from both fractions is respectively 8.3x104 Da for the greater pic and 5.0x102 Da. Fourier transform infrared spectroscopy analysis showed the presence of hydroxyl and carboxyl groups and glycosidic linkages. TLC analysis indicated that the EPS was a heteropolymer composed of fructose, and sucrose as monomeric constituent units. The strain L. plantarum and pathogenic E. coli 3398, St. aureus 745, B. subtilis 6633, S. typhimurium 3591, L. monocytogenes 863 and E. aerogenes 3691 were tested for their growth utilizing the EPS from L. plantarum as the sole carbon source for its possible use as a prebiotic. L. plantarum exhibited growth in the EPS supplied medium compared with sucrose as carbon source, whereas the pathogenic strains did not grow in the EPS-supplied medium. In vitro evaluations showed that, like other reported polysaccharides, this EPS displayed significant prebiotics properties.*

Keywords: Exopolysaccharides, *Lactobacillus plantarum* Ts, prebiotic, lactic acid bacteria, pathogenic

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Introduction

Biopolymers are defined as polymers that are bio-based, biodegradable, or both (Imre & Pukánszky, 2013). Biopolymers produced by live microorganisms, such as various bacteria, yeast, and mold species have gained substantial attention in recent decades due to their possible applications in industry (Moradi et al., 2021). Microbial extracellular polysaccharides are widely used in the food industry and also in the cosmetic and pharmaceutical industries (Korcz et al., 2018). Due to their applicability in the food industry, particular attention has been given to special biopolymers called exopolysaccharides (EPS) produced by lactic acid bacteria (LAB). EPS (exopolysaccharide) is a term first used by Sutherland (Arrage et al., 1995) to describe high-molecular-weight carbohydrate polymers produced by marine bacteria The physiological role of EPS depends on the ecological niches and the natural environment in which microorganisms have been isolated. Bacterial EPSs are believed to play an important role in the environment by promoting survival strategies such as bacterial attachment to surfaces and nutrient trapping, which facilitate processes of biofilm formation and development (Sauer et al., 2022). According to Moradali et al. (2019), bacterial EPSs are believed to play an important role in the environment by promoting survival strategies such as bacterial attachment to surfaces and nutrient trapping, which facilitate processes of biofilm formation and development. Most LAB, especially *Fructilactobacillus, Lacticaseibacillus, Lactiplantibacillus, Lactobacillus, Lactococcus, Latilactobacillus, Lentilactobacillus, Leuconostoc, Limosilactobacillus, Pediococcus, Streptococcus, and Weissella* species are capable of synthesizing a variety of EPS (Angelin & Kavitha, 2020; Zheng et al., 2020). *Lactobacillus* strains of food origin are regarded as safe and their EPSs can be used as prebiotics. Prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health (Glenn et al., 1995). EPSs produced by the food-grade Lactic acid bacteria (LAB) with GRAS (Generally Recognized as Safe) status are an important source of natural alternatives to commercial additives of plant or animal origin. Most of those additives used are chemically modified to improve the rheological properties of the product and hence are not allowed in most European Union countries (German et al., 1999; Idrees et al., 2022). EPS produced by LAB are in a great variety, depending on the type of LAB strains, culture conditions, and medium composition (Korcz & Varga, 2021). L. plantarum is a versatile, Gram-positive, bacterium that can be found in a wide range of habitats such as dairy, meat, and many plant fermentations. The properties of EPSs depend on the composition of the monosaccharides and the connections of the monomers to build the exopolymers (Kodali et al., 2011).

For the commercial and therapeutic importance of the products, is important to provide information on the EPS isolated and purified from a *Lactobacillus plantarum* isolated from Bulgarian Wheat and Rye Flour and has shown prebiotics potential.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Materials and Methods

Materials, Bacterial Strains, and Culture Conditions

Strain *Lactobacillus plantarum* was obtained from the collection of the Department of Biology, Shumen University. Molecular analysis in LAB (lactic acid bacteria) was performed by molecular identification (16S rRNA gene sequencing) in GeXP Genetic Analysis System (Beckman Coulter, USA) (Ibryamova et al., 2019; Ignatova-Ivanova et al., 2020). The *L. plantarum* were cultured in de Man, Rogosa, and Sharpe (MRS) agar medium at 37°C and pH of media was adjusted to 6.5 with 1 M NaOH. The basic media was sterilized in an autoclav at 121°C for 20 min. After wards the carbohydrates supplemented were sterilized by 0.22 μM filters (Manisart®). *Escherichia coli* 3398, *Staphylococus aureus* 745, *Bacillus subtilis* 6633, *Salmonella typhimurium* 3591, *Listeria monocytogenes* 863 and *Enterobacter aerogenes* 3691 are from the Collection of the Department of General and Applied Microbiology at Sofia University. All of the isolated substances were checked for purity and maintained in slants of Luria Bertani (LB) agar. The pathogenic strains were culture in Luria Bertani (LB) agar at 37°C. Modified MRS (mMRS) medium (10 g/l casamino acids, 2 g/l Na_2HPO_4, 5 g/l sodium acetate, 2 g/l triammonium citrate, 0.2 g/l $MgSO_4 \cdot 7H_2O$, 0.2 g/l$MnSO_4 \cdot 4H_2O$, and 1 g/l Tween 80) was used for the study of EPS utilization by LAB strains. Additionally, carbohydrates supplemented 10% sucrose were sterilized using 0.22 μM filters (Manisart®).

Extraction and Quantification of Exopolysaccharides from L. plantarum

L. plantarum strains were cultured in mMRS broth with 10% sucrose at 37°C for 48 h. The culture was centrifuged at 5,000 ×g for 10 min to remove the cells and the supernatant was boiled for 10 min to inactivate enzymes. The supernatant was decolorized with activated carbon in a water bath at 40°C for 30min (Chen et al., 2013). After wards was deproteinized as described previously (Wang et al., 2007). The deproteinized supernatant was subsequently centrifuged at 5,000 ×g for 10 min, mixed with 4 volumes of 95% ethanol, and kept overnight at 4°C. The mixture was centrifuged at 8,000 ×g for 10 min. The pellets were collected and dissolved in sterile distilled water. The solution was centrifuged at 3,000 ×g for 10 min, and so the supernatant was dialyzed against sterile distilled water for 24 h. The EPS solution was dried freezed and stored at 4°C until used and the EPS content was measured by the phenol-sulfuric acid method (Masuko et al., 2005).

Fourier Transform Infrared Spectra of the EPS

Functional groups present in the purified EPS were determined by Fourier transform infrared (FTIR) spectroscopy. The samples selected for infrared analysis were prepared by mixing with spectroscopygrade KBr and than prepared in the form of pellets at a pressure of 1 MPa. All of the pellets were of about 10 mm diameter and 1 mm thickness. The FTIR spectral data were recorded via a Varian 2000 FTIR spectrometer (FT-IR Afinity-1 Shimadzu) as FTIR spectra were recorded covering the region of 4,000–400 cm^{-1}.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

EPS Composition Analysis

The lyophilized EPS (10 mg) was hydrolyzed with 6 M trifluoroacetic acid (TFA) for 6 h at 100°C and TFA was removed using a rotary vacuum evaporator. The hydrolyzed solution was neutralized with 15 M ammonia solution (0.32 ml) (6). The resultant hydrolysate was analyzed by thin-layer chromatography (TLC) to determine the monosaccharide composition. The monosaccharide standard was also treated with 6 M TFA and as described above, neutralized with ammonia. The EPS hydrolysates were separated using Silica Gel 60 TLC. For this we used pre-coated plates (Merck, Germany) developed with a mobile phase of ethylacetate: n-propanol: acetic acid: water (4:2:2:1 (v/v/v/v)). The separated sugars were visualized with an orcinol-sulfuric acid spray reagent.

Carbohydrates Used in This Study

We studied fife types of commercially available carbohydrates presenting different degree of polymerization (DP): FOS (Raftilose P95 from Orafti, Tienen, Belgium) contained 5% glucose, fructose, and sucrose; and 12% DP2, 48% DP4 and35%DP7; GalOS (TOS-PfromYakult,Tokyo,Japan) contained 2% DP2, 48% DP3, 38% DP4 and 12% DP5; GOS (BioEcolians from Solabia, Pantin, France) contained 6% glucose and leucrose, and 24% DP4, 56% DP5, 7% DP6 and 7% DP7; FOS from chicory (Sigma) and comersial β-1,3 glucan from Euglena gracilis (Sigma). Glucose (purity 99%, Merck); Fructose(purity 99%, Merck), Sucrose (purity 99%, Merck), Lactose (purity 99%, Merck), Maltose (purity 99%, Merck) and raffinose (purity 99%, Fluka, Sigma Aldrich, Switzerland) were used as standarts at EPS Composition Analysis.

HPLC analis

The average numerous molecular weight (Mn) and average molecular weight (Mw) of the samples were determined by molecular sieve chromatography (HPLC-SEC) at the University of Food Technology, Plovdiv. The separation was performed on an HPLC ELITE LaChrome chromatograph (VWR Hitachi, Japan) equipped with a Shodex OH PAK 806 MHQ column (8 mm x 300 mm), (Shodex Co., Tokyo, Japan) and an RI detector (VWR Hitachi, Chromaster, 5450, Japan). We used 0.1 M NaNO3 at 30 ° C as the mobile phase at a flow rate of 0.8 ml / min. The column was maintained at 30.0 ± 0,1° C, as all samples (2 mg / ml in 0.1 M $NaNO_3$) were filtered through 0.45 μm, PTFE45/ 25 mm (Isolab, Germany) before injection and 20 μl were injected at each assay. The standard curve was obtained using different standards of known molecular weight pullulins (P-5, P-10, P-20, P-50, P-100, P-200, P-400, P-800). The polydispersity index was calculated (I = Mw / Mn) and it represents a characteristic of polymers (Murdzheva et al., 2016).

Effect of EPSs on Growth of Lactic Acid Bacteria and Enteric Pathogens

The growth and EPS utilization by LAB such as *L. plantarum*, and *Escherichia coli* 3398, *Staphylococus aureus* 745, *Bacillus subtilis* 6633, *Salmonella typhimurium* 3591, *Listeria monocytogenes* 863 and *Enterobacter aerogenes* 3691 were evaluated in mMRS medium with 1% EPS at 37°C for 24 h.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

LAB growth was also analyzed in mMRS with 1% glucose (positive control), as the growth of the pathogenic bacteria *Escherichia coli* 3398, *Staphylococus aureus* 745, *Bacillus subtilis* 6633, *Salmonella typhimurium* 3591, *Listeria monocytogenes* 863 and *Enterobacter aerogenes* 3691 was monitored by using LB medium supplemented with 1% EPS and 1% glucose (positive control) as the carbon source separately. Bacterial growth was measured by a turbidimetric method at 650 nm and calibrated against cell dry-weight using a spectrophotometer (UV/Vis Shimadzu, Japan) and for each experiment, data was analyzed using Excel statistical package.

Statistical Analyses

Experiments were repeated at least three times. The data are expressed as the mean ± standard deviation.

Results and Discussion

Screening of EPS-Producing Microbes

The presence of EPS associated with bacterial cells can be recognized by the formation of colonies in mucous solid medium (Stadler et al., 2010). Colonies showing a slimy appearance were picked and subcultured in mMRS wiht 10% glucose and incubated for 2 days at 37°C. Totally nine isolates were collected from the ganjang samples.Therefore, the presence of a translucent or creamy material involving a mucoid colony is indicative of EPS production potential. When cultivated in a media with high content of saccharides such as 10% sucrose solutions, strain *L. plantarum* synthesizes exopolysaccharides (Fig. 1).

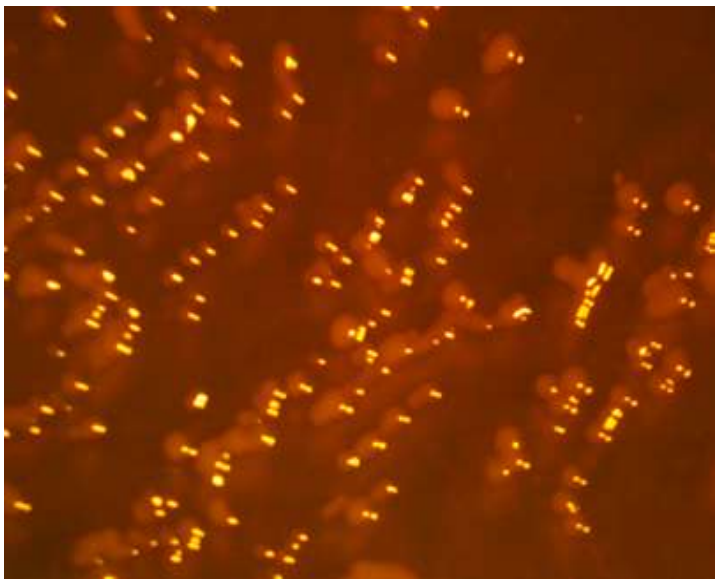


Figure 1. EPSs (exopolysaccharides) produced by *L. plantarum* cultivated in a media containing 10% sucrose, which are secreted in the culture medium

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Quantification of the EPS

The EPS produced by *L. plntarum* Ts strain was quantified by the phenol-sulfuric acid method (Dubois et al., 1956). The results of the analysis showed that starin *L. plantarum* produced EPS amount (9.4 ± 0.8 g/l).

FTIR Spectral Analysis of the EPS from L. plntarum

The FTIR spectrum of the EPS from *L. plantarum* showing the functional groups in the 4,000-400 cm^{-1} region is represented on Fig. 2.

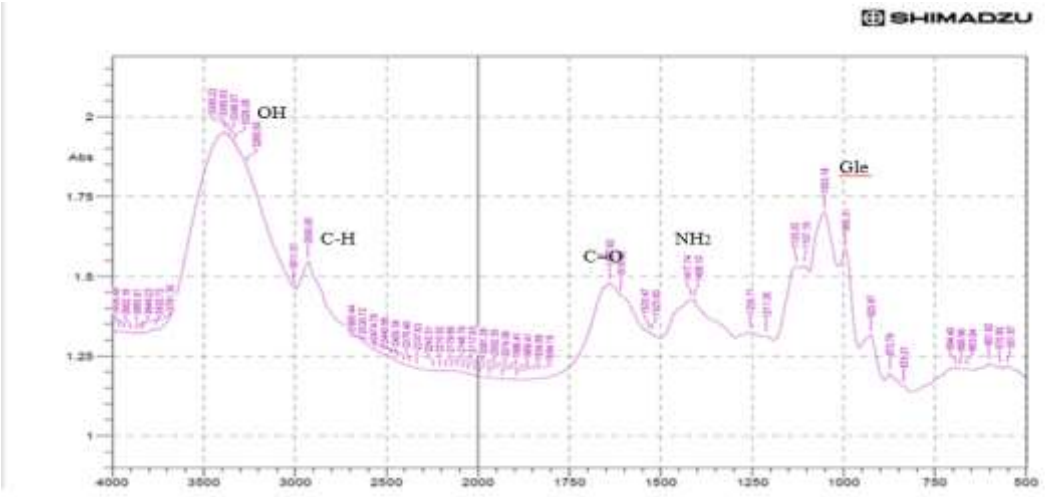


Figure 2. FTIR analysis of the exopolysaccharide preparation from *L. plntarum* Ts

We performed a comparative analysis of the spectrum of the obtained EPS and the spectra of two known OSs - raffinose and glucooligosaccharide. The results are shown in Figure 3.

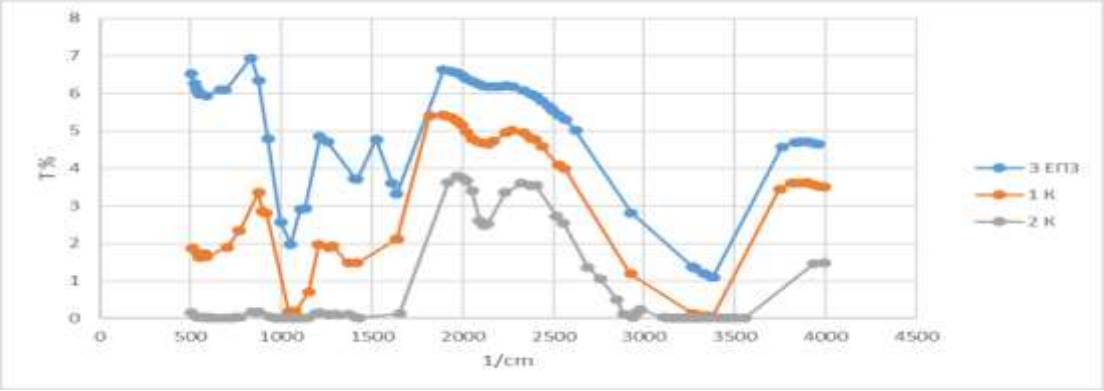


Figure 3. FTIR analysis of an EPS of *L. plantarum* obtained by culturing the strain on 10% sucrose compared with glucooligosaccharide-1K and raffinose-2K

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

There is no complete match between the synthesized *L. plantarum* species with the two standards tested. This suggests that the synthesized FTE has a new unique structure.

***Monosaccharide Composition of the EPS from L. plntarum* Ts.**

Hydrolysis of the EPS with 6 M TFA and subsequent analysis of the EPS hydrolysate by TLC showed the presence of mannose and glucose as principal components of the EPS from *L. plntarum* (Fig. 4).

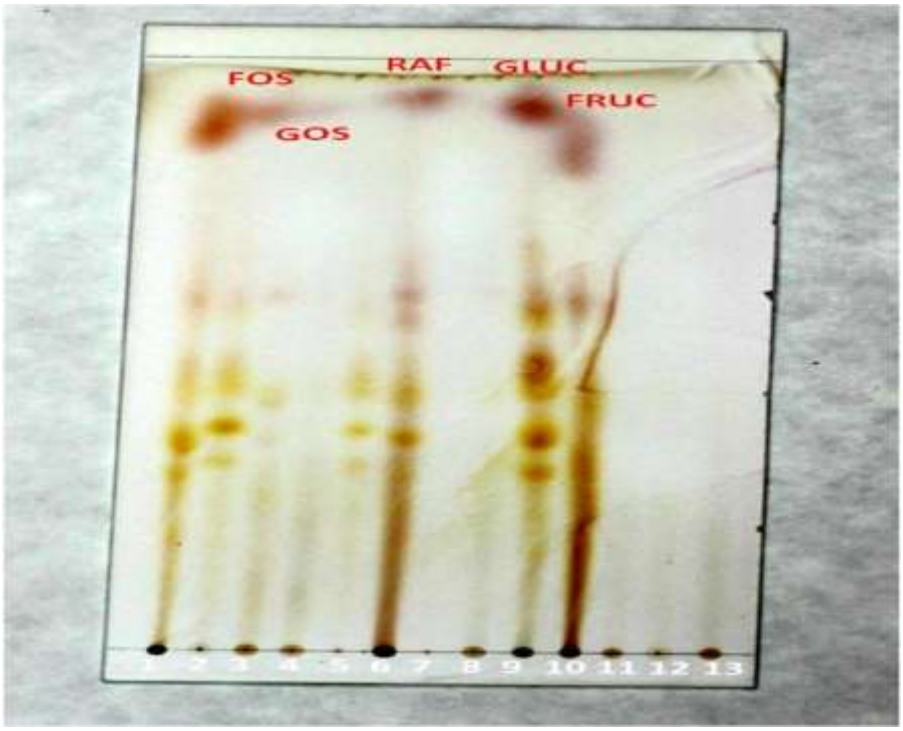


Figure 4. TLC analysis of monosaccharides from *L. plntarum* Ts. exopolysaccharide hydrolysate
Where 1. EPS from *L. plntarum* Ts; 2. FOS; 3. GOS; 4 GalOS; 5 FOS from chicory (Sigma); 6 Raffinose; 7 comersial β-1,3 glucan from Euglena gracilis (Sigma); 8 Glucose; 9. Fructose; 10 Sucrose; 11. Galactose, 12.Lactose; 13 Maltose

Hydrolysis of the EPS with 6 M TFA and subsequent analysis of the EPS hydrolysate by TLC showed the presence of fructose and glucose as principal components of the EPS from *L. plantarum* (Fig. 3). Comparison with selected standards shows similarity as FOS (Raftilose P95) and raffinose, FOS and GOS (Figure 4).

HPLC analysis of EPS

The average numerous molecular weight (Mn) and average molecular weight (Mw) of the purified EPS were determined by molecular sieve chromatography (HPLC-SEC) at UCT Plovdiv. The molecular weights were calculated according to the relative molecular weight of the known molecular weight

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

pullulans (P-5, P-10, P-20, P-50, P 100, P-200, P-400, P-800). The polydispersity index, which is a characteristic of polymers, is calculated by the formula: I = Mw / Mn (11). The results obtained are shown in Figure 5.

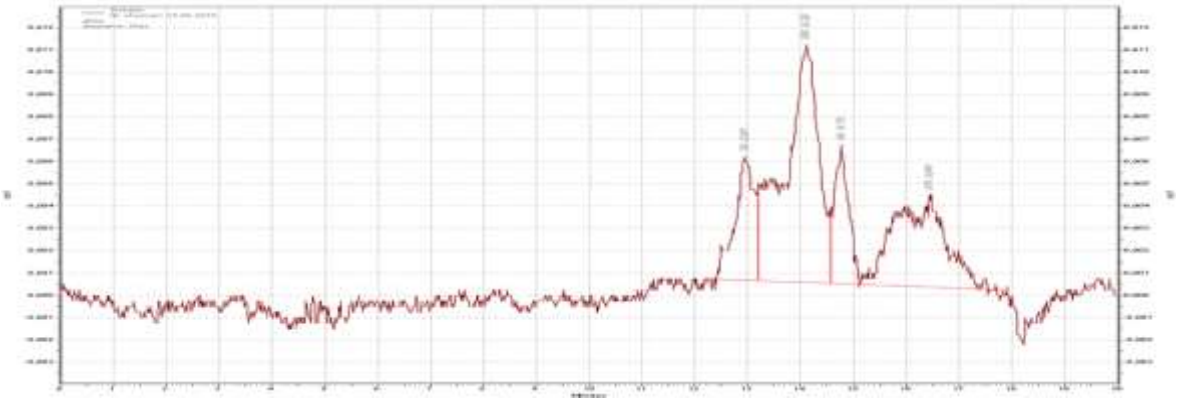


Figure 5. HPLC-SEC

The chromatogram of the EPS appeared in two asymetrical pics. In the sample was observed two fractions – polymer and oligomer. The molecular mass of the EPS from both fractions is respectively $8.3x10^4$ Da for the greater pic $5.0x10^2$ Da.

Effect of the EPS on the Growth of Lactic Acid Bacteria and Enteric Pathogens

Growth of *L. plantarum* strain in modified MRS broth supplemented with carbohydrates EPS utilization by LAB shown in Table 1. The growth of the pathogenic bacteria was monitored using LB medium supplemented with 1% EPS utilization by LAB and 1% sucrose (positive control) as the carbon source separately. Growth was evaluated in terms of maximum optical density at 600 nm and specific growth rate achieved during 24 h fermentation. Growth kinetics on sucrose were used as control.

L. plantarum strain fermented EPS utilization by LAB. Pathogenic strains *E. coli* 3398, *St. aureus* 745, *B. subtilis* 6633, *S. typhimurium* 3591, *L. monocytogenes* 863 and *E. aerogenes* 3691 did not show any growth in EPS-supplied medium compared with sucrose-supplied medium (Table 1), indicating that these strain could not utilize EPS as a carbon source.

The ablity of *L. plantarum* to sintesyze EPS is proven from longterm studies of Bulgarian authors (Ignatova-Ivanova et al., 2010; Ignatova-Ivanova et al., 2017; Ibrjamova et. al, 2018; Doychinova et al., 2019; Doychinova, 2020). The majority of sintesyzed EPS show antimicrobial activity (Petkova et al., 2021a, b, c), and some can be used for inhibition of corrosion processes (Ignatova-Ivanova et al., 2018; Ibrjamova et al., 2018) The function of EPS's depend from the species / strain, which sintesyze them from one site and of the molecular weight from other prespective. Those dependencies are observed and from other authors as well (Ruas-Madiedo et al., 2010; Pryanika et al., 2014). FT-IR is an effective analytical instrument for detecting functional groups and characterizing covalent bonding information.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Table 1. Oligosaccharide Utilization

Strains	Growth on MRS	OD sucrose[a]	Growth on MRS-EPS	OD EPS[a]
L. plantarum Ts.	+	6,9±0,11	+	4,2±0.01
E. coli 3398	+	0,1±0,09	-	0,1±0,11
St. aureus 745	+	0,2±0,02	-	0,1±0,03
B. subtilis 6633	+	0,2±0,08	-	0,2±0,04
S. typhimurium 3591	+	0,1±0,11	-	0,2±0,08
L. monocytogenes 863	+	0,2±0,05	-	0,2±0,02
E. aerogenes 3691	+	0,1±0,08	-	0,2±0,04

[a] Optical density (OD) at 600 nm after 24 h in MRS, MRS-EPS broths. For each experiment, data were ana lyzed using the Excel statistical package. The OD readings and standard deviations were calculated from duplicate samples from three separate experiments.

The technique works on the fact that bonds and groups of bonds vibrate at characteristic frequencies. A small peak around 1,530 cm^{-1} corresponding to an amino group was also observed in the spectrum of EPS. The obvious absorption peak 879.54 cm^{-1} revealed the existence of b-glycosidic bond. The band in the range 1,030–944 cm^{-1}, with minimum at 998 cm^{-1} can be attributed to Glc. The absorption bands at 812, and 879 cm^{-1} indicated that the EPS contained both α and β-type glycosidic linkages in its structure. Similar results were obtained by other authors (Xu et al., 2010). The broad peak at 3331 $cm^{-1}$was an OH stretching peak, the carbonyl (C=0) stretching was at 1670 cm^{-1}, and the peak between 2800 and 3000 $cm^{-1}$was a C-H stretching peak. Absorption at 1110 cm^{-1} was typical for D-glucose in pyranose form, and that at 847 cm^{-1} was indicative of the presence of an R-glycosidic linkage between individual glycosyl residues in EPS. In the region (950- 700 cm^{-1}), polysaccharides are reported to exhibit an obvious characteristic absorption at 812 cm^{-1} that corresponds with the presence of mannose. Такива резултати се наблюдават и от (Kodali et al., 2011).

The carbohydrates show high absorbencies in the region 1,200–950 cm^{-1}, that is within the so-called fingerprint region, where the position and intensity of the bands are specific for every polysaccharide, allowing its possible identification. Polysaccharides present the highest capacity for carrying biological information since they have the greatest potential for structural variability. Presence of glucose and sucrose indicated that the EPS produced by the isolate is a heteropolysaccharide. The biological activities of glucomannans implicate the importance of this polysaccharide extracted from the flour LAB strain which showed probiotic properties.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Mw is a major property of *L. plantarum* WLPL04 FTE that may affect probiotic properties. FW mw from *L. plantarum* WLPL04 is determined to be 6.61 × 10^4 Da, which is lower than FEV (1.1 × 10^5 Da) than *L. plantarum* YW11 (17) and FEV (1.15 × 10^6 Da) of *L. plantarum* C88 (24), but higher than *L. plantarum* BC-25 FEV (1.83 × 10^4 and 1.33 × 10^4 Da) (25). In a parallel study, the Mw of *L. plantarum* ZDY2013 was 5.17 × 10^4 Da by high performance chromatography except size (Zhang et al., 2016).

The EPS from *L. plantarum* showed potential prebiotic property by selectively supporting the growth of LAB. The pathogenic strains were unable to grow in the medium containing EPS as a carbon source, indicating that in the presence of EPS, LAB will be selectively enriched, whereas pathogenic Enterobacteriaceae members will be suppressed. A similar result was observed in the cases of EPS from *Weissella cibaria* A2, *W. confusa* A9, *L. plantarum* A3, and *Pediococcus pentosaceus* 5S4, which selectively enhanced the growth of *Bifidobacterium* and *Lactobacillus/ Enterococcus* groups whereas *Clostridia* were suppressed (Hongpattarakere et al., 2012).

It has been demonstrated in this study that, in the presence of EPS strain *L. plantarum* is capable of fermenting it. Despite significant commercial interest in using oligosaccharides as prebiotic substrates, little is known about how these oligosaccharides are metabolized by LAB and related bacteria. LAB accumulate sugars by secondary active transport (mainly by proton motive force, PMF), the PTS, or an ATP-mediated system. However, more studies should be performed in order to elucidate the pathways of utilization of oligosaccharides in these *Lactobacillus* strains.

Conclusion

In conclusion, *L. plantarum* produced a water soluble heteropolysaccharide, which is composed of sucrose and glucose. Additionally, growth study using EPS as the sole carbon source showed that it could specifically support the growth of LAB while suppressing the growth of *E. coli* 3398, *St. aureus* 745, *B. subtilis* 6633, *S. typhimurium* 3591, *L. monocytogenes* 863 and *E. aerogenes* 3691. These results indicate that the EPS from *L. plantarum* has potential application as a prebiotic in the food industry.

Acknowledgments

This study was partly funded by National Program Young Scientists and Postdoctoral Students.

References

1. Arrage, A. A., Vasishtha, N., Sundberg, D., Bausch, G., Vincent, H. L., White, D.C., On-Line Monitoring of Antifouling and Fouling-Release Surfaces Using Bioluminescence and Fluorescence Measurements during Laminar-Flow, *Journal of Industrial Microbiology*, **1995**, *15*, 277-282.
2. Angelin, J., Kavitha, M., Exopolysaccharides from probiotic bacteria and their health potential *International Journal of Biological Macromolecules* **2020**, *162*, 853-865.

Corresponding author: s.toshkova@shu.bg Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

3. Doychinova, K., Nadezhda, N., Model for contentive and technological integration applied in environmental education. *Chemistry: Bulgarian Journal of Science Education*, **2019**, *28(6)*, 746-761.
4. Doychinova, K. Project-based learning in a model for content and technology integration in environmental education. *Natural Sciences and Advanced Technology Education*, **2020**, *30(4)*, 380-394.
5. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F. Colorimetric method for determination of sugars and related substances, *Anal. Chem*, **1956,** *28(3),* 350-356.
6. European Food Safety Authority EFSA, Statement by the EFSA Panel on Biological Hazards on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 12: Suitability of taxonomic units notified to EFSA until March 2020. *EFSA Journal*, **2020**, 18 p. 6174, 10.2903/j.efsa.2020.6174.
7. Glenn, R., Gibson, M.B., Roberfroid, Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. *The Journal of Nutrition*, **1995**, *125(6)*, 1401–1412.
8. German, B., Schiffrin, E.J., Reneiro, R., Mollet, B., Pfeifer, A., Neeser, J., The development of functional foods: lessons from the gut. *Trends Biotechnol***, 1999,** *17*, 492–499.
9. Hongpattarakere, T., Cherntong, N., Wichienchot, S., Kolida, S., Rastall, R.A. *In vitro* prebiotic evaluation of exopolysaccharides produced by marine isolated lactic acid bacteria. *Carbohydr. Polym.*, **2012**, *87*, 846-852.
10. Ibryamova, S., Ismailov I., Hasanov, H., Ivanov, R., Ignatova-Ivanova, Ts., Functional Characterization of an Exopolysaccharide Produced by *Lactobacillus plantarum* Ts Isolated from Bulgarian Wheat and Rye Flour. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2019**, *10(4)*, 132-138.
11. Ibrjamova, S., Ivanov, R., Ignatova-Ivanova, Ts., Exopolisaccharides from *L. fermentum* Ts as corrosion inhibitors, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2018**, *9(6)*, 366-374. IF 0.38.ISSN 0975-8585.
12. Ignatova-Ivanova, Ts., Doychinova, K., Nescheva, D., Ivanov, R., Investigation of the anticorrosive activity of the species *Lactobacillus plantarum* isolated from home-made cow's yogurt. *UNITECH' 10, Gabrovo*, **2010**, *3*, 489-492.
13. Ignatova-Ivanova, Ts., Ibrjmova, S., Andreeva, A., Ivanov, R., Study of biofilm formation from *Lactobacillus fermentum* S cultivated on different carbohydrates. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2017**, *8(6)*, 282-289.
14. Ignatova-Ivanova, Ts., Ibryamova, S., Ivanov, R., Exopolysaccharides from *Lactobacillus plantarum* Ts as Corrosion Inhibitors. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **2017**, *9(6)*, 1103-1111.

Corresponding author: s.toshkova@shu.bg Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

15. Ignatova-Ivanova, Ts., Ibryamova, S., Ismailov, I., Hasanov, H., Ivanov, R., Structural characterization of an exopolysaccharide produced by *Lactobacillus plantarum* Ts isolated from Bulgarian wheat and rye flour. *International Journal of ecology and development*, **2020**, *35(2)*, 1-12.
16. Idrees, M., Muhammad, I., Naima, A., Zahra, R., Abid, R., Alreshidi, M., Roberts, T., Abdelgadir, A., Khalid, M. T., Farid, A., Olawale, O. A., Ghazanfar, S., Probiotics, their action modality and the use of multi-omics in metamorphosis of commensal microbiota into target-based probiotics. *Front. Nutr.*, 16 September, *Sec. Nutrition and Food Science Technology*. **2022**. https://doi.org/10.3389/fnut.2022.959941.
17. Imre, B., Pukánszky, B., Compatibilization in bio-based and biodegradable polymer blends, *European Polymer Journal*, **2013**, *49*, 1215-1233.
18. Kodali, V.P., Perali, R.S., Sen, R.. Purification and partial elucidation of the structure of an antioxidant carbohydrate biopolymer from the probiotic bacterium Bacillus coagulans RK-02, *J. Nat. Prod.*, **2011**, *74*, 1692-1697. https://doi.org/10.1021/np1008448.
19. Korcz, E., Varga, L., Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. *Trends in Food Science & Technology*, **2021**, *110*, 375-384.
20. Korcz, E., Kerényi, Z., Varga, L., Dietary fibers, prebiotics, and exopolysaccharides produced by lactic acid bacteria: Potential health benefits with special regard to cholesterol-lowering effects, *Food & Function*, **2018**, *9*, 3057-3068.
21. Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S., Lee, Y.C. Carbohydrate analysis by a phenol-sulfuric acid method in microplate format, *Anal. Biochem*, **2005**, *339*, 69-72.
22. Moradali, M.F., Rehm, B.H.A., The Role of Alginate in Bacterial Biofilm Formation. In: Cohen E., Merzendorfer H. (eds) Extracellular Sugar-Based Biopolymers Matrices. *Biologically-Inspired Systems*, **2019**, vol 12. Springer.
23. Moradi, M., Guimarães, J.T., Sahin, S., Current applications of exopolysaccharides from lactic acid bacteria in the development of food active edible packaging, *Current Opinion in Food Science*, **2021**, *40*, 33-39.
24. Murdzheva, D., Petkova, N.T, Todorova, M., Vasilev, I., Ivanov, I., Denev, P. Microwave-assisted synthesis of methyl esters of alginic acids as potential drug carrier. International Journal of *Pharmaceutical and Clinical Research*, **2016**, *8(10)*, 1361-1368.
25. Petkova, N., Arabadzhieva, R., Vassilev, D., Gencheva, G., Tumbarski, Y., Ignatova-Ivanova, Ts., Ibryamova, S., Todorova, M., Koleva, M., Denev, P., Physicochemical Characterization And Antimicrobial Properties Of Inulin Acetate Obtained By Microwave-Assisted Synthesis. *Journal of Renewable Materials*, **2020**, *8(4)*, 365-381. a
26. Petkova, N., Tr., Arabadzhiva, R. D., Tumbarski, Y. D., Todorova, M. M., Hambarlyiska, I. P., Ivanov, I. G., Ibryamova, S. F., Ignatova-Ivanova, Ts. V., Physicochemical Properties and Antimicrobial

Corresponding author: s.toshkova@shu.bg Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License

Activity of Acetylated Chicory Fructooligosaccharides. *Philippine Journal of Science*, **2021**, *150(4)*, 633-642. b

27. Petkova, N., Arabadzhieva, R., Hambarliyska, I., Vassilev, D., Gencheva, G., Tumbarski, Y., Ignatova-Ivanova, Ts., Ibryamova, S., Koleva, M., Denev, P., Ultrasound-Assisted Synthesis of Antimicrobial Inulin and Sucrose Esters with 10-Undecylenic Acid. *Biointerface Research in Applied ChemistryPlatinum*, **2021**, *11(4)*, 12055-12067. c
28. Priyanka, P., Arun, A., & Rekha, P. Sulfated exopolysaccharide produced by *Labrenzia* sp. PRIM-30, characterization and prospective applications. *International Journal of Biological Macromolecules*, **2014**, *69*, 290-295. https://doi.org/10.1016/j.ijbiomac.2014.05.054.
29. Ruas-Madiedo, P., Medrano, M., Salazar, N., Los Reyes-Gavilán, D., Pérez, P., Abraham, A. Exopolysaccharides produced by *Lactobacillus* and *Bifidobacterium* strains abrogate in vitro the cytotoxic effect of bacterial toxinson eukaryotic cells. *Journal of Applied Microbiology*, **2010**, *109(6)*, 2079-2086.
30. Sauer, K., Stoodley, P., Goeres, D.M. et al. The biofilm life cycle: expanding the conceptual model of biofilm formation. *Nat Rev Microbiol*, **2022**, *20*, 608–620.
31. Stadler, R., Wei, L., Furbeth, W., Grooters, M., Kuklinski, A. Influence of bacterial exopolymers on cell adhesion of *Desulfovibrio vulgarison* high alloyed steel: corrosion inhibition by extracellular polymeric substances (EPS). *Mater Corros*, **2010**, *61(12)*, 1008–16.
32. Wang, X., Yuan, Y., Wang, K., Zhang, D., Yang, Z., Xu, P. Deproteinization of gellan gum produced by *Sphingomonas paucimobilis* ATCC 31461. *Biotechnol*, **2007**, *128*, 403-407.
33. Xu, R., Shen, Q., Ding, X., Gao, W., Li, P. Chemical characterization and antioxidant activity of an exopolysaccharide fraction isolated from Bifidobacterium animalis RH. Eur. *Food Res. Technol*, **2010**, *232*, 231–240.
34. Zhang, J., Cao, Y., Wang, J., Guo, X., Zheng, Y., Zhao, W., Mei, X., Guo, T., Yang, Z. Physicochemical characteristics and bioactivities of the exopolysaccharide and its sulphated polymer from *Streptococcus thermophilus* GST-6. *Carbohydrate Polymers*, **2016**, http://dx.doi.org/10.1016/j.carbpol.03.063.
35. Zheng, J.S., Wittouck, S., Salvetti, E., Franz, C.M.A.P., Harris, H.M.B., Mattarelli, P. et al. A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. *International Journal of Systematic and Evolutionary Microbiology*, **2020**, *70*, 2782-2858.

Corresponding author: s.toshkova@shu.bg
Full Paper
DOI: 10.2478/asn-2022-0022
©2022 Stephany Toschkova, published by Sciendo
This work is licensed under the Creative Commons Attribution 3.0 Public License