



Protective Effects of *Lactobacillus plantarum* Exopolysaccharides from Home Made Dairy Products on Obesity

Ahmet Turan Demir^{1,a*}, Bilge Hilal Çadırcı Efe^{1,b}, Ahmet Altun^{2,c}

¹Faculty of Engineering and Architecture, Department of Bioengineering, Tokat Gaziosmanpaşa University, Tokat, Turkey

²Faculty of Medicine, Department of Medical Pharmacology, Sivas Cumhuriyet University, Sivas, Turkey

*Corresponding author

Research Article

History

Received: 05/08/2022

Accepted: 30/09/2022

ABSTRACT

As it is known, obesity is one of the most important health problems of developed and developing countries today. Obesity; endocrine, cardiovascular, respiratory, gastrointestinal (GI), genitourinary, musculoskeletal system such as body systems and psychosocial condition causes many health problems due to the negative effects. The ability of probiotics to support GI health has been recognized for years. Since alternative approaches against obesity can be developed by intervention in the GI system, this study aimed to evaluate the effects of exopolysaccharides (EPS) as an anti-obesity biomaterial isolated from natural products with probiotic characteristics. First, the one with the highest EPS amount among the 10 microorganisms with known probiotic properties was identified by MALDI-TOF and 16s rRNA sequence analysis and named as *Lactobacillus plantarum* BHC007. Then, the monosaccharide composition of the extracted EPS was examined, and its average molecular weight was determined as 221.860±0.006. The effect of EPS on the adipogenesis process was examined qualitatively with Oil Red O dye on the 3T3 cell line and cellular triacylglycerol (TAG) levels. It was concluded that the highest EPS concentration (2.25 mM) inhibited adipogenesis by 75%. Also, 17% inhibition of adipogenesis was achieved at the lowest EPS concentration (0.45 mM). In conclusion, TAG analysis revealed that EPS was suppressed depending on the lipid droplet formation concentration.

Keywords: Exopolysaccharide, probiotic, obesity

Ev Yapımı Süt Ürünlerinde Bulunan *Lactobacillus plantarum* Ekzopolisakkaritlerinin Obezite Üzerindeki Koruyucu Etkileri

Süreç

Geliş: 05/08/2022

Kabul: 30/09/2022

ÖZ

Bilindiği gibi, obezite günümüzde gelişmiş ve gelişmekte olan ülkelerin en önemli sağlık sorunlarından biridir. Obezite; endokrin, kardiyovasküler, solunum, gastrointestinal (GI), genitoüriner, kas-iskelet sistemi gibi vücut sistemleri ve psikososyal durumlara olumsuz etkileri nedeniyle birçok sağlık sorununa neden olmaktadır. Probiyotiklerin GI sağlığı destekleme yeteneği yıllardır bilinmektedir. GI sistemine yapılan müdahale ile obeziteye karşı alternatif yaklaşımlar geliştirilebildiğinden, bu çalışmada, probiyotik karakteristiklere sahip doğal ürünlerden izole edilen bir anti-obezite biyomalzemesi olarak ekzopolisakkaritlerin (EPS) etkilerinin değerlendirilmesi amaçlanmıştır. İlk olarak, bilinen probiyotik özelliklere sahip 10 mikroorganizma içerisinde en yüksek EPS miktarına sahip olanı, MALDI-TOF ve 16srRNA dizi analizi ile tanımlanmış ve *L. plantarum* BHC007 olarak isimlendirilmiştir. Daha sonra, ekstrakte edilen EPS'nin monosakkarit bileşimi incelenmiş ve ortalama moleküler ağırlığı 221,860±0.006 olarak belirlenmiştir. EPS'nin adipogenez süreci üzerindeki etkisi 3T3 hücre hattı üzerinde, Oil Red O boyası ile niteliksel olarak, ayrıca triasilgliserol (TAG) seviyeleri hücresel olarak incelenmiştir. En yüksek EPS konsantrasyonunun (2.25 mM) adipogenez %75 inhibe ettiği sonucuna varılmıştır. Ayrıca, en düşük EPS konsantrasyonunda (0.45 mM) %17 adipogenez inhibisyonuna ulaşıldı. Sonuç olarak, TAG analizi EPS'nin lipid damlacık oluşum konsantrasyonuna bağlı olarak baskılandığını ortaya koymuştur.

License



This work is licensed under
Creative Commons Attribution
4.0 International License

Anahtar sözcükler: Ekzopolisakkarit, probiyotik, obezite

^a ahmetturan.demir1018@gop.edu.tr <https://orcid.org/0000-0002-3464-0466> ^b bilgehilal.cadirci@gop.edu.tr <https://orcid.org/0000-0003-1525-9608>
^c md.ahmetaltun@gmail.com <https://orcid.org/0000-0003-2056-8683>

How to Cite: Demir AT, Çadırcı Efe BH, Altun A (2022) Protective Effects of *Lactobacillus plantarum* Exopolysaccharides from Home Made Dairy Products on Obesity, Cumhuriyet Medical Journal, September 2022, 44 (3): 273-281

Introduction

The relationship between the gut and brain is driven by the network of neurons, chemicals, and hormones. This enteric nervous system is called the “second brain” that controls our hunger, stress, mental health, etc., and is under the control of the gastrointestinal system. Since then, microbial flora in the intestine system has gained importance for human being¹.

Probiotics are defined as “living microorganisms and products containing microbial metabolites which, *in vivo*, affect the normal intestinal bacterial flora by improving its balance, and which are consequently biologically beneficial”^{2,3,4}. The probiotic bacterium, *Lactobacillus (L.) plantarum* which is in generally-recognized-as-safe (GRAS) status according to the US Food and Drug Administration is generally used in food industry applications, including in yogurt, fermented vegetables, and beverages^{5,6}. The reported health benefits of *L. plantarum* is on cholesterol, diarrhea, and irritable bowel syndrome⁷. After growing in glucose or sucrose, *L. plantarum* strains can produce exopolysaccharides (EPS)⁸. EPS are metabolic by-products of microorganisms⁹. EPS are extracellular macromolecules that are secreted by bacteria as a loosely connected slime layer or as a securely bonded capsule. When it comes to protecting cells against desiccation, phagocytosis, cell identification, phage assault, antibiotics or poisonous substances, and osmotic stress, EPS is most important¹⁰. EPS contains sugar residues with proteins like xanthan, dextran, alginate, gellan, levan, cellulose, curdlan, pullulan, succinoglycan, hyaluronic acid, DNA, phospholipids, and non-carbohydrate substituents such as acetate, glycerol, pyruvate, sulfate, carboxylate, succinate and phosphates^{10,11,12,13,14}. Natural polymers have received a lot of interest in the last several decades from the scientific community due to their potential for therapeutic use. Particularly, the EPS isolated from probiotic bacteria that have different carbohydrate compositions has a wide range of advantageous qualities¹⁰. Probiotic EPS are supplemented in the treatment of human disorders such as inflammatory bowel diseases, autoimmune diseases, colon cancer, gastric ulcers, cardiovascular diseases and obesity^{10,15,16}.

Because of the polysaccharide nature of the EPS, *in vitro* anti-diabetic activity of EPS was delayed¹⁰. Recently, the investigations are increasing showing the relation between obesity and EPS. The energy-salvaging capacity of the gut microbiota from dietary ingredients has been proposed as a contributing factor for the development of obesity. This knowledge generated interest in using of non-

digestible dietary ingredients such as prebiotics to manipulate host energy homeostasis¹⁷. Lim et al. (2017) investigate the antiobesity potential of EPS isolated from kefir grains *in vitro* and in a mouse model as compared with β -glucan (BG), a viscous polysaccharide, and reported the EPS and its residue can prevent obesity¹⁸.

This study aimed to show the protective effect of EPS from probiotic bacteria isolated from dairy products on lipogenesis. For this purpose, EPS from best producer isolate was characterized genetically, and purified EPS was exposed to 3T3 mouse fibroblast cells *in vitro* before formation of the adipose tissue.

Materials and Methods

Isolation of the lactic acid bacteria

Lactic acid bacteria from yogurt, kefir, cheese, were isolated by plate pour method after decimal dilutions. The isolated strains were exposed to standard analysis to check pure cultures. MRS media were used for cultivations. All the isolated strains were stored in the culture collection of Tokat Gaziosmanpaşa University Faculty of Engineering and Architecture Bioengineering Department Industrial Microbiology and Biotechnology Laboratory in Turkey.

Determination of the best EPS production

Cell-free supernatants were used to determine the EPS production.

Preparation of cell-free supernatants from lactic acid bacteria (LAB)

The LABs used in the study were first activated in MRS agar (Merck 10660) at 37°C for 48 hours, then inoculated into 100 ml flasks containing 80 ml of MRS broth (Oxoid CM0359). EPS formation was controlled by taking samples from bacteria grown at 32-37°C for 48-72 hours. For this purpose, 2 ml of bacterial culture was taken and suspended in 10 ml of distilled water, and the cells were lysed in a sonic water bath mrc ACP-120H (Hagavish 3, Holon, Israel) at 40 Watt (W), 50 times at 10 seconds (sec) intervals for 5 sec. After the obtained suspension was centrifuged Tehtnica Centric 400R (Železniki, Slovenia) at 12000 rpm for 15 min at 4°C, the supernatants formed were sterilized by filtration through a 0.45 μ m polyethersulfone (PES, ISOLAB) syringe filter. Samples were lyophilized SCANVAC (Bjarkesvej, Denmark) and stored at -80°C Haier BIO-MEDICAL (Qingdao, Shandong, China) until use¹⁹.

Extraction and purification of EPS from cell-free culture supernatants

Zhang and their friends have been implemented for this process by modifying the work in 2016²⁰. EPS from the cell-free culture supernatant was extracted by slowly adding 20 ml of 75% (v/v) cold

ethanol (Merck 100983) to each sample for 24 hours. EPS samples precipitated at the end of the period were collected by centrifugation (Tehtnica Centric 400R) at 12000 rpm for 20 minutes and the resulting debris was dissolved in 150 ml of milliQ water Milli-Q IQ 7003 (Molsheim, France). To remove the proteins in the extract, 50 ml of sample was incubated in 20 ml of sevag reagent [1:4 v/v; 1-butanol (Merck 101988)/chloroform (Merck 102445)] for 2 hours at 125 rpm with shaking Lap Companion SI-300R (Daejeon, Republic of Korea). The precipitated proteins were removed by centrifugation at 5000xg for 20 min (Tehtnica Centric 400R). After centrifugation, the solution containing EPS was dialyzed against 5 l of distilled water for 2 days with three times daily water changes in a dialysis membrane Spectra/Por (New Brunswick, USA) with a molecular weight permeation of 3000 Da.

Control of EPS purity

The phenol sulfuric acid technique was used to determine the total carbohydrate content of the EPS generated²¹. After the bacterial cells were recovered with short spin centrifugation following the dialysis process, 1 ml of 5% phenol (Merck 100206), 5 ml of concentrated H₂SO₄ (Merck 100731) were added to a 1 ml aliquot taken from the supernatant, and the mixture was immediately vortexed IKA 2 (Staufen, Germany). After 20 minutes of incubation at room temperature, absorbance was measured at 490 nm in a spectrophotometer Thermo Scientific Multiskan FC (Singapore). The link between these absorbances recorded for each sample, glucose (Merck 108337) absorbance values as a reference, and the covariates were investigated using regression analysis.

The total protein content of EPS was measured using the Bradford method and an optical density measurement at 595 nm²². ThermoFisher Scientific 23200 ampoules of Bovine Serum Albumin (BSA) Coomassie (Bradford) Protein Assay Kit (Rockford, USA) were used as a protein concentration standard. The experiments were repeated independently and 3 times.

Identification of the best EPS producer microorganism

This procedure was carried out both by using the Bruker Matrix-Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI TOF MS, Billerica, MA USA) Biotyper ID equipment and 16S rRNA analysis. The sample from which single colonies were obtained from the subcultures was taken to the target plate using a sterile toothpick tip. The sample was coated with 1µl cinnamic acid (C₉H₈O₂, Merck 800235) and air-dried as a matrix solution. The loaded target plate was placed in the

device and exposed to short laser pulses, and thus the evaporation of the microorganism and matrix identified on the plate was achieved with laser energy. In this way, the ribosomal proteins of the microorganism are also ionized. The ions are accelerated in an electromagnetic field created by a potential of 20 kilovolts (kV) with the device software, before entering the flight tube. The time of flight (TOF) of the detector located at the end of the flight tube, which is a mass spectrometer, was measured, and based on this TOF information, microorganism identification was performed by matching the mass spectrum of the sample with the spectrum stored in the device's database. Microorganism identification of the same sample was also performed by 16S rRNA analysis. For this purpose, DNA isolation (GeneAll- Clinic SV), PCR processes (WizPure- PCR 2X Master), and purification (Thermo- ExoSAP-IT Express PCR Cleanup Reagents) of PCR products were carried out by the kit protocols by using the relevant kits. Then, DNA sequence analysis was performed with the ABI 3500 XL device (Foster City, CA, USA).

Determination of monosaccharide compositions

Since the highest EPS value was obtained from the 7th sample consequently the analyzes made, further investigations were carried out only on this sample. The monosaccharide composition of EPS in sample 7 was determined using Agilent 1260 High-Performance Liquid Chromatography (HPLC, Santa Clara, USA) equipped with a refractive index detector and Agilent ChemStation software integrated with the instrument.

Fourier-Transformed Infrared (FT-IR) spectroscopy

FT-IR spectroscopy has been accounted to be a significant apparatus for checking the underlying changes of biopolymers²³. Using Tensor II BRUKER FT-IR spectroscopy (Billerica, MA USA), the functional groups contained in pure EPS were identified and the OPUS software integrated with the instrument.

Molecular weight determination

The average molecular weight distribution of purified EPS was determined by Malvern VISCOTEK gel permeation chromatography (GPC, Worcestershire, United Kingdom) using the method of Cui et al²⁴.

Cell culture research

The 3T3 cell line used in the research, 3T3 An1 Swiss albino mouse fibroblasts (mouse), were obtained from the T.C. Ministry of Agriculture and Forestry ŞAP Institute in T25 flask with registration number 950217. 3T3 pre-adipocytes were maintained in sub-confluent cultures in high glucose gibco Dulbecco's Modified Eagle Medium (DMEM 11965092) containing 10% gibco Fetal Bovine Serum (FBS 16000044), 100 U/ml penicillin, and 100

$\mu\text{g/ml}$ streptomycin (gibco 15140122). Cells were grown in T75 flasks at 37°C in a humid atmosphere maintained at 5% Nüve EC 160 CO_2 incubator (Ankara, Turkey). Cells were subcultured 2 or 3 times per week depending on growth rate. For further studies, cells were transferred to 6-well plates, and those in other flasks were frozen-stocked for repeated experiments.

Differentiation into adipocyte cells

For the differentiation of 3T3 cells into adipocyte cells, abcam's protocol was applied²⁵. First, cells were seeded into a 6-well-plate at a density of 3.0×10^3 cells per cm^2 . The medium was changed every 2-3 days, and the cells were grown in DMEM until confluence was reached. To initiate differentiation, DMEM was removed from 2-day-old confluent cells, and 2-3 ml of methyl isobutyl xanthine (Sigma I5879), dexamethasone (Thermo Scientific Acros 230302500), insulin (Biological Industries 41-975-100) "MDI" induction medium per well was added (day 0). On day 3 of the cells' incubation conditions, the MDI induction medium was aspirated, and the medium was replaced with 2-3 ml of insulin medium. On the sixth day following the incubation, insulin medium was aspirated from the cells, and fresh DMEM was added. On day 10, fully differentiated adipocyte cells were obtained. The effects of EPS on the adipogenesis of 3T3 cells were recorded on day 0 of the protocol.

Oil Red O staining

Oil Red O staining monitored differentiation into adipocyte cells for lipid accumulation. For this, Abnova Lipid (Oil Red O) Staining Kit KA4541 was applied. To prepare 0.5% Oil Red O dye, the dye was dissolved entirely in 100% isopropanol (IPA, Sigma-Aldrich W292907) and stored in a sealed brown bottle. The stock dye solution obtained was diluted with ddH_2O at a ratio of 3:2 into a 15 ml falcon tube, covered with aluminum foil, and kept at laboratory temperature for 10 min. Oil Red O dilution solution was filtered into a 50 ml falcon tube with the help of a $0.2 \mu\text{m}$ injector filter. 1 ml of Oil Red O dilution solution was added to the cells. The plate was incubated for 10-20 min with gentle circular shaking to ensure that the cells were entirely and evenly covered. At the end of the period, the Oil Red O dilution solution was aspirated and washed 2-3 times with ddH_2O until the excess dye was no longer visible. Cells were kept covered with ddH_2O while examined under the microscope.

Triacylglycerol (TAG) analysis

Cayman Chemical triglyceride analysis kit protocol (Cayman 10010303, Ann Arbor, MI USA)

was used for cellular TAG level detection. Lipids were extracted from 6-day-old differentiated 3T3 adipocyte cells as previously described for analysis. Before the analysis, the diluted Standard Diluent Analysis Reagent (Item No. 700732) was used to obtain the standard curve during the preparation of the standard. Serial dilution was started by taking $200 \mu\text{l}$ of standard mix (200mg/dl) from tubes. Each time the mix was thoroughly mixed for each tube. Thus, TAG concentrations were obtained with serial dilutions.

At the stage of performing the analysis, first, $10 \mu\text{l}$ of the standard was added to the TAG standard wells, the cell growth medium was added as a control and $10 \mu\text{l}$ of EPS was investigated as an anti-obesity agent. Then, the reaction was started by adding $150 \mu\text{l}$ of the diluted Enzyme Mix (Item No. 10010511) solution in the kit to each well. The plate was then incubated at 37°C for 30 min, and absorbance was measured at 550 nm using a plate reader.

Statistical analysis

Regression and ANOVA (variance) analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM) program version 23. The statistical data reported includes results from at least three biological replicates. All results are expressed as mean \pm SEM. Differences were considered significant at $p < 0.05$.

Results

Determination of EPS production conditions

Temperature, pH, and incubation duration of 10 material samples utilized in the formation of EPS were measured (Table 1).

Control of EPS purity

EPS amounts in the samples were estimated by the phenol-sulfuric acid method. Regression and ANOVA (variance) analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM) program version 23. According to the data obtained from the program, the determination coefficient is $R^2=0.998$, indicating that the model's adaptability is very high.

The linear equation observed that the highest EPS value was obtained from the pickle sample number 7 (Table 1). Further research was carried out only on this sample.

The Bradford method estimated protein amounts in the samples²². Thus, test samples were screened regarding protein content, and it was decided that they were partially pure (Table 1).

Table 1. Some incubation values of material samples

Material	Temperature (°C)	pH	Incubation time (h)	EPS amounts (ppm)	Protein amounts (mg/ml)
1	37	4.61±0.01	48	9.95±0.04	0.0028±0.0002
2	37	4.59±0.03	48	61.12±0.28	0.0016±0.0002
3	37	4.37±0.00	48	72.31±0.87	0.0015±0.0004
4	32	4.13±0.04	72	51.98±1.36	0.0017±0.0006
5	32	3.92±0.05	72	33.26±0.58	0.0022±0.0003
6	32	3.94±0.02	72	82.95±0.51	0.0014±0.0001
7	37	4.54±0.01	48	103.39±0.38	0.0011±0.0001
8	37	4.81±0.06	48	76.59±0.53	0.0014±0.0003
9	37	4.69±0.03	48	38.45±0.73	0.0019±0.0002
10	37	4.96±0.04	48	21.81±0.21	0,0025±0.0001

Determination of monosaccharide compositions

When the HPLC chromatogram of fructose, glucose, galactose, and maltose was examined as a standard in the analysis, it was determined after

9.026, 11.498, 18.677, and 23.315 min, respectively. The fructose, glucose, galactose, and maltose in the composition in EPS were determined after 9.103, 11.514, 18.781 and 23.695 min (Fig. 1).

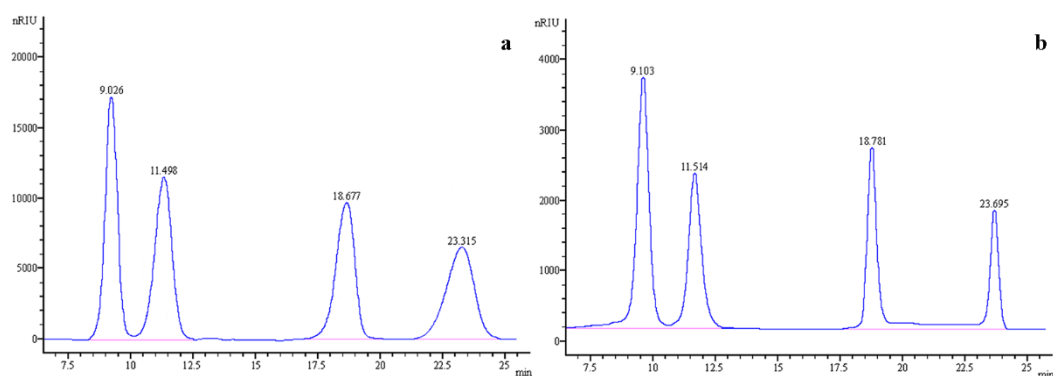


Fig. 1. HPLC chromatogram of EPS. (a) Standard chromatogram. (b) Sample chromatogram

FT-IR spectroscopy

The intense broad stretching peak seen at 3417 cm^{-1} indicates that it contains a significant number of hydroxyl groups. Absorption in this region has rounded features specific to hydroxyl groups²⁶. This suggests that the sample is a polysaccharide. The FT-IR spectra of EPS revealed functional characteristics with two weak C-H stretch peaks at 2921 and 2851 cm^{-1} corresponding to the methyl and methylene groups. The strong absorption observed at 1650 cm^{-1} corresponds to the amide stretch, while the peak at 1414 cm^{-1} can be attributed to the $>\text{C}=\text{O}$ stretch of the COO^- groups and the C-O bond from the COO^- groups^{27,28}. The absence of a peak in the wavenumber range of 1775-1700 cm^{-1} , which is accepted as the fingerprint region for glucuronic acid or diacyl esters, in the produced EPS proved that these compounds were not encountered in the sample. The peak around 1650 cm^{-1} indicates the presence of the C-O group, consistent with the results reported in the

literature²⁷. A large C-O-C, C-O tension was observed at 1200-1000 cm^{-1} , suggesting the presence of carbohydrates²⁹ (Fig. 2).

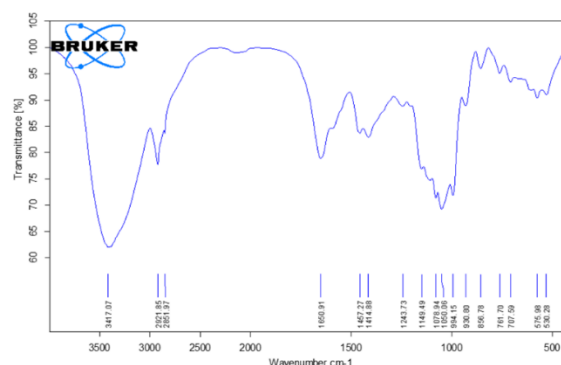


Fig. 2. FT-IR spectrum of EPS

Molecular weight determination

The weight-average molecular weight (M_w) of EPS was analyzed by GPC. Comparing the refractive index databases of purified EPS from sample 7 against the standard, only one symmetrical peak shows that the EPS is homogeneous (Fig. 3).

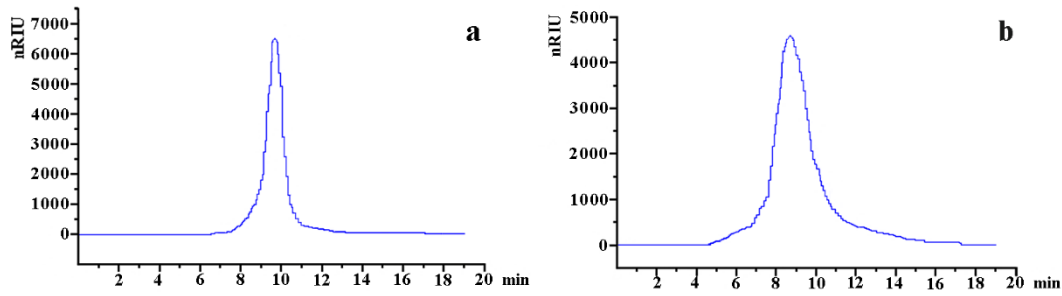


Fig. 3. Gel permeation chromatography of EPS. (a) Standard chromatogram. (b) Sample chromatogram

It has been shown that EPS is a narrow distribution example with a polydispersity of 1.174 with an M_w/M_n ratio approaching 1.00, and the

relative molecular weight distribution can be considered homogeneous. This value also indicates that the sample is of biological origin (Table 2).

Table 2. Molecular weight and relative molecular weight distribution of EPS

Molar moments of mass ($\text{g}\cdot\text{mol}^{-1}$)				Polydispersity	
M_n^a	M_p^b	M_w^c	M_z^d	M_w/M_n	M_z/M_n
188,956±0.02	192,720±0.01	221,860±0.006	315,608±0.01	1.174±0.01	1.670±0.01

^a Number-average molecular weight

^b Peak position molecular weight

^c Weight-average molecular weight

^d z-average molecular weight

Identification of EPS produced microorganism

Both MALDI TOF MS and 16S rRNA analyzes showed that the EPS producing microorganism was

L. plantarum. The isolate is called as *L. plantarum* BHC07 and the phylogenetic tree of microorganisms is showed in Figure 4.

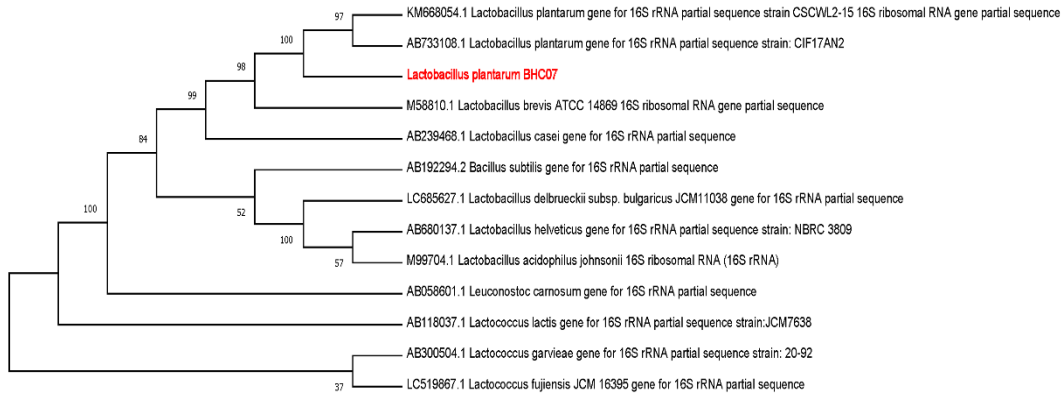


Fig. 4. Gene tree showing the evolutionary distance of the microorganism in sample 7

Cell culture research

Differentiation into adipocyte cells

3T3 cells have been shown to have a fibroblast-like morphology during the developmental phase (Fig. 5a). Twenty-four hours after the addition of the MDI induction medium, the spindle-shaped

morphology of the cells began to change (Fig. 5b). Successful differentiation from 3T3 pre-adipocytes to adipocyte cells was observed with the formation of lipid droplets following the differentiation protocol (Fig. 5c-d).

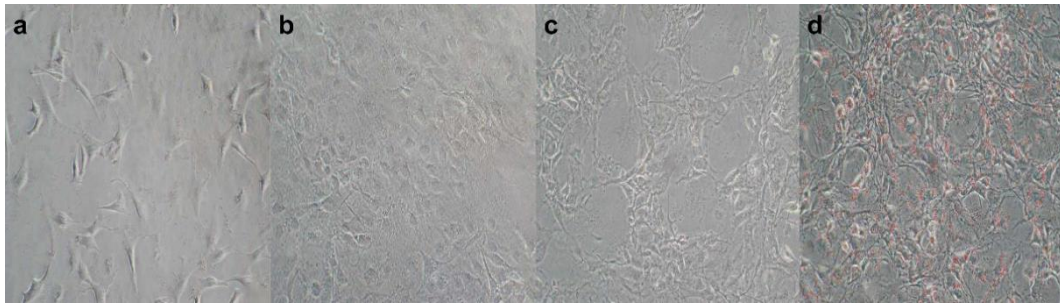


Fig. 5. Differentiation of 3T3 cells into adipocyte cells

Oil Red O staining

Lipid accumulation was evaluated as an indicator of differentiation by staining cells with Oil Red O after differentiation was induced. The effects

of the agents whose concentrations increase from left to right on the cells are shared. Qualitatively, a concentration-proportional lipid accumulation was observed in EPS agent profiles compared (Fig. 6).

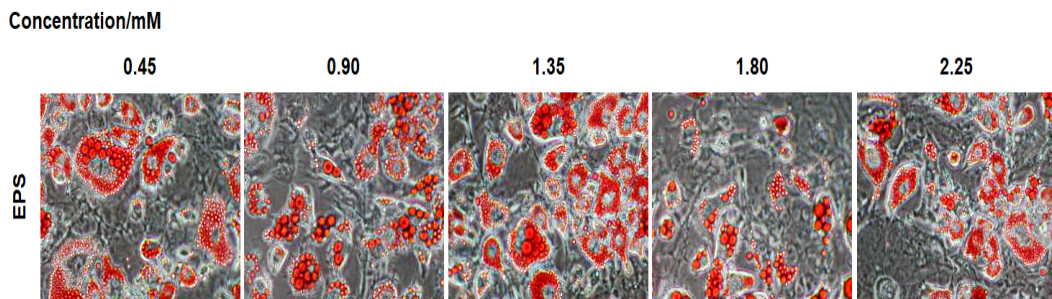


Fig. 6. Oil Red O staining of cells

Triacylglycerol (TAG) analysis

The absorbance averages of the TAG standards measured in three replicates were subjected to regression analysis, and the linear graph with the $y=0.0036x-0.007$ regression equation was obtained in the very high goodness-of-fit model with the $R^2=0.9994$ value of the determination coefficient.

The absorbance values measured for each of EPS agents applied to the cells were evaluated in this equation, and their effects on cellular TAG levels were investigated. While no statistically meaningful difference was regarded for the cell growth medium

application alone, it was observed that there was a statistically meaningful difference between both EPS and control applications ($p<0.05$). It was also regarded that there was a statistically significant difference between each different concentration application of EPS ($p<0.05$). It was concluded that EPS inhibited adipogenesis by 75% at the highest concentrations of 2.25 mM respectively, according to the percentage of control, and 17% at the lowest concentrations of 0.45 mM. Thus, the decrease in TAG levels due to increasing concentration was proven quantitatively (Fig. 7).

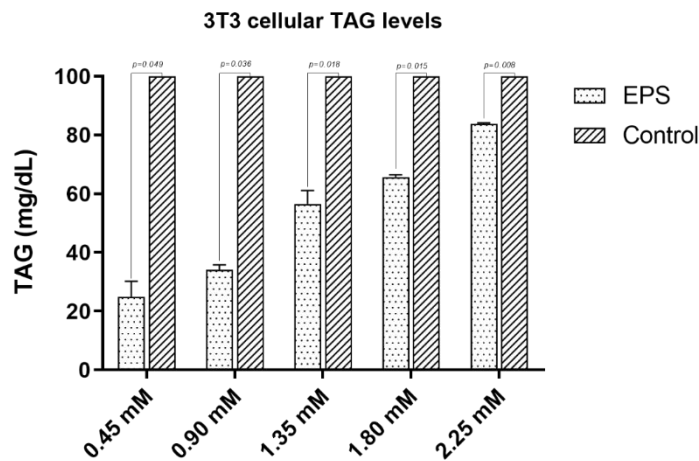


Fig. 7. 3T3 cellular TAG levels

Discussion

EPSs are extracellular carbohydrate polymers created and released by microorganisms and gathered outside cells³⁰. These molecules have various biological effects. In our study, it was investigated whether EPSs synthesized from *L. plantarum* BHC007 have anti-obesity effects.

Different methods and mediums can be used for the synthesis of EPSs. Although studies in the literature show that they are synthesized better in basic mediums, we carried out our syntheses in an acidic medium due to the media and bacteria we used. Considering that the culture medium of LABs, which is the subject of this study, is acidic, EPSs obtained by incubating different concentrations of aqueous solutions of EPSs from *L. plantarum* BHC007. In this study, EPS released into the supernatant after the culture of a live microorganism (*L. plantarum*), whose probiotic character is known, is precipitated with 75% (v/v) cold ethanol. Then it is isolated from cell components by purification processes. It is thought that it will bring a new approach to the literature in the field.

In the current study, after EPS was synthesized, HPLC for monosaccharide composition of EPS, FT-IR spectroscopy for assessing functional groups in EPS, and GPC was used for the average molecular weight distribution of EPS. When we look at the literature, Abedfar et al. found that the associate monosaccharides released from EPS isolated from *L. acidophilus* were galactose, glucose, and maltose, while our sample contained fructose, glucose, galactose, and maltose monosaccharides³¹. The difference in content is due to the absence of any previous study on *L. plantarum*. In the study of Liu et al. in 2017, the FT-IR results for *L. plantarum* WLPL04 and our results are almost similar³². In their study, the M_w of EPS was 5.17×10^4 Da, and in our study, this result was 2.21×10^5 Da. Since these EPS structures are large molecules, it is difficult for them to attach to or enter cells. This situation reduces the efficiency and sometimes biocompatibility of EPSs.

In C57BL/6J mice given a high-fat (HF) diet, the physiological characteristics of water-soluble EPS and their remains after EPS removal (Res) from probiotic kefir were investigated, EPS significantly inhibited 3T3-L1 preadipocyte adipogenesis due to the dose¹⁸. In another study, the fibroblast cell line of 3T3-L1 was used as a cell model for the practicability and assessment of probiotic components in making better animal lipid metabolisms. Extracts from 12 *Lactobacillus* strains have been displayed to remarkably decrease triacylglycerol (TAG) gathering with the enlistment of extreme swelling in 3T3-L1 adipocytes²⁰.

In the results obtained from our study, while the effect on adipogenesis was evaluated qualitatively with Oil Red O staining, the same effect was investigated quantitatively with TAG analysis. In Oil Red O staining, EPS inhibited lipid droplet formation in a concentration-

dependent manner, while the same concentration-dependent effect was observed in TAG analysis. *L. plantarum* seems to be an excellent source to produce the correct form of EPS, which can inhibit adipogenesis *in vitro*. The bioengineering approach we took in the current study is promising, yet we need to expand our study *in vivo* with further studies.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was supported with 100/2000 Ph.D Scholarship within the scope of Turkey Council of Higher Education 100/2000 Biomaterials and Tissue Engineering. In this regard, I would like to thank the Turkey Council of Higher Education.

References

1. Sonnenburg J, Sonnenburg E. The Good Gut: Taking Control of Your Weight, Your Mood and Your Long-Term Health, by Justin and Erica, PhDs. Penguin Press, USA, 2015.
2. Fuller, R. Probiotics in man and animals. J Appl Bacteriol 1989; 66(5): 365-378.
3. Yoshida Y, Seki T, Matsunaka H et al. Clinical Effects of Probiotic *Bifidobacterium breve* Supplementation in Adult Patients with Atopic Dermatitis. Yonago Acta Medica 2010; 53(2): 37-45.
4. Honda, K, Moto, M, Uchida, N, He, F, Hashizume, N. Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice. Journal of Clinical Biochemistry and Nutrition 2012; 51(2): 96-101.
5. Tanganurat, W, Quinquis, B, Leelawatcharamas, V, Bolotin, A. Genotypic and phenotypic characterization of *Lactobacillus plantarum* strains isolated from Thai fermented fruits and vegetables. Journal of Basic Microbiology 2009; 49(4): 377-385.
6. Brinques, GB, Ayub, MAZ. Effect of microencapsulation on survival of *Lactobacillus plantarum* in simulated gastrointestinal conditions, refrigeration, and yogurt. Journal of Food Engineering 2011; 103(2): 123-128.
7. Yang, B, Yue, Y, Chen, Y et al. *Lactobacillus plantarum* CCFM1143 Alleviates Chronic Diarrhea via Inflammation Regulation and Gut Microbiota Modulation: A Double-Blind, Randomized, Placebo-Controlled Study. Frontiers in Immunology 2021; 12(746585): 1-12.
8. Ismail, B, Nampoothiri, KM. Production, purification and structural characterization of an exopolysaccharide produced by a probiotic *Lactobacillus plantarum* MTCC 9510. Archives of Microbiology 2010; 192(12): 1049-1057.

9. Ziadi, M, Bouzaiene, T, M'Hir, S et al. Evaluation of the Efficiency of Ethanol Precipitation and Ultrafiltration on the Purification and Characteristics of Exopolysaccharides Produced by Three Lactic Acid Bacteria, *BioMed Research International* 2018; 1896240: 1-11.
10. Angelin, J, Kavitha, M. Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules* 2020; 162: 853-865.
11. Freitas, F, Alves, VD, Reis, MAM. Advances in bacterial exopolysaccharides: from production to biotechnological applications, *Trends Biotechnol.* 2011; 29(8): 388-398.
12. Nwodo, U, Green, E, Okoh, A. Bacterial Exopolysaccharides: Functionality and Prospects. *International Journal of Molecular Sciences* 2012; 13(12): 14002-14015.
13. De Vuyst, L, Degeest, B. Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews* 1999; 23(2): 153-177.
14. Escárcega-González, CE, Garza-Cervantes, JA, Vázquez-Rodríguez, A, Morones-Ramírez, JR. Bacterial Exopolysaccharides as Reducing and/or Stabilizing Agents during Synthesis of Metal Nanoparticles with Biomedical Applications. *International Journal of Polymer Science* 2018; 7045852: 1-15.
15. London, LEE, Kumar, AHS, Wall, R et al. Exopolysaccharide-Producing Probiotic *Lactobacilli* Reduce Serum Cholesterol and Modify Enteric Microbiota in ApoE-Deficient Mice. *The Journal of Nutrition* 2014; 144(12): 1956-1962.
16. El-Deeb, NM, Yassin, AM, Al-Madboly, LA, El-Hawiet, A. A novel purified *Lactobacillus acidophilus* 20079 exopolysaccharide, LA-EPS-20079, molecularly regulates both apoptotic and NF- κ B inflammatory pathways in human colon cancer. *Microbial Cell Factories* 2018; 17(1): 1-15.
17. Sarbini, SR, Kolida, S, Deaville, ER, Gibson, GR, Rastall, RA. Potential of novel dextran oligosaccharides as prebiotics for obesity management through *in vitro* experimentation. *British Journal of Nutrition* 2014; 112(8): 1303-1314.
18. Lim, J, Kale, M, Kim, DH et al. Antiobesity Effect of Exopolysaccharides Isolated from Kefir Grains. *Journal of Agricultural and Food Chemistry* 2017; 65(46): 10011-10019.
19. Park, JE, Oh, SH, Cha, YS 2013. *Lactobacillus plantarum* LG42 Isolated from Gajami Sik-Hae Inhibits Adipogenesis in 3T3-L1 Adipocyte. *BioMed Research International* 2013; 460927: 1-7.
20. Zhang, Z, Zhou, Z, Li, Y, Zhou, L, Ding, Q, Xu, L. Isolated exopolysaccharides from *Lactobacillus rhamnosus* GG alleviated adipogenesis mediated by TLR2 in mice. *Scientific Reports* 2016; 6(1): 1-14.
21. Dubois, M, Gilles, KA, Hamilton, JK, Rebers, PA, Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry* 1956; 28(3): 350-356.
22. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 1976; 72(1-2): 248-254.
23. Wilson, RH, Goodfellow, BJ, Belton, PS. Fourier transform infrared spectroscopy for the study of food biopolymers. *Food Hydrocolloids* 1988; 2(2): 169-178.
24. Cui, J, Gu, X, Wang, F, Ouyang, J, Wang, J. Purification and structural characterization of an α -glucosidase inhibitory polysaccharide from apricot (*Armeniaca sibirica* L. Lam.) pulp. *Carbohydrate Polymers* 2015; 121, 309-314.
25. Reed, BC, Lane, MD. Insulin receptor synthesis and turnover in differentiating 3T3-L1 preadipocytes. *Proceedings of the National Academy of Sciences* 1980; 77(1): 285-289.
26. Howe, KJ, Ishida, KP, Clark, MM. Use of ATR/FTIR spectrometry to study fouling of microfiltration membranes by natural waters. *Desalination* 2002; 147(1-3): 251-255.
27. Haxaire, K, Maréchal, Y, Milas, M, Rinaudo, M. Hydration of polysaccharide hyaluronan observed by IR spectrometry. I. Preliminary experiments and band assignments. *Biopolymers* 2003; 72(1): 10-20.
28. Ben Salah, R, Jaouadi, B, Bouaziz, A et al. Fermentation of date palm juice by curdlan gum production from *Rhizobium radiobacter* ATCC 6466TM: Purification, rheological and physico-chemical characterization. *LWT-Food Science and Technology* 2011; 44(4):1026-1034.
29. Bremer, PJ, Geesey, GG. An evaluation of biofilm development utilizing non-destructive attenuated total reflectance Fourier transform infrared spectroscopy. *Biofouling* 1991; 3(2): 89-100.
30. Moscovici, M. Present and future medical applications of microbial exopolysaccharides. *Frontiers in Microbiology* 2015; 6(1012): 1-11.
31. Abedfar A, Abbaszadeh, S, Hosseininezhad, M, Taghdir, M. Physicochemical and biological characterization of the EPS produced by *L. acidophilus* isolated from rice bran sourdough. *LWT Food Science and Technology* 2020; 127(109373):1-9.
32. Liu, Z, Zhang, Z, Qiu, L et al. Characterization and bioactivities of the exopolysaccharide from a probiotic strain of *Lactobacillus plantarum* WLPL04. *Journal of Dairy Science* 2017; 100(9): 6895-6905.