Kafkas Univ Vet Fak Derg
21 (2): 229-232, 2015
DOI: 10.9775/kvfd.2014.12116

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Isolation and Identification of an Exopolysaccharide Producer *Streptococcus thermophilus* Strain from Turkish Yogurt

Enes DERTLİ [1]

[1] Department of Food Engineering, Faculty of Engineering, Bayburt University, TR-69000 Bayburt - TURKEY

Article Code: KVFD-2014-12116 Received: 05.08.2014 Accepted: 30.10.2014 Published Online: 07.11.2014

Abstract

Production of exopolysaccharides (EPS) by starter Lactic acid bacteria (LAB) gained special interest due to technological role of these natural polymers. Yogurt samples were collected from different households in Turkey and bacterial strains were isolated and evaluated for EPS production. Strains potentially producing EPS were then identified to species level with 16S rRNA sequencing. An EPS producer *Streptecoccus thermophilus* strain was identified from yogurt samples. EPS was extracted from culture supernatants of *S. thermophilus* and partially purified and structural analysis of the crude EPS by FTIR spectroscopy revealed the presence of typical functional groups related to exopolysaccharides. This study explains the identification of a potential starter culture for yogurt production with EPS production.

Keywords: *Exopolysaccharides, Streptococcus thermophilus, yogurt, 16S rRNA, FTIR*

Yoğurttan Ekzopolisakkarit Üreten *Streptococcus thermophilus* Suşunun İzolasyonu ve Tanımlanması

Özet

Starter Laktik Asit Bakterileri (LAB) tarafından ekzopolisakkarit (EPS) üretimi bu doğal polimerlerin teknolojik rollerinden dolayı son yıllarda önem kazanmıştır. Bu çalışmada yoğurt örnekleri yerel kaynaklardan toplanmış, bakteriler izole edilmiş ve bu türlerin EPS üretimi test edilmiştir. EPS üretim potansiyeli gösteren suşlar 16S rRNA sekanslama tekniği ile suş seviyesine kadar tanımlanmıştır. Bu çalışmalar neticesinde EPS üretimi gösteren bir *Streptecoccus thermophilus* suşu tanımlanmıştır. EPS *S. thermophilus*'un kültür süpernatantından ekstrakte edilmiş, kısmi olarak saflaştırılmış ve FTIR spektreskopi ile gerçekleştirilen yapısal analiz ekstraktın ekzopolisakkaritler ile alakalı tipik fonksiyonel grupları içerdiğini göstermiştir. Bu çalışma yoğurt üretimi için EPS üretme kabiliyetinde olan potansiyel bir starter kültürün tanımlanmasını açıklamaktadır.

Anahtar sözcükler: *Ekzopolisakkaritler, Streptococcus thermophilus, Yoğurt, 16S rRNA, FTIR*

INTRODUCTION

Several lactic acid bacteria (LAB) strains, generally used in fermentation processes as starter cultures, are capable of producing exopolysaccharides (EPS) that can either form a capsule as an outer layer or directly being secreted to the environment [1]. The structure of EPS produced by LAB strains has a wide diversity depending on the sugar monomers that EPS are composed of and those containing only one type of sugar monomer are described as homo-polysaccharides and those containing two or more sugar molecules are described as heteropolysaccharides [2]. EPS of LAB have several ecological functions such as playing important roles on colonization, adhesion, stress resistance, host-bacteria interactions and immunomulation [2]. But EPS are also of special interest in food industry due to their technological roles and their GRAS (generally recognised as safe) status [3].

Yogurt is one of the most consumed fermented milk products worldwide and production of healthier yogurt with less food additives sustaining its technological properties is an important issue matching with consumer's demands. For this reason the use of EPS producing ropy starter cultures in yogurt production is a common practice in order to replace food additives with an improved yogurt viscosity and *in situ* EPS production during yogurt fermentation may also result in a smooth and creamy texture in the final product [4]. Importantly it was shown that these positive effects in technological properties of yogurt is more pronounced when EPS was formed *in situ* rather than when added as an additive [5]. Thus, finding

İletişim (Correspondence)
+90 458 2111153/3225
edertli@bayburt.edu.tr

new EPS producer yogurt starter cultures is crucial in order to develop more desired products as EPS structure, production level of different LAB strains are different and directly affects the functions of EPS [1].

The aim of this study was to isolate and identify an EPS producer yogurt starter culture from Turkish yogurt collected from different households and investigate the EPS structure produced by this potential starter culture.

MATERIAL and METHODS

Isolation of LAB from Yogurt Samples

Yogurt samples (n=8) were randomly collected from four different households in Düzce province of Turkey and samples were stored at 4°C and analysed within 48 h as previously described [6]. Basically, 10 g of yogurt samples were taken aseptically and transferred to separate sterile bags. In order to homogenise yogurt samples, 90 ml of sterile saline solution (0.85%, pH: 7.0) were added to each sample. Serial dilutions were prepared and aliquots of these dilutions were plated to MRS (de Man, Rogosa and Sharpe) and BHI (Brain-heart infusion) agars and incubated at 37 and 42°C for 48 h. Colonies with typical slimy characteristics were randomly selected from MRS and BHI agar plates and tested for Gram stain, cell morphology and catalase reaction as described elsewhere [6]. Potential LAB were selected from these colonies and further investigated for their genotypic identification.

Bacterial Identification by 16S RNA Sequencing

Seven slimy colonies were selected as potential EPS producer LAB cultures using the previously described method [7] and grown in MRS broth and further inoculated to MRS agar to have single pure fresh colonies. Single bacterial colonies were resuspended in 10 ml sterile H_2O and 1 ml aliquots were taken from these suspensions as a DNA template in a PCR reaction that contained 1 µl DNA template, 10 µl 5× PCR buffer for Taq polymerase (Go Taq, Promega), 0.4 µl dNTPs (Bioline), 1 µl of 20 mM primers AMP_F (5'- GAGAGT TTGATYCTGGCTCAG - 3') and AMP_R (5'-AAGGAGGTGATCCARCCGCA - 3') [8], 0.25 µl 5U Taq polymerase and up to 50 µl of sterile H_2O in order to amplify complete 16S rDNA with a final product of c. 1.5 kb. PCR was performed using a thermocycler (Biometra) with the following programme: 95°C for 2 min, 20 cycles of 95°C for 30 s, 55°C for 20 s, and 72°C for 30 s and 72°C for 5 min final extension. PCR products were run on a gel to check the amplication and amplicons were further purified using SureClean kit (Bioline). Sequencing reactions were prepared using primers AMP_F/AMP_R at 1.6 µM concentrations and the ABI Prism BigDye Terminator v3.1 Cycle Sequence Kit (Applied Biosystems) according to the manufacturer's protocol. Sequences obtained were interrogated by using Ribosomal Database Project II [9] and the identities of the isolates were determined on the basis of more than 0.98 matching score.

Isolation of Exopolysaccharides

Exopolysaccharides (EPS) were isolated from bacterial strain using the method described previously [10]. Briefly identified *Streptecoccus thermophilus* strain was grown in 500 ml MRS culture, inoculated at 1% (v/v) with an overnight culture then incubated at 37°C for 2 d. The bacterial supernatant was collected after centrifugation at 6.000 × g for 30 min at 4°C and an equal volume of chilled ethanol was added to the supernatant to precipitate bacterial EPS and stored at 4°C overnight. Sample was centrifuged at 10.000 × g for 30 min at 4°C and the pellet of the precipitates was retained. The sample was resuspended in H_2O with gentle heating (50°C) and EPS was recovered by precipitation upon the addition of 2 volumes of chilled ethanol. After centrifugation at 10.000× g for 30 min at 4°C the resulting EPS was resuspended in distilled H_2O with gentle heating (less than 50°C) followed by dialysis for 72 h (12.000-14.000-Da dialysis membrane) at 4°C, with two changes of H_2O per day. The contents of the dialysis tubing were freeze-dried to provide EPS. This was further purified by dissolving in 10% TCA and stirring overnight. The precipitated protein was removed by centrifugation at 10.000 × g for 15 min at 4°C. The pH of the supernatant was adjusted to 7 with 1 M NaOH and EPS was precipitated again with 2 volumes of chilled ethanol. The pellet was dissolved in distilled water and then lyophilized by freeze drying. The EPS samples were stored at 4°C for further analysis.

FTIR Spectroscopy Analysis of EPS

Fourier transform infrared (FTIR) spectra of the pure capsular EPS isolated from wild type and mutants cell pellets were measured with a FTS 175C Digilab FT-IR spectrometer (Bio-Rad, US) equipped with a MCT detector and a single-reflection diamond ATR sampling accessory (GoldenGate, Specac). The spectra were recorded in the region of 4000-800 cm^{-1} with 128 scans at 4 cm^{-1} resolution and processed by the spectrometer software. The finger-print region of 800-1800 cm^{-1} spectra of EPS samples were analysed in detail.

RESULTS

Identification of EPS Producer LAB Strain Isolated from Traditional Yogurt

Yogurt samples that were collected for this study were produced with traditional methods and it should be noted that bacterial populations from four yogurt samples were low and ranged between 10^5 - 10^6 CFU/ml suggesting the traditional yogurt production conditions were not that appropriate. A total of seven bacterial isolates from thirty isolates were selected as potential EPS producing

LAB strains after morphological and chemical tests and subjected to 16S rRNA sequencing and as can be seen in *Fig. 1*, the 1.5 kb region of 16S gene from each isolate were successfully amplified. From this seven isolates three strains were identified as *Streptecoccus thermophilus* as can be expected and three strains were identified as *Staphylococcus hominis* and one strain were matched with an uncultured bacterium (data not shown). Isolation of *S. hominis* as a slimy colony from yogurt was not that surprising as this strain was isolated from traditionally fermented milk in South Africa and its EPS production for the first time was reported recently [11]. *S. hominis* was not our target strain in this study but it should be noted that this strain could preserve its ropiness for a period of cell transfers [11] which is not always the case for the ropy LAB as genetic biosynthesis mechanism of EPS production is not that stable. After isolation of EPS from the new *S. thermophilus* strain structural analysis has been conducted in order to confirm the polymer as EPS.

Structural Analysis of EPS by FTIR Analysis

In this study structural and functional groups of the crude EPS were determined by FTIR analysis *(Fig. 2)*. The EPS sample showed a wide absorption peak around 3200-3400 cm^{-1}, indicating typical hydroxyl groups (O-H) of polysaccharides suggesting that the analysed sample is a polysaccharide [12]. The peak from 2800 to 2950 cm^{-1} showed a weak C-H stretching frequency for exopolysaccharide of *S. thermophiles* ED1 [13]. The region around 1500-1600 cm^{-1} did not show the intense peak which is assigned to N-H bending and C-N stretching in proteins [14] *(Fig. 2)*. The amide C=O stretching and carboxyl groups were detected from the corresponding peak at 1600-1700 cm^{-1}. The EPS sample showed an intense peak around 1000 cm^{-1} which

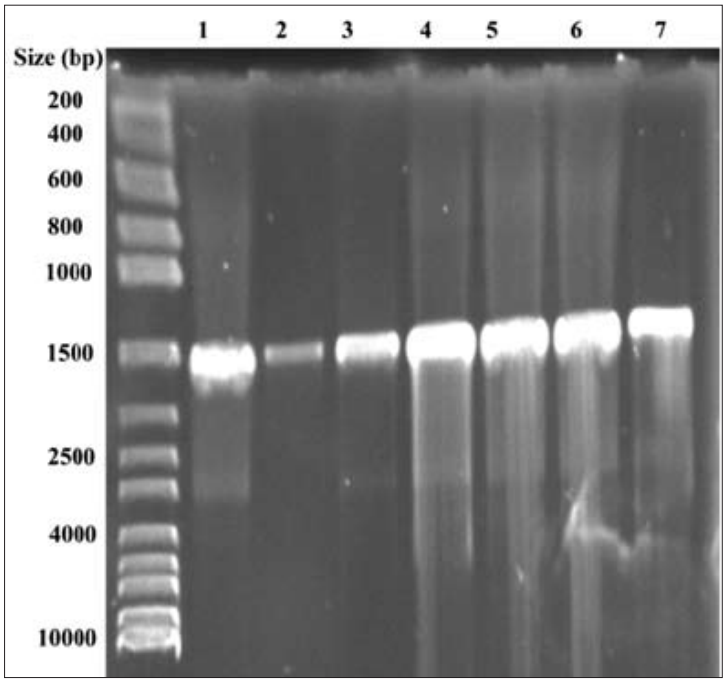
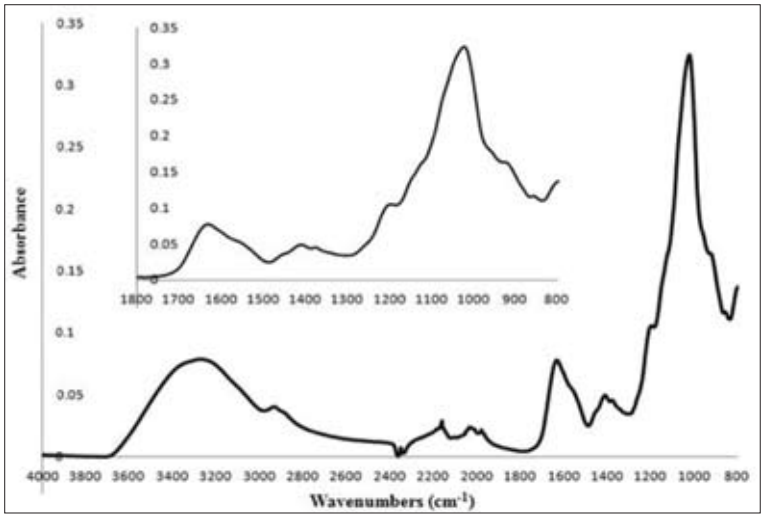


Fig 1. Agarose gel (0.8%) showing the amplication of 16S rRNA genes from seven yogurt isolates

Şekil 1. 16S rRNA geninin 7 yogurt izolatında çoğaltılarak elde edilmesini gösteren agaroz (%0.8) jeli

Fig 2. FTIR spectra of EPS isolated from yogurt isolate *S. thermophilus* strain. The inner figure represents the spectra of the fingerprint region

Şekil 2. Yoğurt izolatı *S. thermophilus* suşundan izole edilen EPS'in FTIR analiz görüntüsü. İçteki şekil parmak izi bölgesinin analiz görüntüsünü vermektedir



indicated the characteristic C-O bond of polysaccharides [13]. Overall FTIR spectrum confirmed the EPS production of yogurt isolate *S. thermophilus* strain.

DISCUSSION

In this study a new *S. thermophilus* strain is identified with EPS production ability as a potential starter culture in yogurt production. It was possible to isolate and identify several other strains from yogurt but our target was finding an EPS producer LAB strain as EPS production in yogurt is critical for yogurt rheology, texture and its microbiological characteristics [1]. Previously several studies also showed the EPS production of *S. thermophilus* strains [15,16] and importantly *S. thermophilus* was the first food related organism in which the *eps* gene cluster was identified [17]. Structural analysis in different studies revealed that *S. thermophilus* strains were able to produce heteropolymeric type EPS in which glucose, galactose, rhamnose, N-acetylgalactosamine and fucose were the sugar monomers comprising the EPS repeating units [15,18-20]. Following the isolation and identification of an EPS producer *S. thermophilus* strain, EPS from culture supernatants of *S. thermophilus* were extracted and subjected to FTIR structural analysis and the polymer was confirmed as an EPS.

Recent interest in food industry especially in dairy industry increased the attention to EPS producer LAB strains due to the technological role of *in situ* EPS production. We should note that more studies are required in order to find new EPS structures and new EPS producer strains as technological performance of EPS is dependent on EPS production levels and EPS structures. Research is ongoing with the identified *S. thermophilus* strain in this study in order to determine its EPS production levels under different conditions and its potential as a new starter culture for yogurt production under industrial scale.

In conclusion, an EPS producer *S. thermophilus* strain was isolated from Turkish yogurt as a potential ropy starter culture and the structural analysis of the crude EPS was analysed by FTIR Spectroscopy. Studies on EPS production levels of this strain under different conditions such as incubation temperature, carbon source and pH are definitely required in order to optimise the EPS production. Additionally identification of the sugar monomers that EPS is composed of as well as the potential *eps* genes is also in our future plans. Moreover technological properties of the yogurt produced with this stain will be studied.

REFERENCES

1. De Vuyst L, Degeest B: Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiol Rev*, 23 (2): 153-177, 1999.

2. Delcour J, Ferain T, Deghorain M, Palumbo E, Hols P: The biosynthesis and functionality of the cell-wall of lactic acid bacteria. *Antonie Van Leeuwenhoek*, 76 (1-4): 159-184,1999. DOI: 10.1007/978-94-017-2027-4_7

3. Badel S, Bernardi T, Michaud P: New perspectives for Lactobacilli exopolysaccharides. *Biotechnol Adv*, 29 (1): 54-66,2011. DOI: 10.1016/j.biotechadv.2010.08.011

4. Guzel-Seydim ZB, Sezgin E, Seydim AC: Influences of exopolysaccharide producing cultures on the quality of plain set type yogurt. *Food Control*, 16 (3): 205-209, 2005. DOI: 10.1016/j.foodcont.2004.02.002

5. Doleyres Y, Schaub L, Lacroix C: Comparison of the functionality of exopolysaccharides produced in situ or added as bioingredients on yogurt properties. *J Dairy Sci*, 88 (12): 4146-4156, 2005. DOI: 10.3168/jds.S0022-0302(05)73100-3

6. Aslim B, Onbasili D, Yuksekdag ZN: Determination of lactic acid production and antagonistic activity against *Helicobacter pylori* of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* strains. *Kafkas Univ Vet Fak Derg*, 17 (4): 609-614, 2011.

7. Ruas-Madiedo PCG, de los Reyes-Gavilan: Invited review: Methods for the screening, isolation, and characterization of exopolysaccharides produced by lactic acid bacteria. *J Dairy Sci*, 88 (3): 843-856, 2005. DOI: 10.3168/jds.S0022-0302(05)72750-8

8. Baker GC, Smith JJ, Cowan DA: Review and re-analysis of domain-specific 16S primers. *J Microbiol Methods*, 55 (3): 541-555, 2003. DOI: 10.1016/j.mimet.2003.08.009

9. Cole JR, Chai1 B, Farris RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM J. M. Tiedjeet JM: The ribosomal database project (RDP-II): Sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res*, 33 (Suppl. 1): D294-D296, 2005. DOI: 10.1093/nar/gki038

10. Horn N, Wegmann U, Dertli E, Mulholland F, Collins SR, Waldron KW, Bongaerts RJ, Mayer MJ, Narbad A: Spontaneous mutation reveals influence of exopolysaccharide on *Lactobacillus johnsonii* surface characteristics. *PLoS One*, 8 (3): e59957, 2013. DOI: 10.1371/journal.pone.0059957

11. Bauer R, Bekker JP, Wyk N, du Toit C, Dicks LMT, Kossmann J: Exopolysaccharide production by lactose-hydrolyzing bacteria isolated from traditionally fermented milk. *Int J Food Microbiol*, 131 (2-3): 260-264, 2009. DOI: 10.1016/j.ijfoodmicro.2009.02.020

12. Kanmani P, Satish Kumar R, Yuvaraj N, Paari KA, Pattukumar V, Arul V: Production and purification of a novel exopolysaccharide from lactic acid bacterium Streptococcus phocae PI80 and its functional characteristics activity *in vitro. Bioresour Technol*, 102 (7): 4827-4833, 2011. DOI: 10.1016/j.biortech.2010.12.118

13. Wang Y, Ahmed Z, Feng W, Li C, Song S: Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. *Int J Biol Macromol*, 43 (3): 283-288, 2008. DOI: 10.1016/j.ijbiomac.2008.06.011

14. Yuan SJ, Sun M, Sheng GP, Li Y, Li WW, Yao RS, Yu HQ: Identification of key constituents and structure of the extracellular polymeric substances excreted by *Bacillus megaterium* TF10 for their flocculation capacity. *Environ Sci Technol*, 45 (3): 1152-1157, 2011. DOI: 10.1021/es1030905

15. Lemoine J, Chirat F, Wieruszeski JM, Strecker G, Favre N, Neeser JR: Structural characterization of the exocellular polysaccharides produced by *Streptococcus thermophilus* SFi39 and SFi12. *Appl Environ Microbiol*, 63 (9): 3512-3518, 1997.

16. Petersen BL, Dave RI, McMahon DJ, Oberg CJ, Broadbent JR: Influence of capsular and ropy exopolysaccharide-producing *Streptococcus thermophilus* on mozzarella cheese and cheese whey. *J Dairy Sci*, 83 (9): 1952-1956, 2000. DOI: 10.3168/jds.S0022-0302(00)75071-5

17. Stingele F, Neeser JR, Mollet B: Identification and characterization of the eps (Exopolysaccharide) gene cluster from Streptococcus thermophilus Sfi6. *J Bacteriol*, 178 (6): 1680-1690, 1996.

18. Bubb WA, Urashima T, Fujiwara R, Shinnai T, Ariga H: Structural characterisation of the exocellular polysaccharide produced by *Streptococcus thermophilus* OR 901. *Carbohydrate Res*, 301 (1-2): 41-50, 1997. DOI: 10.1016/S0008-6215(97)00083-9

19. Doco T, Wieruszeski JM, Fournet B, Carcano D, Ramos P, Loones A: Structure of an exocellular polysaccharide produced by S*treptococcus thermophilus. Carbohydr Res*, 198 (2): 313-321, 1990. DOI: 10.1016/0008-6215(90)84301-A

20. Low D, Ahlgren JA, Horne D, McMahon DJ, Oberg CJ, Broadbent JR: Role of *Streptococcus thermophilus* MR-1C capsular exopolysaccharide in cheese moisture retention. *Appl Environ Microbiol*, 64 (6): 2147-2151, 1998.