



Microbial exopolysaccharide production of *Streptococcus thermophilus* and its antiquorum sensing activity

Demet Genc Karadeniz¹ · Banu Kaskatepe¹ · Merve Eylul Kiymaci² · Kenan Can Tok³ · Mehmet Gumustas³ · Cigdem Karaaslan⁴

Received: 21 September 2020 / Revised: 26 March 2021 / Accepted: 29 March 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Interest in the production of exopolysaccharides by microorganisms has increased in the recent years. Using low-cost product is the main step of microbial production to reduce cost and compete with chemical production. In this work, EPS production of *Streptococcus thermophilus* isolates from yogurt (S2), kefir (S3), and *S. thermophilus* ATCC 19258 (S1) isolate which was used as control strains were investigated by using different fruit pulps. *S. thermophilus* isolates were identified by morphological and 16S sequence analysis. The amount of EPS obtained was measured spectrophotometrically using glucose as standard with phenol sulfuric acid method. All three isolates produced higher amounts of EPS on M17 medium than Nutrient medium. When the fruit pulp was added to the medium, EPS production increased in all three isolates. When different nitrogen sources were added together with fruit pulp juice, EPS production increased. The highest amount of EPS produced by ATCC 19258 strain (21.570 mg/L) and S3 isolate (29.131 mg/L) is the medium where mixed fruit pulp juice and nitrogen source is tryptophan. It has been shown that EPS production is increased by adding fruit pulps to the prepared media. It is thought that apricot pulp can be a good alternative in EPS production especially in the evaluation of wastes. Also, antequorum sensing activity of the highest amount EPS was determined by using *Chromobacterium violaceum* CV026 strain and found effective on violacein pigment inhibition and C6-AHL production of biosensor strain.

Keywords Exopolysaccharide · Phenol–sulfuric acid · Fruit pulp · FTIR · *S. thermophilus* · Antiquorum sensing

Introduction

Microbial polysaccharides are polymers that are secreted to the external environment after they are synthesized or synthesized directly outside the cell by enzymes associated

with the cell wall. These polysaccharides are called exopolysaccharides (EPSs). Interest in microbial production has increased in the recent years because of microorganisms create a viscous solution even in very low concentrations and have pseudoplastic structure (Soyucok et al. 2016).

Microbial exopolysaccharides can be classified according to their chemical structure, molecular weight, functionality, and bonding. They are grouped as homo-polymeric or hetero-polymeric according to their monomeric composition. Homo-polysaccharides contain only one type of monosaccharide, while hetero-polysaccharides are composed of repeating units (Ergene and Avci 2016; Nwodo et al. 2012).

EPSs are produced in response to biotic and abiotic stress factors or to adapt to difficult environmental conditions, and their main task is helping microorganism to protect itself against environmental challenges (Donot et al. 2012). EPS production in microorganisms takes place during or at the end of the logarithmic development phase. In addition, EPS production can be realized as a primary or secondary metabolic product in stationary phase (Ergene and Avci 2016).

Communicated by Erko Stackebrandt.

✉ Banu Kaskatepe
bkaskatepe@ankara.edu.tr

¹ Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ankara University, Ankara, Turkey

² Department of Pharmaceutical Microbiology, Gulhane Faculty of Pharmacy, University of Health Sciences Turkey, Ankara, Turkey

³ Department of Forensic Toxicology, Institute of Forensic Sciences, Ankara University, Ankara, Turkey

⁴ Department of Pharmaceutical Chemistry, Gulhane Faculty of Pharmacy, University of Health Sciences Turkey, Ankara, Turkey

The physiological tasks of microbial EPSs are to protect the microorganism against phagocytosis, phage attacks, antibiotics, toxic metal ions, and osmotic stress (Fukuda et al. 2010).

EPSs have important commercial values in the industry due to their physicochemical properties. The ability of lactic acid bacteria (LAB) to produce a wide range of EPS in various compositions and functionality increases their use in industrial areas (Patel et al. 2012). Microbial EPSs have a wide range of commercial applications in various fields such as food, feed, packaging, chemical, textile, cosmetics, wastewater treatment, agriculture, medicine, and pharmaceutical industries. Although there are a large number of microbial EPS varieties, only a few microbial EPS varieties are commercially available. This is due to the high production costs (Ates 2015). Food-related LABs have generally recognized as safe (Febriani et al. 2010) status. These bacteria were found to be suitable for functional EPS production. LABs that can produce EPS are used in the food industry for making yogurt, cheese, milk acid, and milk desserts (Patel et al. 2012). In the food industry, EPSs are used as thickeners, emulsifiers, and viscosity providers. They are the food thickeners of the new generation due to their role in increasing the consistency and texture of the food (Kanamarpudi and Muddada 2017).

S. thermophilus is a nonpathogenic homo fermentative microorganism used as an initial culture in fermented dairy products. It is also the only type of *Streptococcus* that has been recognized as a Generally Safe Bacteria (Febriani et al. 2010) by the Food and Drug Administration because it has not caused any disease in humans for centuries (Li et al. 2016; Uriot et al. 2017). *S. thermophilus* produces extracellular polysaccharide, vitamin, and bacteriocin (Iyer et al. 2010). EPS production is one of the most important and attractive properties of *S. thermophilus* isolates. The EPS produced by *S. thermophilus* is hetero-polysaccharide. It mainly consists of galactose, glucose, and rhamnose monomers, but the structure also includes N-polymers containing acetyl-galactosamine, fucose, and acetylated galactose (Cui et al. 2017; Laws et al. 2001). EPSs with defined bioactive and probiotic properties produced by this microorganism find use in various medical and pharmaceutical fields as antioxidant, anticancer, anti-inflammatory, and immunomodulatory agents. Owing to the wide use of EPS, the number of studies to increase the exopolysaccharide production of different microorganisms has increased (Bengoa et al. 2018). EPS production of *S. thermophilus* generally depends on the growth rate of the bacteria. However, environmental factors, such as temperature, pH, carbon source, carbon concentration, and the ratio of carbon to nitrogen also have an effect (Shene et al. 2008). In addition, the growth phase, agitation, and aeration affect EPS production (Barcelos et al. 2019).

The fruit pulp is emerging as a by-product from fruit juice factories. Fruit pulps contain high amounts of sugar, and

are considered to be an excellent substrate for many bioprocesses because they are rich in different carbon sources. Fruit pulp is quite cheap. They are also abundant throughout the harvest period (Vendruscolo et al. 2008). The use of cheap fruit pulp instead of the commonly used carbon source glucose or sucrose can result in a lower cost of the final product (Stredansky and Conti 1999).

In this study, it was aimed to investigate the EPS production of *S. thermophilus* isolates from three different sources: kefir, boza, and yogurt and to investigate the effect of different fruit pulps on the production of EPS. For this reason, modified and waste reduced phenol sulfuric acid method was used for the determination of EPS. The other aim of study was to evaluate the antiquorum sensing activity of EPS. There are no studies determining the efficacy of exopolysaccharide on the quorum sensing mechanism, which is known to be an importance in the regulation of bacterial virulence. Therefore, the results of this study will be a preliminary study for other studies to be conducted.

Quorum sensing is a bacterial communication mechanism that regulates gene expression depending on the population density and signal molecule secretion (Papenfort and Bassler 2016), also assumed to be responsible for bacterial virulence, such as motility, enzyme secretion, biofilm formation, etc. (Sakuragi and Kolter 2007). In Gram negative bacteria, quorum sensing occurs via acyl homoserine lactone (AHL) signal molecules. Although *Chromobacterium violaceum* (*C. violaceum*, wild type) is a strain capable of producing short chain AHL and purple colored violacein pigment; mutant *Chromobacterium violaceum* CV026 is a biosensor strain that does not produce AHL and pigments, but can produce violacein pigment by recognizing short-chain AHL molecules (AHL molecules with chain length C4 to C8) (McClellan et al. 1997).

Materials and methods

Isolation and identification of EPS-producing *S. thermophilus* isolates

Three different fermented dairy products of different brands such as kefir, boza, and yogurt were purchased from the supermarket. M17 (Liofilchem, Italy) medium was used for *S. thermophilus* isolation. One g of yogurt and boza samples were weighed and homogenized in 9 mL sterile saline (0.85%) tubes. Zero point one (0.1) mL of homogenized samples were taken and the M17 liquid medium was seeded. As the kefir sample was liquid, it was cultured in M17 medium without mixing with saline. The samples were incubated at 37 °C for 24 h. After incubation, the passage was made from liquid culture to M17 agar and incubated for 48 h. Gram staining and catalase test were performed

to identify bacterial colonies developing in the plates after incubation. Gram (+) and catalase (–) strains were identified by 16S rRNA analysis.

DNA isolations were performed with the Sigma-Gen Elute Bacterial Genomic DNA Kit. Isolated DNAs were visualized in agarose gel electrophoresis and then PCR was performed using 16S rDNA primers. The primary sequences that were used are 5'-AGA GTT TGA TCC TGG CTC AG-3' and 5'-CCG TCA ATT CCT TTG AGT TT-3'. The expected base size is 974 bp. The PCR products that were obtained, were imaged in 1.5% agarose gel electrophoresis, and then it was checked whether the desired band sizes (bp) belonging to the relevant primer were found in the genotypes. After agarose gel electrophoresis, band sizes of PCR products were visualized using the UV imaging system. PCR tapes carried out on the gel were purified using the Promega-Wizard SV Gel Clean-Up System Kit.

S. thermophilus ATCC 19258 standard strain (American Type Culture Collection, Manassas, VA, USA) S1, *S. thermophilus* isolates isolated from yogurt and kefir were recorded as S2 and S3, respectively, and they were named as such in the continuation of the study.

Preparation of fruit pulp juice and determination of their contents

In this study, sour cherry, apricot, and peach pulps were used. These pulps were supplied from the Dimes factory in July 2018 and stored at –20 °C until use. Fruit pulps were boiled by stirring on the heater after being mixed with 1/5 (w/w) pure water before use. After boiling for one hour, it was allowed to cool. Fruit pulps were filtered with filter paper when it reached room temperature, and the fruit pulp juice was filtered again with a 0.22-µm pore diameter membrane filter when it was used in EPS production. In addition, this fruit pulp juice was analyzed by chromatography method in the Duzen North West laboratory.

Media for EPS production

M17 broth (Liofilchem, Italy) and Nutrient Broth (Neogen, UK) were used for the determination of basic EPS production of the isolates. Then, four different media were prepared for EPS production. Magnesium sulfate (Sigma-Aldrich, Germany) (0.2 g/L), monopotassium phosphate (Sigma-Aldrich, Germany) (3 g/L), and sodium chloride (Sigma-Aldrich, Germany) (1 g/L) are the main compounds of all media. Peptone (Difco, USA) (2.5 g/L) was added for medium I, beef extract (Neogen, UK) (2.5 g/L), and tryptophan (Difco, USA) (2.5 g/L) were added for medium II and III, respectively. Peptone (2.5 g/L), beef extract (2.5 g/L), and tryptophan (2.5 g/L) were added together for the fourth media (IV).

Base solution: Magnesium sulfate (0.2 g/L), monopotassium phosphate (3 g/L) and sodium chloride (1 g/L), and distilled water.

Medium I: BS + Peptone (2.5 g/L).

Medium II: BS + Beef extract (2.5 g/L).

Medium III: BS + Tryptophan (2.5 g/L).

Medium IV: BS + Peptone (2.5 g/L) + Beef extract (2.5 g/L) + Tryptophan (2.5 g/L).

Production and isolation of EPS

In order to determine EPS production of the isolates, firstly M17 broth and Nutrient broth were used. Then, EPS production of isolates in prepared media was examined. Four percentage of activated isolates were grown on 10 mL M17 broth medium and incubated at 37 °C for 24–72 h to produce EPS. After incubation, 1 mL of sample was taken into eppendorf tubes and boiled at 100 °C for 15 min and cooled to room temperature. By the boiling process, bacteria and enzymes were inactivated. Three point four (3.4) µL of TCA (trichloroacetic acid) (Carlo Erba Reagents, France) (85% w/v) was added to the sample at room temperature and vortexed to homogenize the sample. Subsequently, the cells were separated by centrifugation at +4 °C for 30 min at 13,000 rpm. After centrifugation, the supernatant was transferred to a sterile eppendorf tube. Approximately, 1 mL of ethanol (Sigma-Aldrich, Germany) was added to over the supernatant. After standing for half an hour, the EPS–protein complex was precipitated by centrifugation at 13,000 rpm for 30 min at +4 °C and pellets were obtained. The supernatant was poured, and about 1 mL of ethanol was added onto the pellet. The pellet was allowed to dissolve in ethanol for half an hour. The samples were then centrifuged for 30 min at 13,000 rpm at +4 °C and after centrifugation, the alcohol was removed to obtain EPS (Degeest et al. 1999; Petry et al. 2000).

Determination of EPS quantity

The colorimetric phenol–sulfuric acid method developed by Dubois and co-workers (Dubois et al. 1956) and Masuko and co-workers (Masuko et al. 2005) was used in combination and with small modifications to calculate the amount of EPS. After the sample pellets were dissolved in 100 µL sterile pure water, the phenol sulfuric acid method was applied. Fifty µL of phenol is added to the samples and vortexed. Subsequently, sulphuric acid is added rapidly to complete the total volume to 2 mL. The samples were allowed to stand at room temperature for 10 min and then thoroughly shaken. Two hundred µL of each sample was loaded onto the microplate and measured at 480 nm with the Clariostar microplate reader (BMG Labtech, Germany). The results of 10 replicate

measurements of the samples were analyzed by the Mars data analysis program (BMG Labtech, Germany).

Validation of the method

The glucose stock solution (Supelco, USA) was prepared at a concentration of 1 mg/mL. A 7-point calibration curve was constructed to calculate the glucose concentration. All standards were measured with 10 replicates, and the precision of this method demonstrated as RSD% values which was found between 1.6 and 4.9. The proposed UV spectrophotometric technique provided a good linearity for sugars with the correlation coefficient (R^2) higher than 0.973. The limit of quantification (LOQ) and limit of detection (LOD) were calculated as 0.3 and 0.9 mg/L respectively.

EPS characterization with FTIR

Different samples of EPS were analyzed by FTIR spectroscopy. Infrared (IR) spectra were obtained using an Agilent Cary 630 Fourier transform IR (FTIR) with Diamond ATR accessory (Agilent Technologies, Santa Clara, CA, USA) (Bramhachari and Dubey 2006).

Determination of the effects of M17 broth and fruit pulp on EPS production

In order to determine the optimal incubation time and appropriate medium, all isolates were inoculated 4% on both nutrient and M17 medium and incubated at 37 °C for 72 h in the incubator. At the end of 24, 48, and 72 h of growth, samples were taken, and the amount of EPS was determined.

The effect of different ratio of fruits pulp was investigated. Two mL and 5 mL of sour cherry, apricot, and peach pulp juice were added separately into the M17 medium so that the total medium was 10 mL. Then, 4% of S1, S2, and S3 isolates were added in this prepared medium. At the end of the incubation period, EPSs were obtained and quantified by the phenol–sulfuric acid method.

Determination of the effect of prepared media on EPS production

The effect of medium I and fruit pulp on EPS production

A medium was formed by adding 5 mL of medium I and 5 mL of pulp juice. Then, 4% of S1, S2, and S3 isolates were cultivated in this prepared medium. At the end of the incubation period, EPSs were obtained and quantified by the phenol–sulfuric acid method.

Effect of prepared media containing different nitrogen sources and fruit pulp on EPS production

Four mL of the prepared media were taken into sterile tubes. Two mL of sour cherry, 2 mL of peach, and 2 mL of apricot pulp juice were added. Thus, three pulp juice together with the prepared media. The total volume of the medium is 10 mL. Then, 4% of S1, S2, and S3 isolates were cultivated in this prepared medium. At the end of the incubation period, EPSs were obtained and quantified by the phenol–sulfuric acid method.

Antiquorum sensing activity determination

In this study, a modified method was used to determine the antiquorum sensing activity. *N*-hexanoyl-L-homoserine lactone inhibition of the highest amount EPS that *S. thermophilus* sourced was investigated by using *C. violaceum* CV026 strain as violace in pigment declining. The fresh culture of *C. violaceum* CV026 at 30 °C for 18 h was taken, and adjusted to Mc Farland 0.5 density (10^8 cfu/mL). A hundred μ L *C. violaceum* CV026, and 50 μ L *N*-hexanoyl-L-homoserine lactone was added to 10 mL soft Luria Bertani agar (0.9%) medium and poured into petri plates after vortexing thoroughly. Pure EPS, from the highest amount produced isolate, was dropped on (15 μ L) agar plates and incubated 30 °C for 48 h. Test was carried out three times (Erdozmez et al. 2018).

Statistical analysis

The results obtained in the studies are given on average. SPSS (Version, 17.0) package program was used for statistical evaluation of the data. The comparison of EPS amounts obtained from prepared media with EPS obtained on M17 medium was made by the Wilcoxon's test (values of $p \leq 0.05$ were considered statistically significant). The study was conducted with double control.

Results and discussion

In the recent years, EPSs secreted by *S. thermophilus* isolates have attracted commercial interest in the dairy industry due to their positive effects on dairy products. It was found that they can form the texture of yogurt and low-fat cheeses by affecting the viscosity, rheology, and mouth feel of fermented products (Zhang et al. 2018).

The aim of this study was to determine the effect of fruit pulps on the production of microbial exopolysaccharide. *S. thermophilus* isolates isolated from kefir and yogurt and standard strain *S. thermophilus* ATCC 19258 were used in the study. The obtained isolates were subjected to catalase

test and found to be catalase-negative. The isolates thought to be *S. thermophilus* were determined on a species basis by 16S rRNA analysis. Isolated DNAs were visualized on agarose gel electrophoresis, and then PCR was performed using 16S rDNA primers. According to 16S rRNA sequence analysis, two isolates obtained from kefir and yogurt samples were found as *S. thermophilus*. Similarity values for S2 and S3 isolates were determined as 99.77% with accession number MG815652.1 and 99.66% with accession number MF540945.1, respectively.

Fruit pulp juice was analyzed by chromatography method. The sugar contents of cherry, apricot, and peach pulp juice obtained from fruit pulps are given in Table 1. When the contents are examined, it is seen that apricot pulp juice contains more amount of sugar in terms of glucose, fructose, and sucrose.

Firstly, EPS production was investigated in M17 and Nutrient media for 3 different incubation periods of 24, 48 and 72 h. All three isolates produced better amounts of EPS on M17 medium than Nutrient Broth. S1 isolate achieved the highest EPS production (1.141 and 4.490 mg/L) at the end of the 24-h incubation period in M17 medium while S2 and S3 isolates produced the highest EPS at the end of 72 h incubation period. The average EPS amount at different incubation times and different media of all isolates is given in Table 2. In a study by Aslim et al. (2005) *Lactobacillus delbruckii* subsp. *bulgaricus* B3, G12, and *S. thermophilus* W22 strains were allowed to incubate in MRS and M17 medium for 5 to 48 h respectively. The highest EPS production was obtained after 18 h of incubation period. After 24 h of incubation, it was found that EPS production started to decrease. In another study, the effect of pH (6.0–8.0), temperature (20–40 °C) and incubation time (12–96) for EPS production of *W. cibaria* GA44 was investigated. The highest amount of EPS was obtained when pH 7.0, incubation temperature was 30 °C and incubation time was 48 h (Adesulu-Dahunsi et al. 2018). EPS production of microorganisms

may vary depending on the type of microorganism, growth curve, and fermentation conditions (Table 3).

When 2 or 5 mL of pulp juice was added to the M17 medium, there was no significant difference between the amounts of EPS obtained. However, all three isolates increased the amount of EPS when fruit pulp juice was added to the medium. When the results were examined, all isolates produced highest EPS in apricot pulp juice. Apricot pulp juice increased EPS production more because it contains more glucose, fructose, and sucrose. Average amount of EPS in M17 medium containing fruit pulp juice is given in Fig. 1. In parallel with our study, it was found that sugar in the medium affected EPS production. Not only the nature of the carbon source but also the combination of sugars and concentration units have been found to have a significant stimulating effect on the synthesis of EPS (Jaiswal et al. 2014). It has been shown that different carbohydrates or mixtures of carbohydrates have significant effects on microbial growth and EPS production (Cui et al. 2017). When the medium I–fruit pulps mixture media were examined, high amounts of EPS production for S1 and S2 isolates were obtained in apricot pulp juice of medium I. S3 isolate produced a higher amount of EPS in the presence of

Table 1 Content of fruit pulp juice

	Sucrose (g/L)	Glucose (g/L)	Fructose (g/L)
Cherry pulp juice	0.08	4.9	2.8
Apricot pulp juice	8.7	15.4	6.3
Peach pulp juice	0.08	13.8	3.8

Table 2 Average EPS amount at different incubation times and different media of all isolates

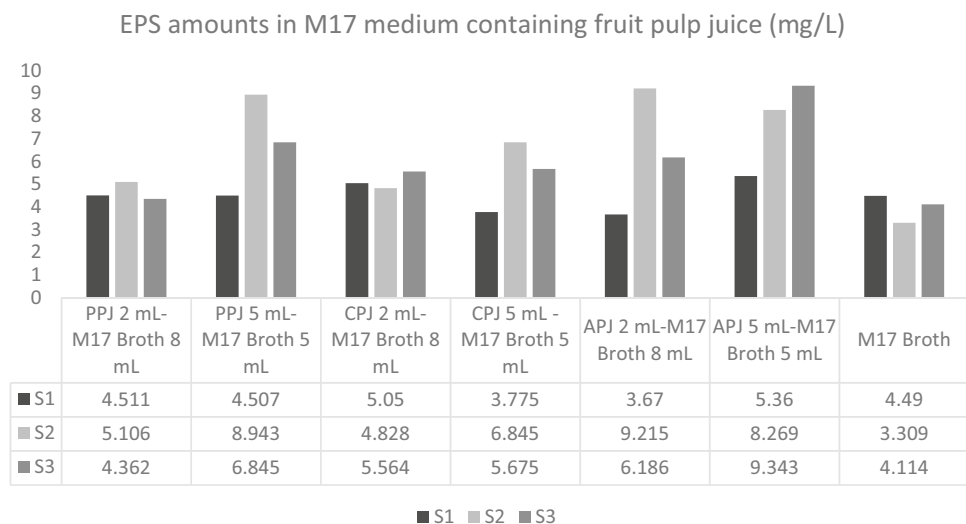
Time (h)	Nutrient medium (mg/L)			M17 medium (mg/L)		
	S1	S2	S3	S1	S2	S3
24	1.141	1.205	1.581	4.490	2.254	1.758
48	1.242	1.418	1.327	2.587	2.780	2.586
72	0.871	1.061	1.809	4.277	3.309	4.114

Bold values indicate the highest amounts of EPS

Table 3 General EPS production of isolates (mg/L)

Medium	Graphs that views	S1	S2	S3
M17	(1)	4.490	3.309	4.114
Nutrient	(2)	1.141	1.061	1.809
M17—Sour cherry pulp 2 mL	(3)	5.050	4.828	5.564
M17—Sour cherry pulp 5 mL	(4)	3.775	6.845	5.675
M17—Apricot pulp 2 mL	(5)	3.670	9.215	6.186
M17—Apricot pulp 5 mL	(6)	5.360	8.269	9.343
M17—Peach pulp 2 mL	(7)	4.511	5.106	4.362
M17—Peach pulp 5 mL	(8)	4.507	8.943	6.845
Medium I—Sour cherry pulp	(9)	4.514	2.948	3.353
Medium I—Apricot pulp	(10)	15.005	15.842	6.568
Medium I—Peach pulp	(11)	9.015	12.489	9.913
Medium I—Mixed fruit pulp	(12)	14.377	19.625	15.031
Medium II—Mixed fruit pulp	(13)	18.392	19.689	19.419
Medium III—Mixed fruit pulp	(14)	21.570	11.121	29.131
Medium IV—Mixed fruit pulp	(15)	11.760	5.775	7.472

Fig. 1 Average amount of EPS in M17 medium containing fruit pulp juice (mg/L). *PPJ* peach pulp juice; *CPJ* cherry pulp juice; *APJ* apricot pulp juice



peach pulp juice in medium I. All three isolates showed the lowest yield in medium prepared with medium I and sour cherry pulp juice. Because cherry pulp contains less sugar than peach and apricot pulp. EPS productions of all isolates are shown in Fig. 2.

S1 and S3 stains produced a high amount of EPS in a medium containing fruit pulp mixture and medium III. The highest amount of EPS was obtained when tryptophan was the source of nitrogen. The S2 isolate produced high amounts of EPS in a mixture medium consist fruit pulp mixture and medium II. S2 prefer beef extract instead of peptone and tryptophan as a nitrogen source. The least amount of EPS was produced in the mixture of fruit pulp juice and medium IV for all isolates. It was found that EPS production decreased when three nitrogen sources were present at the same time for all three

isolates. EPS production in the prepared medium containing different nitrogen sources is given in Fig. 3. All three isolates reduced EPS production in the presence of all nitrogen sources. This is thought to be due to the deterioration of the C/N ratio. In another study, it was shown how important the C/N ratio is. When C/N ratio is 13.6, a high amount of EPS is produced and when C/N ratio is 3.4, a low amount of EPS is produced. It has been shown that the amount of EPS produced decreases as the C/N ratio decreases. EPS production increases with a high C/N ratio (Miqueleto et al. 2010). In a study conducted with *S. thermophilus* ST1, when 0.5% whey was added to skim milk, it increased both bacterial growth and EPS production. It was reported that EPS was produced at 82.70 mg/L with whey supplementation (Zhang et al. 2011). There was no high difference between the three isolates in terms of

Fig. 2 EPS production in the prepared medium 1 (mg/L). *PPJ* peach pulp juice; *CPJ* cherry pulp juice; *APJ* apricot pulp juice

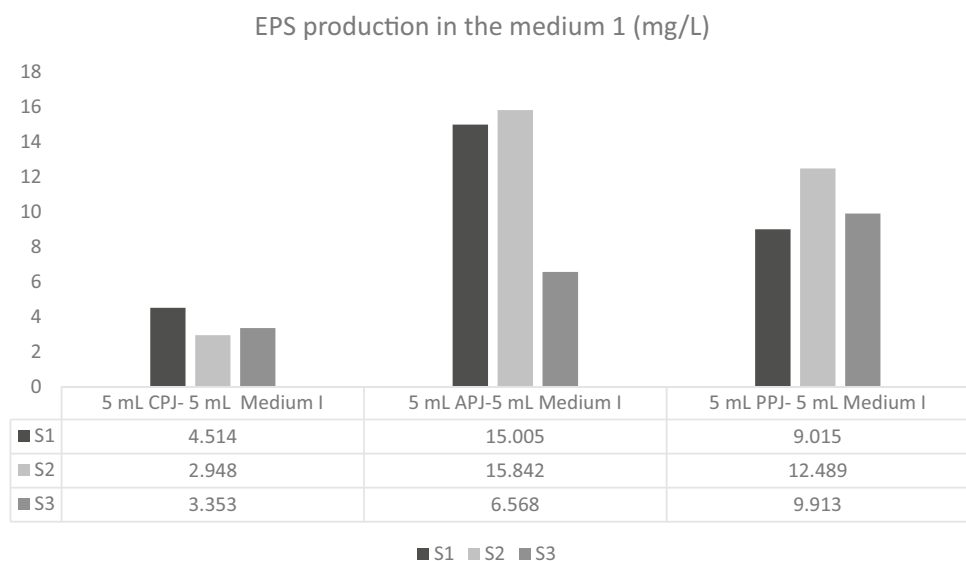
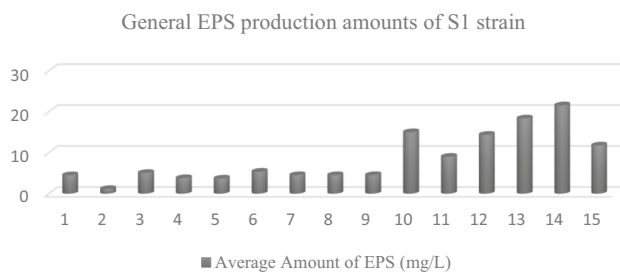
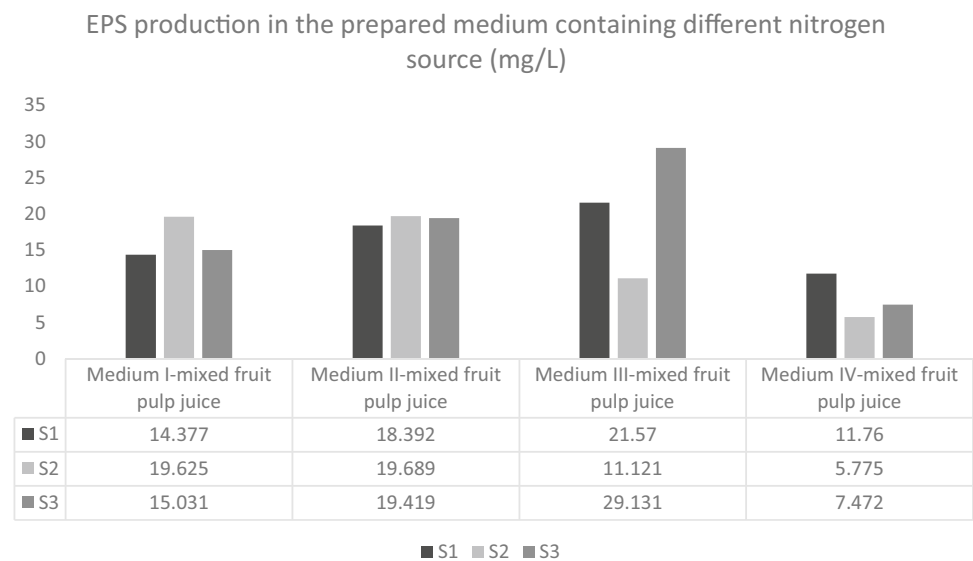
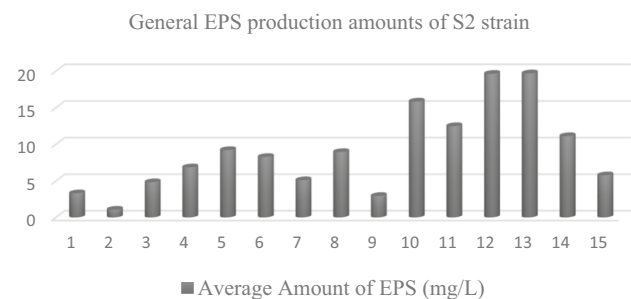
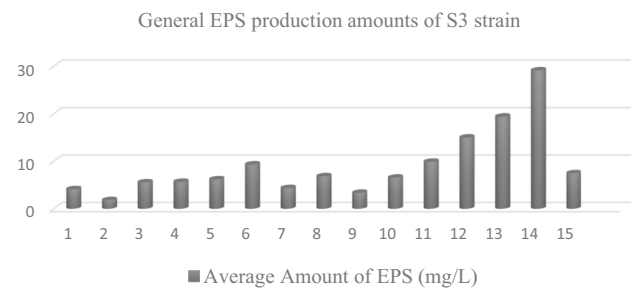


Fig. 3 EPS production in the prepared medium containing different nitrogen source (mg/L)**Fig. 4** General EPS production amounts of S1 isolate**Fig. 5** General EPS production amounts of S2 isolate

EPS production. When the results obtained, EPS production was increased in all three isolates at each stage. The obtained data showed that the media created by adding fruit pulp increased EPS production.

EPS production of S1 and S3 isolates decreased in two medium, increased in 11 medium compared to M17 medium and this increase was statistically significant ($p=0.016$ and $p=0.005$ respectively). EPS production of S2 isolate decreased in 1 medium, increased in 12 medium as compared to M17 medium and this increase was statistically significant ($p=0.002$). General EPS production of the S1, S2, and S3 isolates are shown in Figs. 4, 5, and 6 respectively.

In addition, different samples of EPS were analyzed by FTIR spectroscopy. The example displayed a wide band at around 3287 cm^{-1} attributed to the stretching vibration of the hydroxyl groups of carbohydrates and a C–H stretching band at around 2925 cm^{-1} . The band at 1638 cm^{-1} is associated with the tensile vibration of C=O and corresponds to the characteristic absorption of polysaccharides. All spectra show typical polysaccharide bands at about 1026.9 cm^{-1} within the fingerprint region ($1200\text{--}950\text{ cm}^{-1}$). The FTIR spectrum of the sample EPS is shown in Fig. 7.

**Fig. 6** General EPS production amounts of S3 isolate

The main purpose of this study was to investigate the effect of fruit pulps on EPS production. When the peach, apricot and cherry pulp were added separately into the M17 medium, EPS production increased and the highest EPS production was obtained in apricot medium. When fruit pulp was added to the prepared medium, EPS production was increased than Nutrient broth, M17 broth and M17 medium added to fruit pulp. It has been shown that EPS production is increased by adding fruit pulps to the prepared media. It

Fig. 7 FTIR spectrum of EPS samples

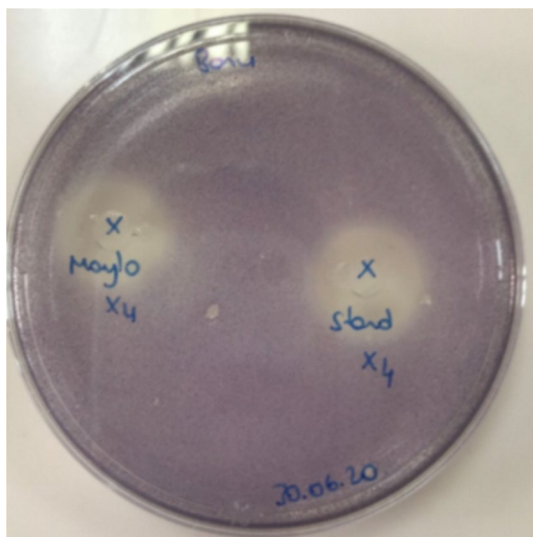
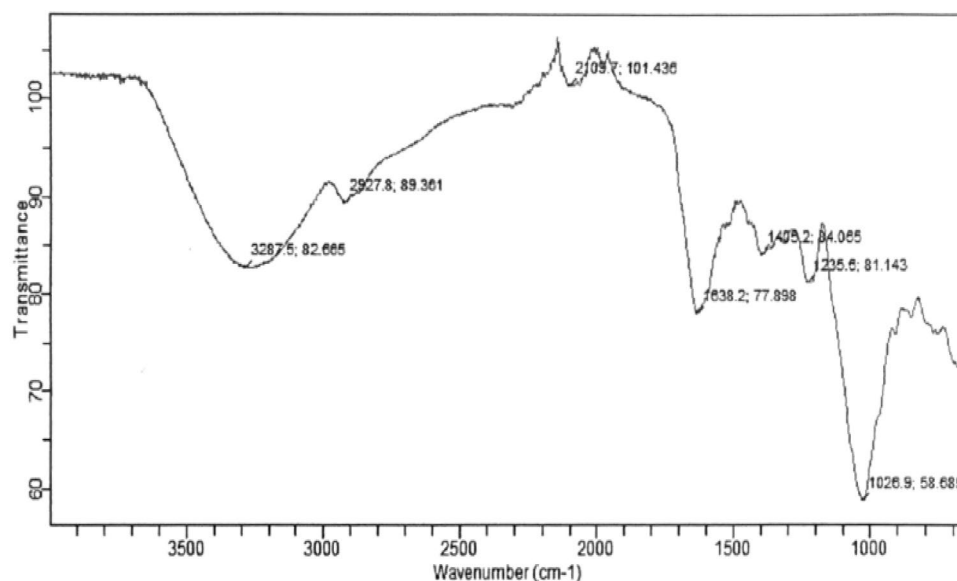


Fig. 8 Violacein pigment inhibition of *C. violaceum* by EPS

is thought that apricot pulp can be a good alternative in EPS production especially in the evaluation of wastes.

In this study, antiquorum sensing activity of the highest amount EPS was investigated and determined that pure EPS had an inhibitory activity on violacein pigment production of biosensor strain in the presence of signal molecules in the environment (Fig. 8). Although the amount of EPS present was small, the CV026 strain had an inhibitory effect on the C6-AHL signal molecule, suggesting that if the EPS to be produced in larger quantities may have an inhibitory effect on gene expression of virulence factors of pathogen microorganisms. In the literature, it was determined that the researchers had studies on the effect of quorum sensing

mechanism on exopolysaccharide production, and that purified exopolysaccharide was not tested on quorum sensing mechanism as an inhibitory agent. Microbial exopolysaccharide is at the forefront as a product that appears as the virulence mechanism of some bacteria and can be used in various industries for different purposes after purification. We think that determination of quorum sensing inhibition in the efficacy studies of the purified exopolysaccharide is also important and it will be a preliminary study for future studies.

Funding This research has been supported by Ankara University Scientific Research Projects Coordination Unit. Project Number: 18L0237005, 2018.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

- Adesulu-Dahunsi AT, Sanni AI, Jeyaram K (2018) Production, characterization and in vitro antioxidant activities of exopolysaccharide from *Weissella cibaria* GA44. LWT Food Sci Technol 87:432–442. <https://doi.org/10.1016/j.lwt.2017.09.013>
- Aslim B, Yuksekdogan ZN, Beyatli Y, Mercan N (2005) Exopolysaccharide production by *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus thermophilus* strains under different growth conditions. World J Microb Biotechnol 21:673–677. <https://doi.org/10.1007/s11274-004-3613-2>
- Ates O (2015) Systems biology of microbial exopolysaccharides production. Front Bioeng Biotechnol 3:200. <https://doi.org/10.3389/fbioe.2015.00200>

- Barcelos MCS, Vespermann KAC, Pelissari FM, Molina G (2019) Current status of biotechnological production and applications of microbial exopolysaccharides. *Crit Rev Food Sci Nutr*. <https://doi.org/10.1080/10408398.2019.1575791>
- Bengoa AA, Llamas MG, Iraporda C, Duenas MT, Abraham AG, Garrote GL (2018) Impact of growth temperature on exopolysaccharide production and probiotic properties of *Lactobacillus paracasei* strains isolated from kefir grains. *Food Microbiol* 69:212–218. <https://doi.org/10.1016/j.fm.2017.08.012>
- Bramhachari PV, Dubey SK (2006) Isolation and characterization of exopolysaccharide produced by *Vibrio harveyi* strain VB23. *Lett Appl Microbiol* 43:571–577. <https://doi.org/10.1111/j.1472-765X.2006.01967.x>
- Cui YH, Jiang X, Hao MY, Qu XJ, Hu T (2017) New advances in exopolysaccharides production of *Streptococcus thermophilus*. *Arch Microbiol* 199:799–809. <https://doi.org/10.1007/s00203-017-1366-1>
- Degeest B, Van Ve VS, De Vuyst L (1999) Process characteristics of exopolysaccharide production by *Streptococcus thermophilus*. *Macromol Symp* 140:43–52
- Donot F, Fontana A, Baccou JC, Schorr-Galindo S (2012) Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohydr Polym* 87:951–962. <https://doi.org/10.1016/j.carbpol.2011.08.083>
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356. <https://doi.org/10.1021/ac60111a017>
- Erdonmez D, Rad AY, Aksoz N (2018) Anti-quorum sensing potential of antioxidant quercetin and resveratrol. *Braz Arch Biol Technol* 61:e18160756
- Ergene E, Avci A (2016) Microbial Exopolysaccharides. *SAÜ Fen Bil Der* 20:193–202
- Febriani Y, Levallois P, Gingras S, Gosselin P, Majowicz SE, Fleury MD (2010) The association between farming activities, precipitation, and the risk of acute gastrointestinal illness in rural municipalities of Quebec, Canada: a cross-sectional study. *BMC Public Health*. <https://doi.org/10.1186/1471-2458-10-48>
- Fukuda K et al (2010) Effects of carbohydrate source on physicochemical properties of the exopolysaccharide produced by *Lactobacillus fermentum* TDS030603 in a chemically defined medium. *Carbohydr Polym* 79:1040–1045. <https://doi.org/10.1016/j.carbpol.2009.10.037>
- Iyer R, Tomar SK, Maheswari TU, Singh R (2010) *Streptococcus thermophilus* strains: multifunctional lactic acid bacteria. *Int Dairy J* 20:133–141. <https://doi.org/10.1016/j.idairyj.2009.10.005>
- Jaiswal P, Sharma R, Sanodiya BS, Bisen PS (2014) Microbial exopolysaccharides: natural modulators of dairy products. *J Appl Pharm Sci* 4:105–109
- Kanamralapudi LRK, Muddada S (2017) Characterization of exopolysaccharide produced by *Streptococcus thermophilus* CC30. *Biomed Res Int*. <https://doi.org/10.1155/2017/4201809>
- Laws A, Gu YC, Marshall V (2001) Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. *Biotechnol Adv* 19:597–625. [https://doi.org/10.1016/S0734-9750\(01\)00084-2](https://doi.org/10.1016/S0734-9750(01)00084-2)
- Li D, Li JX, Zhao F, Wang GH, Qin QQ, Hao YL (2016) The influence of fermentation condition on production and molecular mass of EPS produced by *Streptococcus thermophilus* 05-34 in milk-based medium. *Food Chem* 197:367–372. <https://doi.org/10.1016/j.foodchem.2015.10.129>
- Masuko T, Minami A, Iwasaki N, Majima T, Nishimura S, Lee YC (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal Biochem* 339:69–72. <https://doi.org/10.1016/j.ab.2004.12.001>
- McClellan KH et al (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology* 143(Pt 12):3703–3711. <https://doi.org/10.1099/00221287-143-12-3703>
- Miqueleto AP, Dolosic CC, Pozzi E, Foresti E, Zaiat M (2010) Influence of carbon sources and C/N ratio on EPS production in anaerobic sequencing batch biofilm reactors for wastewater treatment. *Bioresour Technol* 101:1324–1330. <https://doi.org/10.1016/j.biortech.2009.09.026>
- Nwodo UU, Green E, Okoh AI (2012) Bacterial exopolysaccharides: functionality and prospects. *Int J Mol Sci* 13:14002–14015. <https://doi.org/10.3390/ijms131114002>
- Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol* 14:576–588. <https://doi.org/10.1038/nrmicro.2016.89>
- Patel S, Majumder A, Goyal A (2012) Potentials of exopolysaccharides from lactic acid bacteria. *Indian J Microbiol* 52:3–12. <https://doi.org/10.1007/s12088-011-0148-8>
- Petry S, Furlan S, Crepeau MJ, Cerning J, Desmazeaud M (2000) Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii subsp bulgaricus* grown in a chemically defined medium. *Appl Environ Microb* 66:3427–3431. <https://doi.org/10.1128/Aem.66.8.3427-3431.2000>
- Sakuragi Y, Kolter R (2007) Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. *J Bacteriol* 189:5383–5386. <https://doi.org/10.1128/JB.00137-07>
- Shene C, Canquil N, Bravo S, Rubilar M (2008) Production of the exopolysaccharides by *Streptococcus thermophilus*: effect of growth conditions on fermentation kinetics and intrinsic viscosity. *Int J Food Microbiol* 124:279–284. <https://doi.org/10.1016/j.jfoodmicro.2008.03.013>
- Soyucok A, Ekiz T, Basyigit-Kilic G (2016) Ekzopolisakkaritlerin Özellikleri ve Gıda Sanayindeki Önemi. *Nevşehir Bilim Teknol Dergisi* 5:332
- Stredansky M, Conti E (1999) Xanthan production by solid state fermentation. *Process Biochem* 34:581–587. [https://doi.org/10.1016/S0032-9592\(98\)00131-9](https://doi.org/10.1016/S0032-9592(98)00131-9)
- Uriot O, Denis S, Junjua M, Roussel Y, Dary-Mourot A, Blanquet-Diot S (2017) *Streptococcus thermophilus*: from yogurt starter to a new promising probiotic candidate? *J Funct Foods* 37:74–89. <https://doi.org/10.1016/j.jff.2017.07.038>
- Vendruscolo F, Albuquerque PM, Streit F, Esposito E, Ninow JL (2008) Apple pomace: a versatile substrate for biotechnological applications. *Crit Rev Biotechnol* 28:1–12. <https://doi.org/10.1080/07388550801913840>
- Zhang TH, Zhang CH, Li SY, Zhang YC, Yang ZN (2011) Growth and exopolysaccharide production by *Streptococcus thermophilus* St1 in skim milk. *Braz J Microbiol* 42:1470–1478. <https://doi.org/10.1590/S1517-83822011000400033>
- Zhang H et al (2018) Characterization of a yogurt-quality improving exopolysaccharide from *Streptococcus thermophilus* AR333. *Food Hydrocolloid* 81:220–228. <https://doi.org/10.1016/j.foodhyd.2017.12.017>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.