Microbiol. Biotechnol. Lett. (2023), 51(1), 18–25
http://dx.doi.org/10.48022/mbl.2211.11006
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

The Mutant *Lactobacillus plantarum* GNS300 Showed Improved Exopolysaccharide Production and Antioxidant Activity

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Received: November 28, 2022 / Revised: February 21, 2023 / Accepted: February 22, 2023

After random mutagenesis, the mutant *Lactobacillus plantarum* GNS300 showed improved exopolysaccharide production as determined by the quantification of total sugar. The mutant *L. plantarum* GNS300 produced 2.82 g/l of exopolysaccharide which showed 79.62% improved exopolysaccharide production compared with the parental strain. When exopolysaccharide of *L. plantarum* GNS300 was analyzed, the exopolysaccharide is composed of galactose (93.35%) and glucose (6.65%). Through the optimization of fermentation conditions using a bioreactor, 2.93 g/l of exopolysaccharide was produced from 20 g/l of glucose at 35℃, 500 rpm, and 0.1 vvm for 12 h. The mutant *L. plantarum* GNS300 exhibited 69.18% higher antioxidant activity than that from the parental strain, which might be caused by higher exopolysaccharide production. The concentrated supernatant of the mutant *L. plantarum* GNS300 inhibited the growth of gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli, Vibrio parahaemolyticus,* and *Salmonella typhimurium*).

Keywords: ***Lactobacillus plantarum*, exopolysaccharide, transcriptomic analysis, antioxidant activity, antibacterial activity**

Introduction

Lactic acid bacteria (LAB) are microorganisms that produce lactic acid, a type of organic acid, and are present not only in the body but also in various fermented foods [21]. *Lactobacillus plantarum* is a gram-positive LAB, which is frequently found in the human body as well as in fermented foods [14, 23, 33]. The potential health benefits of food-associated *L. plantarum*, including antibacterial, antioxidant, antigen toxicity, anti-inflammatory, and immunomodulatory effects, have been demonstrated in various *in vitro* and *in vivo* studies [11, 30].

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The exopolysaccharide made by LAB is a natural polymer produced to withstand the harsh environment of the human intestine [26]. For this reason, exopolysaccharide produced by LAB is generally recognized as safe [19, 20]. Exopolysaccharide released by LAB can be considered a safe biological polymer and an alternative source of microbial polysaccharides for use in a variety of industries [5]. In addition, recent evidence suggests that exopolysaccharide produced by various LAB strains is involved in potential biological activities, such as antioxidant and antibacterial properties [3, 15, 25, 28]. Therefore, as the demand for natural polymers gradually increases in various industrial fields, research on exopolysaccharide produced by microorganisms has been conducted in recent years [1, 2, 9]. Many microorganisms can synthesize exopolysaccharide and excrete it out

of the cell [8]. Exopolysaccharide has special physicochemical and rheological properties, such as viscosification, stabilization, gelation, and emulsification, and hence, it is a potential chemical substitute [4, 12].

However, the amount of extracellular polysaccharide produced by LAB is usually very small. Therefore, studies are being conducted to increase the yield of exopolysaccharide produced by LAB. Many studies have shown that the amount and characteristics of exopolysaccharide depend heavily on culture conditions and media composition. When lactose was used as a carbon source, exopolysaccharide production by *L. plantarum* KX041 was the highest, followed by glucose, fructose, and sucrose [35]. In another study, exopolysaccharide production by *L. plantarum* SP8 was the highest when glucose was used as a carbon source [38]. It has also been reported that for *Lactococcus lactis* F-mou, fermentation conditions (initial optical density, agitation speed, and incubation period) had stronger influence on exopolysaccharide production than the components of fermentation medium [24].

Therefore, the purpose of this study was to obtain an improved strain with increased yield of total sugar by mutating *L. plantarum* strain-one of the LAB frequently found in various fermented foods. In addition, we confirmed that the mutant *L. plantarum* strain has higher antioxidant activity than the parental strain, which might be caused by higher exopolysaccharide production.

Materials and Methods

Strains and culture conditions

L. plantarum strain isolated from fermented food was used in this study. The pre-culture of the parental strain was cultured at 30℃ and 200 rpm for 24 h in *Lactobacilli* De Man, Rogosa and Sharpe (MRS) medium (Difco Laboratories, USA). To compare the amount of exopolysaccharide, the culture was performed at 35℃ and 200 rpm for 48 h in 5 ml of *Lactobacilli* MRS medium. In addition, the strains selected from 50 ml culture were cultured at 35℃ and 200 rpm for 24 h. All 50 ml cultures were conducted with an initial inoculum of OD 0.5. All the experiments were performed in duplicate and error bars indicate standard deviation.

Random mutagenesis

Random mutagenesis was performed using ethyl methanesulfonate (EMS, Sigma, USA). *L. plantarum* was pre-cultured in *Lactobacilli* MRS medium for 24 h. The cells were collected, and about 1×10^{10} CFU/ml cells were washed with 1 M sodium phosphate buffer (pH 7.2) and EMS was added at a final concentration of 1.0 M. The optimal mutation conditions were determined by incubating the mixture at 30℃ and 200 rpm for 30, 60, 75 or 90 min. Mutagenized cells were plated on the *Lactobacilli* MRS agar medium.

Quantification of total sugar

The culture broth was centrifuged at 12,225 ×*g*, and the supernatant was diluted 50 folds to prepare the sample. The sample (50 µl) was added to a 96-well plate and 150 µl of anthrone reagent (0.2% anthrone in 85% H_2SO_4) was added, and allowed to react at 80℃ for 60 min. After the reaction, the absorbance at OD_{630} was determined using a microplate reader (Biotek, USA). The standard curve was obtained by preparing different concentrations of glucose, and the absorbance values were used to plot the standard curve for quantitative analysis. All experiments were performed in triplicate and error bars indicate standard deviation.

Transcriptomic analysis

RNA sequencing analysis was conducted with the parental strain and mutant *L. plantarum* GNS300 cultured in *Lactobacilli* MRS medium at the early exponential phase. Total RNA was extracted by using AccuPrep® Bacterial RNA Extraction Kit (Bioneer Inc., Korea), following the manufacturer's instruction. The integrity and quality of total RNA were determined by RNeasy Mini Kit (Qiagen, Germany) and an Agilent 2100 Bioanalyzer (Aglient, USA). Sequencing was performed by a commercial service (Macrogen Inc., Korea) using the Illumina HiSeq 4000 sequencer (Illumina Inc., USA).

Optimization of agitation conditions for *L. plantarum* GNS300

To optimize agitation conditions for *L. plantarum* GNS300 using a bioreactor, the pre-culture was performed for 24 h at 200 rpm and 35℃ using *Lactobacilli*

MRS medium. The pre-cured cells were inoculated to the initial OD_{600} of 0.5. Fermentation experiments were carried out under different culture temperatures (30, 32, 35, 37 or 40℃) or different agitation conditions (300, 400, 500, or 600) at 0.1 vvm in a 5.0 L bioreactor (Bio Control & System, Korea), with an initial working volume of 2 L.

Partial purification of exopolysaccharide

After 24 h of cultivation, supernatant was harvested at 4℃ by centrifugation at 8000 ×*g* for 20 min. After trichloroacetic acid was added to the obtained supernatant to a final concentration of 14%, incubation was performed at room temperature at 90 rpm for 30 min. After incubation, centrifugation was performed at 8000 ×*g* at 4℃ for 20 min to obtain a supernatant. After mixing the obtained supernatant with 2 times the volume of absolute ethanol, it was treated at 4℃ for 24 h. After that centrifugation was performed at 8000 ×*g* at 4℃ for 20 min to obtain pellets. The pellet was washed with sterile distilled water, collected overnight and lyophilized to remove water and stored at 4℃.

Analysis of antioxidant activity

The analysis of antioxidant activity of each sample was performed using an oxygen radical absorbance capacity (ORAC) assay kit (Cell biolabs, USA) according to the suggested method. The Parental strain and mutant *L. plantarum* GNS300 was disrupted at 4℃ using a sonicator (amplitude 70%, on 5 sec, off 15 sec, 12 min), and the supernatant obtained by centrifugation at 21124 ×*g* for 10 min was used for analysis. The sample was diluted to an appropriate concentration, 25 µl was added to a 96-well immune plate, and 150 µl of fluorescein solution was added to perform stabilization at 37℃ for 30 min. Thereafter, 25 µl of free radical initiator at a concentration of 80 mg/ml was added to react. Using a microplate fluorometer (Thermo Scientific), after excitation at 485 nm, fluorescence was measured every 1 min at 37℃ for 60 min under the condition of emission at 535 nm, and the reduction rate was measured to analyze the antioxidant activity. For data processing, the area of the curve (AUC) was expressed using the sample addition group and the non-addition group, and then was measured by substituting a calibration curve prepared using standard Trolox (Cell Biolabs, USA). All experiments were performed in triplicate and error bars indicate standard deviation.

Analysis of antibacterial activity

To verify the antibacterial activity of *L. plantarum* GNS300 culture broth, a modified paper disc diffusion assay was performed. Various bacterial strains (*B. cereus, E. coli, S. aureus, V. parahaemolyticus,* and *S. typhimurium*) were pre-cultured in LB medium for 18 h. Next, 1×10^6 cells were spread on fresh LB-agar medium. *L. plantarum* GNS300 culture broth was incubated for 24 h in *Lactobacilli* MRS medium, followed by centrifugation. The supernatant was concentrated 10 folds by freeze drying, and 30 µl was loaded onto a paper disc (0.6 cm in diameter) on the LB-agar medium.

Analytical methods

Cell cultures were periodically sampled, and the cell density was determined by measuring the OD at 600 nm using a GENESYS^{TM} 10S UV-visible spectrophotometer (Thermo Inc., USA). The harvested cells were centrifuged and concentrations of glucose and galactose were analyzed in the resulting supernatant using a high-performance liquid chromatography system (HPLC) (1200 Series, Agilent Inc., USA) using a Rezex ROA-Organic Acid H^+ column (Phenomenex Inc., USA). The column and refractive index detector were maintained at 50℃. A solution of 0.005 N H_2SO_4 was used as the mobile phase at a flow rate of 0.6 ml/min. To determine the monosaccharide composition of exopolysaccharide, 20 mg exopolysaccharide was first hydrolyzed with 1 M sulfuric acid at 100℃ for 3 h. Then, the hydrolyzate was neutralized with 1N NaOH, and analyzed using a HPLC. Statistical analysis was performed with SPSS Version 26 statistic software package.

Results and Discussion

Isolation of the mutant *L. plantarum* GNS300 through random mutagenesis

Random mutagenesis was performed to isolate the mutant *L. plantarum* strains which were capable of producing a higher level of exopolysaccharide than the parental strain. Through the optimization of random mutagenesis conditions, the optimum death rate (99.990–99.999%) was obtained with 1.0 M EMS at 75 min of reaction time. A total of 300 mutant strains

http://dx.doi.org/10.48022/mbl.2211.11006

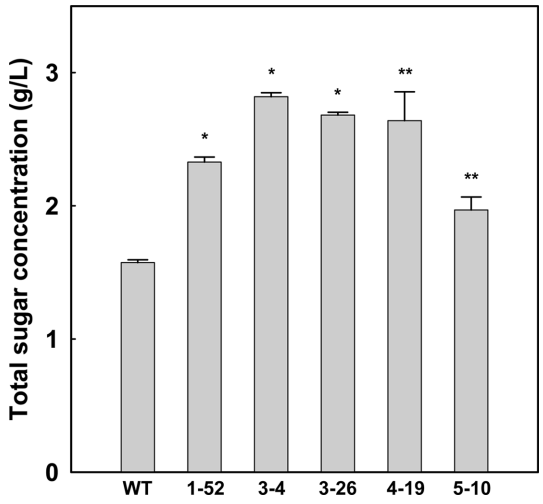

Fig. 1. Comparison of the amount of exopolysaccharide from the parental strain with the selected five mutant strains through random mutagenesis using flask cultivation at 35℃ and 200 rpm for 24 h in *Lactobacilli* MRS medium. (* $p < 0.001$ and ** $p < 0.005$)

obtained under the optimum mutagenesis conditions were cultured in 5 ml of *Lactobacilli* MRS medium at 35℃ and 200 rpm for 48 h. Through the verification of glucose depletion, the quantification of total sugar produced by mutant strains was performed, except for the strains that did not consume any glucose. Of all the strains, five mutant strains with the highest the amount of exopolysaccharide were selected through total sugar quantification. The amount of exopolysaccharide of the five mutant strains were compared with that of the parental strain by using flask cultivation at 35℃ and 200 rpm for 24 h (Fig. 1). While the parental strain consumed 20 g/l of glucose within 24 h and produced 1.57 ± 0.02 g/l of exopolysaccharide, the five mutant strains produced higher the amount of exopolysaccharide (1.97 ± 0.10–2.82 ± 0.03 g/l) from 20 g/l of glucose within 24 h. Of the five mutant strains, the 3–4 strain with the highest the amount of exopolysaccharide was isolated and named as mutant *L. plantarum* GNS300. The mutant *L. plantarum* GNS300 produced 2.82 ± 0.03 g/l of total sugar, which was about 79.62% higher than that from the parental strain.

When exopolysaccharide of *L. plantarum* GNS300 was analyzed, the HPLC spectrum shows that the exopolysaccharide is composed of galactose (93.35%) and glucose (6.65%). According to previous studies, most components of exopolysaccharide by *L. plantarum* consist of galactose and glucose [13]. The exopolysaccharide produced by *L. plantarum* ZDY2013 is mainly composed of galactose (98.3%) [39].

Transcriptomic analysis through RNA sequencing

When transcriptomic analysis was performed through RNA sequencing, the genes associated with glucose uptake and exopolysaccharide synthesis in the mutant *L. plantarum* GNS300 were up-regulated, compared with those in the parental strain. According to a previous study on *L. casei* CRL 87, glucose enters the cell as glucose 6-phosphate through the phosphotransferase system (PTS) and glucose entering through permease is converted into glucose 6-phosphate by glucokinase [22]. As shown in Table 1, genes associated with glucose uptake, such as PTS mannose transporter subunit IIAB, phosphoenolpyruvate-protein phosphotransferase, and glucokinase, were up-regulated by 1.50, 1.48, and 1.78 folds, respectively.

It has been reported that phosphoglucomutase, UDP-glucose pyrophosphorylase, and UDP-galactose 4-epimerase are associated with exopolysaccharide synthesis in *L. sakei* 0-1 [7]. In this study, these genes (UDP-glucose pyrophosphorylase, phosphoglucomutase, and UDP-glucose 4-epimerase) were upregulated by 1.16–1.44 folds in the mutant *L. plantarum* GNS300, compared with those in the parental strain (Table 1). Glucose 6-phosphate is a basic component of the exopolysaccharide synthesis pathway. In particular, glucokinase and UDP-glucose 4-epimerase activities do not occur in mutant

Table 1. Up-regulated transcriptomes of *L. plantarum* GNS300 associated with glucose uptake and exopolysaccharide synthesis compared with the parental strain through RNA-Seq analysis.

	Product	Fold change
Glucose uptake related	PTS mannose transporter subunit IIAB	1.50
	Phosphoenolpyruvate-protein phosphotransferase	1.48
	Glucokinase	1.78
Exopolysaccharide synthesis related	UDP-glucose pyrophosphorylase	1.16
	Phosphoglucomutase	1.44
	UDP-glucose 4-epimerase GalE	1.31

strains that do not synthesize exopolysaccharides [22]. Homologous overexpression of UDP-glucose pyrophosphorylase and phosphoglucomutase genes increased the exopolysaccharide yield of *S. thermophilus* LY03 from 0.17 to 0.31 g/mol [16].

Optimization of fermentation conditions for the production of total sugar by *L. plantarum* GNS300

In order to optimize the culture conditions for increased production of total sugar, flask cultivation of the mutant *L. plantarum* GNS300 was performed at various culture temperatures or various agitation conditions. At the different temperatures, 30, 32, 35, 37, or 40℃, and 200 rpm, the highest the amount of exopolysaccharide was observed at 35℃ (Table 2). However, there were no significant differences in the amount of exopolysaccharide at the different temperatures. According to previous studies, *L. plantarum* KX041 showed the highest yield at 35℃, whereas *L. plantarum* Q823 and EP56 showed higher yield at lower temperature [27, 32, 35]. For further optimization of agitation conditions, the mutant *L. plantarum* GNS300 was cultivated at various agitation conditions, 300, 400, 500, and 600 rpm, and at 35℃ and 0.1 vvm using a bioreactor. As shown in Table 2, the amount of exopolysaccharide did not significantly change when agitation conditions were varied. In a study by Liu *et al*. on *L. acidophilus* ATCC, the agitation condition was not treated as a factor that can significantly affect exopolysaccharide production [18].

Table 2. Optimization of culture temperature and agitation conditions for exopolysaccharide production by the mutant *L. plantarum* GNS300 using bioreactor.

Culture temperatures (℃)	Exopolysaccharide production (g/l)
30	2.64 ± 0.24
32	2.71 ± 0.12
35	2.86 ± 0.15
37	2.78 ± 0.04
40	2.67 ± 0.34
Agitation speeds (rpm)	**Exopolysaccharide production (g/l)**
300	2.77 ± 0.12
400	2.80 ± 0.04
500	2.93 ± 0.02
600	2.85 ± 0.08

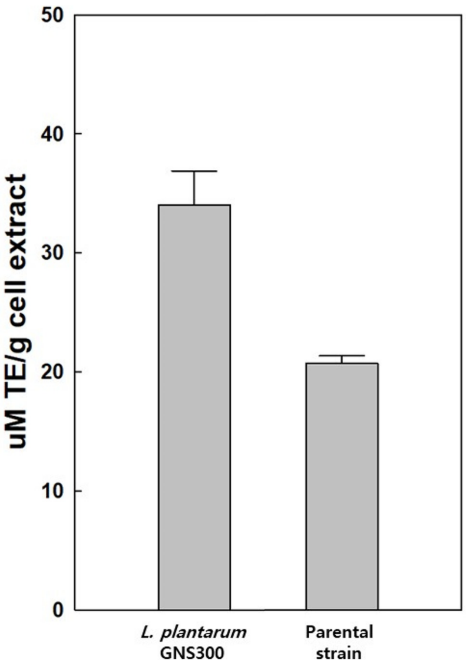

Fig. 2. Comparison of antioxidant activities of the parental strain and the mutant *L. plantarum* GNS300 using the ORAC activity assay. ($p < 0.05$)

High antioxidant activity from the mutant *L. plantarum* GNS300

The ORAC activity assay utilizing the principle that a peroxyl radical oxidizes a fluorescent probe through a hydrogen atom transfer process was used for comparisons of antioxidant activities from the parental strain and the mutant *L. plantarum* GNS300. As shown in Fig. 2, the antioxidant activities of the mutant *L. plantarum* GNS300 was 34.04 ± 2.83 μM TE/g cell extract which is 69.18% higher than that of the parental strain (20.12 ± 0.50 μM TE/g cell extract). Exopolysaccharide isolated from the culture of *L. plantarum* C88 exhibited concentration-dependent scavenging activities against hydroxyl radicals [37]. Wei reported antioxidant activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *L. plantarum* R315 [17]. Lu reported that the exopolysaccharide from *Lactobacillus plantarum* KX041 exhibited good antioxidant activity with high free radical scavenging capacity of ABTS, DPPH, hydroxyl and superoxide free radicals [35]. Besides, many studies show that exopolysaccharide isolated from *Lactobacillus* sp. exhibited high antioxidant activities [29, 31, 36]. The high antioxidant activity

Table 3. Antibacterial activities of the parental strain and the mutant *L. plantarum* GNS300 culture broth against of gram-positive or negative bacteria. The culture broth was 10 times concentrated.

Strain		Clear zone (mm)	
		Parental strain	*L. plantarum* GNS300
Gram positive	*B. cereus*	17.35 ± 0.63	15.05 ± 1.20
	S. aureus	16.40 ± 0.42	16.40 ± 0.85
Gram negative	*E. coli*	14.55 ± 0.49	12.00 ± 0.00
	V. parahaemolyticus	15.45 ± 0.35	15.75 ± 0.63
	S. typhimurium	12.90 ± 0.42	11.80 ± 0.28

*Paper disk diameter 6 mm.

from the mutant *L. plantarum* GNS300 might be caused by 79.62% higher exopolysaccharide production as compared to the parental strain.

Comparisons of antibacterial activities between the parental strain and the mutant *L. plantarum* GNS300

The concentrated supernatant (30 μl) of the mutant *L. plantarum* GNS300 was dispensed into paper discs placed on LB-agar medium and cultured at 37℃ to determine the antibacterial activity. As shown in Table 3, the concentrated supernatant of the mutant *L. plantarum* GNS300 inhibited the growth of gram-positive bacteria (*B. cereus* and *S. aureus*). Clear zones of 15.05 ± 1.20 and 16.40 ± 0.85 cm were obtained for *B. cereus* and *S. aureus*, respectively. In addition, the concentrated supernatant of the mutant *L. plantarum* GNS300 slightly inhibited the growth of gram-negative bacteria, *E. coli, V. parahaemolyticus,* and *S. typhimurium*, with clear zones of 12.00 ± 0, 15.75 ± 0.63, and 11.80 ± 0.28 cm, respectively. *L. plantarum* GNS300 showed the greatest antibacterial activity against *S. aureus*. Both *L. plantarum* GNS300 and the parental strain had antimicrobial activity against various pathogenic strains, which supports the results of other studies [6, 10]. A previous study showed that the antibacterial activity of *L. plantarum* LP1, LP2, and LP3 against *E. coli* was superior to that against *S. aureus* [34].

Conclusion

The LABs are known to produce small quantities of exopolysaccharide; hence, random mutagenesis was used to obtain strains with improved productivity. Among 300 mutants, the mutant strain *L. plantarum* GNS300 was obtained, with 79.62% increase in the production of exopolysaccharide. In addition, the production of exopolysaccharide was increased to 2.93 g/l through the optimization of fermentation conditions using a bioreactor. Transcriptome sequencing revealed that the mutant *L. plantarum* GNS300 showed up-regulation of genes associated with glucose uptake and exopolysaccharide synthesis, compared with the parental strain. The higher exopolysaccharide production of the mutant *L. plantarum* GNS300 could cause higher antioxidant activity than that from the parental strain. In addition, the concentrated supernatant of the mutant *L. plantarum* GNS300 showed the inhibition to various gram-positive bacteria and gram-negative bacteria.

Acknowledgments

This research was supported by research program funded by The Garden of Naturalsolution Co., Ltd (C1016186-01-01).

Conflict of Interest

The authors have no financial conflicts of interest to declare.

References

1. Ale EC, Batistela VA, Correa Olivar G, Ferrado JB, Sadiq S, Ahmed HI*, et al.* 2020. Statistical optimisation of the exopolysaccharide production by *Lactobacillus fermentum* Lf2 and analysis of its chemical composition. *Int. J. Dairy Technol.* **73**: 76-rm87.
2. Almalki MA. 2020. Exopolysaccharide production by a new *Lactobacillus lactis* isolated from the fermented milk and its antioxidant properties. *J. King Saud. Univ. Sci.* **32**: 1272-1277.
3. Arqués JL, Rodríguez E, Langa S, Landete JM, Medina M. 2015. Antimicrobial activity of lactic acid bacteria in dairy products and gut: effect on pathogens. *BioMed Res. Int.* **2015**.
4. Ates O. 2015. Systems biology of microbial exopolysaccharides production. *Front. Bioeng. Biotechnol.* **3**: 200.
5. Banik R, Kanari B, Upadhyay S. 2000. Exopolysaccharide of the gellan family: prospects and potential. *World J. Microbiol. Biotechnol.* **16**: 407-414.
6. Davoodabadi A, Dallal MMS, Foroushani AR, Douraghi M, Harati FA. 2015. Antibacterial activity of *Lactobacillus* spp. isolated from the feces of healthy infants against enteropathogenic bacteria. *Anaerobe* **34**: 53-58.
7. Degeest B, Janssens B, De Vuyst L. 2001. Exopolysaccharide (EPS) biosynthesis by *Lactobacillus sakei* 0-1: production kinetics,

enzyme activities and EPS yields. *J. Appl. Microbiol.* **91**: 470-477.
8. Donot F, Fontana A, Baccou J, Schorr-Galindo S. 2012. Microbial exopolysaccharides: main examples of synthesis, excretion, genetics and extraction. *Carbohydr. Polym.* **87**: 951-962.
9. Ermiş E, Poyraz E, Dertli E, Yılmaz MT. 2020. Optimization of exopolysaccharide production of *Lactobacillus brevis* E25 using RSM and characterization. *Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi*. **24**: 151-160.
10. Fuochi V, Li Volti G, Furneri PM. 2017. Probiotic properties of *Lactobacillus fermentum* strains isolated from human oral samples and description of their antibacterial activity. *Curr. Pharm. Biotechnol.* **18**: 138-149.
11. Garcia-Gonzalez N, Battista N, Prete R, Corsetti A. 2021. Health-promoting role of L*actiplantibacillus plantarum* isolated from fermented foods. *Microorganisms* **9**: 349.
12. Han Y, Liu E, Liu L, Zhang B, Wang Y, Gui M, *et al.* 2015. Rheological, emulsifying and thermostability properties of two exopolysaccharides produced by *Bacillus amyloliquefaciens* LPL061. *Carbohydr. Polym.* **115**: 230-237.
13. Jiang Y, Yang Z. 2018. A functional and genetic overview of exopolysaccharides produced by *Lactobacillus plantarum*. *J. Funct. Foods* **47**: 229-240.
14. Jose NM, Bunt CR, Hussain MA. 2015. Comparison of microbiological and probiotic characteristics of lactobacilli isolates from dairy food products and animal rumen contents. *Microorganisms* **3**: 198-212.
15. Kim H, Kim J-S, Kim Y, Jeong Y, Kim J-E, Paek N-S, Kang C-H. 2020. Antioxidant and probiotic properties of *Lactobacilli* and *Bifidobacteria* of human origins. *Biotechnol. Bioprocess Eng.* **25**: 421-430.
16. Levander F, Svensson M, Rådström P. 2002. Enhanced exopolysaccharide production by metabolic engineering of *Streptococcus thermophilus*. *Appl. Environ. Microbiol.* **68**: 784-790.
17. Li S, Huang R, Shah NP, Tao X, Xiong Y, Wei H. 2014. Antioxidant and antibacterial activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315. *J. Dairy Sci.* **97**: 7334-7343.
18. Liu Q, Huang X, Yang D, Si T, Pan S, Yang F. 2016. Yield improvement of exopolysaccharides by screening of the *Lactobacillus acidophilus* ATCC and optimization of the fermentation and extraction conditions. *EXCLI J.* **15**: 119.
19. Macori G, Cotter PD. 2018. Novel insights into the microbiology of fermented dairy foods. *Curr. Opin. Biotechnol.* **49**: 172-178.
20. Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, *et al.* 2017. Health benefits of fermented foods: microbiota and beyond. *Curr. Opin. Biotechnol.* **44**: 94-102.
21. Mathur H, Beresford TP, Cotter PD. 2020. Health benefits of lactic acid bacteria (LAB) fermentates. *Nutrients* **12**: 1679.
22. Mozzi F, Rollán G, de Giori GS, Font de Valdez G. 2001. Effect of galactose and glucose on the exopolysaccharide production and the activities of biosynthetic enzymes in *Lactobacillus casei* CRL 87. *J. Appl. Microbiol.* **91**: 160-167.
23. Nami Y, Abdullah N, Haghshenas B, Radiah D, Rosli R, Khosroushahi AY. 2014. Assessment of probiotic potential and anticancer activity of newly isolated vaginal bacterium *Lactobacillus plantarum* 5BL. *Microbiol. Immunol.* **58**: 492-502.
24. Nehal F, Sahnoun M, Smaoui S, Jaouadi B, Bejar S, Mohammed S. 2019. Characterization, high production and antimicrobial activity of exopolysaccharides from *Lactococcus lactis* F-mou. *Microb. Pathog.* **132**: 10-19.
25. Parvez S, Malik KA, Ah Kang S, Kim HY. 2006. Probiotics and their fermented food products are beneficial for health. *J. Appl. Microbiol.* **100**: 1171-1185.
26. Patel A, Prajapat J. 2013. Food and health applications of exopolysaccharides produced by lactic acid bacteria. *Adv. Dairy Res.* 1-8.
27. Pingitore EV, Pessione A, Fontana C, Mazzoli R, Pessione E. 2016. Comparative proteomic analyses for elucidating metabolic changes during EPS production under different fermentation temperatures by *Lactobacillus plantarum* Q823. *Int. J. Food Microbiol.* **238**: 96-102.
28. Qian B, Xing M, Cui L, Deng Y, Xu Y, Huang M, Zhang S. 2011. Antioxidant, antihypertensive, and immunomodulatory activities of peptide fractions from fermented skim milk with *Lactobacillus delbrueckii* ssp. bulgaricus LB340. *J. Dairy Res.* **78**: 72.
29. Silva LA, Lopes Neto JHP, Cardarelli HR. 2019. Exopolysaccharides produced by *Lactobacillus plantarum*: technological properties, biological activity, and potential application in the food industry. *Annal. Microbiol.* **69**: 321-328.
30. Son J, Jeong KJ. 2020. Recent advances in synthetic biology for the engineering of lactic acid bacteria. *Biotechnol. Bioprocess Eng.* **25**: 962-973.
31. Sørensen HM, Rochfort KD, Maye S, MacLeod G, Brabazon D, Loscher C, *et al.* 2022. Exopolysaccharides of Lactic acid bacteria: Production, purification and health benefits towards functional food. *Nutrients* **14**: 2938.
32. Tallon R, Bressollier P, Urdaci MC. 2003. Isolation and characterization of two exopolysaccharides produced by *Lactobacillus plantarum* EP56. *Res. Microbiol.* **154**: 705-712.
33. Wang J, Ji H, Zhang D, Liu H, Wang S, Shan D, *et al.* 2011. Assessment of probiotic properties of *Lactobacillus plantarum* ZLP001 isolated from gastrointestinal tract of weaning pigs. *Afr. J. Biotechnol.* **10**: 11303-11308.
34. Wang L, Zhang H, Rehman MU, Mehmood K, Jiang X, Iqbal M, *et al.* 2018. Antibacterial activity of *Lactobacillus plantarum* isolated from Tibetan yaks. *Microb. Pathog.* **115**: 293-298.
35. Wang X, Shao C, Liu L, Guo X, Xu Y, Lü X. 2017. Optimization, partial characterization and antioxidant activity of an exopolysaccharide from *Lactobacillus plantarum* KX041. *Int. J. Biol. Macromol.* **103**: 1173-1184.
36. Xu X, Peng Q, Zhang Y, Tian D, Zhang P, Huang Y, *et al.* 2020. A novel exopolysaccharide produced by *Lactobacillus coryniformis* NA-3 exhibits antioxidant and biofilm-inhibiting properties in vitro. *Food Nutr. Res.* **64**. doi: 10.29219/fnr.v64.3744. eCollection 2020.
37. Zhang L, Liu C, Li D, Zhao Y, Zhang X, Zeng X, *et al.* 2013. Antioxi-

dant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. *Int. J. Biol. Macromol.* **54**: 270-275.
38. Zhang L, Zhao B, Liu C-J, Yang E. 2020. Optimization of biosynthesis conditions for the production of exopolysaccharides by *Lactobacillus plantarum* SP8 and the exopolysaccharides antioxidant activity test. *Indian J. Microbiol.* **60**: 334-345.
39. Zhang Z, Liu Z, Tao X, Wei H. 2016. Characterization and sulfated modification of an exopolysaccharide from *Lactobacillus plantarum* ZDY2013 and its biological activities. *Carbohydr. Polym.* **153**: 25-33.