

Preliminary Study on the Characterization of a Novel Polysaccharide from *Enterococcus faecium* SL60 Isolated from Siyez Sourdough

Siyez Ekşi Mayasından İzole Edilen *Enterococcus faecium* SL60'tan Elde Edilen Yeni Bir Polisakkaritin Karakterizasyonu Üzerine Ön Çalışma

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ABSTRACT

This study reports the production, structural characterization, and preliminary cytotoxicity evaluation of an extracellular polysaccharide (EPS) synthesized by *Enterococcus faecium* SL60, a strain isolated from traditional Siyez (einkorn) sourdough. Analytical techniques including GC-MS, FTIR, XRD, zeta potential, and elemental analysis were applied to characterize the EPS. The purified EPS was found to be a water-soluble, negatively charged, amorphous heteropolysaccharide predominantly composed of mannose and glucose, with minor proportions of rhamnose, arabinose, galactose, and xylose. The total carbohydrate yield was 7.6 g/L. FTIR confirmed the presence of typical carbohydrate functional groups, while zeta potential analysis indicated a moderate surface charge (-9.71 mV), implying colloidal stability. Cytotoxicity evaluation using the CCD-1079Sk fibroblast cell line showed no significant toxicity at concentrations up to 500 µg/mL. These findings highlight the potential of SL60-derived EPS as a safe bioactive compound with possible applications in functional food development and health-related products.

Keywords: Einkorn, *Enterococcus faecium*, Exopolysaccharide, Sourdough

Öz

Bu çalışma, geleneksel Siyez (einkorn) ekşi mayasından izole edilen *Enterococcus faecium* SL60 suşu tarafından sentezlenen bir ekstraselüler polisakkaritin (EPS) üretimini, yapısal karakterizasyonunu ve ön sitotoksikite değerlendirmesini bildirmektedir. EPS'yi karakterize etmek için GC-MS, FTIR, XRD, zeta potansiyeli ve element analizi içeren analitik teknikler uygulanmıştır. Saflaştırılmış EPS'nin, ağırlıklı olarak mannoz ve glikozdan, az oranda da ramnoz, arabinoz, galaktoz ve ksilozdan oluşan, suda çözünür, negatif yüklü, amorf bir heteropolisakkarit olduğu bulunmuştur. Toplam karbonhidrat verimi 7,6 g/L'dir. FTIR tipik karbonhidrat fonksiyonel gruplarının varlığını doğrularken, zeta potansiyeli analizi orta düzeyde bir yüzey yükü (-9,71 mV) göstererek kolloidal kararlılığa işaret etmektedir. CCD-1079Sk fibroblast hücre hattı kullanılarak yapılan sitotoksikite değerlendirmesi, 500 µg/mL'ye kadar konsantrasyonlarda önemli bir toksisite göstermemiştir. Bu bulgular, SL60 türevi EPS'nin fonksiyonel gıda geliştirme ve sağlık ürünlerinde olası uygulamalara sahip güvenli bir biyoaktif bileşik olarak potansiyelini vurgulamaktadır.

Anahtar Kelimeler: Siyez, *Enterococcus faecium*, Ekzopolisakkarit, Ekşi maya

Received Date: 18.02.2025
Revision Request Date: 19.06.2025
Last Revision Date: 18.11.2025
Accepted Date: 04.12.2025
Publication Date: 29.12.2025

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Cite this article: Genç, B. (2026). Preliminary Study on the Characterization of a Novel Polysaccharide from *Enterococcus faecium* Isolated from Siyez Sourdough. *Research in Agricultural Sciences*, 57(1), 48-56. <https://doi.org/10.17097/agricultureatauni.1641981>



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Introduction

Siyez (*Triticum monococcum* L. subsp. *monococcum*) is a diploid hulled wheat domesticated approximately 10,000 years ago in Mesopotamia and the Eastern Mediterranean and found in the Karacadag region of Türkiye (Peng et al., 2011). Siyez wheat draws attention with its lower gluten content and higher protein, amino acid, vitamin, and mineral content than modern wheat. The natural structure of siyez flour enhances the characteristic acidic properties of sourdough, resulting in more flavorful and healthier bread. Furthermore, the gluten in bread made with einkorn sourdough is more digestible (Brouns et al., 2022; Hidalgo & Brandolini, 2014).

Sourdough fermentation is a traditional production method that improves the sensory properties and contributes to nutritional value of the final product (Dimidi et al., 2019; Marco et al., 2017). In recent years, there has been increasing interest in the use of Siyez wheat in sourdough fermentation to discover functional components derived from this unique grain and its sourdough. The researches are ongoing to identify the microbial community in the sourdough of Siyez wheat. The community in sourdough is composed of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus paralimentarius*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus sanfranciscensis*, *Pediococcus pentosaceus*, *Leuconostoc mesenteroides*), and yeast (*Candida humulis*, *Torulaspora delbrueckii*, *Debaryomyces hansenii*, and *Saccharomyces cerevisiae*) (Bansal et al., 2013; Bartkiene et al., 2020; Cizeikiene et al., 2020; Gullo et al., 2003; Meroth et al., 2003; Minervini et al., 2012; Mohammadi-Kouchesfahani et al., 2019; Raimondi et al., 2017; Rice et al., 2020; Saeed et al., 2009).

Lactic acid bacteria from sourdough can produce a variety of bioactive compounds. These bacteria, which can grow in acidic environments, are characterized by production of lactic acid and, in some cases, the production of extracellular polysaccharides. Microbial polysaccharides, which can affect both biological functions and structural properties of fermented products, are macromolecules composed of numerous sugar units produced by microorganisms. These polysaccharides are produced during the metabolism of microorganisms in response to environmental factors (exopolysaccharides) or for biological functions such as cell wall structure (endopolysaccharides). Intrinsic polysaccharides, which serve as structural components of the cell wall, may help protect the cell against environmental stresses. The exopolysaccharides produced and released into the culture medium have generally of high molecular weights. (Ketabi et al., 2008; Lim et al., 2018; Poutanen et al., 2009; Torino et al., 2015).

Polysaccharides produced by lactic acid bacteria have many biological and functional properties. One of these is the probiotic effect. Polysaccharides that cannot be digested in the intestine support the growth of beneficial bacteria. In addition, polysaccharides produced by lactic acid bacteria can adhere to surfaces and form biofilms, thus protecting the microorganism against environmental conditions. Some lactic acid bacteria-derived extracellular polysaccharides are known to exhibit antioxidant and antimicrobial properties. In this way, it constitutes an important potential in food preservation and health (Ciszek-Lenda, 2011; Gerwig, 2019; Torino et al., 2015). Exopolysaccharide profiles of bacteria from Siyez sourdough are one of the components that have not been extensively studied (Kavitake et al., 2023).

The genus *Enterococcus*, a member of the group of lactic acid bacteria, is a genus of bacteria found mainly in the human and animal intestinal flora but also in various food products. *Enterococcus* sp. are generally gram-positive, facultative anaerobic (able to survive in oxygenated media), and lactic acid-producing microorganisms. Members of this genus are known for their role in sourdough fermentation, contributing both to lactic acid production and the synthesis of various bioactive metabolites. Some *Enterococcus* species show probiotic properties. These bacteria can improve digestive health by balancing the intestinal flora. Species such as *Enterococcus faecalis* and *Enterococcus faecium* play an important role in food fermentation and probiotic products.

Recent studies have highlighted the ability of *E. faecium* to produce polysaccharides thought to be important for the texture, stability, and health benefits of fermented products (Franz et al., 2011; Holzapfel et al., 2018; Moreno et al., 2006; Tan et al., 2013; Tilwani et al., 2021). Thus, the originality of this study lies in the isolation, production, and characterization of an exopolysaccharide produced by *Enterococcus faecium* SL60, a strain uniquely obtained from traditional einkorn (Siyez) sourdough which is a cereal-based ecosystem that remains largely unexplored in polysaccharide research. SL60 was selected based on its distinctive mucoid colony morphology, an indicator of high polysaccharide secretion.

Methods

Isolation and Identification of EPS-Producing Strain

Sourdough production from siyez flour (1.75% fat, 64.9% carbohydrate, 9.7% fibre and 9.6% protein; Dogalsan, Ankara, Turkey) was carried out by traditional methods. All the chemicals were purchased from Sigma-Aldrich, Merck (St. Louis, MO, USA), unless otherwise stated. To obtain traditional siyez sourdough, 100 grams of Siyez flour and 100 mL of water were mixed and incubated at 36°C for 120

hours. After fermentation, the fresh water-flour mixture was inoculated with the matured sourdough (1:1 ratio) for five days (Sáez et al., 2018). After the sourdough was matured, serial dilutions were made in sterile physiological water up to a dilution of 10^{-8} to isolate pure colonies. 100 μ L of each dilution was spread on M17 (Merck Milipore) agar plates and incubated at 36°C for 72 hours in anaerobic jars (Merck Milipore) supplemented with Anaerocult®A (Merck Milipore). Isolates were purified according to colony characteristics, and selected colonies were stored at -86°C. Among these isolates, SL60 was selected as the extracellular polysaccharide producer due to its prominent mucoid structure on the agar surface. SL60 was identified by the matrix-assisted laser desorption ionization-time of flight mass spectrometry technique. The extraction procedure was carried out using the ethanol-formic acid method for protein isolation from pure bacterial culture. The identification process was then continued by comparing the spectra obtained with the instrument's flex control software program (Biotyper 3.0; Microflex LT; Bruker Daltonics GmbH, Bremen, Germany) with the Maldi Biotyper Real-Time Classification (RTC) software (Genc et al., 2024).

Extracellular polysaccharide production and purification

Polysaccharide production by SL60 was carried out in M17 broth (with the addition of 20 g/L sucrose) at a 1% (v/v) inoculation rate at 36°C for 72 hours. The biomass was separated by centrifugation (Allegra X30; Beckman Coulter, Brea, CA, USA) at 4500 rpm for 15 minutes. Purification of the polysaccharide was achieved by adding 4% (v/v) trichloroacetic acid (TCA) (Isolab Laborgeräte GmbH, Weinheim, Germany) to a final concentration to remove proteins and contaminants. then 3 volumes of chilled ethanol was added, and the polysaccharide was precipitated overnight at 4°C (Genc et al., 2021). The partially purified polysaccharide was lyophilized.

Total Carbohydrate and UV-Vis Analysis

The polysaccharide was water-soluble. The phenol-sulfur method was used to measure the carbohydrate content (Genc, 2024). Spectrum scanning was performed in the 200-1000 nm range to determine the presence of nucleic acids.

Structural Characterization

The spectrum of functional groups in partially purified polysaccharide samples were determined using Fourier-transform infrared spectrophotometer (FT-IR) (PerkinElmer Spectrum Version 10.5.2). The sample (10 mg) was mixed with potassium bromide (KBr), and pressed into a disk. The whole IR spectrum was analyzed with a scan range of 4000-400 cm^{-1} .

The elemental composition was analyzed to determine the presence of elements in the sample. Analyses were carried

out at the RTU Central Research Laboratory, Application and Research Center.

The zeta potential of the polysaccharide was measured at 25°C through dynamic light scattering analysis by Malvern/Zetasizer Nano Zsp.

Gas chromatography-mass spectrometry (GC-MS model 7890A; Agilent Technologies, Santa Clara, CA, USA) was used to determine the monosaccharide composition through the breakdown of polysaccharide structures and subsequent volatilization of monosaccharides. For this or hydrolysis, 1 mg of polysaccharide was treated with 500 μ L of 2M trifluoroacetic acid at 120°C for 120 min. Reduction and acetylation were conducted by addition of NaBH_4 (20mg) in 1mL of 2M NH_4OH to the residue and the solution was heated at 60°C for 60 min, then neutralized with acetic acid, evaporated to dryness with a stream of N_2 at 40°C, and evaporated five times by adding 500 μ L methanol. The residue was acetylated with 200-250 μ L of pyridine and 200–250 μ L of acetic anhydride at 100-120°C for 20min, partitioned with water and CH_2Cl_2 , and the resulting alditol acetates were analyzed with flame ionization detection (FID) coupled to MS (Silveira et al., 2015). The polysaccharide samples were passed through HP-5 ms capillary GC column with a flow rate of 1.2 mL/min of Helium. The oven temperature was increased to 190°C at 10°C/min. The injection temperature and the transfer line temperature were 250°C while the mass detector temperature and the ion source temperature were 230°C. Standard sugars were glucose, fructose, mannose, xylose, rhamnose, and arabinose.

X-ray diffraction analysis (XRD) of the sample (10 mg) was carried out using the Rigaku SmartLab XRD instrument, with a scanning speed of 2°/min across an angular span from 30° and 100°.

Cytotoxicity Assay

The evaluation of cytotoxicity was carried out at the Drug Administration and Research Center at Istanbul Bezmialem University. To assess cell viability, the MTT assay was employed on the CCD-1079Sk fibroblast cell line at a concentration of 1×10^4 cells/well. The cells were cultured at 37°C for 24 hours with 5% CO_2 . A control group was maintained using DMEM-F12 supplemented with 10% FBS, and 1% penicillin/streptomycin. Results and standard deviations were analyzed with GraphPad Prism 5. All the experiments were triplicate.

Results and Discussion

The isolate SL60, selected based on its pronounced mucoid morphology on M17 agar, was identified as *Enterococcus faecium* SL60 using MALDI-TOF MS analysis (Genc et al., 2024). This phenotype is frequently associated with high

exopolysaccharide production, as the mucoid texture typically results from polysaccharide secretion around bacterial colonies (Franz et al., 2011). The total yield of 7.6 g/L from SL60 aligned with values reported for other *E. faecium* strains. Mesophilic *E. faecium* MC-5 produced 7.76 g/L (± 0.26) polysaccharides in nitrogenous medium (Tilwani et al., 2021). *E. faecium* KT990028 produced extracellular polysaccharides with a total carbohydrate content of 11.2% (de Brito et al., 2024). The result indicates that this strain is a competitive polysaccharide producer under laboratory conditions. However, beyond the quantity, the composition and structural features of the polysaccharide determine its potential application.

Structural Purity and Composition

UV-Vis scanning revealed no absorbance at 260 nm, confirming the absence of nucleic acid contamination, while a small peak at 280 nm suggested a protein likely to be in the polysaccharide structure (Figure 1). These observations were further validated by FTIR spectroscopy conducted before and after trichloroacetic acid (TCA) precipitation. The reduction of amide-related peaks and disappearance of the sulfate-associated band at 1219 cm^{-1} following TCA treatment (Aanniz et al., 2015) confirmed the removal of proteinaceous and charged impurities (Table 1, Figure 2 and Figure 3). However, after TCAP treatment, the existing band at 1660 cm^{-1} indicated the presence of protein in the polysaccharide backbone, and it could be concluded that the extracellular polysaccharide obtained from *E. faecium* SL60 was a heteropolysaccharide. Elemental analysis (Figure 4) also supported these findings by showing a decrease in sulfur content, and indicating the EPS was not sulfated-a property relevant to its bioactivity profile, as sulfating often enhances antioxidant and immunomodulatory functions.

Figure 1.
Spectrum scanning of polysaccharide

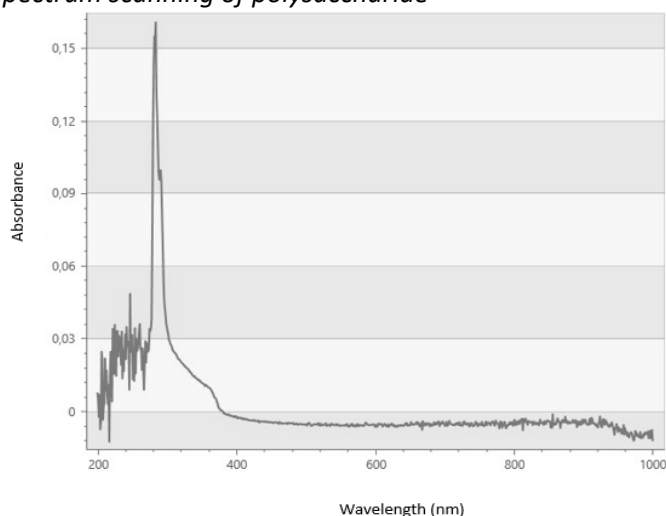


Figure 2.
Transmittance values of functional groups of polysaccharide before trichloroacetic acid precipitation

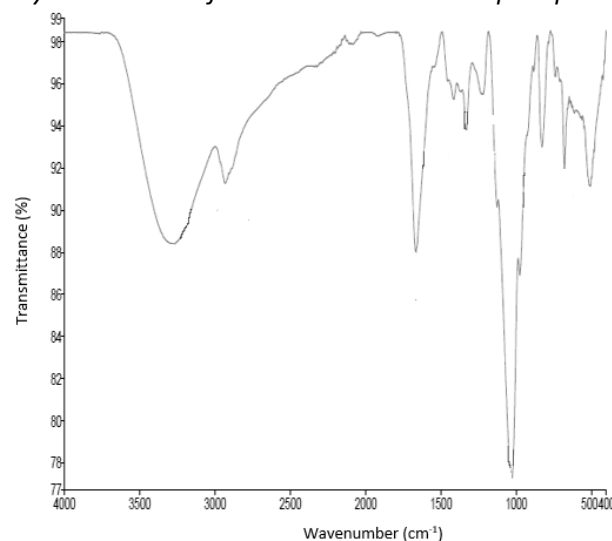


Figure 3.
Transmittance values of functional groups of polysaccharide after trichloroacetic acid precipitation

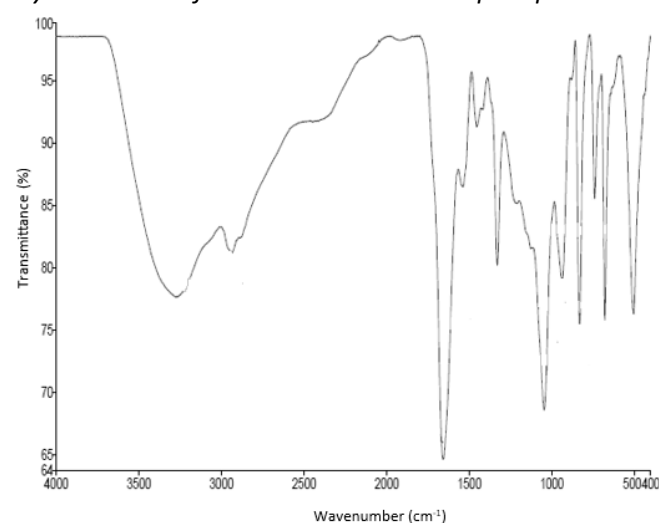


Figure 4.
Elemental composition of polysaccharide from *E. faecium* SL60

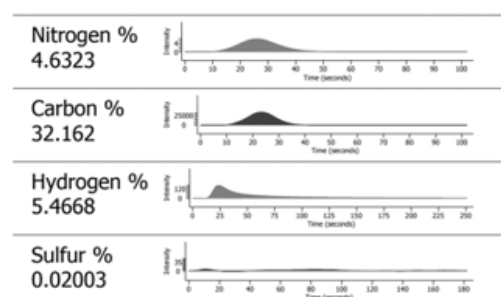
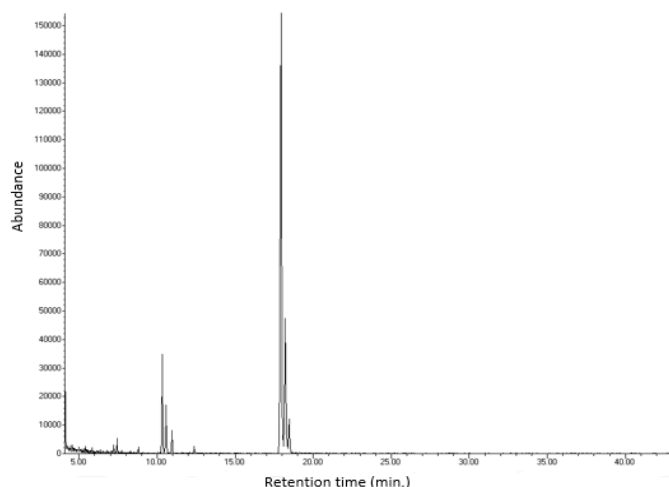


Table 1.
Comparison of polysaccharide functional groups before and after TCAP

Relevant functional groups	Frequency (cm ⁻¹)		Transmittance (%)	
	Before TCAP	After TCAP	Before TCAP	After TCAP
Intracellular hydrogen bond or hydroxyl group (Zhang et al., 2020)	3273.93	3276.28	88.39	77.64
aliphatic C-H, and C-O group (Rani et al., 2017)	2935.55	2937.75	91.28	81.21
C=O stretches of amide bonds (Aanniz et al., 2015)	1665.92	1656.89	87.95	64.63
-OH group (Habib et al., 2022)	1330.47	1328.18	93.72	80.22
S=O stretching vibrations (de Brito et al., 2024)	1219.62	-	95.48	-
C–C vibrations (Tilwani et al., 2021)	1026.50	1044.46	77.27	68.58
α and β configurations in pyranose form (Jing et al., 2015)	826.73	829.03	93.00	75.36
carbohydrate skeletal vibrations (Zhao et al., 2021)	677.20	676.16	91.90	75.82
	512.79	500.97	91.10	76.29

GC-MS analysis identified the polysaccharide as a heteropolysaccharide predominantly composed of mannose (64.1%) and glucose (17.3%), with minor contributions from rhamnose (8.8%), arabinose (4.2%), galactose (3.7%), and xylose (1.9%) (Figure 5). *E. faecium* KT990028 and *E. faecium* EJRM152 produced polysaccharides with similar carbohydrate chains except for xylose, and galactose, respectively (de Brito et al., 2024; Kansandee et al., 2019). Notably, the mannose-rich profile is of particular interest. Mannose residues are often associated with immunoregulatory and prebiotic functions, especially in stimulating beneficial gut microbiota such as *Bifidobacterium* spp. (Gerwig, 2019; Kansandee et al., 2019). Moreover, the presence of rhamnose and galactose residues could facilitate adhesion to epithelial surfaces, enhancing mucosal persistence and biofilm formation which have critical attributes for probiotic-associated polysaccharides (Ciszek-Lenda, 2011).

Figure 5.
Monosaccharide composition of the polysaccharide



When compared to polysaccharides from other *E. faecium* strains, such as KT990028 and EJRM152 (de Brito et al., 2024; Kansandee et al., 2019), the monomer composition of SL60 particularly of xylose and galactose, suggests that it is a polysaccharide that may provide quite diverse bioactivity. This monomeric diversity may also affect the polymer's ability to regulate hydrophilicity, solubility, and viscosity, making it advantageous for food applications as a natural thickener or stabilizer.

Zeta Potential and Colloidal Stability

The zeta potential of the purified polysaccharide was measured as -9.71 mV (± 0.2). This moderately negative charge reflects partial electrostatic stabilization in aqueous solution, which could support dispersion and suspension in food matrices (Xu et al., 2019). While values beyond ± 30 mV are typically considered optimal for colloidal stability, moderate charges can still prevent aggregation in combination with steric hindrance provided by polymeric chains (Zhao et al., 2021). Importantly, the negative charge may also facilitate interactions with positively charged proteins or minerals in food systems, potentially influencing texture or bioavailability. Additionally, from a biological standpoint, negatively charged polysaccharides have been implicated in scavenging free radicals and modulating immune responses, thereby enhancing their functionality in health-promoting foods (de Brito et al., 2024; Gerwig, 2019; Xu et al., 2019).

Crystallinity and Functional Structure

XRD analysis demonstrated that the polysaccharide was amorphous, lacking sharp crystalline peaks (Figure 6) like *E. durans* (Genc et al., 2024). An amorphous configuration is commonly observed in microbial polysaccharides and is often associated with improved solubility, hydration

capacity, and flexibility-traits favorable for texture-modifying applications in bakery and dairy products (Genc et al., 2024; Tilwani et al., 2021). Moreover, the amorphous nature can enhance the polymer's ability to entrap water, stabilize emulsions, and interact with other macromolecules in the food matrix. When interpreted in conjunction with the monosaccharide profile, the amorphous structure likely arises from irregular branching or varied glycosidic linkages, which disrupt regular packing. This structural diversity can be exploited to tailor rheological properties or create controlled-release formulations in nutraceuticals.

Figure 6.

Structural confirmation of the polysaccharide by XRD

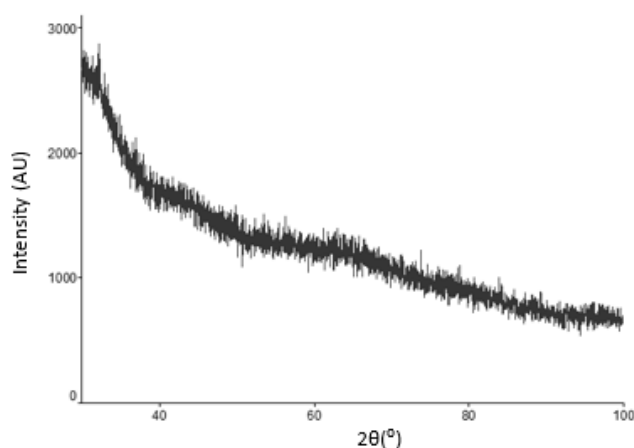
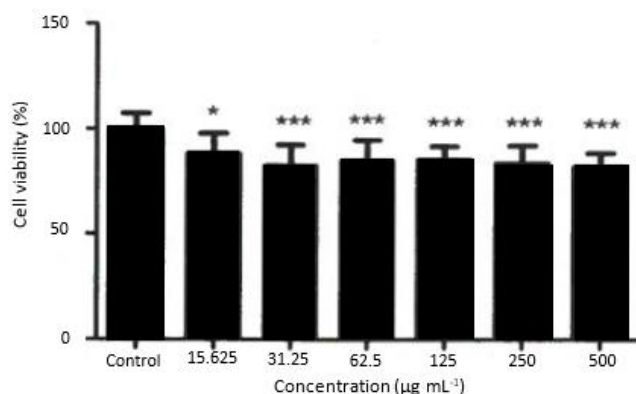


Figure 7.

*Cytotoxicity effect of polysaccharide from *E. faecium* SL60 on human fibroblast cell line (CCD-1079Sk) (* $p < 0.05$, and ** $p < 0.01$)*



Biocompatibility

Cytotoxicity experiments were performed at different concentrations (15.625, 32.25, 62.5, 125, 250, and 500 µg/mL), and MTT assays on CCD-1079Sk fibroblast cells showed no significant cytotoxicity even at polysaccharide concentrations up to 500 µg/mL (Figure 7), indicating high biocompatibility. Although this is a preliminary *in vitro*

evaluation, the absence of cytotoxic effects suggests potential use of this polysaccharide in functional foods or even pharmaceutical excipients. Notably, polysaccharide from *Enterococcus* strains have been studied for applications such as cholesterol reduction, anti-inflammatory activity, and probiotic encapsulation (Holzapfel & Wood, 2014).

Conclusion and Recommendations

This study provides a preliminary structural and biological characterization of a novel polysaccharide from *E. faecium* SL60 isolated from einkorn sourdough. The strain demonstrated a strong ability to produce extracellular polysaccharide, consistent with other *E. faecium* strains. The analyses confirmed the polysaccharide's purity and proteinaceous heteropolysaccharide nature. The absence of a sulfate group may affect its antioxidant capacity, but indicates a relatively clean structural profile suitable for food or biomedical applications.

The monomeric diversity suggests potential for multifunctional bioactivities, including prebiotic effects, epithelial adhesion, and biofilm support desirable in probiotic delivery systems. Also, the moderate negative zeta potential implies sufficient colloidal stability, which may enhance dispersibility in aqueous formulations and interaction with food components.

The amorphous structure observed through XRD supports the potential for improved solubility, water retention, and interaction with other macromolecules, making this polysaccharide a promising candidate for use as a natural thickener, stabilizer, or texturizer in functional food matrices. Furthermore, its lack of cytotoxicity demonstrates favorable biocompatibility, reinforcing its potential in safe food and biomedical applications.

Peer-review: Externally peer-reviewed.

Ethics Committee Approval: This study does not require ethics committee approval.

Conflict of Interest: The author has no conflicts of interest to declare.

Financial Disclosure: This research is a second study derived from Gumüşhane University [BAP- 20.F5119.01.03] project.

Use of Artificial Intelligence: The author declares that not artificial intelligence (AI) or similar programs were used in this research article.

Hakem Değerlendirmesi: Dış bağımsız.

Etik Komite Onayı: Bu çalışma için etik kurul onayına gerek yoktur.

Çıkar Çatışması: Yazar, çıkar çatışması olmadığını beyan etmiştir.

Finansal Destek: Bu araştırma, Gümüşhane Üniversitesi [BAP-20.F5119.01.03] projesinden türetilen ikinci bir çalışmadır.

Yapay Zekâ Kullanımı: Yazar, bu araştırma makalesinde yapay zekâ (AI) veya benzeri programlardan yararlanmadığını beyan etmiştir.

References

- Aanniz, T., Ouadghiri, M., Melloul, M., Swings, J., Elfahime, E., Ibbijbjen, J., Ismaili, M., & Amar, M. (2015). Thermophilic bacteria in Moroccan hot springs, salt marshes and desert soils. *Brazilian Journal of Microbiology*, 46(2), 443-453. <https://doi.org/10.1590/S1517-838246220140219>
- Bansal, S., Singh, A., Mangal, M., & Sharma, S. K. (2013). Isolation and Characterization of Lactic Acid Bacteria from Fermented Foods. *Vegetos*, 26(2), 325-330. <https://doi.org/10.5958/j.2229-4473.26.2.092>
- Bartkiene, E., Lele, V., Ruzauskas, M., Domig, K. J., Starkute, V., Zavistanaviciute, P., Bartkevics, V., Pugajeva, I., Klupsaite, D., Juodeikiene, G., Mickiene, R., & Rocha, J. M. (2020). Lactic Acid Bacteria Isolation from Spontaneous Sourdough and Their Characterization Including Antimicrobial and Antifungal Properties Evaluation. *Microorganisms*, 8(1), Article 64. <https://doi.org/10.3390/microorganisms8010064>
- Brouns, F., Geisslitz, S., Guzman, C., Ikeda, T. M., Arzani, A., Latella, G., Simsek, S., Colomba, M., Gregorini, A., Zevallos, V., Lullien-Pellerin, V., Jonkers, D., & Shewry, P. R. (2022). Do ancient wheats contain less gluten than modern bread wheat, in favour of better health? *Nutrition Bulletin*, 47(2), 157-167. <https://doi.org/10.1111/nbu.12551>
- Ciszek-Lenda, M. (2011). Biological functions of exopolysaccharides from probiotic bacteria. *Central European Journal of Immunology*, 36(1), 51-55.
- Cizeikiene, D., Jagelaviciute, J., Stankevicius, M., & Maruska, A. (2020). Thermophilic lactic acid bacteria affect the characteristics of sourdough and whole-grain wheat bread. *Food Bioscience*, 38, Article 100791. <https://doi.org/10.1016/j.fbio.2020.100791>
- de Brito, L. P., da Silva, E. C., Lins, L. F., de Medeiros, R. S., Silva, F. C. O., Pastrana, L., Cavalcanti, I. D. L., de Britto Lira-Nogueira, M. C., Cavalcanti, M. T. H., & Porto, A. L. F. (2024). Optimization, structural characterization, and biological applications of exopolysaccharide produced by *Enterococcus faecium* KT990028. *International Journal of Biological Macromolecules*, 282, 136926. <https://doi.org/10.1016/j.ijbiomac.2024.136926>
- Dimidi, E., Cox, S. R., Rossi, M., & Whelan, K. (2019). Fermented foods: definitions and characteristics, impact on the gut microbiota and effects on gastrointestinal health and disease. *Nutrients*, 11(8), 1806. <https://doi.org/10.3390/nu11081806>
- Franz, C., Huch, M., Abriouel, H., Holzapfel, W., & Gálvez, A. (2011). *Enterococci* as probiotics and their implications in food safety. *International Journal of Food Microbiology*, 151(2), 125-140. <https://doi.org/10.1016/j.ijfoodmicro.2011.08.014>
- Genc, B. (2024). Production and Characterization of Extracellular Polysaccharide from *Anoxybacillus flavithermus* AAG-61 with Resistance to Sulfamethoxazole and Ofloxacin. *Nepal Journal of Biotechnology*, 12(1), 74-80.
- Genc, B., Karatas, S. M., & Tunç, M. T. (2024). *Enterococcus durans* SL70, A Novel Exopolysaccharide Producer from Traditional Sourdough Fermentation of Einkorn (*Triticum monococcum* L. subsp. *monococcum*). *Food Technology and Biotechnology*, 62(4).
- Genc, B., Taskin, M., & Adiguzel, A. (2021). Exopolysaccharide of *Anoxybacillus pushchinoensis* G11 has antitumor and antibiofilm activities. *Archives of Microbiology*, 203(5), 2101-2118. <https://doi.org/10.1007/s00203-021-02185-x>
- Gerwig, G. J. (2019). Structural Analysis of Exopolysaccharides from Lactic Acid Bacteria. In K. M. (Ed.), *Lactic Acid Bacteria: Methods and Protocols* (Vol. 1887, pp. 67-84). https://doi.org/10.1007/978-1-4939-8907-2_7
- Gullo, M., Romano, A. D., Pulvirenti, A., & Giudici, P. (2003). *Candida humilis*--dominant species in sourdoughs for the production of durum wheat bran flour bread. *International Journal of Food Microbiology*, 80(1), 55-59. [https://doi.org/10.1016/s0168-1605\(02\)00121-6](https://doi.org/10.1016/s0168-1605(02)00121-6)
- Habib, B., Vaid, S., Bangotra, R., Sharma, S., & Bajaj, B. K. (2022). Bioprospecting of probiotic lactic acid bacteria for cholesterol lowering and exopolysaccharide producing potential. *Biologia*, 77(7), 1931-1951. <https://doi.org/10.1007/s11756-022-01058-y>
- Hidalgo, A., & Brandolini, A. (2014). Nutritional properties of einkorn wheat (*Triticum monococcum* L.). *Journal of the Science of Food and Agriculture*, 94(4), 601-612. <https://doi.org/10.1002/jsfa.6382>
- Holzapfel, W., Arini, A., Aeschbacher, M., Coppolecchia, R., & Pot, B. (2018). *Enterococcus faecium* SF68 as a model for efficacy and safety evaluation of pharmaceutical probiotics. *Beneficial Microbes*, 9(3), 375-388. <https://doi.org/10.3920/bm2017.0148>
- Holzapfel, W. H., & Wood, B. J. (2014). *Lactic acid bacteria: biodiversity and taxonomy*. John Wiley & Sons.
- Jing, Y., Zhu, J., Liu, T., Bi, S., Hu, X., Chen, Z., Song, L., Lv, W., & Yu, R. (2015). Structural characterization and biological activities of a novel polysaccharide from cultured

- Cordyceps militaris* and its sulfated derivative. *Journal of Agricultural and Food Chemistry*, 63(13), 3464-3471. <https://doi.org/10.1021/jf505915t>
- Kansandee, W., Moonmangmee, D., Moonmangmee, S., & Itsaranuwat, P. (2019). Characterization and *Bifidobacterium* sp. growth stimulation of exopolysaccharide produced by *Enterococcus faecalis* EJRM152 isolated from human breast milk. *Carbohydrate polymers*, 206, 102-109. <https://doi.org/10.1016/j.carbpol.2018.10.117>
- Kavitake, D., Devi, P. B., Delattre, C., Reddy, G. B., & Shetty, P. H. (2023). Exopolysaccharides produced by *Enterococcus* genus-An overview. *International Journal of Biological Macromolecules*, 226, 111-120. <https://doi.org/10.1016/j.ijbiomac.2022.12.042>
- Ketabi, A., Soleimanian-Zad, S., Kadivar, M., & Sheikh-Zeinoddin, M. (2008). Production of microbial exopolysaccharides in the sourdough and its effects on the rheological properties of dough. *Food Research International*, 41(10), 948-951. <https://doi.org/10.1016/j.foodres.2008.07.009>
- Lim, S. B., Tingirikari, J. M. R., Seo, J. S., Li, L., Shim, S., Seo, J. H., & Han, N. S. (2018). Isolation of lactic acid bacteria starters from Jeung-pyun for sourdough fermentation. *Food Science and Biotechnology*, 27(1), 73-78. <https://doi.org/10.1007/s10068-017-0274-0>
- Marco, M. L., Heeney, D., Binda, S., Cifelli, C. J., Cotter, P. D., Foligné, B., Gänzle, M., Kort, R., Pasin, G., & Pihlanto, A. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology*, 44, 94-102. <https://doi.org/10.1016/j.copbio.2016.11.010>
- Meroth, C. B., Hammes, W. P., & Hertel, C. (2003). Identification and population dynamics of yeasts in sourdough fermentation processes by PCR-denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, 69(12), 7453-7461. <https://doi.org/10.1128/aem.69.12.7453-7461.2003>
- Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., & Gobbetti, M. (2012). Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology*, 78(4), 1251-1264. <https://doi.org/10.1128/AEM.07721-11>
- Mohammadi-Kouchesfahani, M., Hamidi-Esfahani, Z., & Azizi, M. H. (2019). Isolation and identification of lactic acid bacteria with phytase activity from sourdough. *Food Science & Nutrition*, 7(11), 3700-3708. <https://doi.org/10.1002/fsn3.1229>
- Moreno, M. R. F., Sarantinopoulos, P., Tsakalidou, E., & De Vuyst, L. (2006). The role and application of enterococci in food and health. *International Journal of Food Microbiology*, 106(1), 1-24. <https://doi.org/10.1016/j.ijfoodmicro.2005.06.026>
- Peng, J. H., Sun, D., & Nevo, E. (2011). Domestication evolution, genetics and genomics in wheat. *Molecular Breeding*, 28, 281-301.
- Poutanen, K., Flander, L., & Katina, K. (2009). Sourdough and cereal fermentation in a nutritional perspective. *Food Microbiology*, 26(7), 693-699. <https://doi.org/10.1016/j.fm.2009.07.011>
- Raimondi, S., Amaretti, A., Rossi, M., Fall, P. A., Tabanelli, G., Gardini, F., & Montanari, C. (2017). Evolution of microbial community and chemical properties of a sourdough during the production of Colomba, an Italian sweet leavened baked product. *Lwt-Food Science and Technology*, 86, 31-39. <https://doi.org/10.1016/j.lwt.2017.07.042>
- Rani, R. P., Anandharaj, M., Sabhapathy, P., & Ravindran, A. D. (2017). Physiochemical and biological characterization of novel exopolysaccharide produced by *Bacillus tequilensis* FR9 isolated from chicken. *International Journal of Biological Macromolecules*, 96, 1-10. <https://doi.org/10.1016/j.ijbiomac.2016.11.122>
- Rice, T., Sahin, A. W., Lynch, K. M., Arendt, E. K., & Coffey, A. (2020). Isolation, characterisation and exploitation of lactic acid bacteria capable of efficient conversion of sugars to mannitol. *International Journal of Food Microbiology*, 321, Article 108546. <https://doi.org/10.1016/j.ijfoodmicro.2020.108546>
- Saeed, M., Anjum, F. M., Zahoor, T., Nawaz, H., & Sajjad-Ur-Rehman. (2009). Isolation and Characterization of Starter Culture from Spontaneous Fermentation of Sourdough. *International Journal of Agriculture and Biology*, 11(3), 329-332.
- Sáez, G. D., Saavedra, L., Hebert, E. M., & Zárate, G. (2018). Identification and biotechnological characterization of lactic acid bacteria isolated from chickpea sourdough in northwestern Argentina. *LWT*, 93, 249-256. <https://doi.org/10.1016/j.lwt.2018.03.040>
- Silveira, M. L. L., Smiderle, F. R., Agostini, F., Pereira, E. M., Bonatti-Chaves, M., Wisbeck, E., Ruthes, A. C., Sasaki, G.

- L., Cipriani, T. R., Furlan, S. A., & Iacomini, M. (2015). Exopolysaccharide produced by *Pleurotus sajor-caju*: Its chemical structure and anti-inflammatory activity. *International Journal of Biological Macromolecules*, 75, 90-96. <https://doi.org/10.1016/j.ijbiomac.2015.01.023>
- Tan, Q. L., Xu, H. Y., Aguilar, Z. P., Peng, S. S., Dong, S. Q., Wang, B. G., Li, P., Chen, T. T., Xu, F., & Wei, H. (2013). Safety Assessment and Probiotic Evaluation of *Enterococcus Faecium* YF5 Isolated from Sourdough. *Journal of Food Science*, 78(4), M587-M593. <https://doi.org/10.1111/1750-3841.12079>
- Tilwani, Y. M., Lakra, A. K., Domdi, L., Yadav, S., Jha, N., & Arul, V. (2021). Optimization and physicochemical characterization of low molecular levan from *Enterococcus faecium* MC-5 having potential biological activities. *Process Biochemistry*, 110, 282-291. <https://doi.org/10.1016/j.procbio.2021.08.021>
- Torino, M. I., de Valdez, G. F., & Mozzi, F. (2015). Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. *Frontiers in Microbiology*, 6, Article 834. <https://doi.org/10.3389/fmicb.2015.00834>
- Xu, Y., Cui, Y., Yue, F., Liu, L., Shan, Y., Liu, B., Zhou, Y., & Lü, X. (2019). Exopolysaccharides produced by lactic acid bacteria and Bifidobacteria: Structures, physiochemical functions and applications in the food industry. *Food Hydrocolloids*, 94, 475-499. <https://doi.org/10.1016/j.foodhyd.2019.03.032>
- 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 Journal of Microbiology*, 60(3), 334-345. <https://doi.org/10.1007/s12088-020-00865-8>
- Zhao, D., Jiang, J., Liu, L., Wang, S., Ping, W., & Ge, J. (2021). Characterization of exopolysaccharides produced by *Weissella confusa* XG-3 and their potential biotechnological applications. *International Journal of Biological Macromolecules*, 178, 306-315. <https://doi.org/10.1016/j.ijbiomac.2021.02.182>