

EVALUATION OF *BACILLUS LICHENIFORMIS* STRAIN HT3 AS A POTENTIAL PROBIOTIC

Serpil Ugras^{1✉}, Kerem Kizmaz², Dilsah Bahat³

Received on September 19, 2025

Presented by H. Najdenski, Member of BAS, on December 16, 2025

Abstract

The present study investigates the probiotic potential of *Bacillus licheniformis* strain Ht3 (*Bl*-Ht3), which was isolated from thermal spring water. The isolate was taxonomically identified through 16S rDNA sequencing and subsequently subjected to a series of assays to characterize its probiotic attributes. *Bl*-Ht3 exhibited tolerance to a broad range of gastrointestinal conditions; however, it showed limited survivability at highly acidic pH levels (pH 1.0 and 2.0), which represents a critical constraint in terms of gastric passage. Despite this limitation, the strain demonstrated γ -hemolytic activity, and was highly susceptible to all tested antibiotics, fulfilling key safety requirements for probiotic application. In terms of functional properties, the strain exhibited relatively weak autoaggregation capacity (13.04%), which may affect its ability to colonize the intestinal mucosa. Conversely, the coaggregation ability exceeded 35%, suggesting potential for interaction with other microbial species within the gastrointestinal tract, possibly contributing to competitive exclusion of pathogens. Collectively, the findings of this study delineate both the beneficial and limiting characteristics of *Bl*-Ht3 as a probiotic candidate. Future optimization efforts, particularly the application of spore-based formulations, are recommended to enhance acid resistance and improve survivability through the gastric environment. These improvements may further strengthen the strain's utility as a safe and stable probiotic for functional food or pharmaceutical applications.

Key words: *Bacillus licheniformis*, molecular characterization, probiotics

Introduction. According to the World Health Organization (WHO), probiotics are defined as live microorganisms that confer health benefits to the host when administered in adequate amounts. These microorganisms are inherently non-pathogenic and non-toxic in nature [1]. Most probiotic species naturally inhabit the gastrointestinal system (GIS) as part of the commensal microbiota. This microbiota plays a crucial role in various physiological processes, including immune modulation, pathogen inhibition, drug metabolism, and the biosynthesis of essential vitamins [2]. In addition, accumulating evidence suggests that probiotics may be beneficial in the management of several clinical conditions such as diarrhea, inflammatory bowel diseases, metabolic disorders including obesity and type 2 diabetes, cardiovascular diseases, and certain malignancies [3]. The preservation of a balanced and functional gastrointestinal microbiota is therefore fundamental to human health [4].

In recent years, the therapeutic potential of probiotics, along with the growing preference for natural health interventions, has significantly increased scientific interest in this field [5]. However, modern lifestyle factors including irregular dietary habits, insufficient hydration, chronic stress, frequent antibiotic use, smoking, and alcohol consumption disrupt the ecological balance of the intestinal microbiota [6]. Numerous *in vivo* and clinical studies support the notion that restoration of dysbiotic microbiota may contribute to improved host health [7], emphasizing the necessity for external probiotic supplementation.

Current probiotic research predominantly focuses on traditional genera such as *Lactobacillus* spp., *Bifidobacterium* spp., *Propionibacterium* spp., *Streptococcus* spp., and specific *Saccharomyces* spp. [5]. Despite their well-established probiotic properties, these microorganisms exhibit limited survivability under harsh gastrointestinal conditions [8]. The reported survival rates of such traditional probiotics in the gastrointestinal tract range between 1–15%, with some strains showing even lower viability. These limitations have prompted growing interest in alternative probiotic candidates, particularly spore-forming bacteria such as certain *Bacillus* species [9]. Owing to their sporulation capacity, *Bacillus* spp. exhibit enhanced resistance to extreme environmental conditions, thereby offering superior stability and viability compared to conventional probiotic strains [7].

Bacillus species are Gram-positive, aerobic or facultatively anaerobic microorganisms known for their resilience to heat, acid, and other environmental stressors [2, 10]. Although some *Bacillus* strains have been used in probiotic formulations for decades, systematic scientific evaluations have intensified only in the last 15 years. The most extensively studied species include *B. subtilis*, *B. clausii*, *B. cereus*, *B. coagulans*, *B. licheniformis*, *B. pumilus*, and *B. laterosporus*. While generally regarded as safe, specific strains may harbour transferable antibiotic resistance genes or produce enterotoxins and/or emetic toxins, which necessitates thorough safety assessments [11]. The probiotic efficacy of *Bacillus* spp. is pri-

marily attributed to their ability to secrete bioactive metabolites, modulate host immune responses, and produce various digestive enzymes [12].

In this study, a *Bacillus* strain isolated from thermal spring water was subjected to molecular identification and probiotic characterization. The investigation aimed to evaluate its potential applicability as a functional probiotic candidate, considering the increasing significance of spore-forming bacteria in probiotic development.

Material and methods. Bacterial identification. Bacterial isolation was carried out from thermal spring water samples collected in Giresun, Türkiye. Samples were initially cultured in Nutrient Broth (NB) and subsequently plated on Nutrient Agar (NA) to obtain discrete colonies. Pure isolates were obtained through repeated streaking and subculturing, and the resulting monocultures were preserved at -20°C in NB supplemented with 30% (v/v) glycerol for long-term storage. The molecular identification of the bacterial isolates was performed using the method applied by UGRAS et al. [13].

Hemolytic activity. Hemolytic activity of the bacterial isolate was assessed by streaking fresh cultures grown in NB onto Columbia agar plates (Biomérieux) supplemented with 5% (v/v) defibrinated sheep blood. Reference strains *Bacillus pumilus*, *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus epidermidis* were included as controls. Inoculated plates were incubated at 30°C for 48 h [14].

Antibiotic susceptibility testing. The antibiotic susceptibility profile of the bacterial isolate was evaluated using the disk diffusion method against seven antibiotics: tobramycin, erythromycin, azithromycin, ciprofloxacin, cefdinir, imipenem/cilastatin, and streptomycin. This study was carried out following the methodology described by UGRAS et al. [15]. For control and comparison purposes, data previously published by Ugras et al. [15] were re-analyzed in this study. The test was performed in duplicate and repeated twice.

Determination of probiotic properties. The gastrointestinal survivability of the bacterial isolate was assessed through a series of tolerance assays, including acid, pepsin, bile salt, and pancreatin tolerance tests. In this study, the method applied by MARAGKLOUDAKIS et al. [16] was utilized. All experiments were repeated two times in duplicate. The negative control contained only the medium.

Determination of antagonistic activity. The antagonistic activity of the isolate against pathogenic bacteria and yeasts was assessed using the agar well diffusion method. The tested microorganisms included twelve bacterial strains: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Salmonella typhimurium* ATCC 14028, *Klebsiella pneumoniae* ATCC 13883, *Yersinia pseudotuberculosis* ATCC 911, *Enterococcus faecalis* ATCC 29212, *Enterobacter cloacae* ATCC 13047, *Proteus vulgaris* ATCC 13315, *Bacillus subtilis* ATCC 6633, and *Listeria monocytogenes* ATCC 7644, as well as two yeast strains: *Candida albi-*

cans ATCC 90028 and *Candida parapsilosis* (clinical isolate) [15,17]. The test was performed in duplicate and repeated twice.

Determination of aggregation properties. Autoaggregation ability of the bacterial isolate was determined according to the procedure described by Ugras et al. [15]. Autoaggregation was calculated using the following formula:

$$\text{Autoaggregation (\%)} = [(OD_1 - OD_2) / OD_1 \times 100],$$

where OD_1 is the optical density before incubation and OD_2 is the optical density after incubation.

Coaggregation assays were conducted to assess the ability of the probiotic candidate to coaggregate with pathogenic bacteria (*Listeria monocytogenes* and *Escherichia coli*) [15]. Coaggregation was determined using the following formula:

$$\text{Coaggregation(\%)} = [(OD_1 + OD_2 - 2OD_3) / (OD_1 + OD_2)] \times 100,$$

where OD_1 is the optical density of the probiotic sample before incubation, OD_2 is the optical density of the pathogen before incubation, OD_3 is the optical density of the probiotic + pathogen mixture after incubation.

For the determination of aggregation properties, data from Ugras et al. [15] were used as controls and for comparative purposes.

Statistical analysis. The data were analyzed using IBM SPSS version 20.0 (SPSS for Windows, SPSS Inc., Chicago, IL, USA) and presented as mean $\pm$ standard deviation (SD) based on two independent experiments conducted in duplicate.

Results and discussion. Characterization of the bacterial strain.

With the continuously evolving landscape of healthcare, the development of additional or more dynamic treatment modalities for human health has become a critical need in modern medicine [18]. In recent years, the use of probiotics has emerged as a highly beneficial and widely sought-after therapeutic approach among various potential treatment strategies. This growing demand is driven not only by the significant efficacy of probiotics in disease prevention and management but also by the increasing preference for natural health solutions [5]. Current studies suggest that probiotics offer substantial benefits not only in maintaining the balance of the gastrointestinal system but also in managing diseases, reducing the reliance on antibiotics, and supporting oral microbiota balance in the treatment of periodontal diseases. Furthermore, their immunomodulatory, anticancer, and wound healing properties have garnered considerable attention [4,19].

Recent research predominantly focuses on traditional probiotic strains, such as lactic acid bacteria. However, the limitations of these probiotics, particularly their low survival rates under harsh conditions, underscore the necessity for alternative probiotic strains. Consequently, spore-forming probiotic candidates have garnered increasing interest due to their enhanced durability. Among these, non-pathogenic *Bacillus* species are particularly promising. In this context, this study

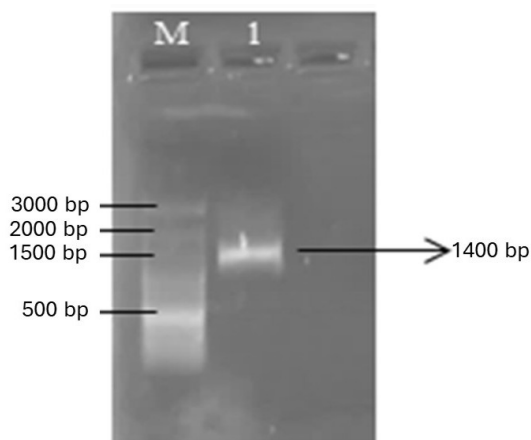


Fig. 1. PCR Profile of the isolate. M: Marker; 1: 1400 bp fragment of the 16S rDNA gene region specific to *Bl*-Ht3

aims to isolate and evaluate a novel *Bacillus* species with potential probiotic properties. As a result of molecular characterization, the bacterium isolated from the thermal spring was identified as *Bacillus licheniformis* strain Ht3 (*Bl*-Ht3). The PCR amplification profile of the strain is presented in Fig. 1.

Hemolytic activity of the bacterium. In this study, the safety profile of the isolate was evaluated through hemolytic activity and antibiotic susceptibility assays. Hemolytic activity of the bacterial isolate was evaluated based on the type of hemolysis exhibited on blood agar. Colonies that produced no visible zone around their growth were classified as non-hemolytic (γ -hemolytic), while those exhibiting a clear zone were classified as β -hemolytic, and those producing a greenish discolouration were considered α -hemolytic [14]. Hemolysis is a common virulence factor among pathogens, leading to conditions like anemia in the host. As hemolysis is a well-known virulence factor among pathogenic microorganisms, the absence of hemolytic activity is considered a prerequisite for safety in probiotic bacteria. The results of the hemolysis assay, *Bl*-Ht3 was determined to be γ -hemolytic, indicating a lack of hemolytic activity and supporting its safety as a probiotic candidate.

Antibiotic susceptibility of the bacterium. Another critical safety criterion for probiotic evaluation is the assessment of antibiotic resistance, as resistance traits can potentially be transferred horizontally between microorganisms, posing a significant public health risk. In this study, the antibiotic susceptibility profile of *Bl*-Ht3 was evaluated against seven commonly used antibiotics. The results were compared with those of *Pediococcus pentosaceus* strain AF2, which we had characterized in previous studies [15]. *Pp*-AF2 was found to be resistant to certain antibiotics. Numerous studies in the literature indicate that such resistance

T a b l e 1

Antibiotic susceptibility profile of *Bl*-Ht3 and the control strain *Pp*-AF2*

Antibiotics	Inhibition zone (mm)	
	<i>Bl</i> -Ht3	<i>Pp</i> -AF2*
Tobramycin (TOB30, 30 µg/disk)	33.33 ± 0.57	00.00 ± 0.00
Erythromycin (E30, 30 µg/disk)	> 50.00	25.00 ± 1.00
Azithromycin (AZM30, 30 µg/disk)	47.00 ± 1.00	19.50 ± 0.41
Ciprofloxacin (CIP30, 30 µg/disk)	> 50.00	00.00 ± 0.00
Cefdinir (CD30, 30 µg/disk)	25.25 ± 1.52	14.50 ± 0.21
Imipenem/Cilastatin (IMC20, 10 µg/10 µg/disk)	> 50.00	26.00 ± 1.52
Streptomycin (S25, 25 µg/disk)	35.55 ± 1.00	00.00 ± 0.00

* – *Pediococcus pentosaceus* strain AF2 [15]

traits in lactic acid bacteria, including *Pediococcus* species, are generally chromosomally encoded and non-transferable. Nevertheless, the underlying resistance mechanisms should still be elucidated. In contrast, the strain *Bl*-Ht3 was susceptible to all tested antibiotics, suggesting no acquired antibiotic resistance and further supporting its safety as a probiotic candidate (Table 1).

Assessment of probiotic properties. The study focused on evaluating pH resistance, with results indicating that *Bl*-Ht3 was not resistant to pH 1.0 and pH 2.0, although some viability was retained after 1 h of exposure. At pH 3.0, the bacterium exhibited approximately 35% viability after 1 h and approximately 19% viability after 3 h (Fig. 2). To assess the isolate's tolerance to pepsin under simulated gastric conditions, *Bl*-Ht3 was exposed to pepsin-containing PBS at pH 2.0 and pH 3.0. At pH 2.0, bacterial viability was determined to be approximately 6.7% at 0 h, decreasing to 1.7% at 1 h, and 1.0% at 3 h. At pH 3.0, initial viability was approximately 64% at 0 h, dropping to 4.0% at 1 h and 3.6% at 3 h (Fig. 3). To evaluate pancreatin tolerance, the viability of *Bl*-Ht3 was assessed under exposure to pancreatin in PBS at pH 7.2 and 8.0. Initial results indicated that the strain exhibited sensitivity to pancreatin, with viability measured at approximately 38%. However, as incubation progressed, an increase in viable colony counts was observed, suggesting that the strain adapted to the enzymatic environment and maintained its survival over time. The bile salt tolerance of *Bl*-Ht3 was evaluated by exposing the strain to different concentrations (0.3%, 0.5%, and 1.0% w/v) of bile salts. Initial results indicated that the strain exhibited sensitivity at all tested concentrations. However, following 4 h of incubation, the strain demonstrated an adaptive response, maintaining viability at 0.3% bile salt concentration, where a slight increase in viable cell counts was observed. In contrast, a decline in viability was noted at 0.5% and 1.0% concentrations; nevertheless, complete loss of viability was not observed at any of the tested

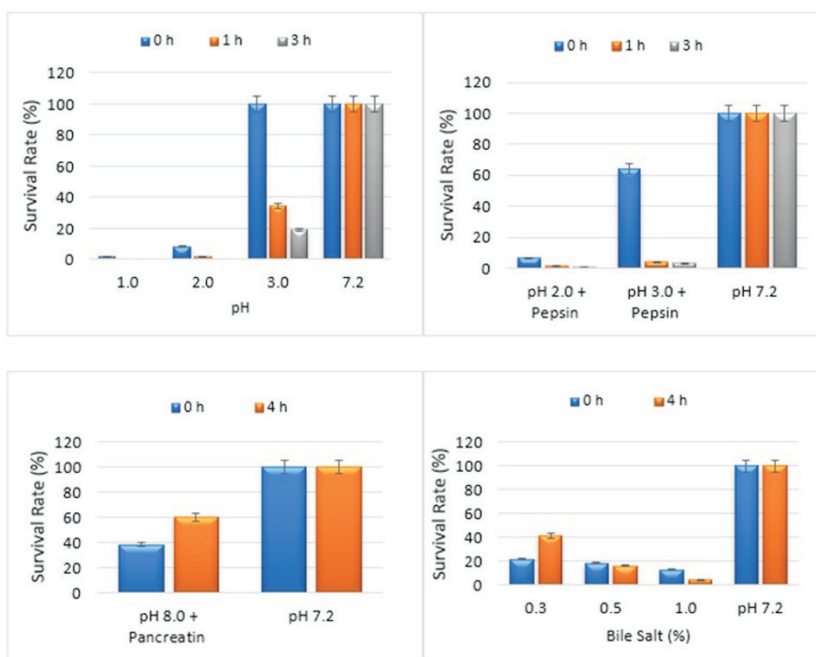


Fig. 2. The ability of transit through the gastrointestinal tract of *BL-Ht3*

levels, indicating a degree of tolerance (Fig. 2). Considering all findings regarding the probiotic properties of the *BL-Ht3* strain, it can be concluded that it is more sensitive to gastrointestinal conditions compared to *Pp-AF2* strain [15].

Overall, the findings of this study indicate that *BL-Ht3*, isolated from thermal spring water, tolerated all simulated gastrointestinal conditions, except under extremely acidic environments (pH 1.0 and 2.0). Such tolerance to gastrointestinal stressors is a highly desirable trait for probiotic candidates. However, the strain's limited tolerance to low pH levels may be considered a drawback. Despite this limitation, extensive research has demonstrated that *Bacillus* species, owing primarily to their capacity for endospore formation, exhibit enhanced resilience under adverse environmental conditions, including heat and gastrointestinal stress, compared to conventional probiotic strains [20]. In this context, the *BL-Ht3* strain, which exhibited probiotic properties in this study, could potentially be formulated in its endospore form to enhance its resistance to gastrointestinal conditions.

Antagonistic and aggregation characteristics of the bacterium. Another key parameter in selecting probiotic candidates is the ability of microorganisms to exhibit antagonistic effects against pathogenic microorganisms. Many probiotic organisms, particularly lactic acid bacteria, produce organic acids such as acetic and formic acid, which lower the intestinal pH, creating an environment that inhibits the growth of pathogenic bacteria. In the present study, the antagonistic activity of *BL-Ht3* was evaluated against 12 bacterial strains and 2

yeast species. Although *Bacillus* species are generally known for their antagonistic activity [20], *Bl*-Ht3 did not exhibit detectable inhibition under the conditions tested. This may be due to strain-specific metabolite production, low metabolite levels, or the influence of environmental factors such as medium composition and pH. In this context, testing the *Bl*-Ht3 bacterial supernatant obtained from different culture media and longer incubation times, concentrating the supernatant, assessing antagonistic activity against a broader range of test bacteria, and modifying the assay method may allow detection and measurement of its antagonistic potential.

Finally, it is crucial for probiotic candidate microorganisms to exhibit aggregation properties, as this is a supportive criterion for evaluating their probiotic potential. Aggregation is classified into two types: autoaggregation and coaggregation. Autoaggregation refers to the self-association of microorganisms of the same species, leading to the formation of cell clusters, whereas coaggregation involves the interaction and aggregation of microorganisms from different species. Probiotics can adhere to intestinal epithelial cells via their autoaggregation ability and prevent the colonization of pathogens through their coaggregation ability. In the present study, it was observed that the autoaggregation ability of *Bl*-Ht3 was relatively low (13.04%), while its coaggregation ability was determined to be 50.21% for *E. coli* and 37.85% for *L. monocytogenes* (Table 2). Comparatively, a previous study involving four different strains, including *Bacillus subtilis* and *B. amyloliquefaciens*, demonstrated that these bacteria exhibited autoaggregation abilities of over 45% after 4 h incubation [20]. In addition, when comparing the aggregation ability of *Bl*-Ht3 with that of *Pp*-AF2, it can be stated that *Bl*-Ht3 exhibits lower auto-aggregation capacity, while its co-aggregation ability, particularly with *E. coli*, appears as a closer value. Although the strain exhibited relatively low auto- and co-aggregation capacities, this limitation does not preclude its probiotic potential, as aggregation is a supportive rather than essential probiotic criterion.

T a b l e 2
Aggregation capacity

Auto-aggregation (%)		Co-aggregation (%)	
		<i>E. coli</i>	<i>L. monocytogenes</i>
<i>Bl</i> -Ht3	13.04	50.21	37.85
<i>Pp</i> -AF2*	73.44	56.80	57.38

* – *Pediococcus pentosaceus* strain AF2 [15]

Probiotics are defined as live microorganisms that, when administered in sufficient quantities, provide health benefits to the host. While traditional probiotics are widely used in therapeutic applications, their viability under gastrointestinal conditions is often limited, with notably low survival rates. These limitations

highlight the need for the identification and development of alternative probiotic strains that offer enhanced stability and resilience. In this context, *Bacillus* species have attracted significant attention due to their ability to form spores and their robust resistance to harsh environmental conditions.

In this study, *Bacillus licheniformis* (*Bl*-Ht3), isolated from hot spring water, was identified as a potential probiotic candidate. While the bacterium's reliability increases its potential as a probiotic, its sensitivity to certain gastrointestinal conditions may reduce its overall probiotic potential. However, similar reductions in viability are observed in traditional probiotics as well. Notably, *Bl*-Ht3 possesses the ability to sporulate, and it is anticipated that its resistance can be enhanced through further investigation of spore formulation in future studies. A spore form capable of withstanding gastrointestinal conditions, while transitioning to the vegetative form in the small intestine where essential molecules such as sugars, nucleosides, and amino acids are present, can ensure both its stability and safety.

REFERENCES

- [1] SZYDŁOWSKA A., B. SIOŃEK (2023) Probiotics and postbiotics as the functional food components affecting the immune response, *Microorganisms*, **11**(1), 104, <https://doi.org/10.3390/microorganisms11010104>.
- [2] KANDIL A. (2021) The effects of a probiotic (*Bacillus clausii*) in acute kidney injury in a rat model of LPS induced endotoxemia, *Eur. J. Biol.*, **80**(1), 48–53, <https://doi.org/10.26650/EurJBiol.2021.931755>.
- [3] PANWAR H., D. CALDERWOOD, I. R. GRANT et al. (2016) *Lactobacilli* possess inhibitory activity against dipeptidyl peptidase-4 (DPP-4), *Ann. Microbiol.*, **66**(1), 505–509, <https://doi.org/10.1007/s13213-015-1129-7>.
- [4] ABATENH E., B. GIZAW, Z. TSEGAY et al. (2018) Health benefits of probiotics, *J. Bacteriol. Infect. Dis.*, **2**(1), 8–27.
- [5] CAO J., Z. YU, W. LIU et al. (2020) Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases, *J. Funct. Foods*, **64**(1), 103643, <https://doi.org/10.1016/j.jff.2019.103643>.
- [6] KAMARLI ALTUN H., E. AKAL YILDIZ (2017) Relationship between prebiotics-probiotics and diabetes mellitus, *Turk. J. Life. Sci.*, **2**(1), 149–156.
- [7] ELSHAGHABEE F. M. F., N. ROKANA, R. D. GULHANE et al. (2017) *Bacillus* as potential probiotics: status, concerns, and future perspectives, *Front. Microbiol.*, **8**, 1490, <https://doi.org/10.3389/fmicb.2017.01490>.
- [8] KELLER D., S. VERBRUGGEN, H. CASH et al. (2019) Spores of *Bacillus coagulans* GBI-30, 6086 show high germination, survival and enzyme activity in a dynamic, computer-controlled in vitro model of the gastrointestinal tract, *Benef. Microbes.*, **10**(1), 77–87, <https://doi.org/10.3920/BM2018.0037>.
- [9] HYRONIMUS B., C. L. MARREC, A. H. SASSI, A. DESCHAMPS (2000) Acid and bile tolerance of spore-forming lactic acid bacteria, *Int. J. Food Microbiol.*, **61**(2–3), 193–197, [https://doi.org/10.1016/s0168-1605\(00\)00366-4](https://doi.org/10.1016/s0168-1605(00)00366-4).

- [10] KHATRI I., G. SHARMA, S. SUBRAMANIAN (2019) Composite genome sequence of *Bacillus clausii*, a probiotic commercially available as Enterogermina®, and insights into its probiotic properties, BMC Microbiol., **19**(1), 307, <https://doi.org/10.1186/s12866-019-1680-7>.
- [11] CUTTING S. M. (2011) *Bacillus* probiotics, Food Microbiol., **28**(2), 214–220, <https://doi.org/10.1016/j.fm.2010.03.007>.
- [12] SAVITRI L. P. (2021) Probiotics for human health. In: Goel G., Kumar A. (Eds) Advances in Probiotics for Sustainable Food and Medicine, Microorganisms for Sustainability, Springer, Berlin, 181–212, https://doi.org/10.1007/978-981-15-6795-7_8.
- [13] UGRAS S. (2017) Characterization of a thermophilic lipase from *Bacillus licheniformis* Ht7 isolated from Hayran thermal springs in Giresun, Rom. Biotechnol. Lett., **22**(1), 12297–12306.
- [14] MONIKA S., V. KUMAR, A. KUMARI et al. (2017) Isolation and characterization of lactic acid bacteria from traditional pickles of Himachal Pradesh, India, J. Food Sci. Technol., **54**(7), 1945–1952, <https://doi.org/10.1007/s13197-017-2629-1>.
- [15] UGRAS S., A. FIDAN, P. A. YOLDAS (2024) Probiotic potential and wound healing activity of *Pediococcus pentosaceus* strain AF2 isolated from *Herniaria glabra* L. which is traditionally used to make yogurt, Arch. Microbiol., **206**(3), 115, <https://doi.org/10.1007/s00203-024-03831-w>.
- [16] MARAGKLOUDAKIS P. A., G. ZOUMPOPOULOU, C. MIARIS et al. (2006) Probiotic potential of *Lactobacillus* strains isolated from dairy products, Int. Dairy J., **16**(3), 189–199, <https://doi.org/10.1016/j.idairyj.2005.02.009>.
- [17] GÜMRÜKÇÜOĞLU N., S. UĞRAŞ, H. I. UĞRAŞ, U. ÇAKIR (2012) Synthesis, extraction and antibacterial studies of some new bis-1,2,4-triazole derivatives part II, J. Incl. Phenom. Macrocycl. Chem., **73**(1–4), 359–367, <https://doi.org/10.1007/s10847-011-0072-x>.
- [18] JIANG S., L. CAI, L. LV, L. LI (2021) *Pediococcus pentosaceus*, a future additive or probiotic candidate, Microb. Cell Factories, **20**, 45, <https://doi.org/10.1186/s12934-021-01537-y>.
- [19] VICARIO M., A. SANTOS, D. VIOLANT et al. (2013) Clinical changes in periodontal subjects with the probiotic *Lactobacillus reuteri* prodentis: a preliminary randomized clinical trial, Acta Odontol. Scand., **71**(3–4), 813–819, <https://doi.org/10.3109/00016357.2012.734404>.
- [20] JLIDI M., I. AKREMI, A. H. IBRAHIM et al. (2022) Probiotic properties of *Bacillus* strains isolated from the gastrointestinal tract against pathogenic Vibriosis, Front. Mar. Sci., **9**, 884244, <https://doi.org/10.3389/fmars.2022.884244>.

¹Department of Pharmaceutical Microbiology, Hamidiye Faculty of Pharmacy, University of Health Sciences, Istanbul, Türkiye
e-mail: serpilkus@gmail.com, <https://orcid.org/0000-0002-1867-5781>

²Department of Biology, Institute of Science, Duzce University, Duzce, Türkiye
e-mail: kizmazkerem@gmail.com, <https://orcid.org/0009-0008-0146-839X>

³Department of Biology, Faculty of Science and Art, Duzce University, Duzce, Türkiye
e-mail: dilsah210899@gmail.com, <https://orcid.org/0009-0003-5599-345x>