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# Probiotic characterization of *Limosilactobacillus fermentum* BGHV110 strain and its influence on innate immune response in *Caenorhabditis elegans*

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## Abstract

Probiotic lactobacilli exhibit the potential to promote health benefits for the host. Thanks to its numerous beneficial effects on human health, *Limosilactobacillus fermentum* stood out as an excellent candidate for the development of commercial probiotic preparations aiming to prevent community-acquired infections. In this study, several *in vitro* tests, including biofilm formation assay, assessment of antibiotic susceptibility, survival in simulated gastrointestinal tract conditions and attachment to intestinal Caco-2 cells, were used to estimate the safety and probiotic potential of *L. fermentum* BGHV110 strain. Additionally, *Caenorhabditis elegans* was used as an *in vivo* model system for the evaluation of *L. fermentum* BGHV110 influence on the host's innate immune response. The results revealed that *L. fermentum* BGHV110 strain showed an excellent capability to survive harsh conditions of the gut, to attach to intestinal Caco-2 cells and to stimulate conserved p38 MAPK immunity pathway and expression of the *clc-1* claudin-like gene and antimicrobial peptides in *C. elegans* in order to enhance the immune response against pathogens. Finally, *L. fermentum* BGHV110 showed no virulence traits and susceptibility to tested antibiotics, confirming its safety status which enables it to be applied as a future probiotic.

**Key words:** *Limosilactobacillus fermentum*, probiotic, innate immune response, gastrointestinal tract, *Caenorhabditis elegans*

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## Introduction

Interaction between the complex microbial community in the gut which forms intestinal microbiota and the host is essential for the regulation of homeostasis of the gastrointestinal tract (GIT) (1). Gut bacteria are responsible for multiple physiological processes such as nutrient digestion, protection against pathogens, proper epithelial barrier function, and immunomodulation, and therefore manipulation of the intestinal microbiota represents a potential strategy for the prevention and treatment of different diseases (2, 3, 4). These mutually beneficial interactions essential for the host's wellbeing could be further potentiated by the application of probiotics, commonly used microbiota-based therapeutics. Probiotics are defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (5). The most common feature of probiotics is the ability to enhance the immune response and prevent community-acquired gastrointestinal infections (6). Innate immune pathways in the gut are kept in the primed state to instantly respond to microbe-associated molecular patterns (MAMPs), such as peptidoglycans, teichoic acids, lipopolysaccharides, dsRNA, flagellin, and microbial polysaccharides (7). Because of their simplicity and nonspecific detection of MAMPs, these pathways are highly conserved through evolution and their orthologs could be found from invertebrates (e.g., nematodes, fruit flies) to complex organisms (8). In mammals, the activation of mucosal intestinal immunity usually induces the expression of different genes responsible for the recruitment of immune cells (dendritic cells and macrophages), stimulation of IgA production by plasma cells from lamina propria, but also activation of Paneth secretory cells specialized in the production of antimicrobial peptides (AMP) including defensins, lysozymes, or C-type lectins similar to those involved in the *Caenorhabditis elegans* response to pathogens (9).

*C. elegans* is a bacterivore nematode which has been used as a model organism to study response to infection by several bacterial pathogens. Conserved signalling pathways identified to be essential for a worm's defence against intestinal pathogens include the PMK-1 pathway corresponding to mammalian p38 mitogen-activated protein kinase (MAPK) pathway and the DBL-1 pathway similar to transforming growth factor β (TGF-β) signalling (10). Due to the high similarity of the *C. elegans* digestive system to mammalian intestines, in terms of expression of epithelial tight junction proteins (TJP) like claudins (e.g., claudin-like in Caenorhabditis, CLC-1) and above-mentioned immunity pathways that control the expression of AMP, *C. elegans* became an excellent model to study probiotic-host interaction in general (11, 12). Recent evidence from experiments conducted on the *C. elegans* model implies that several stress-response mechanisms, including the HLH-30/TFEB dependent autophagy (13), canonical PMK-1/p38 MAPK immunity (8), SKN-1/NRF2 mediated antioxidative response (14), and serotonin signalling (15) could be triggered by different strains of commensal lactobacilli resulting in increased longevity of worms.

*Limosilactobacillus fermentum* is a heterofermentative gram-positive bacterium from the lactic acid bacteria (LAB) group of the Firmicutes phylum. Reported beneficial

roles of *L. fermentum* are mainly related to the prevention or treatment of gastrointestinal disorders including intestinal infection, immunomodulation in colitis and Crohn's disease, reduction of colorectal cancer risk, and hepatoprotective effect against drug-induced toxicity and alcoholic liver disease (6). Based on the described effects, *L. fermentum* were used for the development of various probiotic preparations commercially available on the USA market (6); however, probiotic preparations in Serbia are predominantly composed of *Lactobacillus acidophilus* and *Lactiplantibacillus plantarum* strains. Our previous results described the promising probiotic potential of *L. fermentum* BGHV110 strain, highlighting its ability to activate protective cellular autophagy, a clearance process for the recycling of damaged organelles and misfolded proteins, resulting in a beneficial effect for the host which can be used to design novel probiotic preparations (13, 16).

Here, we demonstrated that *L. fermentum* BGHV110 strain possesses excellent probiotic features for further health promoting applications, and that it showed the capability to stimulate conserved p38 MAPK immunity pathway and expression of TJP and AMP in *C. elegans* necessary for host defence against intestinal infection and invading microbes.

## Materials and Methods

### Bacterial Strain and Culture Condition

*Limosilactobacillus fermentum* BGHV110 was used in this study (16). The strain was cultivated overnight at 37° C in deMan-Rogosa-Sharpe (MRS) broth (Sigma-Aldrich) under anaerobic conditions. The *Escherichia coli* OP50 strain for worms' maintenance was cultivated in Luria Bertani (LB) medium overnight at 37° C aerobically. *Enterococcus faecium* BGZLM1-5 was cultivated in GM17 medium overnight at 37° C.

### Antibiotic Susceptibility Testing

Minimal inhibitory concentrations (MICs) were determined by microdilution assay (17). Antibiotics used in the assay were: ampicillin (2 mg/L), gentamicin (16 mg/L), kanamycin (64 mg/L), streptomycin (64 mg/L), erythromycin (1 mg/L), clindamycin (4 mg/L), tetracycline (8 mg/L), and chloramphenicol (4 mg/L) proposed by EFSA for obligate heterofermentative lactobacilli, including *L. fermentum* (17). Microdilutions were made in the Hi-Sensitivity Test Broth (HiMedia, India). The final CFU per well was $5 \times 10^6$. Cell density was monitored after 24 h incubation at 37 °C with 5% $CO_2$ at 600 nm using a spectrophotometer Plate Reader Infinite 200 pro (MTX Lab Systems, Vienna, Austria).

### Biofilm Formation

Biofilm formation assay was done according to Macovei et al. (18), with minor modifications. The microtiter plates (Sarstedt, Germany) were filled with 180 µl Hi-Sensitivity Test Broth (HiMedia) medium and 20 µL of overnight grown culture of *L.*

*fermentum* BGHV110 (adjusted to 0.5 McFarland units). After 24 h incubation at 37 °C with 5% $CO_2$, microtiter plates were washed with phosphate-buffered saline (PBS) and incubated for 30 min at 65 °C for the drying process. Formed biofilm was dissolved by using 0.1% crystal violet (HIMedia, India), and absorbance was measured at 595 nm using a Plate Reader Infinite 200 pro (MTX Lab Systems). The strains were categorized as no biofilm producer (OD 595 ≤ 0.2), weak biofilm producer (OD 595 0.2 - 0.7), strong biofilm producer (OD 595 0.7 - 1.4), and very strong biofilm producer (OD 595 ≥ 1.4). *En. faecium* BGZLM1-5 was used as a positive control.

**Survival in Simulated Gastrointestinal Tract Conditions**

The survival of *L. fermentum* BGHV110 strain during the passage through GIT was performed in a model of simulated GIT conditions described by Sánchez et al. (19). *L. fermentum* BGHV110 strain, which was grown overnight in MRS medium, was centrifugated at 5000 × g for 10 min, washed in 0.9% NaCl and resuspended in gastric juice (125 mM NaCl, 7 mM KCl, 45 mM NaHCO3, 0.3% pepsin (Sigma), pH 2). After a 90 min challenge, bacterial cells were pelleted and resuspended in duodenal juice (1% bile salt (Sigma), pH 8). After 10 min of incubation, bacteria were collected with centrifugation and resuspended in intestinal juice (0.3% bile salt, 0.1% pancreatin ("Pancreas acetone powder porcine Type I", Sigma), pH 8) for a 120 min challenge. Serial 10× dilutions in 0.9 % NaCl were made after 0, 90, 110 and 180 min of bacterial incubations and plated on MRS agar plates, which were incubated at 37 °C for 24 h. The results were expressed as colony forming units (CFU)/mL of survived bacterial cells after every challenge to GIT juices.

**Adherence to intestinal cells**

The intestinal Caco-2 cells were used to estimate the adhesion ability of *L. fermentum* BGHV110 strain. The Caco-2 cell line was maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine, all purchased from Thermo Fisher Scientific. The cells were seeded in 24-well plates ($1 \times 10^5$ cells/well) and cultivated until a monolayer was formed. Overnight bacterial culture was washed with PBS and resuspended in the DMEM media without antibiotics. The bacterial suspension was added to the cells in a 1:10 ratio. Following co-incubation for 2 h at 37 °C and 5% $CO_2$, the cells were gently washed with PBS and detached with 0.25% Trypsin–EDTA solution (Sigma). The bacteria before adhesion and the bacteria collected after adhesion were diluted in PBS and plated on MRS agar plates. The results were expressed as a % of adhesion, calculated as CFU/mL of adhered bacteria/CFU/mL of added bacteria × 100.

***Caenorhabditis elegans* maintenance and treatment**

*C. elegans* wild-type N2 (Bristol) strain were maintained on nematode growth medium (NGM) plates seeded with *E. coli* OP50 strain at 20 °C by using standard protocols (8). Worms' synchronization was done from a population of egg-bearing

worms by using 0.5 M NaOH and 1% Na-hypochlorite solution, followed by washing with M9 buffer. Eggs were plated to OP50 seeded NGM plates in order to obtain synchronized L1 animals. For the treatments, the overnight culture of *L. fermentum* BGHV110 strain was pelleted by centrifugation at 5000 × g for 10 min at room temperature, washed twice with PBS, resuspended in LB medium and seeded on NGM plates. The control treatment was prepared by seeding an overnight culture of *E. coli* OP50, grown in LB, on NGM plates. Age-synchronized worms in the L4 larval stage were transferred on *E. coli* OP50 NGM plates or *L. fermentum* BGHV110 containing NGM plates, and incubated overnight.

### RNA isolation and quantitative real-time PCR (qRT-PCR)

After overnight treatment, worms were collected with M9 buffer and washed three times to remove the remaining bacteria. Total RNA was isolated using a Trizol reagent by following the manufacturer's protocol (Thermo Fisher Scientific). Genomic DNA contamination was removed by using RapidOut DNA Removal Kit according to the manufacturer's protocol (Thermo Fisher Scientific). Reverse transcription was performed with RevertAid Reverse Transcriptase (Thermo Fisher Scientific) with 0.5 µg of isolated RNA, random hexamers (Thermo Fisher Scientific), and RiboLock RNase inhibitor (Thermo Fisher Scientific) used in the reactions. Quantitative real-time PCR was performed with an IC Green qPCR Universal Kit (NIPPON Genetics, Düren, Germany) under the following conditions: 2 min at 95 °C activation, 40 cycles of 5 s at 95 °C and 30 s at 60 °C in Line-Gene 9600 Plus Real-Time PCR (Hangzhou Bioer Technology). The results were normalized by using the housekeeping *act-1* gene. All used primers are listed in Table I and were purchased from Thermo Fisher Scientific. For each condition, three independent replicates were used.

### Statistical Analysis

All values are presented as mean ± standard deviation (SD). Student's t-test was used to compare the differences between the control and treatment groups, while one-way ANOVA followed by the Tukey post hoc test was used for multiple comparisons. P value lower than 0.05 was considered statistically significant. The statistical analysis and graphs were done in GraphPad Prism version 8.0.0 for Mac, GraphPad Software, San Diego, California USA.

## Results and Discussion

### The safety of *Limosilactobacillus fermentum* BGHV110 strain as a potential probiotic

Traditionally long application in human nutrition listed *Lactobacillus* species on the QPS (qualified presumption of safety) list. However, based on the new evidence which pointed out the increased prevalence of antibiotic resistance among different dairy lactobacilli (20), we first tested the susceptibility of *L. fermentum* BGHV110 to recommended concentrations of clinically relevant antibiotics according to guidelines

**Table I** Primers used for the analysis of the gene expression in *Caenorhabditis elegans*
**Tabela I** Prajmeri korišćeni za analizu ekspresije gena kod *Caenorhabditis elegans*

| Primer name | Primer sequence 5′–3′ | Reference |
|---|---|---|
| *tir-1* forward | CCGACCACCAAAGAAATGCC | 8 |
| *tir-1* reverse | CTTGGTCCACCGATGCTTCT | |
| *pmk-1* forward | ACTTCATCCGACTCCACGAG | 8 |
| *pmk-1* reverse | CAGCAGCACAAACAGTTCCA | |
| *lys-1* forward | GGATCTGGAGCATTCGACACA | This work |
| *lys-1* reverse | GCTGGGGAGGTAACCTGAATC | |
| *lys-8* forward | TTGTCCGTGCATACAACCCA | This work |
| *lys-8* reverse | TCCTTGCTTGCTTGAAGCCG | |
| *dbl-1* forward | TTTTGCGGCGAACAAATCGT | 8 |
| *dbl-1* reverse | TTCGCTGTTGCCTGTTTGTG | |
| *clc-1* forward | CCACTCACCCTCTTTGCAGT | 8 |
| *clc-1* reverse | CGAGTATCCAAGCTGCGAGT | |
| *act-1* forward | TGCAGAAGGAAATCACCGCT | 13 |
| *act-1* reverse | TGCAACGAGAGCAACTGAAC | |

provided by EFSA. We demonstrated that *L. fermentum* BGHV110 is susceptible to ampicillin, gentamicin, erythromycin, clindamycin, tetracycline, and chloramphenicol, while resistant to streptomycin and kanamycin (Table II). According to the previous study, most lactobacilli are intrinsically resistant to aminoglycoside antibiotics, which could explain the obtained results (21). However, the genome of *L. fermentum* BGHV110 should be sequenced for the final confirmation of the absence of acquired or transferable antibiotic resistance genes (21). On the other hand, the potential risk of transfer of antibiotic resistance genes could be reduced by following the novel postbiotic trend in probiotic supplementation, which proposes the use of unviable inactivated bacteria or bacterial metabolites as active ingredients which mimic the beneficial effect of probiotics (22).

Additionally, we investigated the potential of *L. fermentum* BGHV110 to produce biofilm as a common virulent characteristic of different pathogenic bacteria. Biofilm is a structured membrane composed of a polysaccharide matrix, proteins, and other elements, and containing microorganisms, with a complex internal arrangement and channels that facilitate the transport of nutrients within the network which enables pathogen survival in harsh conditions (23). Our results showed that the investigated strain does not have the ability to produce a biofilm on a plastic substrate, suggesting the absence of virulence traits of *L. fermentum* BGHV110 (Figure 1). Overall, these results demonstrated that *L. fermentum* BGHV110 does not possess any of the tested virulent traits. However, genome sequencing is needed for final confirmation of its safety status.

**Table II** Susceptibility of *Limosilactobacillus fermentum* BGHV110 strain to selected antibiotics

**Tabela II** Osetljivost *Limosilactobacillus fermentum* BGHV110 soja na odabrane antibiotike

| Antibiotics (mg/L) | Amp | Gen | Kan | Str | Ery | Cli | Tet | Chl |
|---|---|---|---|---|---|---|---|---|
| MIC value | 2 | 16 | 64 | 64 | 1 | 4 | 8 | 4 |
| Result | < 2 | < 16 | ≥ 64 | ≥ 64 | < 0.5 | < 2 | < 4 | < 2 |

Note: MIC - Minimal inhibitory concentration, Amp - ampicillin, Gen - gentamicin, Kan - kanamycin, Str - streptomycin, Ery - erythromycin, Cli - clindamycin, Tet - tetracycline, and Chl - chloramphenicol

Napomena: MIC - minimalna inhibitorna koncentracija, Amp - ampicilin, Gen - gentamicin, Kan - kanamicin, Str - streptomicin, Ery - eritromicin, Cli - klindamicin, Tet - tetraciklin i Chl - hloramfenikol

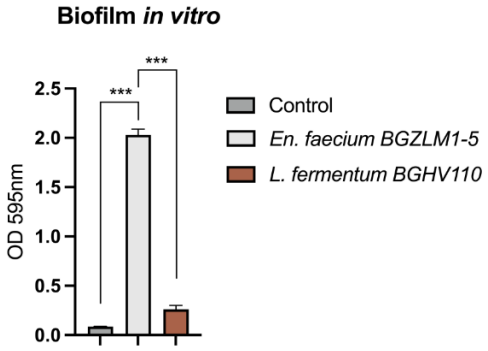


**Figure 1.** ***Limosilactobacillus fermentum*** **BGHV110 does not have potential to form biofilm *in vitro*. Cristal violet assay showing biofilm forming potential of *L. fermentum* BGHV110 strain in comparison with *Enterococcus faecium* BGZLM1-5 used as a positive control. Data are presented as the mean ± SD from results obtained from three independent experiments. One-way ANOVA followed by the Tukey *post hoc* test for multiple comparisons was used (***p<0.001).**

**Slika 1.** ***Limosilactobacillus fermentum*** **BGHV110 nema potencijal za formiranje biofilma *in vitro*. Kristal violet test pokazuje potencijal formiranja biofilma soja *L. fermentum* BGHV110 u poređenju sa *Enterococcus faecium* BGZLM1-5 izolatom koji je korišćen kao pozitivna kontrola. Rezultati su predstavljeni kao srednja vrednost ± SD iz rezultata dobijenih iz tri nezavisna eksperimenta. Jednofaktorska ANOVA i Tukey *post hoc* test su korišćeni za poređenje tretmana (***p<0,001).**

## *Limosilactobacillus fermentum* BGHV110 tolerates intestinal juices during simulated gastrointestinal passage

The first step for establishing host-microbe interaction in the gut is the survival of indigenous bacteria in the gastrointestinal environment and potential gut colonization (5).

Therefore, based on the FAO/WHO guidelines, we further evaluated the capability of *L. fermentum* BGHV110 strain to survive simulated transit through the gastrointestinal tract. Results revealed that *L. fermentum* BGHV110 tolerated passage through all gut compartments, including the stomach, duodenum and small intestines (Figure 2). Overnight culture of *L. fermentum* BGHV110 contained $1.8 \times 10^8$ CFU/mL of bacteria. After 90 min of exposure of the overnight grown culture to gastric juice, bacterial counts slightly decreased to $6.5 \times 10^7$ CFU/mL. As expected, this trend of decreased viability continued after 10 min of incubation in duodenal juice with $1.3 \times 10^7$ CFU/mL of viable bacteria. Finally, after exposure to intestinal juice for 120 min, the bacteria count returned to its initial level, with $1.5 \times 10^8$ CFU/mL bacteria. As reported, the presence of digestive enzymes and bile acids negatively influences the survival of bacteria during transit through the intestine (24). *L. fermentum* BGHV110 exhibited increased sensitivity to pepsin, low pH and high bile acid concertation (1%), resulting in a 1 log decrease of bacterial counts compared to the initial concentration. However, with a dilution of digestive enzymes and bile salts concentrations which occurs in small intestines, *L. fermentum* BGHV110 recovered to its initial count. A similar result was obtained for *L. fermentum* TCUESC01, showing a slight decrease in viable bacteria after exposure to pepsin and bile acids, even with the addition of 10% of milk as a protecting agent (25). This result suggests that *L. fermentum* BGHV110 survives unfavorable conditions of the GIT, making it a desirable candidate for the development of probiotic preparation, without the need to use the gastro-resistant coated pharmaceutical formulations to enable the survival of probiotic bacteria.

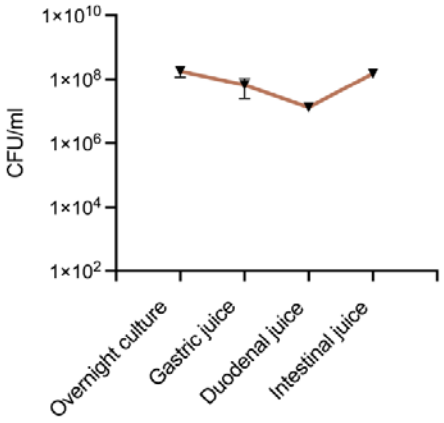


**Figure 2.** ***Limosilactobacillus fermentum*** **BGHV110 survives simulated gastrointestinal tract conditions. Colony forming units (CFU)/mL of *L. fermentum* BGHV110 strain after exposure to gastric, duodenal and intestinal juices presented as the mean ± SD from results obtained from three independent experiments.**

**Slika 2.** ***Limosilactobacillus fermentum*** **BGHV110 preživljava simulirane uslove gastrointestinalnog trakta. Jedinica formiranja kolonija bakterija po ml (CFU)/ml *L. fermentum* BGHV110 soja nakon izlaganja želudačnom, duodenalnom i crevnom soku predstavljeno kao srednja vrednost ± SD iz rezultata dobijenih iz tri nezavisna eksperimenta.**

### *Limosilactobacillus fermentum* BGHV110 shows good adhesion properties to Caco-2 cells and strengthens the epithelial barrier in *Caenorhabditis elegans* model

The ability of probiotic strains to adhere to gut mucosa is an important feature for the selection of future probiotics (5). Therefore, we used Caco-2 cells as an *in vitro* model for the intestinal epithelium, to estimate the percentage of bacterial binding. *L. fermentum* BGHV110 showed good adhesion properties of 7.7 % ± 2.02 % of binding, which was almost comparable with the standard probiotic strain *Lacticaseibacillus rhamnosus* GG (ATCC 53103), often used in commercially available probiotics with binding ability of 9.7 % ± 3.3 % (26). Additionally, as binding ability is species-dependent, the obtained result for *L. fermentum* BGHV110 is similar to that reported for *L. fermentum* BIF-19 strain, estimated to be around 8.78 % ± 0.74 % (27). This result implies that a sufficient number of administrated probiotics will come into close contact with host receptors with the potential to activate different cellular pathways in the gut mucosa.

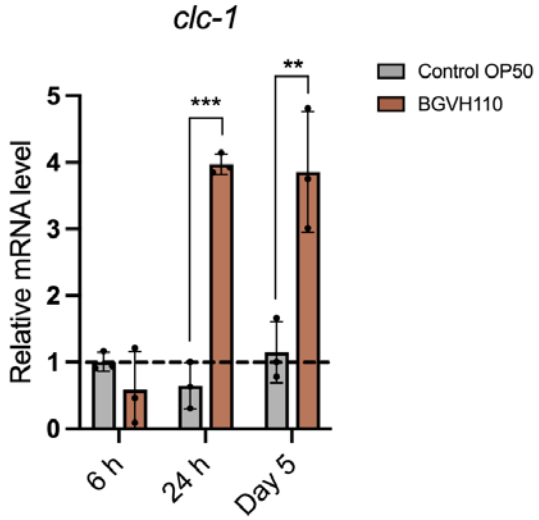


**Figure 3.** ***Limosilactobacillus fermentum* BGHV110 stimulates expression of tight junction protein in *Caenorhabditis elegans*. The expression of the claudin-like *clc-1* gene was measured by qRT-PCR in *C. elegans* after 6 h, 24 h and 5 days of treatment with *L. fermentum* BGHV110 relative to *E. coli* OP50 control. All data are presented as mean ± SD and Student's t-test was used to compare the treated group relative to control (***p<0.001).**

**Slika 3.** ***Limosilactobacillus fermentum* BGHV110 stimuliše ekspresiju proteina tesnih međućelijskih veza u *C. elegans*. Ekspresija gena sličnog klaudinu *clc-1* merena kvantitativnom PCR metodom kod *C. elegans* nakon 6 h, 24 h i 5 dana tretmana sa *L. fermentum* BGHV110 u odnosu na *E. coli* OP50 kontrolu. Svi rezultati su predstavljeni kao srednja vrednost ± SD i Studentov t-test je korišćen za poređenje tretirane grupe u odnosu na kontrolu (***p<0,001).**

As the interaction between bacterial macromolecules and host receptors is essential for the beneficial effect of probiotics (4), we next evaluated the effect of *L. fermentum* BGHV110 on the *clc-1* gene expression, a human ortholog of claudin TJP in the *C. elegans* model (11). Worms fed with *L. fermentum* BGHV110 for 6 h showed no changes in transcription levels of the *clc-1* gene, in comparison with *E. coli* OP50 used as a standard laboratory food. However, prolonged treatment for 24 h and 5 days showed significant upregulation of *clc-1* transcripts, suggesting that *L. fermentum* BGHV110 has the potential to straighten the epithelial barrier (Figure 3). This observation is in line with the reported results for *Latilactobacillus curvatus* BGMK2-41 strain, which showed PMK-1 dependent induction of the *clc-1* genes in *C. elegans*, thus providing higher resistance of treated worms to pathogens (8). Additionally, literature data showed that another strain of *L. fermentum*, labelled as KBL375, exhibits the potential to upregulate TJPs like E-cadherin or Claudin 3 in mice with dextran sulfate sodium-induced colitis, suggesting that probiotic lactobacilli could maintain epithelial barrier integrity not only in infection, but also in inflammation (28).

### *Limosilactobacillus fermentum* BGHV110 triggers conserved p38 MAPK immunity pathway essential for *Caenorhabditis elegans* defence against pathogens

Next, we focused on the evaluation of conserved immunity pathways and AMP production in the *C. elegans* model. We examined the expression of immune-related genes at three time points, starting with 6 h treatment. After 6 h of feeding, no changes in the mRNA transcription of tested genes were observed **(**Figure 4A**)**. Furthermore, since we had identified an elevation of *clc-1* expression after 24 h, we fed worms for 24 h and the expression results revealed elevated transcript levels of PMK-1/p38 MAPK immunity pathway genes, including the *tir-1* and *pmk-1*, as well as the effector *lys-1* gene which encodes a human ortholog of AMP **(**Figure 4B**).** Finally, worms treated for 5 days with *L. fermentum* BGHV110 maintained a high level of transcription of *tir-1* and *lys-1* immunity genes, with additional activation of the DBL-1 pathway, and another worm AMP effector *lys-8* **(**Figure 4C**)**. These results are consistent with our previous finding that *L. curvatus* BGMK2-41 probiotic strain can trigger PMK-1/p38 MAPK immunity to survive lethal *Staphylococcus aureus* and *Pseudomonas aeruginosa* intestinal infection in *C. elegans* (8). Moreover, this study brings new findings about probiotic-mediated activation of lysozyme-like genes *lys-1* and *lys-8* expressed mainly in the intestines, implying potential indication of *L. fermentum* BGHV110 strain in gastrointestinal infections caused by both gram-positive and gram-negative bacteria. It has been shown that some probiotic lactobacilli have the potential to tune the innate immune response and transiently boost proinflammatory cytokines production (29). In mammals, p38 MAPK controls the synthesis of proinflammatory cytokines (IL-1β, TNF-α and IL-6), induction of enzymes important for the innate immune defence such as COX-2 and iNOS, and induction of endothelial adherent proteins along with other inflammatory related molecules necessary for combating incoming infections (30). For example,

*Lacticaseibacillus casei* ATCC27139 was shown to significantly upregulate proinflammatory cytokines by mice splenocytes via p38 MAPK signalling pathways (31). On the other hand, the activation of DBL-1 pathways pointed out that this strain could increase the production of TGF-β cytokine, an important inflammation regulator in mammals, highlighting the dual role of *L. fermentum* BGHV110 in fine-tuning between pro- and anti-inflammatory responses. For some lactobacilli there is evidence that they can trigger the TGF-β signalling pathway in *C. elegans* to mediate *S. aureus* resistance (32).

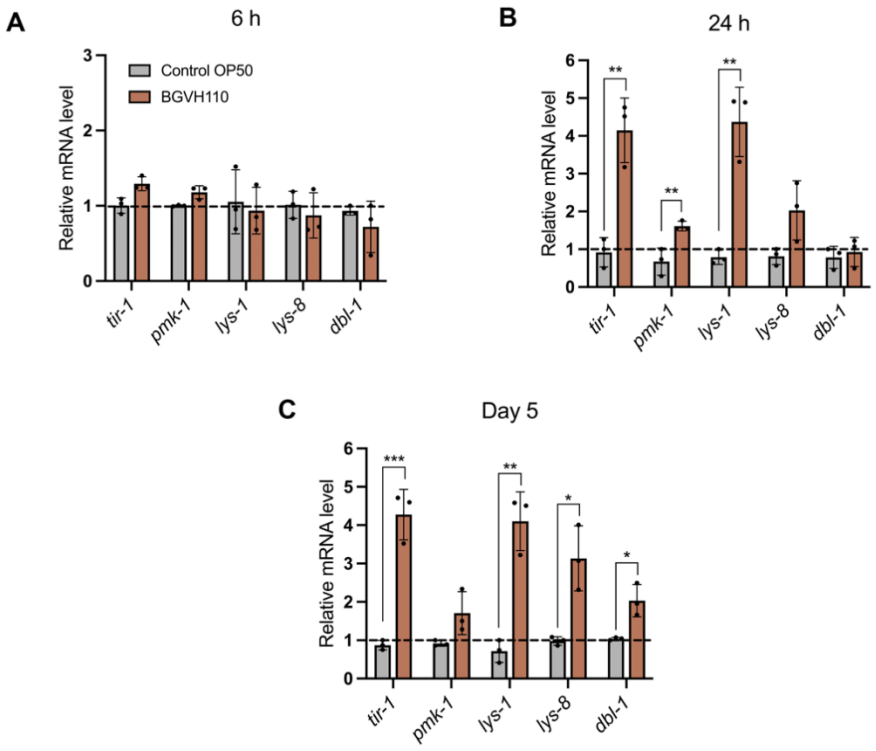


**Figure 4.** ***Limosilactobacillus fermentum*** **BGHV110 activates immunity pathways and AMPs in** ***Caenorhabditis elegans*****. The expression of the immune-related genes (*tir-1, pmk-1, dbl-1*) and AMPs (*lys-1, lys-8*) measured by qRT-PCR in *C. elegans* after 6 h, 24 h and 5 days of treatment with *L. fermentum* BGHV110 relative to *E. coli* OP50 control. All data are presented as mean ± SD and the Student's t-test was used to compare the treated group relative to control (*p<0.05, **p<0.01, ***p < 0.001).**

**Slika 4.** ***Limosilactobacillus fermentum*** **BGHV110 aktivira imunske puteve i antimikrobne peptide u** ***C. elegans*****. Ekspresija gena povezanih sa imunskim odgovorom (*tir-1, pmk-1, dbl-1*) i gena koji kodiraju antimikrobne peptide (*lys-1, lys-8*) merena kvantitativnom PCR metodom kod *C. elegans* nakon 6 h, 24 h i 5 dana tretmana sa *L. fermentum* BGHV110 u odnosu na *E. coli* OP50 kontrolu. Svi rezultati su predstavljeni kao srednja vrednost ± SD i Studentov t-test je korišćen za poređenje tretirane grupe u odnosu na kontrolnu (*p<0,05, **p<0,01, ***p < 0,001).**

Overall, having in mind all the collected results of beneficial effects of *L. fermentum* BGHV110 strain from this and previous studies, we highlight its potential to be used for the prevention or treatment of gastrointestinal infections, with the additional possibility for it to be tested for the treatment of other diseases, especially those related to liver damage and lipid metabolism.

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# Probiotička karakterizacija soja *Limosilactobacillus fermentum* BGHV110 i njegov uticaj na urođeni imunski odgovor kod *Caenorhabditis elegans*

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## Kratak sadržaj

Probiotički laktobacili pokazuju potencijal da pozitivno deluju na zdravlje domaćina. *Limosilactobacillus fermentum* se zahvaljujući brojnim korisnim efektima na zdravlje ljudi izdvojio kao odličan kandidat za razvoj komercijalnih probiotičkih preparata koji imaju za cilj prevenciju širenja infektivnih bolesti. U ovoj studiji, korišćeno je nekoliko *in vitro* testova, uključujući test formiranja biofilma, test procene osetljivosti na antibiotike, test preživljavanje u simuliranim uslovima gastrointestinalnog trakta i test adhezije na intestinalne Caco-2 ćelije, za procenu bezbednosti i probiotičkog potencijala soja *L. fermentum* BGHV110. Dodatno, *Caenorhabditis elegans* je korišćen kao *in vivo* model sistem za procenu uticaja *L. fermentum* BGHV110 na urođeni imunski odgovor domaćina. Rezultati su pokazali da soj *L. fermentum* BGHV110 poseduje odličnu sposobnost da preživi nepovoljne uslove u crevima, da se veže za intestinalne Caco-2 ćelije i da stimuliše evolutivno konzervisani p38 MAPK imunski put i ekspresiju gena sličnog klaudinu *clc-1* i antimikrobnih peptida u *C. elegans* u cilju jačanja imunskog odgovora na infekciju. Dodatno, *L. fermentum* BGHV110 je pokazao odsustvo faktora virulencije i osetljivost na testirane antibiotike, što je potvrdilo njegov bezbednosni status u skladu sa kojim se može primeniti kao budući probiotik.

**Ključne reči:** *Limosilactobacillus fermentum*, probiotik, urođeni imunski odgovor, gastrointestinalni trakt, *Caenorhabditis elegans*