Agricultura Scientia 23: No 1 17-28(2026)
https://doi.org/10.18690/agricsci.23.1.3

University of Maribor Press

Effect of Selected Probiotic Strains and their Combinations on the *SGLT1* and *GLUT2* Gene Expressions in Porcine Intestinal Epithelial Cells

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ABSTRACT

Intestinal morphology and glucose transporter expression are key determinants of glucose absorption and homeostasis. While probiotics beneficially modulate the gut microbiota, their role in regulating glucose transporter gene expression remains unclear. This study aimed to evaluate the effects of selected single- and multi-strain probiotic bacteria on the expression of sodium-driven glucose cotransporter 1 (*SGLT1*) and facilitative glucose transporter 2 (*GLUT2*) in porcine intestinal epithelial CLAB cells. Cells were treated with four selected probiotic strains (*Lactobacillus acidophilus* (ATCC 4356), *Lacticaseibacillus rhamnosus* GG (ATCC 53103), *Lactiplantibacillus plantarum* PCS 20, and *Lactiplantibacillus plantarum* PCS 26) at varying concentrations and in different combinations. Although none of the observed changes were statistically significant, *SGLT1* expression was consistently upregulated across various treatments, whereas *GLUT2* showed a more variable but predominantly downregulated response, suggesting the latter as a more susceptible target for this research. These findings indicate that selected probiotic strains may differentially regulate key intestinal glucose transporters, suggesting a potential role in modulating glucose absorption and homeostasis. Ultimately, this study offers new insights into probiotic–host interactions.

Keywords: probiotics, glucose transporters, gene expression, CLAB cells

INTRODUCTION

Probiotics are live microorganisms which, when administered in adequate quantities, confer beneficial effects on the host's health (Hill et al., 2014) The most commonly used probiotic strains belong to lactic acid bacteria and bifidobacteria, typically isolated from traditional fermented foods and human-derived sources such as the gastrointestinal tract, faeces, and breast milk (Hyrslova et al., 2023; Navarré et al., 2024) The beneficial effects of probiotics are attributed to mechanisms such as modulation of the gut microbiota, competitive adherence to mucosal and epithelial surfaces, strengthening the intestinal barrier, and regulation of immune and inflammatory responses (Liu et al., 2022; Mercado-Monroy et al., 2026). Many of these mechanisms are mediated through the regulation of gene expression in specific tissues, particularly the intestine and liver (Li et al., 2016). In addition, their positive effects on metabolic regulation have positioned probiotics as promising candidates for the prevention and management of metabolic disorders (Shen et al., 2024). However, their effects should not be generalised, as they are typically strain-specific and may differ even within a single species (Maslennikov et al., 2026; Rahmannia et al., 2024).

Species of lactic acid bacteria comprise microorganisms crucial during the early development of the microbiota and immune system (Heczko et al., 2024). As probiotics, they alleviate digestive discomfort, control inflammation and allergic responses, exert anti-obesity and antioxidative effects, reduce insulin resistance and increase carbohydrate metabolism (Shah et al., 2024). Due to extensive research on the effects of probiotics, numerous studies have been conducted using animals, specifically pigs, as they share many physiological similarities with humans (Rossi & Mainardi, 2025). Several strains belonging to the *Lactobacillus* genus has been shown to improve gut health in pigs by modulating intestinal microbiota, enhancing barrier function, and inhibiting pathogenic bacteria. Additionally, they contribute to improved growth performance and immune responses (Barba-Vidal et al., 2019; Rossi & Mainardi, 2025). However, most studies have been

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Received May 2026; Received in revised form June 2026; Accepted June 2026

conducted *in vivo*, while the underlying molecular mechanisms of probiotics remain insufficiently understood. Although single-strain probiotics confer health benefits, multi-strain formulations may enhance efficacy through synergistic and additive effects but may also exhibit inter-strain antagonism. The eradication of *Helicobacter pylori* was more effective when using a mixture containing *Lacticaseibacillus rhamnosus* than with the single strain alone(McFarland, 2021). Piglets given multi-strain probiotics had greater weight gain, improved feed efficiency, and less attachment of enterotoxigenic *Escherichia coli* (ETEC) F4 than single-strain and control group (Kwoji et al., 2021). In weaning piglets, multi-strain probiotics improved growth efficiency by enhancing gut integrity and microbiome balance (Huang et al., 2024).

Glucose uptake across the apical brush border of epithelial cells is mainly mediated by the sodium-dependent cotransporter SGLT1, which couples glucose transport with sodium ions (Koepsell, 2020; Sano et al., 2020). It is then exported across the basolateral membrane primarily via GLUT2, a low-affinity, high-capacity uniporter that facilitates diffusion into the systemic circulation (Shen et al., 2024; Sun et al., 2023). The regulation of monosaccharide transporters is influenced by multiple factors, including circadian rhythms, intake of carbohydrate-rich meals, dietary carbohydrate composition, hormonal and neuronal signals, as well as pathological conditions and surgical interventions (Koepsell, 2020). Furthermore, the effect of various probiotic strains on glucose transport and metabolism has also been proposed in some *in vitro* studies. Hsieh et al. (2020) reported that *Ligilactobacillus salivarius* AP-32 and *Limosilactobacillus reuteri* GL-104 directly downregulated monosaccharide transporter expression in Caco-2 cells. Short-term exposure of intestinal cells to bacterial supernatant reduced glucose accumulation in study of Rooj et al. (2010). Administration of five probiotic strains decreased the expression of *SGLT1* and *GLUT2 i*n diabetic mice (Wang et al., 2020). *Lactiplantibacillus plantarum* PCS26 and *Lactobacillus acidophilus* decreased the *GLUT2* gene expression in CLAB and CaCo-2 cells, respectively, resulting in glucose absorption reduction (Primec et al., 2021). However, the effect of selective probiotic strains on both receptors has so far been tested only at the single-strain level.

Although multiple factors involved in modulating the expression of glucose transporters have been identified, the potential role of probiotics remains an area of growing scientific interest. Due to the strain-specific nature of probiotic effects and the predominance of *in vivo* studies and studies, focusing only on single strains, this *in vitro* study aims to evaluate the potential of multi-strain probiotics *Lactobacillus acidophilus* (LA), *Lacticaseibacillus rhamnosus* GG (LGG) (ATCC 53103), *Lactiplantibacillus plantarum* PCS 20 (PCS 20), and *Lactiplantibacillus plantarum* PCS 26 (PCS 26) on *SGLT1* and *GLUT2* gene expression in a porcine intestinal epithelial cell line, as well as to assess the effects of probiotic concentration.

MATERIALS AND METHODS

Cell culture growth and maintenance

Non-carcinogenic porcine-derived enterocytes (CLAB) (Cencič and Langerholc, 2010; Gorenjak et al., 2012; Primec et al., 2021) were cultured in 25 cm^2 adhesive-bottom cell culture flasks (Nunc EasYFlask, ThermoFisher, Denmark) using Advanced Dulbecco's Modified Eagle Medium (DMEM; Gibco, Paisley, UK). The medium was supplemented with penicillin (100 IU/mL) and streptomycin (0.1 mg/mL) (both from Sigma-Aldrich, Steinheim, Germany), 2 mM L-glutamine (Life Technologies), and 5% fetal bovine serum (FBS; Gibco, Paisley, UK) as a growth supplement. Cells were incubated under controlled conditions at 37 °C in a humidified atmosphere containing 5% CO_2. The medium was renewed as required every 2–3 days, and cells were routinely passaged several times prior to the initiation of the experiment.

Cultivation and maintenance of probiotic strains

The probiotic bacterial strains *Lactobacillus acidophilus* (LA) (ATCC 4356), *Lacticaseibacillus rhamnosus* GG (LGG) (ATCC 53103), *Lactiplantibacillus plantarum* PCS 20 (PCS 20), and *Lactiplantibacillus plantarum* PCS 26 (PCS 26) were cultivated in De Man, Rogosa and Sharpe (MRS) broth, (Merck, Darmstadt, Germany), and incubated in a microaerophilic atmosphere at 35 °C using an anaerobic chamber. Subculturing into fresh MRS broth was performed daily or every second day. For the experiment, bacterial strains propagated at least three times were used.

Cytotoxicity test

Cell viability following exposure to selected probiotic strains was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were seeded at a concentration of 5×10^5 cells/mL in flat-bottom 96-well plates (Corning Costar, Thermo Scientific, MA, USA), followed by incubation for 24 h under controlled humidified conditions (37 °C, 5% CO_2). After incubation cells were washed once with antibiotic-free medium. Probiotic cultures grown in MRS broth were centrifuged, and the bacterial pellets were resuspended in DMEM supplemented with 2 mM L-glutamine, without antibiotics. Plated cells were treated in triplicate with 100 µL of probiotic suspensions at various concentrations and combinations, as well as with a medium control. After 6 h incubation, cells were washed twice with

antibiotic-free DMEM, and 200 µL of MTT solution was added to each well (final concentration 0.5 mg/mL). Plates were shaken for 5 minutes and incubated for 3 h (37 °C, 5% CO_2) to allow formazan crystal formation. The MTT solution was then removed, plates were air-dried overnight, and formazan crystals were dissolved the next day in 100 µL of 0.04 % HCl in isopropanol. After shaking for 5 minutes and incubation at room temperature for 15–20 min, absorbance was measured at 570 and 630 nm using an Infinite® M1000 PRO spectrophotometer (Tecan Trading AG, Switzerland). Cell viability was calculated from the difference between absorbance values at 570 nm and 630 nm. The absorbance values of the wells containing probiotic strains were expressed as a percentage of the absorbance of the control, with the control set at 100%.

Experiment design

CLAB cells were cultured in a 12-well plate (Corning Incorporated, Thermo Scientific, MA, USA) at 4×10^5 cells per well. After 48 h of incubation at 37 °C, the cells had reached confluence and the medium was removed. Then, the cell layer was washed twice with sterile phosphate-buffered saline (PBS) (Sigma Aldrich, Burlington, MA, USA). Probiotic bacteria were resuspended in DMEM cell culture medium supplemented with 2 mM L-glutamine without antibiotics. Subsequently, 1 mL of each probiotic strain suspension at different concentrations (1 to 4×10^7 CFU/mL) alone or in different combinations was applied to the cells in three replicates. Culture medium without bacteria was used as a control treatment. Cells exposed to probiotics were incubated for 6 h at 37 °C in an atmosphere containing 5% CO_2. The lowest concentration (1×10^7 CFU/mL) was selected based on concentrations reported in previous *in vitro* studies (Gorenjak et al., 2014; Huang & Zheng, 2010; Yoon et al., 2013), while other higher concentrations (2, 3 or 4×10^7 CFU/mL) were adjusted to enable evaluation of single- and multi-strain effects at equivalent total bacterial concentrations.

Isolation of RNA

After incubation, total RNA was extracted using the NucleoSpin® TriPrep kit according to the manufacturer's instructions (Macherey-Nagel GmbH & Co. KG, Germany).

Concentration and purity of isolated RNA

The concentration of isolated RNA was determined using a NanoQuant™ plate (Tecan Group Ltd., Männedorf Switzerland), in combination with an Infinite® M1000 PRO spectrophotometer (Tecan Trading AG, Switzerland). The concentration and purity of the isolated RNA were assessed by measuring optical density (OD) at 260 nm and evaluating the OD260/OD280 ratio, respectively.

Transcription of RNA into complementary DNA (cDNA) by reverse transcription

After assessment of RNA concentration and purity, RNA samples were diluted with sterile, deionized RNAse-free PCR-grade water to a final concentration of 200 ng/µL. Reverse transcription to complementary DNA (cDNA) was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions.

Real-time polymerase chain reaction (qPCR) and gene expression analysis

For qPCR, 2 µL of diluted cDNA (5 ng/µL) were combined with 8 µL of reaction mixture consisting of Maxima SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA USA), forward and reverse primers (0.3 µM), and sterile PCR-grade water. The annealing temperature was set to 60°C for all primer pairs. qPCR was performed using a LightCycler® 96 real-time PCR system (Roche, Basel, Germany). Amplification specificity was verified by melting curve analysis following each qPCR run. The sequences of target and reference *mRNA* genes, obtained from the PubMed Nucleotide Database (https://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/) and the AceView477 database, are listed in Table 1. Primers for the *Beta-2-microglobulin* (*B2M*) reference gene were derived from previously published reference sequences (Wang, et al., 2016), while primers for both target genes and reference gene *POLR2A* were designed using the bioinformatics tool ClustalW2 (http://www.ebi.ac.uk/Tools/) and online bioinformatics tool IDT OligoAnalyzer (https://eu.idtdna.com/pages/tools/oligoanalyzer). Primers for all genes investigated in this study were supplied by

Sigma-Aldrich. Prior to qPCR analysis using the LightCycler Real-Time PCR System (Roche, Basel, Germany), primer annealing temperatures and PCR amplicon specificity were verified by this system and agarose gel electrophoresis.

Table 1: Primer sequences and sequence accession numbers

Gene	Primer sequence 5'→ 3'	Accession number
B2M porcine	CAAGATAGTTAAGTGGGATCG TGGTAACATCAATACGATTTC	(S. Wang et al., 2016)
	Amplicon size: 161 bp	
POLR2A porcine	ACCATCAAGCGAGTGCAGTT TCGGTTGTCTCTGGGTATTTG Amplicon size: 95 bp	XM_005669224.1
SGLT1 porcine	AGTGGGCAGCTCTTCGATTA CCAGCCCAATCATACATCCT Amplicon size: 148 bp	NM_001164021.1
GLUT2 porcine	ATTCTTTGGTGGGATGCTTG ATGAGATGGTCCCAATTTCG Amplicon size: 118 bp	NM_001097417.1

B2M: Beta-2-microglobulin; *POLR2A*: Polymerase II subunit A; *SGLT1*: sodium-driven glucose transporter 1; *GLUT2*: facilitative glucose transporter 2

Data normalization and interpretation

qPCR data were normalized by calculating the geometric mean of the expression levels of two reference genes, *POLR2A* and *B2M*. The expression of target genes was calculated relative to control samples, and minimum and maximum error values were reported. Data were used in linear normalized form ($2^{-\Delta Ct}$) for statistical analysis and in relative expression form ($2^{-\Delta\Delta Ct}$) (Livak & Schmittgen, 2001a) for graphical representation. To facilitate biological interpretation, expression changes of approximately two-fold increase or decrease relative to the control group were considered potentially biologically relevant, as is common in gene expression studies.

Statistical data processing

For the reference gene stability and cytotoxicity tests, data were statistically analysed using the IBM SPSS Statistics computer program (Version 31.0; IBM Inc., Armonk, NY, USA). All data were initially tested for normality with the Shapiro-Wilk test. To assess reference gene stability, $2^{-\Delta Ct}$ values (Livak and Schmittgen, 2001) derived from the raw data were subjected to Levene's test for homogeneity of variances, followed by either ANOVA or the Kruskal-Wallis test, as appropriate. Reference gene stability was assessed for each batch separately. For the cytotoxicity assay, datasets were evaluated using the Mann-Whitney U test.

Data from gene expression analysis were statistically analysed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Statistical analysis was performed in the R environment 4.5.3 (R Core Team, Vienna, 2025). Comparison of receptor gene expression between groups was carried out using the brglm2 R package and bias-reduced binomial logistic regression to adjust for experiment batch effects. The presence of repeated observations and batch-related variation made bias-reduced binomial logistic regression a suitable analytical approach. The significance threshold (α) was set at 0.05, and P-values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of different concentrations of selected probiotic strains on *SGLT1* gene expression

After verifying the viability of the cells after exposure to probiotic strains and stability of the reference genes in treated cells, we analysed the effects of the single probiotic strains LGG, LA, PCS 26 and PCS 20 in four different concentrations (1-4 × 10^7 CFU/mL) on the expression of *SGLT1*. Results are shown in Figure 1. None of the observed changes were statistically significant. Compared to the control, probiotic strains LGG and LA showed a similar expression pattern. *SGLT1* expression *was* decreased at a concentration of 1 × 10^7 CFU/mL, while the other three concentrations increased gene expression, with biologically relevant increase observed at the 3 × 10^7 CFU/mL (2.17-fold) for LGG and 4 × 10^7 CFU/mL for LA (2.27-fold) compared to the control. PCS 26 upregulated *SGLT1* expression in all four concentrations compared to the control. The highest increase in gene expression was observed at 4 × 10^7 CFU/mL (1.92-fold), nearly reaching biological relevance. Moreover, the increase in *SGLT1* gene expression after LA and PCS 26 treatment was proportional to the increase in probiotic concentration.

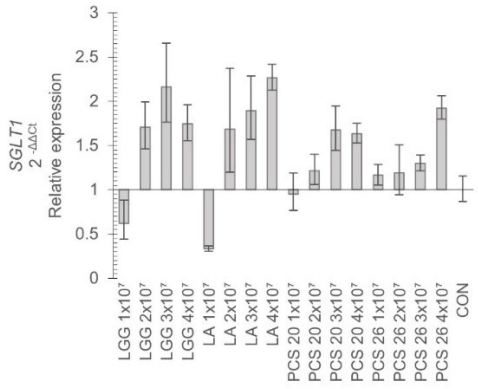

Figure 1: Sodium-glucose transporter 1 (*SGLT1*) relative expression in CLAB cells following 6 h incubation with

single-strain probiotic bacteria at different concentrations (1 - 4 × 10^7 CFU/mL). Results are presented as median values (geometric means) from three independent replicates with ranges. Statistical significance (* P<0.05) compared to control was determined using bias-reduced binomial logistic regression. LA: *Lactobacillus acidophilus*; LGG: *Lacticaseibacillus rhamnosus* GG; PCS 20: *Lactiplantibacillus plantarum* PCS 20; PCS 26: *Lactiplantibacillus plantarum* PCS 26.

Effect of different concentrations of selected probiotic strains on *GLUT2* gene expression

In contrast to *SGLT1* expression, treatment with probiotic strains at different concentrations mostly reduced *GLUT2* gene expression (Figure 2). However, none of the observed changes in expression reached statistical significance. Treatment with LGG led to decreased gene expression compared to the control at all four concentrations, with concentration 1 × 10^7 CFU/mL reaching biological relevance (1.99-fold). Gene expression increased progressively with higher concentrations, indicating a concentration-dependent rise. *GLUT2* expression in cells treated with LA showed a variable response across concentrations. The lowest concentration produced the greatest decrease in *GLUT2* expression (2.00-fold). Treatment with PSC 26 produced the most variable effects among all concentrations, however, none reached biological relevance.

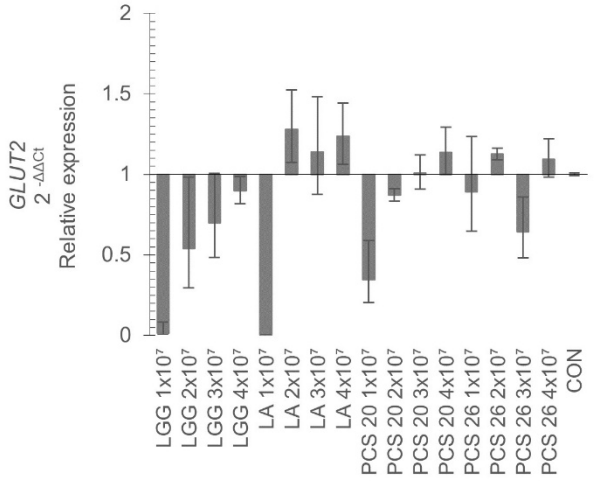

Figure 2: Facilitative glucose transporter 2 (*GLUT2*) relative expression in CLAB cells following 6 h incubation with single-strain probiotic bacteria at different concentrations (1 - 4 × 10^7 CFU/mL). Results are presented as median values (geometric means) from three independent replicates with ranges. Statistical significance (* P<0.05) compared to control was determined using bias-reduced binomial logistic regression. LA: *Lactobacillus acidophilus*; LGG: *Lacticaseibacillus rhamnosus* GG; PCS 20: *Lactiplantibacillus plantarum* PCS 20; PCS 26: *Lactiplantibacillus plantarum* PCS 26.

Effect of selected double-strain probiotic combinations on *SGLT1* and *GLUT2* gene expression

None of the observed changes in *SGLT1* and *GLUT2* gene expression after the addition of double-strain probiotic combinations were statistically significant or reached the two-fold threshold used to define biological relevance (Figure 3). *SGLT1* gene expression increased in almost all treatments, compared to the control, except for the PCS 26/PCS 20 probiotic strain combination. In contrast, *GLUT2* expression was downregulated in all combination treatments compared to the control.

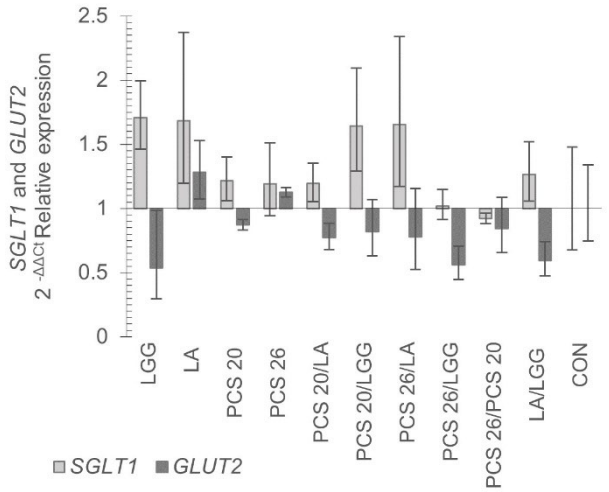

Figure 3: Effect of selected double-strain probiotic combinations on sodium-glucose transporter 1 *(SGLT1)* and facilitative glucose transporter 2 *(GLUT2)* gene expression. The results show *SGLT1* and *GLUT2* gene expression after 6 h incubation of cells with selected single-strain probiotic bacteria and double-strain probiotic combinations at the same final concentration (2 × 10^7 CFU/mL). Results are presented as median values (geometric means) from three independent replicates with ranges. Statistical significance (* P<0.05) compared to control was determined using bias-reduced logistic regression. LA: *Lactobacillus acidophilus*; LGG: *Lacticaseibacillus rhamnosus* GG; PCS 20: *Lactiplantibacillus plantarum* PCS 20; PCS 26: *Lactiplantibacillus plantarum* PCS 26.

Effect of selected triple-strain probiotic combinations on *SGLT1* and *GLUT2* gene expression

Expression of the *SGLT1* gene was upregulated in all probiotic treatments, both single- and triple-strain (Figure 4). However, none of the observed changes was statistically

significant. Compared to the control, the highest and biologically relevant increase in *SGLT1* gene expression was observed after the addition of LGG (2.17-fold), followed by the combinations LGG/LA/PCS 26 (2.02-fold) and LGG/LA/PCS 20 (2.01-fold).

GLUT2 gene expression (Figure 4) was reduced in all treatments except LA addition; however, these changes were neither statistically significant nor biologically relevant.

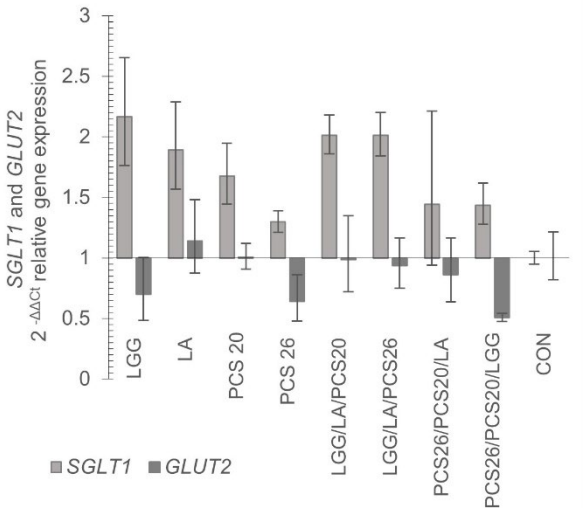

Figure 4: Effect of selected triple-strain probiotic combinations on sodium-glucose transporter 1 *(SGLT1)* and facilitative glucose transporter 2 *(GLUT2)* gene expression in CLAB cells. The results show *SGLT1* and *GLUT2* gene expression after 6 h incubation of cells with single-strain probiotic bacteria and triple-strain probiotic combinations at the same final concentration (3×10^7 CFU/mL). Results are presented as median values (geometric symbols) from three independent replicates with ranges. Statistical significance (* P<0.05) compared to control was determined using bias-reduced binomial logistic regression. LA: *Lactobacillus acidophilus*; LGG: *Lacticaseibacillus rhamnosus* GG; PCS 20: *Lactiplantibacillusplantarum* PCS 20; PCS 26: *Lactiplantibacillus plantarum* PCS 26.

Effect of four-strain probiotic combination on *SGLT1* and *GLUT2* gene expression

The addition of all four tested probiotic strains, either individually or as a four-strain combination, resulted in statistically non-significant *SGLT1* gene upregulation compared to the control (Figure 5). Specifically, LA and PCS 26 increased *SGLT1* expression by 2.27- and 1.92-fold, respectively. The four-strain combination produced the highest upregulation of the *SGLT1* gene (2.34-fold) compared to the control. Based on the predefined criterion of a ≥2-fold change, these responses were considered biologically relevant.

Conversely, a statistically non-significant upregulation or downregulation of *GLUT2* expression was observed following the addition of a single strain and the four-strain combination (Figure 5); however, none of the responses were biologically relevant.

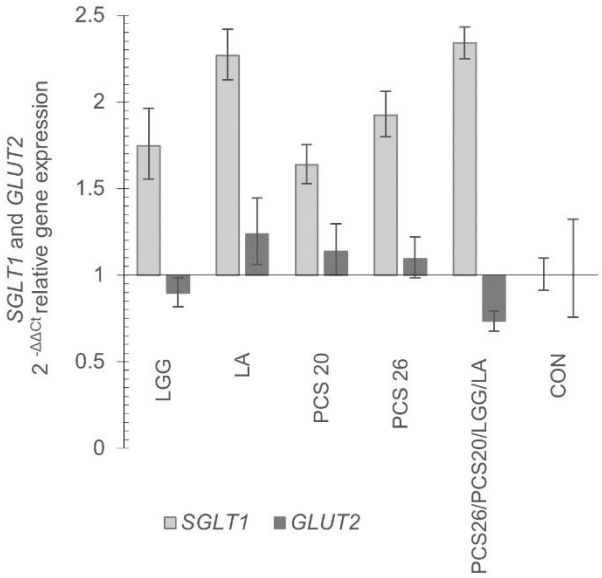

Figure 5: Effect of four-strain probiotic combination on sodium-glucose transporter 1 *(SGLT1)* and facilitative glucose transporter 2 *(GLUT2)* gene expression in CLAB cells. The results show *SGLT1* and *GLUT2* gene expression after 6 h incubation of cells with single-strain probiotic bacteria and a four-strain probiotic combination at the same final concentration (4×10^7 CFU/mL). Results are presented as median values (geometric means) from three independent replicates with ranges. Statistical significance (* P<0.05) compared to control was determined using bias-reduced binomial logistic regression. LA: *Lactobacillus acidophilus*; LGG: *Lacticaseibacillus rhamnosus* GG; PCS 20: *Lactiplantibacillus plantarum* PCS 20; PCS 26: *Lactiplantibacillus plantarum* PCS 26.

DISCUSSION

The gut microbiome is an important regulator of glucose homeostasis, and its alteration by metabolic diseases such as diabetes or by diet may contribute to its dysregulation (Howard et al., 2022). Intestinal morphology and glucose transporter expression are key in early glucose metabolism *in vivo* (Yamamoto et al., 2024). The absorption of monosaccharides is primarily mediated by the Na^+/D-glucose cotransporter 1 (*SGLT1*) and the facilitative transporter *GLUT2* (Koepsell, 2020). *SGLT1*, located in the apical membrane of the small intestine (Sano et al., 2020) transports glucose with two Na^+ ions via the Na^+/K^+-ATPase gradient (Dominguez Rieg & Rieg, 2019) and is a potential

therapeutic target for type 2 diabetes (Gromova et al., 2021). GLUT2, a low-affinity, high-capacity uniporter in the basolateral membrane, exports glucose into the bloodstream (Röder et al., 2014; Thorens, 2015) Both transporters are regulated by dietary carbohydrates and hormones such as leptin and glucagon-like peptide-1 (GLP-1) (Chan & Leung, 2015), and are upregulated in diabetes, increasing glucose absorption (Koepsell, 2020).

As *SGLT1* and *GLUT2* are key regulators of glucose transport and homeostasis, this study investigated their expression after treatment with selected probiotic strains at different concentrations and in combinations. Probiotics have been shown to beneficially affect gut microbiota, SCFA production, incretin secretion, intestinal barrier integrity, and glucose transport (Pintarič & Langerholc, 2022). Yan et al. (2019) report that *Lactobacillus acidophilus* treatment in mice regulated the expression of genes related to glucose and lipid metabolism. In CLAB cells, *Lactobacillus* strains upregulated *SGLT1*, while three of four strains downregulated *GLUT2* (Primec et al., 2021). Unlike their study, which reported statistically significant associations, our study did not identify statistically significant findings. Differences in study design and data structure required a different analytical approach.

Determining optimal probiotic dosage is crucial, as beneficial effects occur only when administered in "adequate amounts," underscoring the need to define effective concentrations. Our results showed that single-strain probiotic treatment at different concentrations produced strain-specific effects among the selected *Lactobacillus* strains (Figure 1 and 2). The lowest concentration (1×10^7 CFU/mL) was selected after checking published *in vitro* studies (Gorenjak et al., 2014; Huang & Zheng, 2010; Yoon et al., 2013). At this concentration, three strains (LGG, LA, PCS 20) downregulated *SGLT1* (Figure 1), unlike Primec et al. (2021), although higher doses showed similar effects. Single-strain probiotics generally reduced *GLUT2* expression (Figure 2), potentially lowering intestinal glucose transport and postprandial glycaemia - this aligns with findings by Röder et al. (2014) and Schmitt et al. (2017) on impaired glucose handling with *GLUT2* deficiency. Notably, the lowest concentrations of LGG, LA, and PCS 20 caused the strongest *GLUT2* downregulation, while LGG and PCS 20 showed dose-dependent increases thereafter. Dose-response studies were mostly conducted *in vivo*. Szajewska et al. (2013) showed in *in vivo* studies that *Lacticaseibacillus rhamnosus* GG was more effective in treating acute gastroenteritis in children at doses $\geq 10^{10}$ CFU/day compared with lower doses. After reviewing the literature, Ouwehand (2017) found no consistent evidence for a dose–response effect on probiotic efficacy. Among the very limited *in vitro* studies reported to date, Bao et al. (2023) showed that *Lactiplantibacillus plantarum* dose-dependently inhibited *Streptococcus mutans* and *Candida albicans*. At 10^8 CFU/mL it significantly downregulated virulence genes ($p < 0.05$) and achieved the strongest antibacterial and antifungal effects in dual- and multi-species models.

Testing probiotic combinations (Figures 3–5) reflects strain-specific effects - mixtures may yield additive or synergistic benefits, driving use of multi-strain products, although antagonism is possible. In their review, Ouwehand et al. (2018) found no convincing evidence for either synergy or antagonism, leaving these assumptions unconfirmed. Chapman et al. (2011) found probiotic mixtures improved gut function, diarrhoea, immunity, IBD, and *Helicobacter pylori* treatment, outperforming single strains in 75% of studies. Furthermore, in terms of inhibiting pathogens, a 14-strain mix best inhibited *Enterococcus faecalis*, while a 3-strain *Lactobacillus* mix inhibited *Escherichia coli* the most (Chapman et al., 2013). However, they found no superiority over single strains, contrasting Timmerman et al. (2004), who suggested multispecies formulations may offer synergistic advantages, as well as strain compatibility.

Due to the lack of comparable *in vitro* studies, direct comparison of our results was not possible. Although the results revealed no statistically significant changes, we still evaluated combined probiotic effects and potential interaction patterns, comparing them with single-strain treatments at the same concentrations (2, 3, or 4×10^7 CFU/mL).

All single strains and most double combinations upregulated *SGLT1* (Figure 3), except for the PCS 26/PCS 20 combination, which slightly downregulated it. LGG/PCS 20 showed no change versus LGG alone, while LGG/PCS 26 slightly reduced *SGLT1* expression, suggesting no clear synergy. LA alone induced stronger *SGLT1* upregulation than LA/PCS 20, indicating a possible interaction or limited contribution of LA in the mixture. PCS 26/LA behaved similarly to LA alone, suggesting minimal PCS 26 effect or inhibition by LA. In contrast, PCS 20/PCS 26 and LA/LGG combinations reversed individual effects and downregulated *SGLT1*, indicating possible inhibitory interactions. *GLUT2* was downregulated in all double-strain combinations, despite LA and PCS 26 alone causing upregulation. The strongest combination effect was seen in PCS 26/LA combination, where downregulation contrasted with single-strain upregulation. LGG alone caused the strongest single-strain *GLUT2* downregulation, while its combinations showed no major additional effect, suggesting limited contribution of added strains. PCS 20/LGG showed weaker decreases compared to LGG alone, again indicating a potential inhibitory interaction between strains.

Similar to double-strain treatments, all triple-strain combinations and single strains upregulated *SGLT1*, while *GLUT2* was mostly downregulated (except LA and PCS 20 alone) and less pronounced (Figure 4). Combined treatments showed more heterogeneous responses with generally attenuated *SGLT1* induction. However, a more consistent

downregulation of *GLUT2* was observed. Notably, combinations with PCS 26 showed the most pronounced shift towards reduced gene expression, suggesting a potential strain-specific modulatory effect in multi-strain formulations. The strongest *GLUT2* downregulation among triple-strain combinations was seen with PCS 26/PCS 20/LGG. Since PCS 20 alone had no effect, this may suggest a combined (possibly additive) effect of LGG and PCS 26. In fact, in double-strain treatments, PCS 26/LGG showed the greatest *GLUT2* downregulation (Figure 3), indicating that these two strains may be mainly responsible for the gene expression reduction. In contrast, LGG/LA/PCS 20 showed almost no effect, despite LGG alone producing the strongest *GLUT2* downregulation among single strains, suggesting possible suppression of LGG by PCS 20 and/or LA.

Finally, compared to single-strain probiotics at a concentration of 4×10^7 CFU/mL, *SGLT1* upregulation was the greatest after treatment with the four-strain combination (Figure 5), suggesting that combining all tested strains produces an additive effect. For *GLUT2* expression, the greatest decrease was also observed with the combination of all four strains. Interestingly, among single strains, only LGG reduced gene expression, while all others increased it. Since lower *GLUT2* expression may reduce intestinal glucose absorption and blood glucose levels, selected strain combinations appear more promising for modulating gene expression.

CONCLUSION

In conclusion, our results, although statistically non-significant, suggest that the selected probiotic strains may differentially modulate intestinal glucose transporters *in vitro* in the porcine CLAB epithelial cell line. Furthermore, these findings represent a novel contribution and address a gap in the current research literature. To confirm our results and clarify the underlying mechanisms of interaction between the selected probiotic strains in CLAB cells, further studies are required, including protein-level and *in vivo* investigations focusing on actual glucose absorption into the bloodstream and its effect on glucose homeostasis.

ACKNOWLEDGEMENT

Research was founded by Slovenian research and innovation agency programme P1-0164 "Research for improvement of safe food and health".

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Vpliv selektivnih probiotičnih sevov in njihovih kombinacij na izražanje genov *SGLT1* in *GLUT2* v prašičjih črevesnih epitelijskih celicah

IZVLEČEK

Črevesna morfologija in izražanje glukoznih prenašalcev sta ključna dejavnika glukozne absorpcije in homeostaze. Čeprav probiotiki ugodno spreminjajo črevesno mikrobioto, njihova vloga pri uravnavanju izražanja genov glukoznih prenašalcev ostaja nejasna. Namen te študije je bil oceniti učinke izbranih enosevnih in večsevnih probiotičnih bakterij na izražanje genov *SGLT1* (od natrijevih ionov odvisni prenašalec 1) in *GLUT2* (od natrijevih ionov neodvisni prenašalec 2) v prašičjih črevesnih epitelijskih celicah CLAB. Celicam so bili dodani štirje izbrani probiotični sevi (*Lactobacillus acidophilus* (ATCC 4356), *Lacticaseibacillus rhamnosus* GG (ATCC 53103), *Lactiplantibacillus plantarum* PCS 20, and *Lactiplantibacillus plantarum* PCS 26) v različnih koncentracijah in v različnih kombinacijah. Čeprav nobena od opaženih sprememb ni bila statistično značilna, je bilo izražanje gena *SGLT1* skozi večino obravnavanj povečano, medtem ko je *GLUT2* pokazal bolj spremenljiv, a pretežno zmanjšan odziv, kar kaže na to, da je slednji dovzetnejša tarča za dotično raziskavo. Ugotovitve študije kažejo, da izbrani probiotični sevi različno uravnavajo ključne črevesne prenašalce glukoze, kar nakazuje na njihovo potencialno vlogo pri spremembi glukozne absorpcije in homeostaze. Študija tako ponuja nove vpoglede v interakcije probiotikov in gostitelja.

Ključne besede: probiotiki, prenašalci glukoze, izražanje genov, celice CLAB