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ORIGINAL PAPER

Mucin pre-cultivated *Lactobacillus reuteri* E shows enhanced adhesion and increases mucin expression in HT-29 cells

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Abstract Adhesion of probiotic bacteria to the mucus layer lining the gastrointestinal tract is necessary for its effective colonisation and specific therapeutic effects. Enrichment of growth medium with mucin might stimulate bacterial adhesion, probably by increasing the expression of surface structures responsible for bacteria-gut epithelia and/or mucus interactions. The aim of this study was to determine if pre-cultivation of potentially probiotic strain *Lactobacillus reuteri* E (LRE) with mucin stimulates its adherence to colon cell line HT-29 and if the increased adhesion modulates mucin expression in these cells. The mucin-producing HT-29 cell line was co-cultivated for 2 h with LRE grown in MRS broth or MRS broth enriched with pig gastric mucin (LRE + M). The adherence ability of LRE was evaluated microscopically and by plate counting. The relative gene expression was measured by qPCR. Pre-cultivation of LRE in mucin enriched medium significantly increased its adhesion to 14 days HT-29 in comparison with LRE by both methods (28.64% vs. 23.83%, evaluated microscopically, and $14.31 \pm 3.95 \times 10^6$ - CFU ml^{-1} vs. $8.54 \pm 0.43 \times 10^6$ CFU ml^{-1}, evaluated by plate counting). MUC2, MUC5AC, and IL-10 were significantly upregulated after co-cultivation with LRE + M in comparison to LRE and control group (lactobacilli-free HT-29). Obtained results suggest that pre-cultivation of lactobacilli with mucin may not only stimulate their adhesion abilities but also promote their effectiveness to modulate the pathways involved in the pathophysiology of some diseases, e.g., with defective mucin synthesis in ulcerative colitis or colorectal cancer.

Keywords Adhesivity · HT-29 · *Lactobacillus reuteri* · Mucin · Probiotics

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Introduction

Probiotics are defined as "live microorganisms which when administered in adequate amounts, confer a health benefit on the host" (Hill et al. 2014). Most of these health benefits are targeting gastrointestinal disorders, such as post-antibiotic diarrhea, irritable bowel syndrome or ulcerative colitis (UC). Possible mechanisms of probiotics action may be the modulation of the immune system, exclusion of pathogens or healing of damaged mucosa. Adhesion of probiotics to the intestinal mucosa is considered important for achieving such effects (Monteagudo-Mera et al. 2019). Studying bacterial adhesivity is, therefore, one of the most important parameters in the selection of new potentially probiotic strains.

The ability to adhere to mucus covering intestinal mucosa is currently best described in genus *Lactobacillus*. Many studies indicate that bacterial surface proteins are the main structures responsible for lactobacilli-mucus interactions (Nishiyama et al. 2016). The binding site has shown to be formed by galactosyl residues of oligosaccharides present in mucin glycoproteins, the main building unit of mucus (Mukai et al. 1998). Many of adhesion-related proteins were described in lactobacilli, e.g., mucus binding protein (MUB), mucus adhesion-promoting protein A (MapA) or elongation factor Tu (EF-Tu) (Van Tassell and Miller 2011). The relationship between these proteins and mucin was shown in the study where the cultivation of *Lactobacillus plantarum* 423 with mucin upregulated the expression of genes coding adhesion proteins (Ramiah et al. 2007). It was also proved that the MRS medium supplemented with mucin stimulated mucus binding ability in various *Lactobacillus reuteri* strains (Jonsson et al. 2001).

Mucin glycoproteins are the principal molecules that give mucus its characteristic attributes. In the gut, they are essential for the homeostasis maintenance as well as for the protection of gut epithelium against pathogens. The main gel-forming mucins located in intestine are MUC2 and MUC5AC (McGuckin et al. 2011). Changes in mucins expression or their chemical structure are associated with various diseases such as UC, some types of cancer or eye disorders (Dhanisha et al. 2018). The importance of mucin proteins is demonstrated in gene-edited mice, where *Muc2*-deficient mice spontaneously develop colitis or colorectal cancer (Velcich et al. 2002; Van der Sluis et al. 2006).

Mucins are also crucial for commensal microbiota. Oligosaccharide residues are providing binding sites for microbial adhesins, and some bacteria utilise them as an energy source (Juge 2012). The link between probiotic bacteria and mucin was implied in the study where *Escherichia coli* Nissle 1917 increased the expression of various mucin proteins in HT-29 cells. Such an effect may be responsible for its health benefits in UC (Hafez 2012).

The aim of this study was to find out if supplementation of the cultivation medium with mucin stimulates adhesivity of *L. reuteri* E (LRE) to HT-29 cells and if increased adhesion may modulate mucin expression in these cells. The desired effect should be a logical consequence of increased numbers of adhered bacteria, which may potentially affect target cells (Fig. 1).

Materials and methods

Origin and cultivation of bacterial strain

L. reuteri E was isolated from stomach mucosa of breast-fed lamb (breeding station Očová, Slovakia) and identified and investigated for probiotic properties (Bilková et al. 2008, 2011; Májeková et al. 2015; Greifová et al. 2017). The bacterial strain was cultivated in MRS broth (VWR, USA; LRE) or MRS broth enriched with gastric mucin (0.1%; Sigma, Germany; LRE + M) at 37 °C in anaerobic conditions for 18 h.

Cultivation conditions of cell line HT-29

Colon adenocarcinoma cell line HT-29 (ECACC91072201; European Collection of Cell Culture, kindly gifted by Dr. Z. Kozovská) was maintained in enriched DMEM medium (4.5 mg glucose ml^{-1}, 1000 U penicillin ml^{-1}, 1000 µl streptomycin ml^{-1}, 10% fetal bovine serum, 0.3 mg glutamine ml^{-1}; Sigma, Germany) in 12-well cultivation plates in humidified CO_2 atmosphere (5%) at 37 °C. The initial number of cells seeded into one well was $3.2 \times 10^5/3.65$ cm^2 (80% confluence), the experiments were performed after 100% confluency was reached. The cultivation medium was changed every second day. Antibiotics in the medium were omitted 24 h prior to experiments.

Adhesion assay

The adhesion rate was investigated by two methods: microscopy and plate counts. HT-29 cells were seeded and maintained 24 h or 14 days as described above. Cells were washed twice with PBS and LRE suspended in DMEM without antibiotics in 1.5×10^8 - CFU ml^{-1} was added. Cells were co-incubated 2 h (5% CO_2 atmosphere at 37 °C). After co-incubation, non-adhered bacteria were washed away with PBS twice.

For the microscopic method, HT-29 cells were seeded onto a glass coverslip and cultivated 24 h or 14 days. After wash, cells were fixed with methanol/acetic acid mixture (3:1; both Centralchem, Slovakia) for 5 min and stained with May–Grünwald and Giemsa–Romanowski dyes (both Centralchem, Slovakia). Coverslips were dried standing in wells of

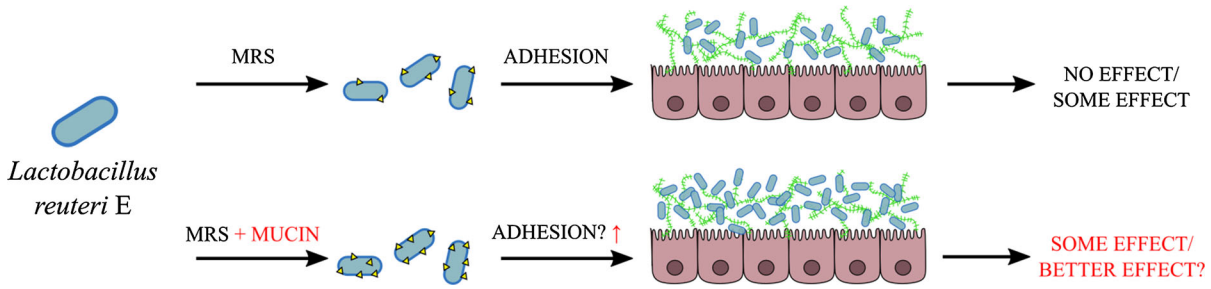

Fig. 1 Hypothesis of adhesion enhancement by mucin. If pre-cultivation of lactobacilli with mucin induces the expression of surface adhesion proteins, the adhesion should be stimulated. The higher number of adhered bacteria may affect the underlying cells with better effectivity. MRS—cultivation medium de Man, Rogosa, Sharpe

cultivation plate. Mixtures of acetone (Laboratórny Servis, Slovakia) and xylene (Lachema, Czech Republic) (1:0, 1:1, 1:2, 1:4, 0:1) were used for draining after which coverslips were attached to slide with DPX (Sigma, Germany). Experiments were done in two replicates. The percentage of HT-29 cells (1000 cells) adhered with at least one lactobacillus was counted.

For plate counting method 14 days HT-29 culture was used. Adhered lactobacilli were detached with trypsin solution, serially diluted in physiological saline and seeded onto MRS agar (VWR, USA) in three replicates. Colonies were counted after cultivation at 37 °C in anaerobic conditions for 24 h. Experiments were done in three parallels.

Gene expression studies

Lactobacilli After 18 h of cultivation the bacterial cells were collected, and RNA was isolated for expression studies.

HT-29 14 days old HT-29 cells were co-incubated with LRE and LRE + M (1.5×10^8 CFU ml^{-1}), lactobacilli-free HT-29 were used as a control (CON). After 2 h of co-cultivation the RNA was isolated for expression studies.

Isolation of bacterial RNA

Total RNA from LRE was isolated by PureLink™ RNA Mini Kit (Invitrogen, USA). Briefly, approximately 1×10^9 bacterial cells were pelleted by centrifugation and mixed with 100 µl of lysozyme solution (10 mg lysozyme ml^{-1} in RNase free water, 10 mM Tris-HCl, 0.1 mM EDTA). Then 0.5 µl of 10% SDS solution was added and the mixture was incubated for 5 min before adding 350 µl of freshly prepared Lysis Buffer. Cells were homogenized by passing 5 times through a 20-gauge needle attached to an RNase-free syringe. Lysates were centrifuged at 12,000×*g* for 2 min and supernatants were transferred to clean RNase-free microcentrifuge tubes. Samples were afterward bound to Spin Columns (provided by the manufacturer), washed with wash buffers I&II, both twice and eluted to 30 µl of RNase free water. The RNA samples were then treated by DNase (DNA-free™ Kit, Ambion, USA) to remove all residual DNA. Quality of isolated RNA was verified by electrophoresis in 0.8% agarose gel and quantified by spectrophotometry on Epoch microplate spectrophotometer (Biotek, USA).

Isolation of HT-29 RNA

Total RNA from HT-29 cells was isolated by the RNazol® RT kit (Molecular Research Center, USA). Briefly, the cell culture medium was discarded and 0.5 ml of RNazol® was added to the cells immediately. The mixture was passed through pipette tip for few times and moved to a sterile 1.5 ml tubes. 0.2 ml of sterile water was added to each sample and the tubes were vortexed for 15 s followed by 15 min of incubation at room temperature. After incubation, the samples were centrifuged at 12,000×*g* for 15 min at 4 °C. 0.5 ml of supernatants were transferred to the new 1.5 ml tubes, mixed with an equal amount of isopropanol and incubated for 10 min at room temperature. After incubation, the samples were centrifuged at 12,000×*g* for 15 min at 4 °C. Isopropanol was discarded and RNA was washed twice with 80% ethanol and centrifuged at 8000×*g* for 5 min at 4 °C. RNA was resuspended in 30 µl of nuclease-free water. Quality of isolated RNA was verified by

electrophoresis in 0.8% agarose gel and quantified by spectrophotometry on Epoch microplate spectrophotometer (Biotek, USA).

Quantitative PCR

500 ng of total bacterial or cell culture RNA was transcribed to cDNA by PrimeScript RT Reagent Kit (Takara, Japan). Expressions of studied genes were quantified by qPCR using thermocycler QuantStudio™ 3 (Applied Biosystems, Thermo Fisher Scientific, USA) using HOT FIREPol EvaGreen qPCR Mix Plus (Solis BioDyne, Estonia) and gene-specific primers for MUC2, MUC5AC, IL-10, MUB, MapA, EF-Tu, 18S rRNA and 16S rRNA (Table 1). The primers were designed using Primer3 or PrimerBlast and checked by OligoAnalyzer 1.0.3.

The PCR program consisted of initial denaturation at 95 °C for 15 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. All experiments were conducted in duplicates along with no-template control. Relative mRNA expressions of studied genes were analysed using the ΔΔCt value method (Winer et al. 1999). PCR products were evaluated by melting curve analysis and gel electrophoresis to confirm the specific amplification. As endogenous control, genes for 18S rRNA and 16S rRNA were used for HT-29 and LRE, respectively.

Statistical analysis

For comparison of relative values between two experimental groups of adherence assay evaluated microscopically, the Student´s unpaired *t* test was used, and *p* values were expressed.

The values from all other measurements represent the means of 3 separate experiments ± standard deviation. The data were analysed using GraphPad Prism statistical analysis. Statistical significances were determined by ANOVA and unpaired *t* test with Welch's correction.

Results and discussion

In this study, we focused on the stimulation of LRE adhesivity to the gut cell line HT-29 and its possible effects on mucin mRNA expression in these cells. Previous experiments showed that LRE harbours at least three genes coding adhesion surface proteins

Table 1 PCR primers used in this study

Target gene	Primer pair	Primer sequence (5′ → 3′)	Target purpose	References
18S rRNA	18SrRNA-for	TGTGATGCCCTTAGATGTCC	HT-29	This study
	18SrRNA-rev	GATAGTCAAGTTCGACCGTC		
MUC2	MUC2-for	GACCCGCACTATGTCACCTT	HT-29	This study
	MUC2-rev	GGACAGGACACCTTGTCGTT		
MUC5AC	MUC5AC-for	CCAGCTCTGTGGCTTACTCC	HT-29	This study
	MUC5AC-rev	TCGGAGGTGGATATTGAAGG		
IL-10	fIL-10	AAGCCTGACCACGCTTTCTA	HT-29	This study
	rIL-10	GCTCCCTGGTTTCTCTTCCT		
16S rRNA	LR16S1F	TTTGGCTATCACTCTGGGA	LRE	Bolado-Martínez et al. (2012)
	LR16S2R	CCGAAACCCTTCTTCACTC		
MUB	qMUB2f	ACGCCAGACAATCCAAAAAC	LRE	This study
	qMUB2r	GCGTAAATTCAGCCTTCTGC		
MapA	mapA_pbf	GCAGTTGCTAAAAAGATGGGCT	LRE	This study
	mapA_pbr	CCCGTTCAGGTGTCTGTGTA		
EF-Tu	ef-tu_p3f	GACGCTTTGGAGTTGGGATG	LRE	This study
	ef-tu_p3r	ACGATTTCCCTGGTGACGAT		

IL-10, interleukin 10; EF-Tu, elongation factor Tu; LRE, *Lactobacillus reuteri* E; MapA, mucus adhesion promoting protein A; MUB, mucus binding protein; MUC, mucin

(MUB, MapA, and EF-Tu; unpublished data). Those proteins were extensively studied in various studies dealing with lactobacilli adhesion, and their presence might be essential for lactobacilli adhesion on mucosal surfaces (Nishiyama et al. 2016). At first, we used qPCR to find out if mucin presence in the cultivation medium affects the expression of above mentioned genes. We investigated three different concentrations of mucin: 0.01%, 0.1%, and 1% with 0.1% giving the best results (data now shown). After 18 h of cultivation of LRE in MRS enriched with 0.1% mucin (LRE + M) we observed significantly increased mRNA expression of MUB, while expression of MapA was significantly decreased and expression of EF-Tu remained unchanged (Fig. 2). In some *Lactobacillus fermentum* strains Archer et al. (2018) observed MUB upregulation in the presence of 0.01% mucin, while 0.05% concentration down-regulated MUB expression. MUB primary function is associated with bacterial adhesion. MapA was referred to be the principal adhesion factor in *L. reuteri* 104R (Miyoshi et al. 2006). Elsewhere was proved MapA´s pleiotropic function, its degradation products have antimicrobial properties (Bøhle et al. 2010). MapA also functions as a component of ABC transporters (Miyoshi et al. 2006). EF-Tu is a primary translational elongation factor; however, it demonstrates adhesive properties, too. Proteins with more functions are classified as so-called moonlighting proteins. The most of the Embden–Meyerhof pathway enzymes are described as moonlighting proteins with adhesive function (Kainulainen and Korhonen 2014). When Archer et al. (2018) compared the expression of MUB and two other genes (fibronectin-binding protein and sortase genes) responsible for adhesion, the trend seemed to be a strain and bacterial density dependent. One of the tested strains, *L. fermentum* MMC 2759, preferably upregulated MUB over the other genes.

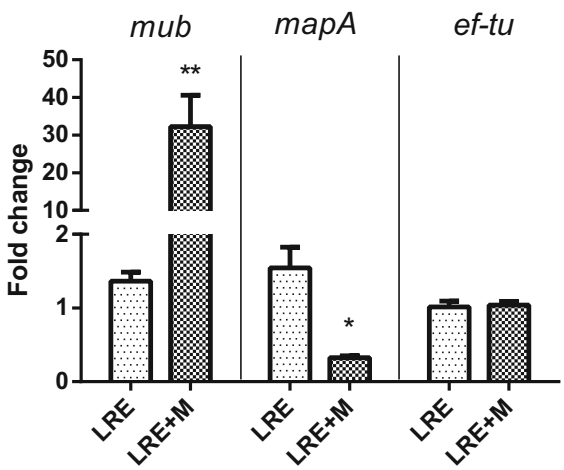

Fig. 2 Relative mRNA expression of MUB, MapA, EF-Tu in LRE and LRE + M recorded by qPCR. LRE + M showed significantly increased expression of *mub* gene (**$p < 0.01$), expression of MapA was decreased (*$p < 0.05$), expression of EF-Tu was not affected. EF-Tu—elongation factor Tu; LRE—*Lactobacillus reuteri* E; LRE + M—*L. reuteri* pre-cultivated in mucin enriched medium; MapA—mucus adhesion promoting factor A; MUB—mucus binding protein

As an in vitro model for studying the adhesive properties of LRE and the possibility of increasing this ability, we selected human intestinal cell line HT-29. Our previous results (Kiňová Sepová et al. 2018) showed that 1 h of co-cultivation may not be sufficient for the complete development of interaction between investigated cells, therefore 2 h long co-cultivation was used. For microscopic evaluation of adhesion, HT-29 cells of different maturity were used: 24 h and 14 days. Results showed the significantly better adhesion of LRE to the 14 days cells, 23.83% versus 0.59% under standard cultivation conditions, and 28.64% versus 0.92% in the case of LRE + M (both $p < 0.001$; Table 2). Therefore, in further experiments, the older HT-29 culture was employed. Moreover, the expression of MUC2 and MUC5AC genes in 14 days HT-29 cells was significantly higher in comparison to 24 h culture (data not shown). The higher adhesion ability of LRE to the 14 days HT-29 cells could be explained by increased production of mucins (Altamimi et al. 2016), in spite of not fully differentiated cells. The mucin enrichment of cultivation medium resulted in significantly improved

Table 2 Adhesivity of *L. reuteri* E (LRE) and *L. reuteri* E pre-cultivated in mucin enriched MRS medium (LRE + M) to HT-29 cells of different maturity (24 h vs. 14 days) after 2 h of co-cultivation. Microscopically evaluated percentage of HT-29 cells adhered with at least one bacterial cell. Mucin pre-cultivation significantly enhanced adherence of LRE ($p < 0.01$ and $p < 0.001$ for 24 h and 14 days HT-29 culture, respectively)

	24 h (%)	14 days (%)
LRE	0.59	23.83
LRE + M	0.92	28.64

adhesion ability of LRE to HT-29 cell line in both tested cases (24 h $p < 0.01$; 14 days $p < 0.001$).

For the determination of living LRE cells adhering to the HT-29 surface, plate counts were performed (Fig. 3). Again, the LRE + M showed significantly higher adhesion ($14.31 \pm 3.95 \times 10^6$ CFU ml^{-1}) than LRE ($8.54 \pm 0.43 \times 10^6$ CFU ml^{-1}).

Mucin supplemented medium significantly increased the adhesion ability of LRE to intestinal cell line HT-29, detected by both methods used. According to Celebioglu et al. (2017) different carbon supplies possibly manifest in different adhesion molecules expression. In their experiments, elongation factor G was more copious in the presence of cellobiose and less numerous in mucin presence. On the other hand, another moonlighting protein pyruvate kinase was more abundant in mucin supplementation and less abundant when growing on cellobiose. This may also explain the differences in expression of herein studied moonlighting proteins (EF-Tu and MapA) and MUB, which play a primary role in bacterial adhesion.

Mucus, and especially mucin proteins and their glycans, protect human mucosa against infections by various mechanisms (Linden et al. 2008; Cobo et al. 2015; Wheeler et al. 2019). In UC, mucin production and/or secretion in the colon is decreased, contributing to inflammation, which is fundamental in UC pathogenesis (Dorofeyev et al. 2013). Some probiotics seem

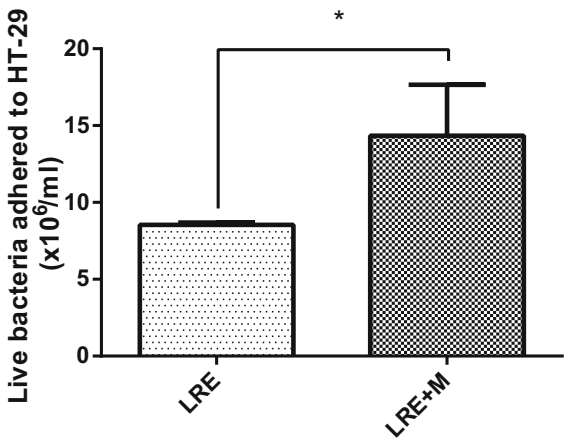

Fig. 3 Adhesivity of LRE and LRE + M to HT-29 cells after 2 h of co-cultivation evaluated by plate counts. Mucin enriched medium significantly enhanced adherence of LRE (*$p < 0.01$). LRE—*L. reuteri* E; LRE + M—*L. reuteri* pre-cultivated in mucin enriched medium

to be effective in inducing remission of UC, however, the exact mechanism of this effect is currently not known (Derwa et al. 2017). One hypothesis might be the ability of some probiotic strains to affect mucus metabolism. Soluble protein p40 produced by *L. rhamnosus* GG induced expression of Muc2 and therefore the thickness of the mucus layer in mice intestine. This is done by transactivation of the epithelial growth factor receptor, which is involved in the regulation of mucin production (Wang et al. 2014). Similarly, the expression of MUC2 was stimulated in human enterocytes (Caco-2) by *Lactobacillus paracasei* CBA L74 fermented milk (Paparo et al. 2018). VSL#3®, a mixture of various strains of lactobacilli, bifidobacteria, and *Streptococcus thermophilus*, reduced intestinal inflammation and improved epithelial barrier function in *Muc2*-deficient mice (Kumar et al. 2016). Authors correlate these effects with stimulation of expression and secretion of Muc2 in the intestines of rats fed the same preparation (Caballero-Franco et al. 2007). An interesting relationship between mucus and inflammation was proved in an extensive study. The authors found that MUC2 protein fed to *Muc2*-deficient mice might regulate intestinal inflammation through mechanisms of immunotolerance involving dendritocytes (Shan et al. 2013).

To test the ability of LRE and LRE + M to change the expression of genes coding mucin proteins we used the method of qPCR. After co-cultivation of HT-29 with lactobacilli, we detected significantly increased levels of MUC2 mRNA in the LRE + M group compared to LRE ($p < 0.05$) and CON ($p < 0.01$). Non-stimulated LRE did not affect MUC2 mRNA expression in HT-29 (Fig. 4a). These results demonstrate that the ability of LRE to affect MUC2 expression was achieved only by mucin pre-cultivation.

In the case of MUC5AC, we observed significantly increased levels of mRNA in both LRE and LRE + M groups in comparison to CON. However, LRE + M increased MUC5AC mRNA expression in HT-29 with higher statistical significance than LRE ($p < 0.01$ vs. $p < 0.05$, respectively; Fig. 4b). This indicates that mucin pre-cultivation enhanced the effect of LRE on the expression of this gene.

One of the commonly-tested markers of the immunomodulatory effect of probiotics is the production of IL-10, as it is an important anti-inflammatory

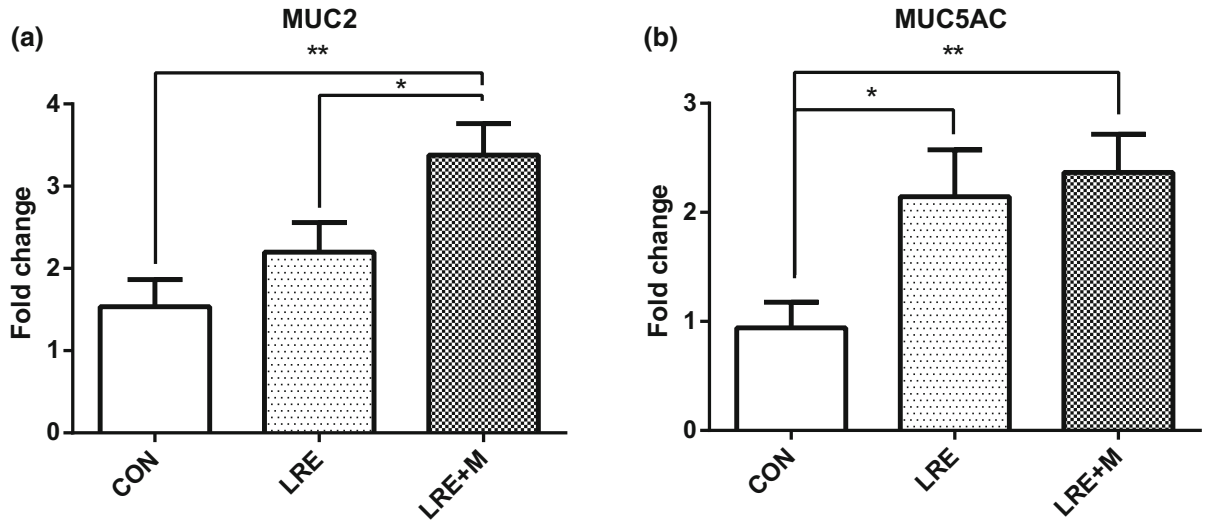

Fig. 4 Relative mRNA expression of mucins MUC2 (**a**) and MUC5AC (**b**) in HT-29 recorded by qPCR. LRE + M significantly increased expression of MUC2 after 2 h of co-cultivation compared to control group (CON; $^{**}p < 0.01$) and LRE group ($^{*}p < 0.05$). Expression of MUC5AC was significantly increased by both LRE + M ($^{**}p < 0.01$) and LRE ($^{*}p < 0.05$) compared to CON. CON—control group; LRE—*L. reuteri* E; LRE + M—*L. reuteri* E pre-cultivated in mucin enriched medium; MUC—mucin

regulator (Gao et al. 2012; Azad et al. 2018). However, IL-10 might also be associated with mucin production or secretion. Schwerbrock et al. (2004) compared germ-free *IL-10* gene knockout mice with germ-free wild type ones before and after induction of colitis using commensal bacteria. *IL-10* gene knockout mice developed colitis and demonstrated significantly lowered Muc2 synthesis and levels, while wild type mice remained healthy. Moreover, *Muc2/IL-10* double knockout mice develop more severe colitis than *Muc2* or *IL-10* lacking mice (Van Der Sluis et al. 2008). IL-10 production induced by probiotic bacteria was described in various experimental models—mice (Jeon et al. 2012), sows (Laskowska et al. 2019), cell cultures (Sichetti et al. 2018). A possible mechanism responsible for this effect is the interaction of pattern recognition receptors (e.g. TLRs) located on the host cell with microbe-associated molecules, for example, surface proteins (Plaza-Diaz et al. 2019). Xiong et al. (2018) recently produced a recombinant MUB protein which promoted IL-10 upregulation and secretion in Caco-2 and Raw 264.7 cells, probably by the above-mentioned mechanism. Since mucin induced the MUB expression in investigated lactobacillus strain, we tested its influence on IL-10 regulation.

We detected significantly increased levels of IL-10 mRNA in HT-29 co-cultivated with both LRE and LRE + M. But as in the case of MUC5AC, LRE + M upregulated IL-10 with higher statistical significance than LRE ($p < 0.01$ vs. $p < 0.05$, respectively; Fig. 5). Mucin pre-cultivation might, therefore, enhance the effect of LRE also in this gene.

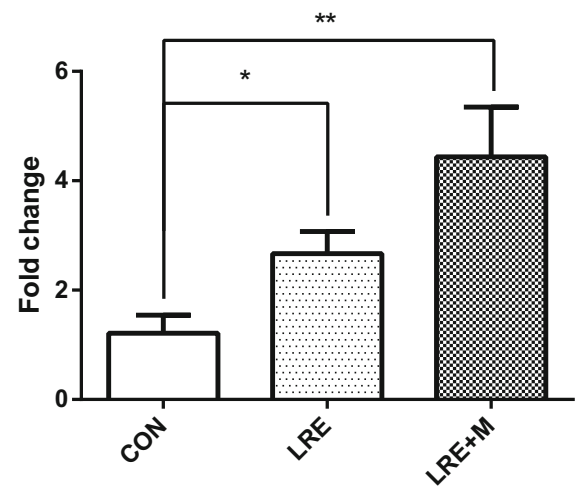

Fig. 5 Relative mRNA expression of IL-10 in HT-29 recorded by qPCR. Expression of IL-10 was significantly increased by both LRE + M ($^{**}p < 0.01$) and LRE ($^{*}p < 0.05$) compared to CON. CON—control group; LRE—*L. reuteri* E; LRE + M—*L. reuteri* E pre-cultivated in mucin enriched medium

It is clear that the adhesive abilities of probiotics are crucial for their effectiveness and various methods were used to stimulate these features. Hsueh et al. (2014) increased the low adhesive activity of *Lactobacillus casei* ATCC 393 to Caco-2 cells by cloning the collagen-binding protein from *L. reuteri* Pg4. Stimulated adhesion of this strain also increased its competition ability with pathogens in adhesion to Caco-2 cells. Adhesion of lactobacilli may be enhanced by altering the gut environment, too. Bustos et al. (2012) modified the adhesion of two lactobacilli strains to intestinal cell lines by co-cultivating both of them in the presence of flavan-3-ols. Celebioglu et al. (2017) achieved increased adhesion of *Lactobacillus acidophilus* NCFM to HT-29 cells by pre-cultivation with some prebiotic carbohydrates or mucin.

We demonstrated that the pre-incubation of lactobacilli with mucin stimulates their adherence to human gut HT-29 cells in vitro. It may potentially lead to their better "bioavailability" in the gut in vivo. Furthermore, mucin stimulated lactobacilli increased mucins (MUC2 and MUC5AC) and IL-10 expression in HT-29. All these substances may mediate immunomodulation, which can be relevant in some diseases treatment. Although the underlying mechanism is yet not known, we suppose that the modification of selected genes expression is caused by both, higher expression of MUB proteins on the bacterial surfaces, and increased numbers of bacteria adhered to the human cells. To shed more light on the topic, further research should be conducted.

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Author contributions All authors contributed to the study conception and design.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate All participants agree with participation.

Consent for publication All authors read and approved the final manuscript.

Availability of data and material The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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