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Restorative effects of *Acetobacter ghanensis* on the pathogenicity of gliadin-induced modulation of tight junction-associated gene expression in intestinal epithelial cells

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Ethics Committee Approval
This article is not a study with human participants. There are no experiments on animals. This article does not contain any studies on human participants or animals performed by the author. The study does not require any ethical permissions since it is an in vitro study.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: At present, a gluten-free diet is the only efficient way to treat celiac disease (CD). The development of novel approaches to lessen or counteract the pathogenic effects of gluten remains crucial for the treatment of CD. The aim in this investigation was to examine the restorative effects of *Acetobacter ghanensis* as a novel probiotic against gliadin-induced modulation in the barrier integrity of an intestinal epithelial cell (IEC) model (Caco-2).

Methods: Fully differentiated Caco-2 cell monolayers were subjected to enzymatically digested gliadin with a pepsin and trypsin (PT) in the presence or absence of *A. ghanensis* for 90 min. The relative amounts of zonulin, zonula occludens-1 (ZO-1), claudin-1, and occludin mRNA expression were determined by quantitative real-time polymerase chain reaction (qRT-PCR). Transepithelial electrical resistance (TEER) was evaluated to monitor the barrier integrity of cell monolayers. Statistical analyses were carried out using one- or two-way ANOVA followed by Tukey's post-hoc analysis for multiple pairwise comparisons.

Results: A significant upregulation (4.7-fold) of zonulin was noted in the PT-gliadin treated Caco-2 cells in comparison with the untreated controls (P<0.001). Conversely, gliadin-induced zonulin expression was markedly downregulated in the Caco-2 cells following exposure to *A. ghanensis* in the presence of PT-gliadin (P<0.001). Furthermore, prominent decreases in the mRNA expression levels of ZO-1 (45%) and occludin (40%) were seen in the PT-gliadin exposed Caco-2 cells compared to the untreated control cells (P<0.001). PT-gliadin in the Caco-2 cells did not significantly alter the mRNA levels of claudin-1 (P=0.172). Similarly to zonulin expression, the decreasing effect of PT-gliadin on ZO-1 was completely attenuated in the PT-gliadin-administrated Caco-2 cells following exposure to *A. ghanensis* (P<0.001).

Conclusion: *A. ghanensis* restored the pathogenicity of PT-gliadin on intestinal barrier integrity.

Keywords: celiac disease, gluten, probiotic, *Acetobacter ghanensis*, caco-2 cells

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Introduction

Celiac disease (CD) is a chronic inflammatory disorder induced by the cells of the natural and acquired immunity in genetically predisposed individuals following consumption of dietary prolamins such as gluten, hordein, and secalin in wheat, barley, and rye, respectively [1]. The disease is characterized by malabsorption due to progressive degrees of inflammation in the intestinal epithelial cells (IECs) resulting in the flattening of the villi, which are specialized cells that carry out absorption of nearly all nutrients. Besides intestinal symptoms, CD is characterized by various signs, namely iron deficiency anemia, osteoporosis, night blindness, muscle weakness, and weight loss [2]. Furthermore, CD is correlated with raised risk for other autoimmune disorders including itchy skin disease (dermatitis herpetiformis), alopecia, and type-1 diabetes [3,4].

Gliadin, a main ingredient of wheat gluten, has been implicated as the primary cause of the increased IEC permeability and inflammatory processes associated with CD [5,6]. Under physiological conditions, the homeostasis of the integrity of the IECs as the physical blockage against exterior factors is facilitated by tight connections between each enterocyte through tight junction (TJ) proteins containing claudin, occludin, and zonula occludens (ZO) [7]. In genetically vulnerable people having human leukocyte antigen (HLA)-DQ2 and/or HLA-DQ8 alleles, however, the interaction of gliadin with chemokine receptor CXCR3 on the apical location of enterocytes initiates a signaling cascade that results in TJ disassembly [8]. During this process, the synthesis of zonulin, a crucial protein contributing to the regulation of TJs in IECs, is upregulated and it is liberated to the gut lumen following exposure to dietary gliadin. The binding of zonulin to protease activated receptor 2 (PAR2) and epidermal growth factor receptor (EGFR) in IECs then leads to the dismantling of TJ proteins from the junctional structure by activating the phosphorylation processes of target proteins including ZO followed by impairment in barrier integrity and function [9,10].

At present, a gluten-free diet is the only efficient way to treat CD. Thus, the investigation of novel therapeutic strategies to lessen or counteract the pathogenic effects of gluten remains crucial for the treatment of CD. In the last few years, there has been a rapid rise in interest in applications of various probiotics that confer health benefits. Specifically, *in vivo* and *in vitro* investigations have shown promising results from the use of probiotic strains including *Bifidobacterium and Lactobacillus* to treat CD [11,12].

In the current study, the novel probiotic strain examined, *Acetobacter ghanensis*, was obtained from kefir, a home-made fermented dairy product; its probiotic characteristics based on antibiotic resistance, antimicrobial activity, hydrogen peroxide and hydrogen sulfide production, and endurance in an extremely acidic environment. However, the physiological health benefits of *A. ghanensis* remain uncertain. The current research was thus undertaken to investigate the potential restorative effects of *A. ghanensis* against gliadin-induced intestinal barrier dysfunction due to modulation in the expression levels of genes encoding TJ-related associated proteins participating in the formation and integrity of IECs *in vitro*.

Materials and methods

Cell cultivation and treatment conditions

Caco-2 cells obtained from the American Type Culture Collection (ATCC) were cultivated in minimal essential medium (Corning™ cellgro™; 10-010-CV) comprising 15% (v/v) fetal bovine serum (Sigma; F3135), streptomycin (10,000 µg/mL), and 1% penicillin (10,000 IU/mL) and an antibiotic mixture (Corning™ cellgro™; 30-002-CI), 1% nonessential amino acid mixture (Corning™ cellgro™; 25-025-CI), and 1% sodium pyruvate (Corning™ cellgro™; 25-000-CI) at 37 °C in a humidified 5% CO_2/O_2 atmosphere. For the experimental setup, Caco-2 cells plated at a concentration of 1×10^5 viable cells were grown as monolayers on 12-mm collagen-coated membrane inserts (Corning® Transwell®) and cultivated for 21 days for full differentiation. The cultivation medium was replaced every 2 days. Before the experimental treatment, fully differentiated Caco-2 cells were kept in complete medium without antibiotic for 2 days. PT-gliadin obtained from wheat was prepared as described previously [13]. For the experimental setup Caco-2 cells were treated with or without the addition of viable *A. ghanensis* suspension (1×10^8 CFU/mL), and then the cells were incubated for 90 min. Untreated Caco-2 cells were also included as a control of the experimental approaches.

Total RNA extraction, cDNA synthesis, and qRT-PCR analysis

Total RNA extraction was carried out using RNAzol® RT reagent (Molecular Research Center, Inc.) in accordance with the manufacturer's directions following the experimental approaches employed for Caco-2 cells for 90 min. An iScript cDNA synthesis kit (Bio-Rad; 170889) was used to synthesize complementary DNA (cDNA) from the isolated RNA samples as described in the manufacturer's instructions.

To assess the relative abundances of the gene products of interest and a housekeeping gene, cyclophilin A (*CYPA*), expressed at comparatively steady levels among the experimental groups as an internal control qRT-PCR method, was applied using a LightCycler® 2.0 real-time PCR system and SYBR® Green Master Mix (Bio-Rad). Comparative C_T ($2^{-\Delta\Delta CT}$) analysis was conducted to assess the relative mRNA expression levels of target genes normalized to *CYPA* mRNA levels. Sequences of gene-specific primer pairs used for qRT-PCR are described as reported previously [14].

Bacterial cultivation

Acetobacter ghanensis, the species examined in the present study, was previously obtained from kefir, a home-made fermented dairy product manufactured without the use of commercial starter culture, and was identified by DNA sequencing. The bacterial stock maintained at -80 °C was thawed at room temperature. It was then plated on a cultivation medium containing HS broth and left to stand at 32 °C for 72 h. After centrifugation at 10,000 × *g* for 10 min, the bacterial cells were collected in the pellet and then washed twice with sanitized phosphate-buffered saline (PBS) with re-centrifugation and were resuspended in MEM cell culture medium. A Den1B McFarland densitometer was used to count bacterial cell numbers.

Evaluation of transepithelial barrier resistance in Caco-2 cells

For *in vitro* monitoring of the barrier integrity of IEC monolayers in real time, TEER values were evaluated at 30-min periods for a total of 90 min using an epithelial volt/ohm meter (World Precision Instruments) as described earlier [15]. Only TEER values greater than 250 Ω/cm^2 were considered acceptable, indicating fully formed IEC barrier integrity with TJs. The TEER levels of the IEC monolayers at the beginning were reflected to be TEER values of 100%.

Statistical analysis

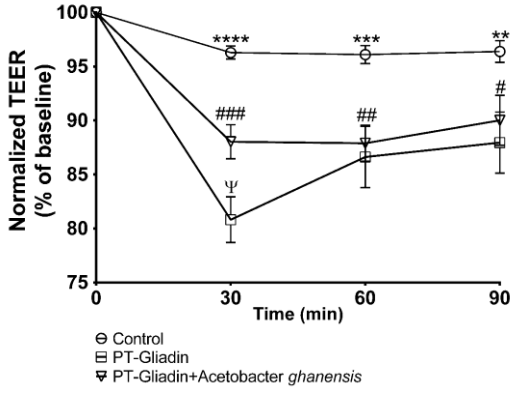
Data from the gene expression analysis and TEER values were analyzed by one- and two-way ANOVA, respectively, using GraphPad Prism (version 6.0 for Windows). Tukey's multiple-comparison post-hoc test was used to determine the differences between the experimental groups. The data were presented as the mean (SEM) of duplicate measures established in two distinct experiments with three replicates. A *P*-value of 0.05 or less was considered significant.

Results

Effects of *Acetobacter ghanensis* on PT-gliadin-induced impairment in the barrier integrity of Caco-2 cells

The exposure of fully differentiated Caco-2 cell monolayers to PT-gliadin led to a considerable decrease of 20% in TEER values in comparison to the untreated control cell monolayers at 30 min ($P<0.001$) (Figure 1). Although the TEER values of the Caco-2 cells exposed to PT-gliadin started to increase over time, they were significantly lower than those of the untreated control cells at 60 and 90 min ($P=0.005$ and $P=0.002$, respectively). In contrast, TEER levels were significantly greater in the *A. ghanensis*-treated Caco-2 cells in the presence of PT-gliadin compared to the PT-gliadin treated Caco-2 cells exposed to PT-gliadin alone at 30 min ($P=0.009$). However, no significant alterations in TEER values were obtained between the PT-gliadin-treated groups in the presence or absence of *A. ghanensis* at 60 and 90 min ($P=0.852$ and $P=0.658$, respectively). Although *A. ghanensis* attenuated PT-gliadin-dependent decreases in TEER values at 30 min, the TEER values were still significantly lower in comparison to the control Caco-2 cell monolayers ($P=0.003$).

Figure 1: The restorative effects of *A. ghanensis* on the IEC barrier.



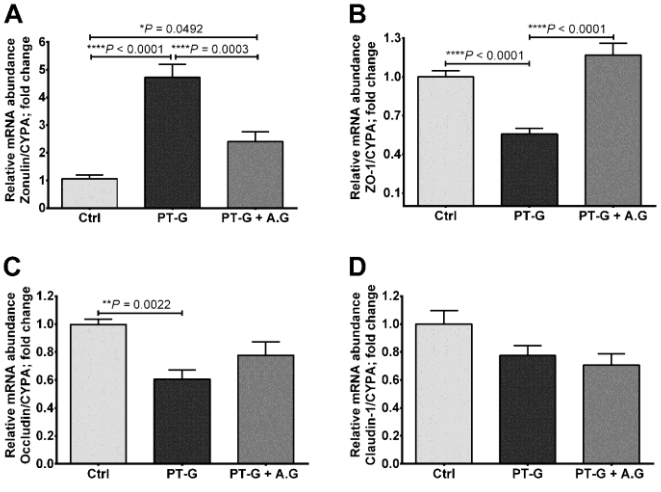
TEER levels were ascertained in PT-gliadin exposed Caco-2 cells with or without addition of *A. ghanensis* (10^8 CFU/mL). $^{**}P=0.019$, $^{***}P=0.001$, and $^{****}P<0.001$ (untreated control group vs. PT-gliadin treated group); $^{\#}P=0.024$, $^{\#\#}P=0.002$, and $^{\#\#\#}P=0.003$ (untreated control group vs. *A. ghanensis* and PT-gliadin co-administrated group); $^{\Psi}P=0.009$ (*A. ghanensis* and PT-gliadin co-administrated group vs. PT-gliadin treated group) at the same measurement time intervals.

Effects of *Acetobacter ghanensis* on TJ gene expression

The mRNA expression of zonulin was significantly upregulated by approximately 4.7-fold when Caco-2 cells were administered with PT-gliadin for 90 min in comparison to untreated control cells ($P<0.001$) (Figure 2A). The increasing impact of PT-gliadin on the mRNA levels of zonulin was significantly suppressed by 50% when *A. ghanensis* was administered to Caco-2 cells in the presence of PT-gliadin ($P<0.001$). Although the presence of *A. ghanensis* attenuated the increasing effects of gliadin, the zonulin expression was still significantly greater (2-fold) than that of the untreated control group ($P=0.049$).

Significant downregulation of ZO-1 (45%) and occludin (40%) was noted in the Caco-2 cells after incubation with PT-gliadin compared to the untreated control cells ($P<0.001$ and $P=0.002$, respectively) (Figure 2B). Importantly, the ZO-1 mRNA expression level in the Caco-2 cells following exposure to *A. ghanensis* and PT-gliadin was significantly higher, by 57%, compared to those of the Caco-2 cells subjected to PT-gliadin alone ($P<0.001$). Taken together, these findings indicate that administration of *A. ghanensis* to Caco-2 cells in the presence of PT-gliadin prevented PT-gliadin-stimulated alterations in the zonulin mRNA levels (~50%) and ZO-1 (~100%) mRNA. Although the mRNA levels of occludin in the *A. ghanensis* and gliadin exposed Caco-2 cells were 28% higher than those of the PT-gliadin-administrated Caco-2 cells, these differences did not reach statistical significance ($P=0.217$) (Figure 2C). Again, while the mRNA expression levels of claudin-1 in the PT-gliadin exposed Caco-2 cells were slightly lower (23%) than those of the untreated control cells, the change was not statistically significant ($P=0.172$) (Figure 2D). On the other hand, administration of *A. ghanensis* to the Caco-2 cells did not change claudin-1 expression in those cells ($P=0.803$).

Figure 2: Expression of the genes involved in the regulation of IEC barrier permeability and function.



The mRNA expression levels of zonulin (A), ZO-1 (B), occludin (C), and claudin-1 in PT-gliadin treated Caco-2 cells in the presence or absence of *A. ghanensis* (10^8 CFU/mL) for 90 min. Ctrl: Untreated control; PT-G: a pepsin and trypsin digested wheat gliadin; A.G: *Acetobacter ghanensis*.

Discussion

Studies have revealed that the incidence of CD has risen dramatically over the last two decades worldwide [16,17]. Although the current global prevalence of CD is considered to be about 1-2%, most individuals with CD (80-90%) are

undiagnosed but still experience high disease burden [18]. Nutrient deficiency-related diseases are commonly seen in patients with CD due to inappropriate inflammatory response in the IECs, which build a chemical and physical barrier in addition to roles in nutrient absorption. Intestinal permeability is controlled by the collaborations of proteins involved in the establishment of TJs between neighboring IECs. In 2008, Lammers et al. [8] elucidated the mechanism that increases the levels of zonulin proteins in IECs following exposure to gliadin, which leads to loss of barrier integrity and permeability, by regulating junctional structures between epithelial cells. In the last decade, scientific attention towards the use of probiotics in therapeutic cure of CD has increased dramatically because of their general health benefits [19-21]. Understanding the mechanisms by which probiotics reduce the toxicity of gluten peptides for the intestinal epithelial barrier in CD is essential for developing efficient approaches for health promotion.

In the present study, we demonstrated *in vitro* that *A. ghanensis*, a novel probiotic strain, attenuated the pathogenicity of PT-gliadin peptides in IECs by decreasing the modulatory impacts of gliadin on the expression of genes essential in the development of TJs. Consistent with our findings, previous reports [9,22,23] demonstrated that the mRNA expression levels of zonulin were upregulated in Caco-2 cells following PT-gliadin exposure. The early effects of gliadin on zonulin release were shown in rat IECs (IEC-6 cell line) by Clemente et al. [22]. In 2006, Drago et al. [9] revealed that exposure to gliadin induced rapid synthesis of zonulin in IEC-6 and Caco-2 cells. An *ex vivo* study conducted by Hollon et al. [23] showed increasing effects of gliadin on zonulin expression in the intestinal biopsy explants of patients with active CD. Collectively, the outcomes of these investigations provide evidence that the modulatory role of zonulin in the formation of TJ proteins is interconnected with CD. In 2014, Orlando et al. [24] reported that the expression levels of zonulin in Caco-2 cells rapidly rose and reached the highest levels following PT-gliadin exposure for 60 min. It was also shown that the expression levels of zonulin decreased after 60 min of incubation in a time-dependent manner. In line with that observation, it was reported that TEER values started to increase after 90 min. Similar observations were reported by Lindfors et al. [25] and Silano et al. [26], who found an increasing tendency for reduced TEER values in Caco-2 cells following incubation with PT-gliadin after 60 min of incubation. Consistent with these findings, we demonstrated that PT-gliadin treatment for 90 min caused a marked increase in the expression levels of zonulin. Since zonulin is a key regulator in the formation of TJs, the elevated levels of zonulin led to a notably rapid decrease in the TEER values of Caco-2 cells exposed to PT-gliadin for 30 min, but the TEER values increased following longer incubation times, consistent with the findings of previous reports.

In the present study, we showed that Caco-2 cells exposed to PT-gliadin exhibited significant decreases in the mRNA expression levels of ZO-1 and occludin in line with elevated levels of zonulin and decreased levels of TEER, but no significant alteration was found in the release of claudin-1. Similar outcomes were reported by Sander et al. [27], who demonstrated that exposure of Caco-2 cells to PT-gliadin (1 mg/mL) resulted in low expression levels of ZO-1 but did not alter claudin-1 protein expression.

The present study was designed to assess the protective roles of *A. ghanensis* against gliadin-induced modulation of TJ formation in IECs. Notably, the administration of *A. ghanensis* to PT-gliadin treated Caco-2 cells decreased the mRNA expression levels of zonulin compared to PT-gliadin incubated Caco-2 cells. On the other hand, the decreasing effect of PT-gliadin on ZO-1 expression was completely attenuated in Caco-2 cells following *A. ghanensis* exposure. This observation supports findings from an earlier investigation in which the zonulin synthesis stimulated by gliadin in Caco-2 cells was suppressed by subsequent *Lactobacillus rhamnosus* GG exposure [24]. It was also reported that the presence of *Lactobacillus rhamnosus* GG in Caco-2 cells incubated with PT-gliadin increased the release of occludin and ZO-1 expression but did not cause a considerable difference in the expression levels of claudin-1 [24]. Similar findings from an *in vivo* study reported by Orlonda et al. [28] demonstrated that exposure of newborn Wistar rats fed with gliadin to the same probiotic strain attenuated the gliadin-induced decrease in the expression levels of ZO-1, occludin, and claudin-1 at the mRNA and protein levels. Similarly, Bhat et al. [29] revealed that the expression levels of ZO-1 and occludin were upregulated by subsequent incubation of Caco-2 cells with *L. rhamnosus* (LR: MTCC-5897). Other research, performed by Anderson et al. [30], showed that the expression levels of TJ proteins visualized by fluorescent microscopy including ZO-1/2 and occludin were higher in Caco-2 cells exposed to *Lactobacillus plantarum* MB452 than in untreated control cells.

In our previous report, we demonstrated that PT-gliadin exposure of Caco-2 cells co-cultivated with peripheral blood mononuclear cells (PBMCs) collected from patients with CD caused a rapid and significant reduction in TEER levels with simultaneous rises in the mRNA expression levels of zonulin in parallel with decreases in the expression levels of occludin and ZO-1 [14]. On the other hand, the administration of *A. ghanensis* to Caco-2 cells co-cultured with PBMCs in the presence of PT-gliadin attenuated the gliadin-induced changes related to intestinal barrier formation and integrity [14]. We also revealed that *Acetobacter ghanensis* was able to metabolize gluten peptides in a medium supplemented with gluten as the major nitrogen supply for survival. The gluten-digesting features of *A. ghanensis* may explain why it can counteract the toxic consequences of gliadin peptides for the maintenance of intestinal homeostasis. A previous study evaluating proteolytic activities against gluten by bacteria localized in the human small intestine demonstrated that a minimum of 85 bacterial strains mainly belonging to the genus *Lactobacillus* and the phylum Firmicutes can digest gluten peptides [31]. This is consistent with the observation that *Lactobacillus* species are involved in the proteolytic digestion of gluten peptides throughout the fermentation process of wheat flour with sourdough [32,33].

Limitation

In the present investigation, the mRNA expression levels of zonulin and TJ associated gene expression levels were determined after 90 min of PT-gliadin exposure with or without addition of *A. ghanensis.* PT-gliadin induced a significant increase in intestinal permeability following incubation with PT-

gliadin for 30 min, and intestinal permeability tended to decrease and then became stable over time (60 min and 90 min). Thus, the expression levels of zonulin as a key regulator of gut permeability at 30 and 60 min of the experimental treatments could be considered to provide a mechanistic explanation for increased intestinal permeability at the early time point. Furthermore, even though no marked alteration in the expression of claudin-1 was observed among the treatment groups, claudin-2, which is another mediator of leaky gut, should be investigated to test the conclusion.

Conclusion

The evidence provided by the present study has emphasized the potential suppressive characteristics of *A. ghanensis* on gliadin-induced cellular responses through alterations in the expression of genes involved in the maintenance of IEC barrier permeability. The gluten-digesting properties of *A. ghanensis* offer a possible explanation for the improvements in maintaining the TJs between IECs. However, more precise mechanistic data from further *in vivo* and *in vitro* investigations and clinical trials are needed to fully elucidate the functional significance of *A. ghanensis in vivo*.

References

1. Lindfors K, Ciacci C, Kurppa K, Lundin KEA, Makharia GK, Mearin ML, et al. Coeliac disease. Nat Rev Dis Primers. 2019;5(1):3. doi: 10.1038/s41572-018-0054-z.
2. Uche-Anya E, Lebwohl B. Celiac disease: clinical update. Curr Opin Gastroenterol. 2021;37(6):619-24. doi: 10.1097/MOG.0000000000000785.
3. Jalilian M, Jalali R. Prevalence of celiac disease in children with type 1 diabetes: A review. Diabetes Metab Syndr. 2021;15(3):969-74. doi: 10.1016/j.dsx.2021.04.023.
4. Naveh Y, Rosenthal E, Ben-Arieh Y, Etzioni A. Celiac disease-associated alopecia in childhood. J Pediatr. 1999;134(3):362-4. doi: 10.1016/s0022-3476(99)70466-x.
5. Chirdo FG, Auricchio S, Troncone R, Barone MV. The gliadin p31-43 peptide: Inducer of multiple proinflammatory effects. Int Rev Cell Mol Biol. 2021;358:165-205. doi: 10.1016/bs.ircmb.2020.10.003.
6. Falcigno L, Calvanese L, Conte M, Nanayakkara M, Barone MV, D'Auria G. Structural Perspective of Gliadin Peptides Active in Celiac Disease. Int J Mol Sci. 2020;21(23). doi: 10.3390/ijms21239301.
7. Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med. 2018;50(8):1-9. doi: 10.1038/s12276-018-0126-x.
8. Lammers KM, Lu R, Brownley J, Lu B, Gerard C, Thomas K, et al. Gliadin induces an increase in intestinal permeability and zonulin release by binding to the chemokine receptor CXCR3. Gastroenterology. 2008;135(1):194-204.e3. doi: 10.1053/j.gastro.2008.03.023.
9. Drago S, El Asmar R, Di Pierro M, Grazia Clemente M, Tripathi A, Sapone A, et al. Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scand J Gastroenterol. 2006;41(4):408-19. doi: 10.1080/00365520500235334.
10. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 2012;1258:25-33. doi: 10.1111/j.1749-6632.2012.06538.x.
11. Håkansson Å, Andrén Aronsson C, Brundin C, Oscarsson E, Molin G, Agardh D. Effects of. Nutrients. 2019;11(8). doi: 10.3390/nu11081925.
12. Marasco G, Cirota GG, Rossini B, Lungaro L, Di Biase AR, Colecchia A, et al. Probiotics, Prebiotics and Other Dietary Supplements for Gut Microbiota Modulation in Celiac Disease Patients. Nutrients. 2020;12(9). doi: 10.3390/nu12092674.
13. Gujral N, Suh JW, Sunwoo HH. Effect of anti-gliadin IgY antibody on epithelial intestinal integrity and inflammatory response induced by gliadin. BMC Immunol. 2015;16:41. doi: 10.1186/s12865-015-0104-1.
14. Doguer C, Akalan H, Tokatlı Demirok N, Erdal B, Mete R, Bilgen T. Protective effects of *Acetobacter ghanensis* against gliadin toxicity in intestinal epithelial cells with immunoregulatory and gluten-digestive properties. Eur J Nutr. 2022. doi: 10.1007/s00394-022-03015-6.
15. Gulec S, Collins JF. Silencing the Menkes copper-transporting ATPase (Atp7a) gene in rat intestinal epithelial (IEC-6) cells increases iron flux via transcriptional induction of ferroportin 1 (Fpn1). J Nutr. 2014;144(1):12-9. doi: 10.3945/jn.113.183160.
16. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World J Gastroenterol. 2012;18(42):6036-59. doi: 10.3748/wjg.v18.i42.6036.
17. Ludvigsson JF, Murray JA. Epidemiology of Celiac Disease. Gastroenterol Clin North Am. 2019;48(1):1-18. doi: 10.1016/j.gtc.2018.09.004.
18. Makharia GK, Singh P, Catassi C, Sanders DS, Leffler D, Ali RAR, et al. The global burden of coeliac disease: opportunities and challenges. Nat Rev Gastroenterol Hepatol. 2022;19(5):313-27. doi: 10.1038/s41575-021-00552-z.
19. Su GL, Ko CW, Bercik P, Falck-Ytter Y, Sultan S, Weizman AV, et al. AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders. Gastroenterology. 2020;159(2):697-705. doi: 10.1053/j.gastro.2020.05.059.
20. Seiler CL, Kiflen M, Stefanolo JP, Bai JC, Bercik P, Kelly CP, et al. Probiotics for Celiac Disease: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Am J Gastroenterol. 2020;115(10):1584-95. doi: 10.14309/ajg.0000000000000749.
21. Cristofori F, Indrio F, Miniello VL, De Angelis M, Francavilla R. Probiotics in Celiac Disease. Nutrients. 2018;10(12). doi: 10.3390/nu10121824.
22. Clemente MG, De Virgiliis S, Kang JS, Macatagney R, Musu MP, Di Pierro MR, et al. Early effects of gliadin on enterocyte intracellular signalling involved in intestinal barrier function. Gut. 2003;52(2):218-23. doi: 10.1136/gut.52.2.218.
23. Hollon J, Puppa EL, Greenwald B, Goldberg E, Guerrerio A, Fasano A. Effect of gliadin on permeability of intestinal biopsy explants from celiac disease patients and patients with non-celiac gluten sensitivity. Nutrients. 2015;7(3):1565-76. doi: 10.3390/nu7031565.
24. Orlando A, Linsalata M, Notarnicola M, Tutino V, Russo F. Lactobacillus GG restoration of the gliadin induced epithelial barrier disruption: the role of cellular polyamines. BMC Microbiol. 2014;14:19. doi: 10.1186/1471-2180-14-19.
25. Lindfors K, Blomqvist T, Juuti-Uusitalo K, Stenman S, Venäläinen J, Mäki M, et al. Live probiotic Bifidobacterium lactis bacteria inhibit the toxic effects induced by wheat gliadin in epithelial cell culture. Clin Exp Immunol. 2008;152(3):552-8. oi: 10.1111/j.1365-2249.2008.03635.x.
26. Silano M, Vincentini O, Luciani A, Felli C, Caserta S, Esposito S, et al. Early tissue transglutaminase-mediated response underlies K562(S)-cell gliadin-dependent agglutination. Pediatr Res. 2012;71(5):532-8. doi: 10.1038/pr.2012.4.
27. Sander GR, Cummins AG, Henshall T, Powell BC. Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins. FEBS Lett. 2005;579(21):4851-5. doi: 10.1016/j.febslet.2005.07.066.
28. Orlando A, Linsalata M, Bianco G, Notarnicola M, D'Attoma B, Scavo MP, et al. GG Protects the Epithelial Barrier of Wistar Rats from the Pepsin-Trypsin-Digested Gliadin (PTG)-Induced Enteropathy. Nutrients. 2018;10(11). doi: 10.3390/nu10111698.
29. Bhat MI, Sowmya K, Kapila S, Kapila R. Potential Probiotic Lactobacillus rhamnosus (MTCC-5897) Inhibits Escherichia coli Impaired Intestinal Barrier Function by Modulating the Host Tight Junction Gene Response. Probiotics Antimicrob Proteins. 2020;12(3):1149-60. doi: 10.1007/s12602-019-09608-8.
30. Anderson RC, Cookson AL, McNabb WC, Park Z, McCann MJ, Kelly WJ, et al. Lactobacillus plantarum MB452 enhances the function of the intestinal barrier by increasing the expression levels of genes involved in tight junction formation. BMC Microbiol. 2010;10:316. doi: 10.1186/1471-2180-10-316.
31. Herrán AR, Pérez-Andrés J, Caminero A, Nistal E, Vivas S, Ruiz de Morales JM, et al. Gluten-degrading bacteria are present in the human small intestine of healthy volunteers and celiac patients. Res Microbiol. 2017;168(7):673-84. doi: 10.1016/j.resmic.2017.04.008.
32. Greco L, Gobbetti M, Auricchio R, Di Mase R, Landolfo F, Paparo F, et al. Safety for patients with celiac disease of baked goods made of wheat flour hydrolyzed during food processing. Clin Gastroenterol Hepatol. 2011;9(1):24-9. doi: 10.1016/j.cgh.2010.09.025.
33. Francavilla R, De Angelis M, Rizzello CG, Cavallo N, Dal Bello F, Gobbetti M. Selected Probiotic Lactobacilli Have the Capacity to Hydrolyze Gluten Peptides during Simulated Gastrointestinal Digestion. Appl Environ Microbiol. 2017;83(14). doi: 10.1128/AEM.00376-17.

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