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ORIGINAL ARTICLE

Tight junctions expression is affected by active, inactive, and derivatives of *Akkermansia muciniphila*

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ABSTRACT

Background and Objectives: Tight junctions (TJs) in the gastrointestinal tract are comprised of various junctional proteins including Occludin and Zonula Occludens (ZO-1) that have a critical role in epithelial barrier function. Gut microbiota and their derived metabolites can maintain and regulate gut epithelial barrier integrity.

Materials and Methods: In the present study, the effects of active, heat-inactivated, cell-free supernatant, and outer membrane vesicles (OMVs) of *Akkermansia muciniphila* were evaluated on the expression of *occludin* and ZO-1 genes in Caco-2 cell line by quantitative real-time PCR.

Results: Data have shown that both forms of the active (metabolically active, growing, and dividing state), and heat inactivated (by exposure to 56°C for 20 minutes) forms of the bacteria and the cell-free supernatant could affect the expression of *occludin* and ZO-1 genes ($P < 0.05$). OMVs significantly increased the expression of the *occludin* gene but had no effects on the expression of ZO-1.

Conclusion: *Akkermansia muciniphila* and its derived metabolites might have the potential to be used in the pharmaceutical and medicinal fields as probiotic, paraprobiotic and postbiotic agents to prevent metabolic and inflammatory diseases; Although, further research is needed to understand their interactions within the complex gut microbiome and to evaluate potential side effects or risks associated with their use.

Keywords: *Akkermansia muciniphila*; Tight junctions; Zonula occludens-1; Occludin

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INTRODUCTION

Human microbiota refers to a community of diverse microorganisms that are colonized in different organs of the body and have shown a symbiotic relationship with their host (1). They induce many beneficial effects on health and homeostasis of human body. The gastrointestinal tract (GIT) contains the largest number of microbiotas among the rest of the organs. Gut microbiota performs outstanding functions in the host body including vitamin, nutrition production, anti-inflammatory and anti-oxidant activities, pathogen protection, metabolic regulation, and gut barrier integrity (1, 2). The maintenance of the gut barrier integrity is a critical factor for supporting gastrointestinal and neurological health (1-3). Gut microbiota dysbiosis may lead to several metabolic (4, 5), gastrointestinal (4), cardiovascular and neurologic disorders which might be the consequence of leaky gut conditions (6, 7). In leaky gut syndrome, the structure and functions of the tight junction proteins such as Occludin and Zonula Occludens (ZO-1) are altered and may increase the permeability of the gut epithelial barrier (3, 6, 7).

Mucus layer is secreted by goblet cells (8) and serves as a selective barrier for transition of nutrients through epithelial cells. It also protects the epithelial tissue from mechanical damages, pathogenic factors and any harmful and/or toxigenic materials (8). There are numerous mucus colonizing bacteria which can decompose mucin as the carbon, nitrogen source (9) and produce short chain fatty acids (SCFAs) including butyrate, propionate and acetate which can activate signaling pathways followed by stimulation of immune and metabolic systems (10).

Among the mucus-colonizing bacteria, *Akkermansia muciniphila* is an anaerobic gram-negative bacterium that colonizes the mucus layer of the gastrointestinal tract and constitutes approximately 3-5% of the human gut microbiota. Its abundance is negatively correlated with metabolic disorders such as obesity, diabetes, and intestinal inflammation (11, 12). Maintaining gut barrier integrity is crucial for preventing these conditions, with tight junction proteins such as Zonula Occludens-1 (ZO-1) and Occludin playing essential roles. These proteins regulate paracellular permeability and contribute to epithelial defense mechanisms. Dysregulation of ZO-1 and occludin leads to increased gut permeability, often referred to as “leaky gut,” which is implicated in various metabolic and inflammatory diseases (13, 14). Therefore, investigating the effects of *A. muciniphila* and its derivatives on the expression of ZO-1 and *occludin* in intestinal cells is important to understand their potential role in preserving epithelial barrier function and supporting gut health.

Furthermore, in order to minimize the side effects of inflammatory and resistance of antibiotics, administration of postbiotics and paraprobiotics (11-14) is considered to substitute the alive bacteria which may exacerbate the inflammation conditions. Bacterial cell free supernatant and outer membrane vesicles (OMVs) represent the postbiotics and heat inactivated form of the bacterium considered as a paraprobiotic. All mentioned biotics (15) have shown efficient interactions with the host and could alleviated the symptoms of the disease (16-18).

In this study, we investigated and compared the effects of active, heat inactivated, and derivatives of *A. muciniphila* on the expression of Occludin and ZO-1 (regarded as tight junction proteins) in Caco-2 cells which represents the best model for studying tight junctions in intestinal cells (18).

MATERIALS AND METHODS

Bacterial culture. *Akkermansia muciniphila* (ATCC BAA-835), obtained from the DSMZ institute (German Collection of Microorganisms and Cell Cultures GmbH), was cultured using brain heart infusion (BHI) agar medium (Quelab, Canada) supplemented with 0.5% mucin-type III (Sigma-Aldrich, St. Louis, Missouri, USA), 5 μg/ml hemin, 1 μg/ml menadione (18), and 0.05% L-cysteine (19) in an anaerobic atmosphere containing 80% N2, 10% H2, and 10% CO2 at 37°C for 3 to 7 days as described previously (20). Macro-and microscopic examinations (gram staining) were done for primarily confirmation and the purity of *A. muciniphila* cultures was identified using a polymerase chain reaction (PCR) assay for 16s ribosomal ribonucleic acid (rRNA). (Primers sequences is shown in Table 1 (20). PCR conditions were as follows: initial denaturation at 95°C for 5 minutes; followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 45 seconds, and extension at 72°C for 60 seconds; with a final extension at 72°C for 10 minutes. The reaction was then held at 4°C.

Cell-free supernatant was separated from the above-mentioned bacterial culture at an optical den-

Table 1. Characteristics and sequences of primers used in qRT- PCR and *A. muciniphila* 16s rRNA primers sequences

Primer Name	Primer sequence	Product Size (bp)	Annealing Tm (°C)	Reference
A. muciniphila 16s rRNA	F: CAGCACGTGAAGGTGGGGAC	316	59	(26)
	R: CCTTGCGGTTGGCTTCAGA			
GAPDH	F: AACAGCCTCAAGATCATCAGCAA	120	55	(27)
	R: GATGGCATGGACTGTGGTCAT			
ZO-1	F: ACCAGTAAGTCGTCCTGATCC	128	55	(28)
	R: TCGGCCAAATCTTCTCACTCC			
occludin	F: GACTTCAGGCAGCCTCGTTAC	132	55	(29)
	R: GCCAGTTGTGTAGTCTGTCTCA			

sity of 1.5 in 600_{nm} after centrifuging in 8000 g (5 min), adjusting the pH to 7.4, and then filtering (2.22 nm). The collected supernatant was stored at -70°C until usage (20). All experiments were carried out in Pasteur institute of Iran.

OMVs isolation and confirmation. *A. muciniphila* bacteria were inoculated in above mentioned BHI broth medium (12×10^8 CFU/ml of bacteria = 4 McFarland). After overnight growth of bacteria (approximately OD 600_{nm} = 1.5), OMVs were isolated as follows sequentially: centrifugation at 6000 g at 4°C, twice washing of the pellets with phosphate-buffered saline (PBS), suspending of the pellets with 9% sodium chloride solution, centrifugation at 6000 g at 4°C for an hour, suspending of the pellets with ethylenediaminetetraacetic acid and sodium deoxycholate (Sigma Aldrich, USA) buffers, centrifugation at 20,000 g at 4°C for an hour, and finally twice ultracentrifugation at 125,000 g for 2 h. Isolated OMVs were stored in sucrose 3% at -70°C until usage (19).

The integrity, morphological characterization and size of OMVs were determined using transmission electron microscopy (TEM; PHILIPS EM 208, Netherlands). Firstly, Samples were adsorbed onto carbon-coated copper grids for 1-2 minutes, negatively stained with 2% phosphotungstic acid (pH 7.0), air-dried and examined at 100 kV. Vesicles integrity, size and morphology were assessed by measuring multiple individual particles using ImageJ software. The biological activity of OMVs was assessed indirectly by analyzing their effects on the expression of ZO-1 and *occludin* genes in Caco-2 cells using qRT-PCR.

OMVs were quantified by analyzing the protein content using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). Samples were mixed with SDS-PAGE loading buffer containing SDS and β-mercaptoethanol, then heated at 90-100°C for 10 minutes for proteins denaturation. The sample were run on a 4% stacking and a 12% resolving gel alongside pre-stained molecular weight markers. Electrophoresis was performed at a constant voltage, and gels were stained with Coomassie Brilliant Blue to visualize protein bands (20).

Cell culture. The human colorectal adenocarcinoma-derived Caco-2 cell line (IBRC C10094) was purchased from the Iranian Biological Resource Center, Tehran, Iran. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Bioidea, Iran) with 10% fetal bovine serum (FBS; Bioidea, Iran), 1% nonessential amino acids (Bioidea, Iran), and 1% penicillin–streptomycin (Bioidea, Iran), and incubated at a humidified atmosphere containing 5% CO2 at 37°C (21, 22). In the present study, Caco-2 cells were seeded at a density of 5×10^5 cells on six-well plates (Sorfa, China) and divided into 12 groups (20). The PBS group was treated with PBS. The sucrose group was treated with sucrose 3%. The BHI group was treated with BHI broth. Caco-2 cells were treated with PBS, sucrose, and BHI as vehicle, buffer, and medium controls, respectively, to account for potential effects of solvents and media. While appropriate, we acknowledge that additional biologically relevant controls could enhance the interpretation of treatment effects. MOI 10, 50, and 100 groups were infected by active bacteria at the multiplicity of infection (MOI) of 10, 50, and 100 ratios, respectively (i.e., 10, 50, and 100 bacteria per cell). The MOI values (10, 50, and 100) were selected based on previous studies involving *A. muciniphila* or its derivatives in intestinal epithelial cell models, where similar MOIs effectively modulated tight junction protein expression without cytotoxicity. Inactive 10, 50, and 100 groups were

infected by inactivated bacteria at the MOI of 10, 50, and 100 ratios, respectively (4, 19, 22, 23). OMV 50 and 100 groups were treated with 50 and 100 µg/mL OMVs, respectively. The supernatant group was treated with 7% (v/v [medium/cell-free supernatant]) cell-free supernatant. For the inactivation, bacteria were incubated at 56°C for 20 minutes (21, 24, 25). The PBS, sucrose, and BHI groups were considered as control groups. All groups were incubated at 37°C in the presence of 5% CO2 for 24 hrs.

RNA extraction and cDNA synthesis. Total RNA molecules from 12 groups were extracted using the RNX-Plus kit (Sinacolon, Tehran, Iran) according to the manufacturer's recommendation. Extracted RNA was treated by DNase for 1 h at 37°C to remove any trace of DNA. The quality and integrity of extracted RNA were evaluated by 2% agarose gel electrophoresis, where the presence of distinct 28S and 18S rRNA bands was considered indicative of intact RNA. Spectrophotometric analysis Nanodrop 2000 (Thermo Fisher Scientific, USA) was used to assess purity, and RNA samples with A260/A280 ratios between 1.8 and 2.0 were used for cDNA synthesis. Reverse transcription was carried out on one microgram of total RNA using a cDNA synthesis kit (Parstous, Tehran, Iran) as described by the manufacturer (19).

Quantitative real-time PCR. Real-time RT-PCR was run on a LightCycler®96 SW 1.1instrument (Roche, Germany) using SYBR Green PCR master mix (Parstous, Tehran, Iran). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a reference gene in order to normalize *occludin* and ZO-1 gene expression levels. The real-time program was as follows: initialization at 94°C for 10 min; amplification for 40 cycles with denaturation at 95°C for 15 s, annealing of each set of primers for 30 s, extension at 72°C for 30 s; and final extension at 72°C for 10 min. To determine the specificity of amplification, dissociation curve analysis was performed for all selected genes. Changes in the fold number were determined by calculating the $2^{-\Delta\Delta Ct}$. The sequence of primers has shown in Table 1.

Statistical analysis. Each experimental condition was tested in two independent biological replicates, with each sample analyzed in technical triplicates. Statistical analyses were performed using GraphPad Prism software (version 8.4.3; GraphPad Software Inc., San Diego, CA, USA). Comparisons between two groups were conducted using an independent sample t-test, and comparisons among more than two groups were assessed using one-way ANOVA followed by Tukey's multiple comparison test. A p-value less than 0.05 was considered statistically significant.

RESULTS

Bacterial confirmation. PCR analysis of 16s rRNA confirmed the presence of *A. municiphila* bacteria (Fig. 1). Columns 1 and 4 represented 16s rRNA specific band (316 bp), number 2 was 100 bp molecular weight marker and 3 was a negative control.

The protein content of the OMVs was confirmed using SDS-PAGE. In addition, TEM observation showed the presence of *A. municiphila*-derived OMVs with diameters ranging from 30 to 300 nm (Fig. 2).

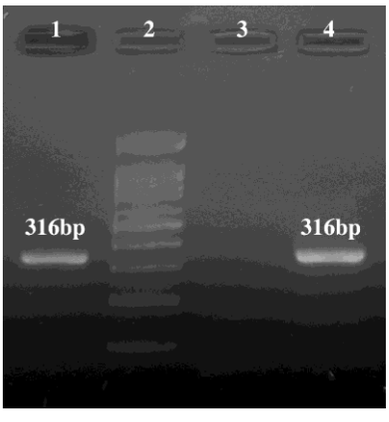

Fig. 1. PCR analysis of 16s rRNA of *A. municiphila* bacteria

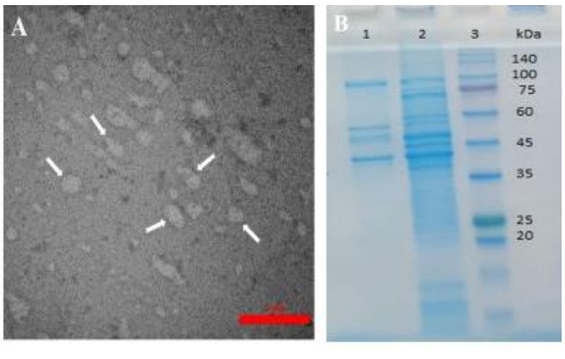

Fig. 2. A. TEM Imaging in x7000. OMVs (size range:30-300 nm) are shown by white arrows. B. SDS PAGE analysis of the protein content of *A. muciniphila* OMVS SDS-PAGE.(1) OMVs proteins (2) supernatant (3) molecular weight Protein Marker (20).

Real-time PCR analysis. As shown in Fig. 3, compared with the PBS control group, the mRNA levels of ZO-1 markedly increased at MOI 10 and MOI 50 (P=0.002 and P<0.0001, respectively) of the active form of the bacterium. In contrast, ZO-1 expression significantly decreased at MOI 50 of the inactivated form (P = 0.01). This divergence suggests that bacterial viability may play a critical role in modulating tight junction gene expression at this concentration. No significant changes in ZO-1 expression were observed at MOI 100 for either form, or at MOI 10 of the inactivated form. Our data also showed that OMVs at any tested concentration did not significantly alter ZO-1 mRNA levels compared with the sucrose control (P>0.05). However, cell-free supernatant at 7% concentration significantly increased ZO-1 expression in Caco-2 cells (P = 0.007) relative to the BHI control.

Regarding the *occludin* gene, MOI 10 of both active and inactive bacterial forms significantly increased mRNA levels compared with PBS (P=0.0005 and P<0.0001, respectively), while no significant changes were observed at MOIs 50 and 100 (P>0.05). OMVs at 50 µg/mL significantly increased *occludin* expression compared with the sucrose control (P=0.01), but the 100 µg/mL dose had no effect. Similarly, the cell-free supernatant at 7% significantly increased *occludin* gene expression (P = 0.005) compared to the BHI control group.

DISCUSSION

Gut microbiota has been mentioned as an effective factor in host metabolism and metabolic disruption regulation. Numerous Agents such as probiotics, prebiotics, postbiotics and even antimicrobial medicines are able to influence the host metabolism by modification of gut microbiota pattern (30). Mucus layer

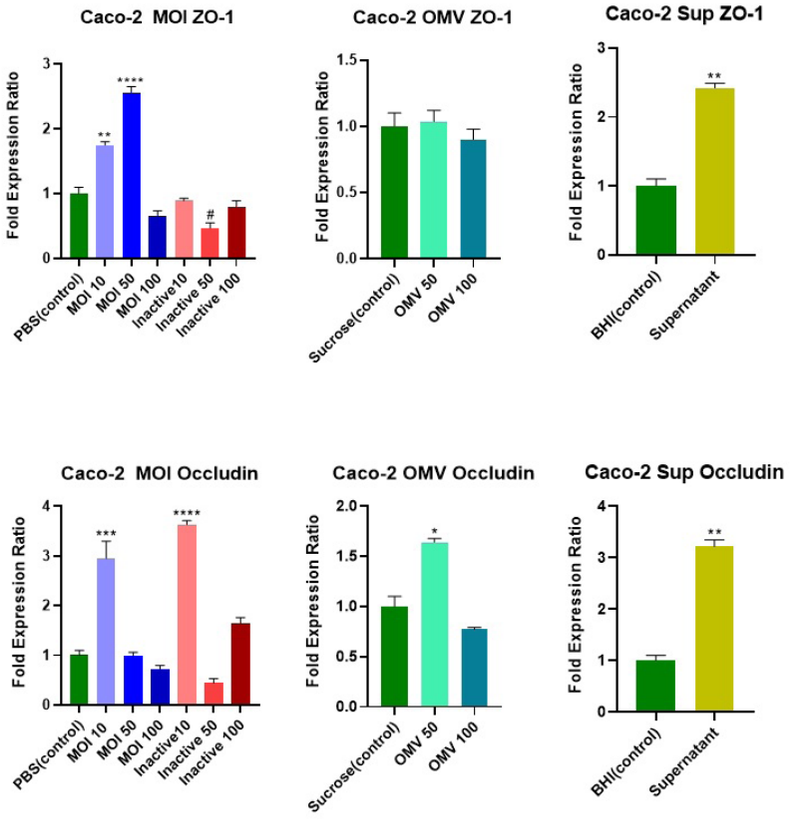

Fig. 3. Effects of active, inactive (MOIs 10, 50 and 100), and derivatives (50 and 100 100 µg/mL of OMVS and 7% cell free supernatant) of *A. muciniphila* on transcription level of ZO-1 and Occludin in Caco-2 cells. Significancy is evaluated in comparison with control. Data are shown as the mean ± SEM. (*) and (#) represent significant increase and decrease, respectively. */# P<0.05, **P<0.01, ***P< 0.001, and ****P<0.0001 by one-way ANOVA and t-test statistical analysis. MOI: multiplicity of infection, OMV: outer membrane vesicle, Supernatant: cell free supernatant.

with protective effect covering all over the gastrointestinal tract, has colonized bacteria -as an interaction site- which are able to use mucin as a carbon source (9). One of these mucin degrading bacteria is called *Akkermansia muciniphila* which is a gram-negative bacterium that includes 3-5% of the human gut microbiota (24). Evidence confirms that this bacterium is involved in regulation of gut barrier and it has role in metabolic and homeostatic procedures (1-4). In recent years, due to the introduction potential of this bacterium as a next generation probiotic, it has attracted more attentions (24). Since, on the basis of previous studies, the frequency of this bacterium is decreased in conditions such as obesity, diabetes, intestinal inflammation, and liver diseases. This can cause the alteration in gut barrier function and has been identified that alive and pasteurized forms of bacteria and their related Amuc-100 protein, revive the gut barrier function in a probable interaction with TLR-2 and re-expression of tight junction proteins (31). *A. muciniphila* is mostly colonized in gastrointestinal tract, and according to the previous studies, it was observed that under leaky gut conditions, the probable harmful effect of this bacterium was substituted and inversed by its OMVs treatment; in a way that extracted OMVs could alleviate the intensity of the disease (16, 32). In conditions of leaky gut, the bacterium and its derivatives might transmit through gut barrier and enter the other organs (4).

In 2008, Cani et al. brought up the probability that changes in repartition and colonization of two tight junction proteins called Zonula Occludens (ZO-1) and Occludin in the intestinal tissue is related to more permeability of rodents GIT suffering from obesity and diabetes (33). In 2010, Muccioli et al. reported that the gut permeability is affected by the endocannabinoid system function and activation of CB1 receptor causes more gut permeability which can induce obesity and diabetes. They also found that the inhibition of CB1 receptor is correlated with improvement in distribution and colonization of tight junction proteins (specially ZO-1 and Occludin) and gut barrier integrity. The results obtained from gut permeability control by CB1 receptor, was representative of a new ECB mechanism in pathogenesis of inflammation related to obesity (systemic and hepatic) (34). Ashrafian et al., in their studies from 2019 to 2021, reported that active *A. muciniphila*, particularly its heated, pasteurized form and its outer membrane vesicles (OMVs), positively affect the expression of ZO-1 and *occludin* genes, thus promoting gut barrier stability (24, 35). In a similar study in 2018, Chelakkot et al. and their team found that *A. muciniphila*-extracted OMVs increased the expression of tight junction proteins, decreased body weight, and improved metabolic functions (4). Plovier et al. (2017) also declared that *A. muciniphila* could regulate tight junction proteins, such as Occludin and Claudin-3, and affect TLR2, leading to improved gut barrier integrity and metabolic functions (12). In this study, the effects of *A. muciniphila* and its derivatives on the expression of ZO-1 and *occludin* were investigated. Results indicated that the active form of the bacterium, at MOI 10 and 50, increased the transcription level of ZO-1. Furthermore, a 7% concentration of cell-free supernatant enhanced the mRNA level of this gene. It was noticeable that MOI 50 of the heat inactivated form of the bacterium reduced the expression of the mentioned gene. In a parallel study, we investigated the effects of mentioned bacterium and its derivatives on the expression of endocannabinoid associated genes namely CB1, CB2 receptors, and fatty acid amide hydrolase (FAAH). We observed that the heat inactivated form of the bacterium could increase the mRNA level of both CB1 and CB2 genes which was probably due to more translocation of bacterial metabolites, immune system activation and inflammation condition (20). Heat-inactivated bacteria, particularly at an MOI of 50, upregulate the transcription levels of CB1 and CB2 genes. This upregulation may induce the downregulation of ZO-1 expression, possibly through the increased mRNA levels of these receptors, which are influenced by the microbial associated molecular patterns (MAMPs) of the bacterium. According to the obtained results of *occludin* gene shown in Fig. 3, both active and heat inactivated forms of the bacterium could increase the transcription level of this gene in MOI 10. These results suggest that the application of heat-inactivated bacteria, which enhances *occludin* mRNA levels, may reduce gut permeability and its associated consequences, thereby improving gut barrier integrity and potentially modulating IBS. In contrast, 50 μg/mL concentrations of OMVs and a 7% (v/v) concentration of cell-free supernatant positively affected the expression of this gene, supporting the use of this bacterium and its derivatives as pro-, para-, and postbiotics in various treatments. The multiplicity of infection (MOI) values used in this study (10, 50, and 100) were selected based on

previous research demonstrating that similar concentrations of *A. muciniphila* and its derivatives can effectively modulate tight junction protein expression in intestinal epithelial cell models without inducing cytotoxicity (4, 23, 24). Although our study did not focus on dose-dependency, these MOIs were sufficient to reveal differences in the expression of ZO-1 and *occludin*, allowing for comparison of biological responses across treatments. Notably, although both MOI 10 and MOI 50 of the active bacterium significantly upregulated ZO-1 expression, the inactivated form at MOI 50 led to a significant downregulation. This apparent contradiction may reflect the influence of bacterial viability on tight junction regulation, possibly due to differences in surface protein conformation, metabolic products, or interaction with host pattern recognition receptors. Previous studies have also noted that heat inactivation can modulate the host response differently from live bacteria, likely due to structural or functional alterations (4, 19, 24). These findings emphasize the importance of considering bacterial viability when evaluating their impact on intestinal barrier function.

Although OMVs were characterized based on size, protein content, and TEM morphology, and their biological activity was demonstrated via changes in tight junction gene expression, further functional analyses such as surface protein profiling, membrane integrity assays, and immunological evaluations are warranted in future studies to fully elucidate their mechanistic roles and therapeutic potential. This study was conducted in vitro using Caco-2 cells, which, while widely used, may not fully reflect the complexity of the in vivo intestinal environment. Moreover, although gene expression changes were assessed, corresponding protein-level evaluations and functional permeability assays were not included and should be considered in future research. Further in vivo validation and mechanistic studies are also recommended to confirm and expand upon these findings.

REFERENCES

1. Hugon P, Lagier J-C, Colson P, Bittar F, Raoult D. Repertoire of human gut microbes. *Microb Pathog* 2017; 106: 103-112.
2. Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? *Diabetes Care* 2010; 33: 2277-2284.
3. Vajro P, Paolella G, Fasano A. Microbiota and gut-liver axis: a mini-review on their influences on obesity and obesity-related liver disease. *J Pediatr Gastroenterol Nutr* 2013; 56: 461-465.
4. Chelakkot C, Choi Y, Kim D-K, Park HT, Ghim J, Kwon Y, et al. *Akkermansia muciniphila*-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. *Exp Mol Med* 2018; 50(2): e450.
5. Karl JP, Margolis LM, Madslien EH, Murphy NE, Castellani JW, Gundersen Y, et al. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *Am J Physiol Gastrointest Liver Physiol* 2017; 312: G559-G571.
6. Forsythe P, Kunze W, Bienenstock J. Moody microbes or fecal phrenology: what do we know about the microbiota-gut-brain axis? *BMC Med* 2016; 14: 58.
7. Teixeira TF, Collado MC, Ferreira CL, Bressan J, Peluzio Mdo C. Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutr Res* 2012; 32: 637-647.
8. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009; 61: 75-85.
9. Sundin J, Aziz I, Nordlander S, Polster A, Hu YOO, Hugerth LW, et. al. Evidence of altered mucosa-associated and fecal microbiota composition in patients with Irritable Bowel Syndrome. *Sci Rep* 2020; 10: 593.
10. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; 461: 1282-1286.
11. Dao MC, Everard A, Aron-Wisnewsky J, Sokolovska N, Prifti E, Verger EO, et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* 2016; 65: 426-436.
12. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017; 23: 107-113.
13. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. *Physiol Rev* 2011; 91: 151-175.
14. Suzuki T. Regulation of intestinal epithelial permeability by tight junctions. *Cell Mol Life Sci* 2013; 70: 631-659.
15. Wegh CA, Geerlings SY, Knol J, Roeselers G, Belzer C. Postbiotics and their potential applications in early life nutrition and beyond. *Int J Mol Sci* 2019; 20: 4673.
16. Ahmadi Badi S, Moshiri A, Fateh A, Rahimi Jamnani F, Sarshar M, Vaziri F, et al. Microbiota-derived ex-

tracellular vesicles as new systemic regulators. *Front Microbiol* 2017; 8: 1610.
17. Moradi M, Tajik H, Mardani K, Ezati P. Efficacy of lyophilized cell-free supernatant of *Lactobacillus salivarius* (Ls-BU2) on *Escherichia coli* and shelf life of ground beef. *Vet Res Forum* 2019; 10: 193-198.
18. Elhenawy W, Debelyy MO, Feldman MF. Preferential packing of acidic glycosidases and proteases into *Bacteroides* outer membrane vesicles. *mBio* 2014; 5(2): e00909-14.
19. Vaezijoze S, Irani S, Siadat SD, Zali M. Modulation of satiety hormones by *Bacteroides thetaiotaomicron, Bacteroides fragilis* and their derivatives. *AMB Express* 2025; 15: 41.
20. Ghaderi F, Sotoodehnejadnematalahi F, Hajebrahimi Z, Fateh A, Siadat SD. Effects of active, inactive, and derivatives of *Akkermansia muciniphila* on the expression of the endocannabinoid system and PPARs genes. *Sci Rep* 2022; 12: 10031.
21. Ashrafian F, Shahriary A, Behrouzi A, Moradi HR, Keshavarz Azizi Raftar S, Lari A, et al. *Akkermansia muciniphila*-derived extracellular vesicles as a mucosal delivery vector for amelioration of obesity in mice. *Front Microbiol* 2019; 10: 2155.
22. Yaghoubfar R, Behrouzi A, Zare Banadkoki E, Ashrafian F, Lari A, Vaziri F, et al. Effect of *Akkermansia muciniphila, Faecalibacterium prausnitzii,* and their extracellular vesicles on the serotonin system in intestinal epithelial cells. *Probiotics Antimicrob Proteins* 2021; 13: 1546-1556.
23. Noori P, Sotoodehnejadnematalahi F, Rahimi P, Siadat SD. *Akkermansia muciniphila* and its extracellular vesicles affect endocannabinoid system in in vitro model. *Digestion* 2025; 106: 338-348.
24. Ashrafian F, Behrouzi A, Shahriary A, Ahmadi Badi S, Davari M, Khatami S, et al. Comparative study of effect of *Akkermansia muciniphila* and its extracellular vesicles on toll-like receptors and tight junction. *Gastroenterol Hepatol Bed Bench* 2019; 12: 163-168.
25. Chatterjee D, Chaudhuri K. Association of cholera toxin with Vibrio cholerae outer membrane vesicles which are internalized by human intestinal epithelial cells. *FEBS Lett* 2011; 585: 1357-1362.
26. Zhai R, Xue X, Zhang L, Yang X, Zhao L, Zhang C. Strain-specific anti-inflammatory properties of two *Akkermansia muciniphila* strains on chronic colitis in mice. *Front Cell Infect Microbiol* 2019; 9: 239.
27. Ebnerasuly F, Hajebrahimi Z, Tabaie SM, Darbouy M. Effect of simulated microgravity conditions on differentiation of adipose-derived stem cells towards fibroblasts using connective tissue growth factor. *Iran J Biotechnol* 2017; 15: 241-251.
28. Yang C, Yao C, Tian R, Zhu Z, Zhao L, Li P, et al. miR-202-3p regulates Sertoli cell proliferation, synthesis function, and apoptosis by targeting LRP6 and cyclin D1 of Wnt/β-catenin signaling. *Mol Ther Nucleic Acids* 2019; 14: 1-19.
29. Cen J, Feng L, Ke H, Bao L, Li LZ, Tanaka Y, et al. Exosomal thrombospondin-1 disrupts the integrity of endothelial intercellular junctions to facilitate breast cancer cell metastasis. *Cancers (Basel)* 2019; 11: 1946.
30. Hur KY, Lee M-S. Gut microbiota and metabolic disorders. *Diabetes Metab J* 2015; 39: 198-203.
31. Cani PD, de Vos WM. Next-generation beneficial microbes: the case of *Akkermansia muciniphila*. *Front Microbiol* 2017; 8: 1765.
32. Kang C-S, Ban M, Choi E-J, Moon H-G, Jeon J-S, Kim D-K, et al. Extracellular vesicles derived from gut microbiota, especially *Akkermansia muciniphila*, protect the progression of dextran sulfate sodium-induced colitis. *PLoS One* 2013; 8(10): e76520.
33. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet–induced obesity and diabetes in mice. *Diabetes* 2008; 57: 1470-1481.
34. Muccioli GG, Naslain D, Bäckhed F, Reigstad CS, Lambert DM, Delzenne NM, et al. The endocannabinoid system links gut microbiota to adipogenesis. *Mol Syst Biol* 2010; 6: 392.
35. Ashrafian F, Keshavarz Azizi Raftar S, Shahryari A, Behrouzi A, Yaghoubfar R, Lari A, et al. Comparative effects of alive and pasteurized *Akkermansia muciniphila* on normal diet-fed mice. *Sci Rep* 2021; 11: 17898.