

Time and strain-specific downregulation of intestinal *EPAS1* via miR-148a by *Bifidobacterium bifidum*

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ABSTRACT

Scope: Bifidobacteria play a role in intestinal homeostasis but molecular mechanisms remain under-investigated. The aim of this study was to assess if probiotic *Bifidobacterium* strains alter expression of intestinal microRNA and downstream target gene response.

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Methods and results: The expression of miR-148a and its validated target *EPAS1* (endothelial PAS domain protein 1) was analyzed in Caco-2 cells and mice cecum in response to *Bifidobacterium bifidum* MIMBb75, *B. bifidum* NCC390 or *B. longum* NCC2705. *In vitro*, exposure to *B. bifidum* MIMBb75, but not to *B. bifidum* NCC390 or *B. longum* NCC2705, increased the expression of miR-148a after 1 and 4 hours ($P < 0.01$), but not after 24h. *In vivo*, *B. bifidum* MIMBb75 administration to C57BL/6J mice increased miR-148a expression in the cecum after 2 but not 14 days ($P < 0.05$). The increase of miR-148a was accompanied by a decrease of *EPAS1* expression in Caco-2 cells and cecum ($P < 0.05$). Silencing of miR-148a reversed *B. bifidum* MIMBb75-dependent downregulation of *EPAS1*.

Conclusion: This study shows an early response of intestinal cells to *B. bifidum* MIMBb75 through miR-148a modulation. This brings a new concept of strain- and time-dependent bifidobacteria-host crosstalk via microRNA. Probiotic *B. bifidum* MIMBb75 may help attenuating *EPAS1* overexpression associated with intestinal inflammation.

1 Introduction

Bifidobacteria are autochthonous inhabitants of human and animal intestinal tracts, representing 80 and 25 % of the infant and adult microbiota, respectively (Reviewed in [1]). This genus comprises widely used probiotics whose benefits are either considered common probiotic core benefits, such as their ability to improve gut microbiota composition [2, 3] and inhibit pathogen adhesion [4], or are strain specific, particularly, their capability to modulate the host-immune system [5]. This is in line with the ability of bifidobacteria to elicit host responses in a species- and strain- dependent manner [5-7]. *Bifidobacterium bifidum* MIMBb75 is a probiotic strain isolated from healthy human feces [8], that resides in cecal-

colonic regions and modulates the composition of the gut microbiota in mice [9]; in a double-blind placebo-controlled study, it was shown to significantly reduce irritable bowel syndrome (IBS) symptoms and improve general quality of life [10]. *B. bifidum* MIMBb75 has stronger ability to adhere to intestinal cells *in vitro* compared to other probiotics such as *B. longum* NCC2705 and *Lactobacillus rhamnosus* GG [11]. *B. bifidum* MIMBb75 possesses several surface proteins that could enable interaction with host cells. These include the bifidobacterial outer protein (BopA), likely involved in the adhesion process [8], and the murine lytic enzyme TgaA, expressed on the outer surface of *B. bifidum* MIMBb75 cells and found to activate dendritic cells and trigger IL-2 production *in vitro* [12, 13]. However, the response of the host to *B. bifidum* MIMBb75 remains largely uncharacterized, particularly at the molecular level. Gene expression regulation is a main mechanism underpinning the continuous interaction between intestinal tissues and microbiota. The coexistence of prokaryotic and eukaryotic cells in the intestine results in a microbiota-dependent gene expression program in host cells [14, 15]. Not surprisingly, this is affected by probiotic administration, including bifidobacteria [16]. We and others have shown that the intestinal microbiota-dependent gene regulation may occur at the post-transcriptional level, via microRNA, in colon and cecum [17, 18]. MicroRNAs (miRNAs) are 20-22 nucleotides long, non-coding RNAs regulating more than 60% of the human transcriptome [19]. MiRNAs have emerged as fine-tuners of host gene expression contributing to intestinal homeostasis (Reviewed in [20]). Like mRNA, miRNAs are expressed in a time-dependent manner [21, 22], acting as a response timer to a variety of stimuli. The time-specific response can happen early (within a few hours) such as in gastric epithelial cells exposed to *Helicobacter pylori* (6 hours) [23] or can be delayed (within days), such as in T lymphocytes where miR146a levels were increased after 8 days to stimulation with PMA (phorbol myristate acetate) and

ionomycin, reaching their highest level of expression after 20 days [24]. MiRNA responses to commensal or probiotic bacteria have not been largely investigated. We found that the presence of the microbiota impacts the intestinal miRNA signature including expression of miR-148a [18]. MiR-148a is a member of the *miR-148/152* family (reviewed in [25]) known to play an important role in the regulation of the immune response [26]. Here, we studied the impact of probiotic *B. bifidum* MIMBb75 on intestinal expression of miR-148a and the downstream target gene response *in vitro* and *in vivo*.

2 Materials and methods

Bifidobacteria

B. bifidum MIMBb75, *B. bifidum* NCC390 and *B. longum* NCC2705 were grown overnight at 37°C in MRS broth (Difco, Detroit, MI, USA) supplemented with 0.05% L-cysteine hydrochloride (cMRS) in an anaerobic chamber (Coy Laboratory Products; Grass Lake, MI, USA) containing an atmosphere of 90% N₂, 5% H₂ and 5% CO₂. Prior to use, cells were harvested at 3000 × g for 10 min, washed twice with phosphate-buffered saline (PBS) and quantified microscopically using Neubauer Bright-Line Hemacytometer (Hausser Scientific, Horsham, PA) and by plating on cMRS Agar.

Caco-2 cell culture, co-incubation and transfection experiments

Caco-2 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and were routinely cultured at 37°C in Dulbecco's modified Eagle's medium (DMEM), as previously described [8]. Prior to co-incubation with *Bifidobacterium* strains, the cells were seeded on 6-well culture plates and grown for two weeks post-confluency [8]. The fully differentiated Caco-2 monolayers were washed with PBS buffer and incubated in parallel with each of the test strain re-suspended in DMEM vehicle without antibiotics, supplemented with 100 mM HEPES (GIBCO, Life Technologies, Carlsbad, CA, USA) at

bacteria:cell ratio of 10:1 for 1, 4 and 24h. Vehicle alone was used as a control. After incubation, cells were washed with PBS, scraped off the bottom of the wells, snap-frozen in liquid nitrogen and stored at -80°C until RNA extraction. Cell viability was determined by Trypan Blue exclusion test (GIBCO, Life Technologies, Carlsbad, USA), whereas bacterial viability was evaluated by plating on cMRS Agar both at the time of addition and after incubation with the cells. Experiments were conducted in triplicates.

For transfection with miRNA inhibitors, Caco-2 cells were seeded on 6-well culture plates at a density of 10^6 cell/well to reach 60 to 80% of confluency at the time of transfection. Cells were transfected with 10 nM of mirVana miR-148a inhibitor (Assay ID: MH10263), mirVana let-7c miRNA inhibitor (positive control, Assay ID: 4464080) (Invitrogen, Life Technologies, Carlsbad, USA), or corresponding vehicle alone (Mock) using lipofectamine RNAiMAX transfection agent and Opti-MEMI reduced serum medium (Invitrogen, life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. The transfection efficiency was evaluated with Block-iT Fluorescent Oligo (Invitrogen, Life Technologies, Carlsbad, USA) according to the manufacturer's protocol. At 72 h post-transfection, miR-148a inhibitor- or mock- transfected cells were incubated with either *B. bifidum* MIMBb75, prepared as described above, at bacteria:cell ratio of 10:1 or vehicle alone, for 1, 4 and 24h.

In vivo probiotic studies

Two studies were conducted. The first, which has been partially described in [9], used twenty eight 5–6 weeks old C57BL/6J male mice purchased from Jackson's Laboratory (Sacramento, CA, USA) and housed at the Division of Comparative Medicine, University of Toronto. After one week acclimatization, mice were randomized into two groups (n=14/group) to receive 0.2 ml of PBS containing 10^8 colony forming units (CFU) of *B.*

bifidum MIMBb75 cells or PBS only daily for 2 or 14 days, via intragastric gavage. The second study used twelve C57BL/6J male mice at the same age and from the same origin as above. Mice received either *B. bifidum* MIMBb75 or PBS for 2 days (n=4-8/group). Viability and quantity of *B. bifidum* MIMBb75 used for gavage were confirmed by plating on cMRS Agar. Cecal tissues and contents were collected immediately after dissection on ice and stored at -80°C. All animal experiments were approved by the animal ethics committee at the University of Toronto (Protocol number: 20010228). Microbiota composition analysis was by qPCR using TaqMan assays (Supporting Information Table S1), as we previously described [9].

RNA extraction and real-time quantitative PCR

Total RNA was isolated from Caco-2 cells and mouse cecum tissues using the *mirVana*TM miRNA isolation kit (Ambion, Life Technologies, Carlsbad, USA), as per the manufacturer's instructions. miRNA and mRNA expression analysis was performed as we described previously [18] (miR-148a, Assay ID: 000470; *EPAS1*, Assay IDs: Hs01026149_m1 and Mm01236112_m1 for Caco-2 cells and mouse cecum, respectively). MiRNA gene expression data were normalized to the endogenous controls RNU6B (Assay ID: 001093) and sno-135 (Assay ID: 001230) for Caco-2 cells and mouse cecum, respectively. Gene expression was normalized to *ACTB* (Assay IDs: Hs01060665_g1 and Mm02619580_g1 for Caco-2 cells and mouse cecum, respectively). Fold changes were calculated using the $2^{-\Delta\Delta Ct}$ method [27].

Identification of microRNA targets

Putative and experimentally validated targets of miR-148a were identified *in silico* using the miRWalk database which integrates seven different algorithms [28].

Statistical analysis

Statistical significance of miRNA and mRNA expression in Caco-2 cells was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Significance of differential miRNA and gene expression mouse cecum was assessed with the Mann-Whitney test. GraphPad Prism software V5.0 (La Jolla, CA, USA) was used for all statistical analyses and values of $p < 0.05$ were considered significant. Data are presented as mean \pm SD.

3 Results

Bifidobacteria modify miR-148a expression in a strain- and time- dependent manner in vitro

To investigate the miRNA response to bifidobacteria in intestinal cells, Caco-2 cells were incubated for 1, 4 or 24 hours with *B. bifidum* MIMBb75 or *B. bifidum* NCC390 or *B. longum* NCC2705. Caco-2 cells viability was $\geq 93\%$ and viable counts of the three strains were in the range of 2 to 9×10^7 CFU/well at all time points (data not shown). *B. bifidum* MIMBb75 elicited an increase in miR-148a expression after 1 and 4 hours (3.5 ± 1.3 and 2.7 ± 0.7 fold changes, respectively, compared to the control, $P < 0.01$) but not after 24 hours. This increase was more pronounced at the earliest time point. MiR-148a did not change in response to *B. bifidum* NCC390 and to *B. longum* NCC2705 (Figure 1).

B. bifidum MIMBb75 modifies intestinal miR-148a expression in vivo

To determine whether *B. bifidum* MIMBb75 modulates host intestinal miRNA expression *in vivo*, we first used tissue samples from a study we had previously conducted, where mice were daily gavaged with 10^8 CFU of *B. bifidum* MIMBb75 or PBS (as a control) for 2 or 14 days [9]. In the cecum of mice receiving probiotic, *B. bifidum* counts corresponded to 5.4 ± 0.3 log CFU/g of contents at day 2, while *C. coccoides* levels were significantly decreased compared to the PBS gavaged mice (8.2 ± 0.1 versus 7.2 ± 0.7 log CFU/g of contents,

P=0.01) (Supporting Information Figure S1); other taxa known to be represented in the gut microbiota, including *C. leptum* group, were not affected (data not shown). Bacterial counts at day 14 were previously published and showed the same pattern with significantly increased *B. bifidum* counts and decreased *C. coccoides* levels in the cecum of mice receiving probiotic [9]. *B. bifidum* counts increased after 14 days of continuous administration; there was no within group difference between *C. coccoides* counts at the two time points. The caecal expression of miR-148a increased after two days of probiotic treatment (1.4 ± 0.1 fold change, P=0.002) but not after 14 days (0.9 ± 0.3 fold change) (Figure 2 A). Data at day 2 were confirmed in a second independent study showing *B. bifidum* counts of 6.0 ± 0.4 log CFU/g of cecum content and a significant decrease of *C. coccoides* counts in mice receiving probiotics compared to the control (6.0 ± 0.4 versus 4.4 ± 0.2 log CFU/g of contents, P=0.001) (Supporting Information Figure S1). MiR-148a expression was significantly increased (1.7 ± 0.2 fold change, P=0.005) (Figure 2 B). These data follow a similar pattern as in Caco-2 cells where the expression of host miR-148a is altered in response to *B. bifidum* MIMBb75 administration with an early response that differs from that at later time-points.

Identification of miR-148a targets

To address the role of miR-148a in host intestinal response to probiotic *B. bifidum* MIMBb75, its gene targets were identified using seven different algorithms. Over 1760 predicted putative targets for miR-148a were found by at least five of these (Supporting Information Table S2); 78 experimentally validated targets were identified including *EPAS1* (Supporting Information Table S3). This gene encoding for endothelial PAS domain protein 1 (also known as Hypoxia-inducible factor-2 alpha, HIF-2 α), was identified by five of the seven interrogated algorithms. Previous studies using mesenchymal stem cells had

experimentally verified that miR-148a controls the expression of *EPAS1* [29]. Thus, this gene was selected for additional investigation *in vitro* and *in vivo*.

MiR-148a impacts expression of EPAS1 in intestinal epithelial cells exposed to B. bifidum MIMBb75a

To determine if miR-148a regulates the expression of its target gene *EPAS1* in Caco-2 cells in response to *B. bifidum* MIMBb75, miR-148a was inhibited for 3 days. Then, miR-148a-inhibited or mock transfected Caco-2 cells were incubated with *B. bifidum* MIMBb75 for 1, 4 and 24 hours and miR-148a and *EPAS1* expression levels were measured by RT-qPCR. To ensure the miRNA inhibition was successful, miR-148a expression levels were also determined in intestinal cells transfected with miR-148a-inhibitor and incubated in the presence of vehicle alone (DMEM without antibiotics, supplemented with 100 mM HEPES) for 1, 4 and 24 hours. Silencing of miR-148a was efficient since cells transfected with miR-148a inhibitor had a 50 % decrease in miR-148a expression compared to control (miR-148a inhibitor) (Figure 3). In cells transfected with miR-148a inhibitor and co-incubated with *B. bifidum* MIMBb75 (miR-148a inh+BB75), silencing was overcome at 4 and 24 hours, showing the ability of *B. bifidum* MIMBb75 to increase the expression of miR-148a. In line with experiments in Figure 1, miR-148a increased after 1 and 4 hours of co-incubation of mock-transfected cells with *B. bifidum* MIMBb75 (BB75 only).

As shown in Figure 4A, inhibition of miR-148a significantly affected *EPAS1* expression compared to the control (miR-148a inhibitor), with more than 200% increase in gene expression in the miR-148a silenced group in response to the probiotic strain at 4 hours (miR-148a inh+BB75) ($P < 0.001$). This gene was also found to be significantly downregulated in the cecum of mice gavaged with *B. bifidum* MIMBb75 for 2 days (0.8 ± 0.1 fold change,

P=0.004) (Figure 4B). This indicates that down-regulation of *EPAS1* gene expression by *B. bifidum* MIMBb75 is mediated by miR-148a.

4 Discussion

In this study, we showed that the expression of miR-148a is altered in intestinal cells in response to bifidobacteria in a strain and time-dependent manner. In particular, exposure to *B. bifidum* MIMBb75 induces an early upregulation of miR-148a both *in vitro* and *in vivo*, which disappears at later time points. Very few studies have investigated the effect of selected probiotic on miRNA expression. *L. rhamnosus* GG was found to increase the expression of miR-155 and decrease miR-146a in human dendritic cells [30]. *Escherichia coli* Nissle 1907 increased tight junction protein expression in T84 cells via miR-595, miR-483 and miR-203 downregulation [31]. Heat-treated *L. paracasei* NCC 2461 downregulated miR-27a in peripheral blood mononuclear cells [32]. *Enterococcus faecium* NCIMB 10415 upregulated miR-423-5p and downregulated its target gene *IGLC* (immunoglobulin lambda light C region) in the intestine of piglets [33]. These studies show that different probiotics elicit different miRNA responses. Though, none of them examined the effects of different probiotics on the same miRNA species. We found that, opposite to *B. bifidum* MIMBb75, *B. longum* NCC2705 and *B. bifidum* NCC390 did not affect miR-148a expression. The molecular mechanisms behind this strain-specific response are not clear. Recently, *B. bifidum* MIMBb75 whole genome sequencing revealed the presence of a gene named *tgaA* coding for a murein lytic enzyme TgaA [12]. This peptidoglycan-degrading enzyme was shown to exert an immunomodulatory effect by inducing DCs activation and IL-2 production through its C-terminal CHAP (cysteine histidine-dependent amidohydrolase/peptidase) domain in a strain-dependent manner, with *B. bifidum* MIMBb75 showing the strongest effect [13]. Therefore, it is tempting to speculate that miRNA-mediated *B. bifidum* MIMBb75-host

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interaction depends on the CHAP domain of TgaA. Two extracellular proteins encoded by *B. bifidum*, the sortase-dependent pili and the type IV tight adherence locus have been shown to mediate its interaction with the host [34-36]. However, opposite to TgaA, these proteins are encoded by genes identified in *B. bifidum* core genome [37], and are thus unlikely to explain strain specific responses. Microbial metabolites such as short chain fatty acids may regulate host gene expression epigenetically [38] offering a possible alternate mechanism for host miRNA modulation. The microbiota is a modifier of the intestinal miRNA response to pathogen exposure, in that the expression of miR-148a was reduced in the intestine of conventional, but not germ-free mice, infected with *Listeria monocytogenes* [39]. *B. bifidum* MIMBb75 modifies the microbiota, resulting in increased bifidobacteria and decreased *C. coccoides* counts (this study and [9]). Though, this is already evident at day 2, with no differences between day 2 and 14. Thus, there could be a “direct” effect of *B. bifidum* MIMBb75 which is not mediated by the microbiota. Though, given its complexity and because the effect of *B. bifidum* MIMBb75 administration on its composition and activity have not been completely elucidated yet, future studies including microbiota profiling are necessary.

MiRNAs respond to bacterial stimuli in a time-dependent manner. For example, pri-miR-147 increased in murine peritoneal macrophages after 2, but not 6 hours of exposure to lipopolysaccharide [40] and the expression of miR-146a in gastric epithelial cells increased between 6-9 hours after *H. pylori* infection, but decreased after 24 h [23]. However, the deferred response to bacterial exposure has not been investigated in the literature. Early responses have been deemed essential in innate immune responses where a short reaction time is critical for resistance for example to a pathogenic microorganism. On the other hand, delayed responses may play a role in adaptive immunity [24]. Thus, our data suggest that the

intestine may adapt to *B. bifidum* MIMBb75 following continued exposure. The time of exposure to a probiotic is a critical factor in clinical practice because it dictates the administration protocol. A time-dependent response following probiotic exposure was found *in vitro* and *in vivo* (mouse cecum) for *B. bifidum* PRL2010 impacting the immune response via alteration of the gene expression signature of HT-29 cells with more consistent changes after 4, rather than 1 or 2, hours of co-incubation[41], and *in vivo* for *Lactobacillus reuteri* 100-23 impacting inflammatory pathways after 6 but not 21 days of colonization [42]. Taken together with our findings, these data suggest that there may be an immediate intestinal response to probiotic exposure which may vary from that resulting from repetitive re-inoculation. This concept aligns with a study showing that, opposite to continuous supplementation, pulse administration of probiotics maintains high expression levels of mucin 3 in the jejunum and ileum of rats [43].

Based on *in silico* analysis, several genes were identified as potential targets of miR-148a, including experimentally validated *EPAS1*. This is a hypoxia-inducible transcription factor of the basic helix-loop-helix/PAS domain family, also known as Hypoxia Inducible Factor-2 α (HIF-2 α) [44]. High levels of EPAS1 have been associated with digestive system cancers (Reviewed in [45]), IBD [46] and colitis [47]. In a mouse model, EPAS1 was found to activate mediators of inflammation such as TNF- α and to promote colonic inflammation and spontaneous colitis after *Citrobacter rodentium* infection or dextran sulfate sodium (DSS) administration [47]. Moreover, it has a role in activating iron transporters [48] and intestinal knockout of EPAS1 significantly decreased tissue iron accumulation, due to a decrease in iron absorptive genes, in a mouse model of sickle cell disease (SDC) [49]. Therefore, intestinal EPAS1 has been suggested as a therapeutic target for IBD [47], SCD [49] and different types of cancers [50]. Here, we found that *B. bifidum* MIMBb75 increased miR-

148a resulting in down-regulation of *EPAS1* in colonic cells. This suggests that *B. bifidum* MIMBb75 could be considered for maintenance of, or to restore, intestinal homeostasis.

In conclusion, our study brings a new concept of strain specific bifidobacteria-host crosstalk mediated by microRNA. Our findings show that *B. bifidum* MIMBb75 stimulates gene expression changes in the host at both the transcriptional and miRNA levels in a time-dependent manner. Although speculative at this stage, the use of *B. bifidum* MIMBb75 may be considered to inhibit EPAS1 function in intestinal cells and as a consequence help in preventing or mitigating colitis and other diseases. Molecular antagonists of EPAS1 have been identified [51], providing therapeutic opportunities to its inactivation in disease. Our study is the first to show an alternative way to achieve EPAS1 inhibition in intestinal cells that is mediated by miRNA in response to a probiotic. This warrants further research to better elucidate the mechanisms involved. In addition, timing of probiotic administration may play an important role in clinical practice.

Author contributions

EMC conceived the study; AT, NS, SA, SG and EMC designed the experiments; AT, NS, JC and SA conducted the experiments; AT and EMC wrote the manuscript; all authors contributed to, read and approved the manuscript; EMC had primary responsibility for the study.

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Figure legends

Figure 1. Strain- and time-specific expression of miR-148a in Caco-2 intestinal cells in response to bifidobacteria.

Relative expression in intestinal Caco-2 cells incubated in presence of *Bifidobacterium* strains for 1, 4 and 24 hours. Data are presented as relative fold change in Caco-2 cells co-incubated with one of the three strains (*B. bifidum* MIMBb75, *B. bifidum* NCC390 or *B. longum* NCC2705) compared to sterile vehicle with delta Cts normalized to RNU6B. Significance is based on ANOVA plus Tukey's multiple comparisons test. * $P < .05$, ** $P < .01$ and *** $P < .001$. The average of three biological replicates with corresponding standard deviations are presented.

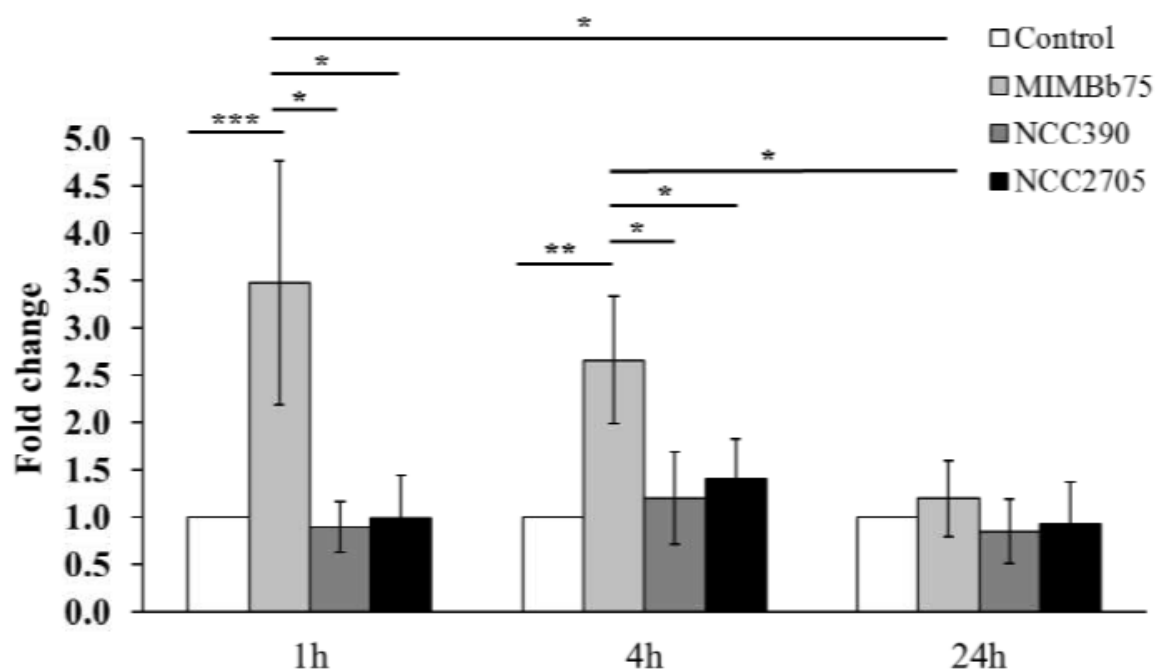


Figure 2. The expression of miR-148a increases early in mouse cecum following administration of *B. bifidum* MIMBb75

Relative expression in mice cecum following intragastric gavage of either *B. bifidum* MIMBb75 or sterile PBS for two and fourteen days in study 1 (A) and or for two days in study 2 (B). Data are presented as relative fold change in *B. bifidum* MIMBb75 versus control group, with delta Cts normalized to snoRNA-135. Significance is based on non-parametric Mann-Whitney test. * $P < .05$, ** $P < .01$ and *** $P < .001$. The average of 4 to 7 mice per group corresponding standard deviations are presented.

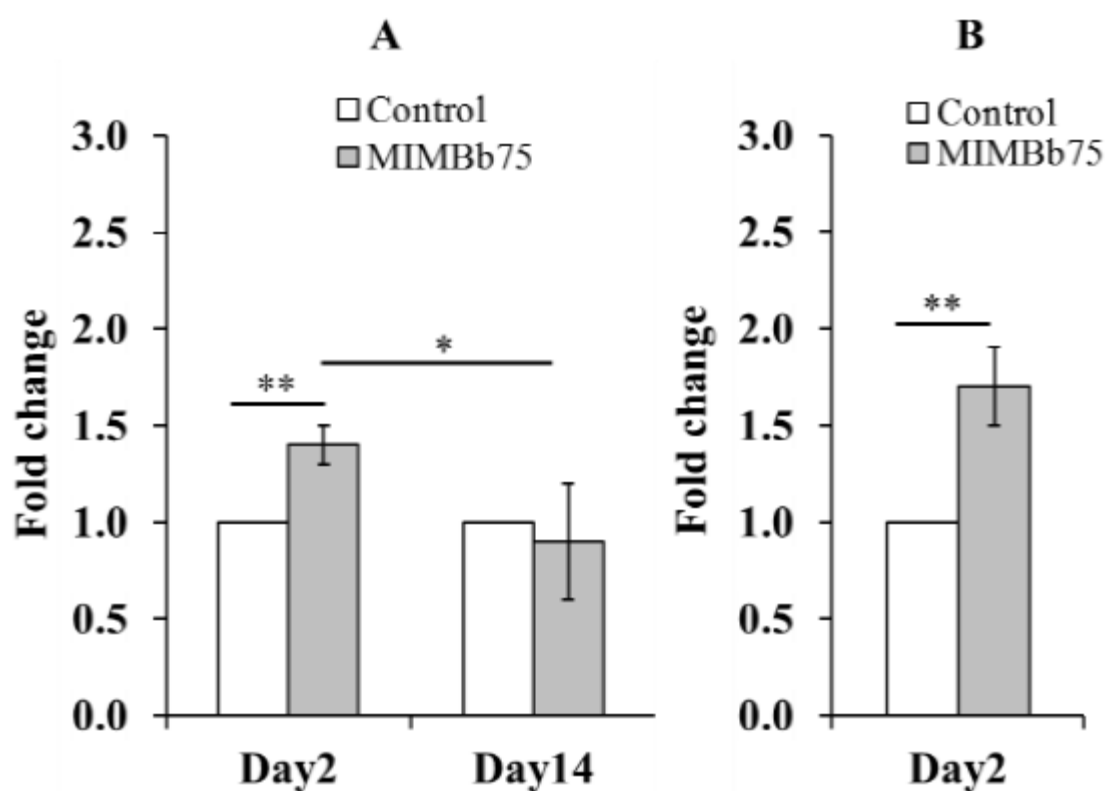


Figure 3. *B. bifidum* MIMBb75 modifies the expression of silenced miR-148a in Caco-2 cells

Relative expression of miR-148a in transfected Caco-2 cells co-incubated *B. bifidum* MIMBb75 or sterile vehicle for 1, 4 and 24 hours. Data are presented as relative fold change in a) miR-148a inhibitor transfected Caco-2 cells incubated with vehicle only (miR-148a inhibitor), b) miR-148a inhibitor transfected Caco-2 cells incubated with *B. bifidum* MIMBb75 (miR-148a inh+MIMBb75) and c) mock transfected Caco-2 cells incubated with *B. bifidum* MIMBb75 (MIMBb75 only), with delta Cts normalized to RNU6B. Significance is based on ANOVA plus Tukey's multiple comparisons test. * $P < .05$, ** $P < .01$ and *** $P < .001$. The average of three biological replicates with corresponding standard deviations are presented.

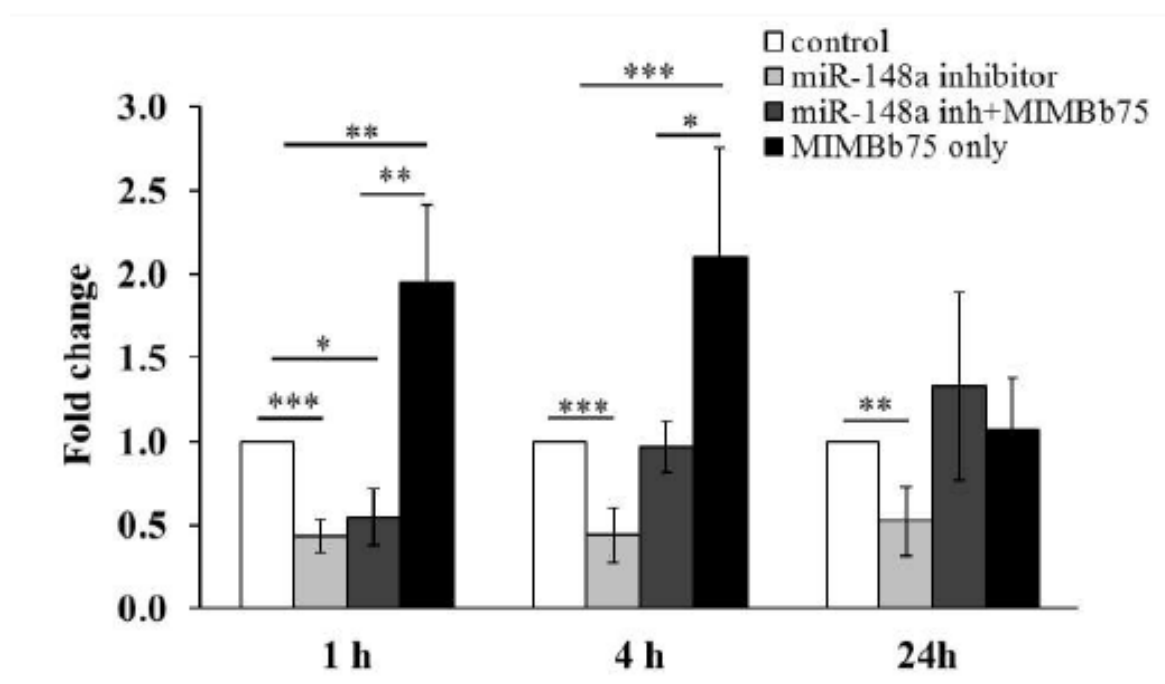
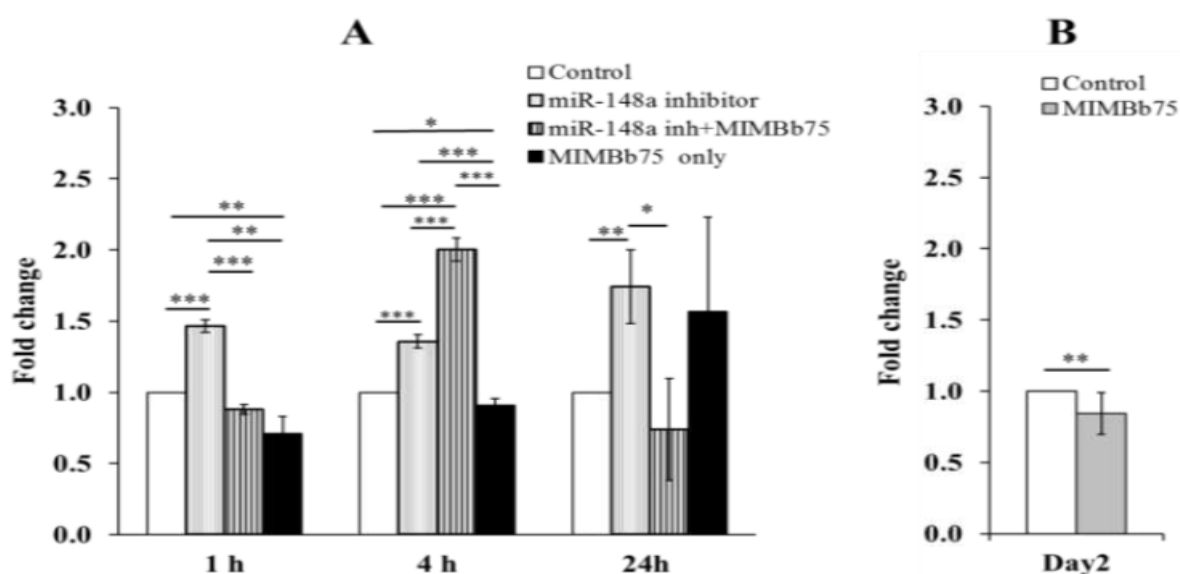


Figure 4. *B. bifidum* MIMBb75 downregulates *EPAS1* in intestinal cells via miR-148a in a time-dependent manner.

Relative expression of *EPAS1* in transfected Caco-2 cells co-incubated with *B. bifidum* MIMBb75 or sterile vehicle for 1, 4 and 24 hours (A). Data are presented as relative fold change in a) miR-148a inhibitor transfected Caco-2 cells incubated with vehicle only (miR-148a inhibitor), b) miR-148a inhibitor transfected Caco-2 cells incubated with *B. bifidum* MIMBb75 (miR-148a inh+MIMBb75) and c) mock transfected Caco-2 cells incubated with *B. bifidum* MIMBb75 (MIMBb75 only), with delta Cts normalized to RNU6B. Significance is based on ANOVA plus Tukey's multiple comparisons test. *P<.05, **P<.01 and ***P<.001. The average of three biological replicates with corresponding standard deviations are presented. Relative expression in mice cecum receiving either *B. bifidum* MIMBb75 or sterile PBS by intragastric gavage for two days (B). Data are presented as relative fold change in *B. bifidum* MIMBb75 versus control group, with delta Cts normalized to *Actb*. Significance is based on non-parametric Mann-Whitney test. *P<.05. The average of 4 to 6 mice per group corresponding standard deviations are presented.



Graphical abstract

We studied if probiotic bifidobacteria alter intestinal microRNA and downstream target gene response. We found that intestinal cells respond to *Bifidobacterium bifidum* exposure via increased expression of miR-148a in a time-dependent manner. At 1 hour, increase of miR-148a results in decreased expression of its target gene endothelial PAS domain protein 1. EPAS1 is involved in intestinal cancers and inflammation; thus, *B. bifidum* could provide a strategy to support homeostasis via microRNA.

