Probiotic lactobacilli mediated changes in global epigenetic signatures of human intestinal epithelial cells during *Escherichia coli* challenge

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Abstract

Host genome environment association is critical for proper development and functioning of an individual. Microbiota and probiotics within the cellular vicinity may serve such critical stimuli that can bring out different epigenetic mediated host responses. The aim of present study was to explore the changes in epigenetic signatures of Caco-2 cells by probiotic strains (*Lactobacillus rhamnosus* MTCC 5897: LR, *Lactobacillus fermentum* MTCC 5898: LF and their mixture: RF), respectively, or during challenge with *Escherichia coli* (ATCC 14948) using exclusion, competition and displacement assays. Adenocarcinoma intestinal epithelial Caco-2 cells were treated with LR, LF, RF and *E. coli* for 6 h, respectively. Caco-2 cells were also challenged with *E. coli* and probiotic lactobacilli during exclusion, competition and displacement assays. Finally, global epigenetic modifications by acetylation of H4 and H3 histone proteins and DNA methylation patterns were determined. Probiotic-treated Caco-2 cells displayed significant ($p < 0.01$) reduction in percent global H4 and H3 acetylation, respectively, in contrast to their elevated ($p < 0.05$) levels after *E. coli* infection. On the other hand, a remarkable ($p < 0.01$) decrease in percent H4 and H3 acetylation were observed when *E. coli* were excluded, competed or displaced by lactobacilli strains. No changes in the global DNA methylation patterns were observed in Caco-2 cells after exposure to probiotic strains or *E. coli*, respectively, but surprisingly, their levels increased significantly ($p < 0.05$) when lactobacilli-treated cells were challenged with *E. coli* during exclusion or competition than displacement assays. Probiotic *L. rhamnosus* and *L. fermentum* modulated the host epigenetic signatures via global histone acetylation individually or during *E. coli* challenge by exclusion, competition and displacement assays. Whilst on the other hand changes in global DNA methylation patterns were obtained significantly during probiotic treatment with *E. coli* in exclusion and competition protocols.

Keywords DNA methylation · Epigenome · Histone acetylation · Lactobacilli · Caco-2

Introduction

Epigenetics is the study of mitotically and meiotically heritable changes in gene function that are independent of DNA sequence (Bhat and Kapila 2017). The epigenome or the overall epigenetic state of an organism is just as important to normal development as is the contribution of the parent genome.

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The relationship between individual genotype and the environment is very essential for the proper host functioning and any perturbation in this association may have lethal consequences (Feinberg 2008; Tollefsbol 2017). Epigenomic reprogramming of the host genome is involved in various life processes such as development, regeneration and postpartum life of higher eukaryotic organisms (Al Akeel 2013). Environmental factors like gut microbiota and their metabolites, nutrients, toxins, infections and hypoxia can have profound effects on the epigenetic signature which may trigger host susceptibility to different disorders (Safronova and Morita 2010; Vel Szic et al. 2015). Epigenetics is therefore considered to be at the epicentre of modern medicine as it helps in understanding the disease aetiology (Feinberg 2008; Tollefsbol 2017). Studies have shown that the bioactive components and gut microbes alter the epigenetic signatures of

host cells through a variety of mechanisms which induced wide host responses ranging from inflammatory cascades to gut–brain axis modulation (Bhat and Kapila 2017; Zhou et al. 2017). The major epigenetic modifications that alter accessibility of DNA to transcriptional machinery and influence gene expression are acetylation, methylation, phosphorylation, biotinylation and RNA interference (Wang et al. 2018). These processes involve chromatin uncoiling and recoiling by the addition and removal of specific chemical groups which subsequently influence the host cells (Wong et al. 2017). Therefore, methylated DNA, acetylated proteins, micro RNA (miRNA), specific substrates, cofactors and enzymes involved in various biochemical reactions associated with these processes could serve as important biomarkers for detection of various diseases (Qureshi et al. 2010; Tollefsbol 2017).

Recently, it has been found that various infectious agents like Epstein-Barr virus, hepatitis viruses B and C, human papilloma virus, polyomaviruses, *Streptococcus bovis*, *Chlamydia pneumoniae*, *Campylobacter rectus* and *Helicobacter pylori* induce their lethal consequences through epigenetic changes (Oka et al. 2011). Similarly, the metabolic imbalances and inflammatory cascades have been linked to aberrant epigenetic patterns in the host cells (Safronova and Morita 2010). Even imbalanced gut microbiome (dysbiosis) set in aberrant epigenetic changes in the host cells that are linked with progression of many diseases such as systemic immune diseases including IBD, multiple sclerosis (MS) and autoimmune diseases (Rogers et al. 2016; Rooks and Garrett 2016; Lee et al. 2017b). The positive manipulation or restoration of altered gut microbiome through transplantation of the healthy gut microbiome or probiotics is therefore considered the best remedy to overcome such health problems and could constitute an effective therapeutic strategy. Probiotic and their metabolites in particular have been recently under tremendous consideration as these form important part of host gut environment and thus may have massive influence on host epigenome. However, the mechanistic understanding of such modifications is still in infancy and thus lot need to be done to comprehend such changes.

The present study was therefore conducted to understand the epigenetic mechanism/modification viz. global H3 and H4 acetylation and DNA methylation induced by two indigenous bacterial probiotic strains *Lactobacillus rhamnosus* (LR: MTCC 5897) and *Lactobacillus fermentum* (LF: MTCC 5898). Both the lactobacilli strains has been previously characterised for various probiotic attributes and have shown different efficacies (Sharma et al. 2014a; Sharma et al. 2014b; Saliganti et al. 2015). *L. rhamnosus* was reported to alleviate the ovalbumin induced allergy in newborn mice at suckling weaning transition and enhance immune functions with reduced oxidative stress in ageing mice as well resistance to *E. coli* infection (Sharma et al. 2014a; Saliganti et al. 2015).

On the other hand *L. fermentum* in the form of fermented milk showed potential to boost immunity, improve antioxidant capacity and resistance against pathogenic infection in aged murine model (Sharma et al. 2014b). Besides, both the probiotic strains showed ability to prevent the *E. coli*-induced aberration in the intestinal barrier integrity under in vitro conditions (in press). Therefore, it was necessary at the moment to investigate how the probiotic lactobacilli strains interferes with the host H3 and H4 acetylation and DNA methylation patterns when incubated individually with host intestinal epithelial cells or during exclusion, competition and displacement assays in presence of *E. coli* as an inflammatory agent.

Material and methods

Epithelial cell culture

The colonic adenocarcinoma enterocytes like Caco-2 cells were procured from the National Centre for Cell Science (NCCS, Pune, India) and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum along with 1% antibiotic solution (containing 1% streptomycin and penicillin G each and amphotericin 0.03%) and incubated at 37 °C in 10% CO₂ humidified atmosphere. The confluent cells with 20–30 passage numbers were used for this study.

Bacterial strains and their propagation

The probiotic *L. rhamnosus* and *L. fermentum* used in the present study were previously isolated from house hold curd and faeces of a 10-month-old infant, respectively. Both bacteria were grown overnight at 37 °C in Mann-Rogosa-Sharpe (MRS) broth (Himedia Laboratories, Mumbai India), centrifuged at 3000 rpm to pellet down the bacteria and washed thrice with saline. Final density of bacteria was set at 10⁹ CFU/mL in antibiotic-free DMEM for experiments.

On the other hand, *E. coli* (ATCC 14948) used to induce the inflammation was obtained from National Collection of Dairy Cultures (NCDC; NDRI, Karnal Haryana) and was grown in nutrient broth overnight at 37 °C on the day of experiment. The final count of *E. coli* bacteria was set also at 10⁹ CFU/mL in antibiotic-free DMEM for the experimental purpose.

Histone acetylation assay

The effect of probiotic lactobacilli on the histone acetylation of intestinal cells was determined by assessing the global histone 4 (H4) and histone 3 (H3) acetylation levels. To conduct the acetylation study, probiotic cultures *L. rhamnosus* and *L. fermentum* were grown overnight in MRS broth, whereas

E. coli was propagated in nutrient broth. On the day of experiment, live probiotic strains *L. rhamnosus* (LR: 1×10^9 CFU/ml), *L. fermentum* (LF: 1×10^9 CFU/ml) and their mixture (RF: LR: 1×10^9 CFU/ml + LF: 1×10^9 CFU/ml) as well as *E. coli* (1×10^9 CFU/ml) were suspended in antibiotic-free DMEM and incubated separately with confluent Caco-2 cells for 6 h. After incubation, cells were rinsed thrice with PBS (pH 7.4) and were processed for the assessment of global changes in histone H4 and H3 acetylation levels using EpiQuik global histone H3 and H4 acetylation assay kits. These epigenetic changes were also determined in challenge study when *E. coli* were excluded, competed or displaced with lactobacilli. In exclusion mode of treatment, Caco-2 cells were pre-treated with respective probiotic lactobacilli strains or their mixture for 6 h followed by removal of lactobacilli containing media and then exposing to *E. coli* for same duration. In competition assay, Caco-2 cells were simultaneously treated with either of probiotic strains individually or their mixture with *E. coli* for 6 h. Whereas in displacement assay, intestinal cells were pre-exposed to *E. coli* for 6 h, and then after aspirating the media, cells were incubated further for 6 h with probiotic *L. rhamnosus*, *L. fermentum* individually or their mixture, respectively. The 6-h duration of exposure of probiotic lactobacilli and *E. coli* with 10^9 CFU/ml dose was selected on the basis of optimal expression of tight junctional genes associated with barrier functions during temporal exposure of probiotic strains or *E. coli* to Caco-2 cells ([Data in supplementary file](#)).

Histone extraction and quantification of H4 and H3 acetylation levels

The intestinal epithelial Caco-2 cells exposed to either probiotic lactobacilli or *E. coli* and untreated cells were lysed and subjected to H3 and H4 histone quantification by using EpiQuik global histone H3/H4 acetylation assay kits (Epigentek Group, Farmingdale, NY, USA), respectively, according to the manufacturer's instructions. Briefly, the treated or untreated cells were initially rinsed thrice with PBS (pH 7.4) and detached from the adherent surface by gentle pipetting. The cells were homogenised with diluted lysis buffer ($200 \mu\text{l}/10^6$ cells) and incubated on ice for 5 min. Cell debris containing nuclei were obtained by centrifugation (12,000 rpm) followed by addition of lysis buffer (20 μl) and extraction/glycerol (60 μl) solution. After 5-min incubation, supernatant was collected by centrifugation and added with 100% TCA solution (1:4) to precipitate the protein. The protein pellet obtained was washed with acetone twice and then air dried before dissolving it in distilled water (20 μl).

The changes in the H3 and H4 levels were estimated in histone extracts using the manufacturer's instructions. Briefly, the protein concentration in each control or treated sample was estimated by Bradford's method and was adjusted

to 200 ng/ μl . Equal volume (5 μl) of protein solution was spread in the central area of each well of 96-well plate (Epigentek, USA) with blank wells having histone buffer (5 μl). For the positive controls, standard acetylated histone H3 and H4 (60 $\mu\text{g}/\text{ml}$) after dilution to 20–120 ng/ μl were added. The plate was incubated at 37 °C for 60–90 min for drying and later 150 μl of blocking buffer was added. To each well, 50 μl of diluted capture antibody (1:100) was added and was incubated (1 h, RT) on an orbital shaker (75 rpm). After proper washing, 50 μl of diluted HRP-conjugated detection antibody (1:1000 diluted) was added to each well and the plate was kept for 30-min incubation at room temperature. The wells were again rinsed with wash buffer and 100 μl of developing solution was added. Finally, reaction was blocked by adding stop solution (50 $\mu\text{l}/\text{well}$) and plates were read at 450 nm (Epoch, BioTek, USA). The results were expressed as relative H3 and H4 acetylation percentage between treated and untreated control epithelial cells.

DNA methylation assay

The change in the DNA methylation patterns of human intestinal cells upon exposure to probiotic lactobacilli and *E. coli* as described above was determined by measuring the global DNA methylation status of treated and untreated cells according to manufacturer's instructions. Briefly, treated and untreated Caco-2 cells were washed thrice in PBS (pH 7.4) and cellular lysate was obtained by addition of 10% SDS (50 μl) for 30 min at room temperature. To the cell lysate, 5 M NaCl (200 μl) and of phenol/chloroform/isoamyl alcohol solution (25:24:1, 100 μl) was added for phase separation and top aqueous phase containing DNA was collected. To pellet down DNA, aqueous phase was added with ice cold iso-propanol (500 μl) and mixed gently followed by incubation at -20 °C for 30 min. The DNA pellet was obtained by centrifugation and washed, air dried and dissolved in nuclease-free water (20 μl). To remove any traces of RNA contamination, RNase I (1 μl , 10 U/ μl) was added to DNA solution and was then processed immediately or stored at -20 °C for later use.

The change in global DNA methylation was estimated by Methylamp global DNA methylation quantification ultra kit (Epigentek Group Inc., USA) following the manufacturer's instructions. Briefly, the DNA concentration in each control or treated sample was adjusted to 80 ng/ μl . Each well was added with 28 μl of DNA binding solution followed by addition of 2 μl of DNA (160 ng/well). Similarly, positive control wells were added with 2 μl of standard diluted samples, whereas negative control wells added with same volume of negative control DNA. The plate was incubated at 37 °C for 40 min followed 30–40-min incubation at 60 °C to evaporate and dry the wells. To each dried well, blocking buffer (150 μl) was added and incubated at 37 °C for 30 min. Each well was

aspirated and rinsed thrice with $1\times$ wash buffer followed by addition of capture antibody ($1:1000$ with $1\times$ wash buffer). After primary antibody incubation (1 h, RT), each well was again washed and added with diluted HRP-conjugated detection antibody ($50\ \mu\text{l}$, $1:5000$) for 30 min at room temperature. After washings, each well was added with enhancer solution ($50\ \mu\text{l}$) for 30 min followed by developing solution ($100\ \mu\text{l}$) and the plate was incubated for 1–5 min away from light for colour development. Finally, horseradish peroxidase catalysed reaction was impeded with stop solution ($50\ \mu\text{l}$) and the plate was read at 450 nm. The results were expressed as percent (%) DNA methylation levels between treated and untreated Caco-2 cells.

Statistical analysis

Data were analysed using the GraphPad Prism (version 5.01). The results were presented as means $\pm$ SEM (standard errors of the mean). One-way analysis of variance (ANOVA) was used for statistical analysis using Tukey-Kramer post hoc test to know the statistical significance at $p < 0.05$ for multiple comparison amongst means.

Results

Global histone acetylation and DNA methylation of intestinal cells upon exposure to probiotic lactobacilli and *E. coli*

Human intestinal Caco-2 cells were treated with probiotic *L. rhamnosus* (1×10^9 CFU/ml), *L. fermentum* (1×10^9 CFU/ml) and their mixture (RF: *L. rhamnosus* + *L. fermentum*) and *E. coli* (1×10^9 CFU/ml), respectively, for 6 h to assess their effect on the global histone acetylation.

Treatment of intestinal epithelial cells with LR LF and RF significantly ($p < 0.01$) reduced the relative H4 acetylation to 56.7 ± 8.1 , 45.6 ± 7.4 and $57.9 \pm 11.3\%$, respectively, compared to untreated control cells (Fig. 1a). Likewise, in case of H3 acetylation, treatment of LR, LF and RF to intestinal Caco-2 also depicted significant ($p < 0.05$) suppression by 56.4 ± 2.3 , 67.0 ± 10.4 and $61.6 \pm 12.6\%$, respectively, in the relative global acetylation level compared to untreated cells (Fig. 1b). On the contrary, both H4 and H3 acetylation enhanced significantly ($p < 0.05$) by 145.2 ± 12.5 (Fig. 2) and $179.3 \pm 25.2\%$ (Fig. 3), respectively, when confluent Caco-2 cells were exposed separately to live *E. coli* for 6 h.

The treatment of human intestinal Caco-2 cells with either of the probiotic *L. rhamnosus*, *L. fermentum* or their mixture for 6 h showed statistically insignificant changes in relative global DNA methylation percentages compared to untreated cells as represented in Fig. 1c. Similarly insignificant

modifications were observed in DNA methylation status by *E. coli* exposure alone (Fig. 4).

Impact of probiotic lactobacilli treatment on global histone acetylation during challenge with *E. coli*

In exclusion assay, pre-treatment of intestinal Caco-2 cells with LR, LF and RF followed by *E. coli* significantly ($p < 0.01$) reduced the percent H4 acetylation levels to 28.5 ± 2.8 , 30.7 ± 3.1 and 44.7 ± 5.1 , respectively (Fig. 2a), compared to *E. coli*-treated (145.2 ± 12.5) cells. Similarly, the simultaneous incubation of inflammatory agent *E. coli* with probiotic strains (LR, LF and RF) on Caco-2 cells during competition assay remarkably ($p < 0.01$) suppressed (52.0 ± 12.0 , 57.8 ± 13.9 and $42.9 \pm 7.8\%$) the global H4 acetylation, respectively (Fig. 2b), compared to cells inflamed with *E. coli* alone ($145.18 \pm 12.5\%$). Likewise, displacement of *E. coli* by LR, LF and RF on intestinal epithelial Caco-2 cells during posttreatment with probiotic strains significantly ($p < 0.01$) diminished global acetylation of H4 histone proteins by 29.0 ± 5.1 , 31.2 ± 6.3 and $66.2 \pm 5.0\%$, respectively, compared to $145.2 \pm 12.5\%$ acetylation with *E. coli* (Fig. 2c).

In case of global H3 acetylation, the epigenetic modifications have shown significant ($p < 0.05$) reduction of 71.0 ± 27.1 , 64.0 ± 18.5 and $73.4 \pm 23.2\%$, respectively (Fig. 3a), on pre-incubation with LR, LF and RF during exclusion as compared to $179.3 \pm 25.2\%$ by *E. coli* alone. Similarly during competition assay, H3 acetylation levels in host Caco-2 cells also diminished significantly ($p < 0.01$) to 74.6 ± 15.9 , 39.2 ± 6.9 and $30.5 \pm 9.4\%$, respectively (Fig. 3b). In the same manner, H3 acetylation patterns of Caco-2 cells declined significantly ($p < 0.05$) to 80.3 ± 9.5 , 53.2 ± 8.0 and $75.3 \pm 10.3\%$ when displacement of *E. coli* was attempted with respective probiotic lactobacilli treatments (Fig. 3c).

Global DNA methylation status of human intestinal cells challenged with *E. coli*

Pre-exposure of Caco-2 cells to probiotic *L. rhamnosus*, *L. fermentum* and their combination significantly ($p < 0.05$) elevated the global DNA methylation to 131.9 ± 11.5 , 145.2 ± 4.9 and $128.8 \pm 5.0\%$, respectively, compared to $83.9 \pm 2.6\%$ when cells were exposed to *E. coli* alone as an inflammatory agent (Fig. 4a). On other hand, concurrent treatment of LR and RF with *E. coli* considerably ($p < 0.01$) enhanced the DNA methylation by 205.6 ± 27.8 and $286.0 \pm 27.8\%$, respectively, compared to *E. coli* control. However, co-treatment of LF with *E. coli* had insignificant effect on DNA methylation of intestinal Caco-2 cells compared to *E. coli* exposed cells or control cells (Fig. 4b). Interestingly, in case of displacement assay, none of probiotic strains or their combination was found to alter the global methylation epigenetic signatures of

Fig. 1 Effect of probiotic lactobacilli (*L. rhamnosus*: LR, *L. fermentum*: LF and their mixture: RF) on global epigenetic signatures of human intestinal epithelial cells. **a** H4 acetylation. **b** H3 acetylation. **c** DNA

methylation. Values are expressed as mean $\pm$ SEM ($n = 3$ for each treatment) and values with different superscript letters are significantly different at $p < 0.01$

host intestinal epithelial cells compared to *E. coli*-exposed Caco-2 cells (Fig. 4c).

Discussion

Epigenetics predominantly emphasise on the mechanisms that could mould the chromatin structures and regulate gene expression with stability by adapting cells to the environment without changing the nucleotide sequence (Canani et al. 2012). In a broader context, there is a mounting interest in probiotics to understand their involvement in epigenetic mechanisms so that more specific and efficacious therapeutic strategies can be developed in the prevention and treatment of different diseases. Epigenetic mechanisms integrate environmental changes at the cellular level, thereby enabling cellular plasticity (Flavahan et al. 2017). There are basically three distinct but intimately interacting epigenetic mechanisms that can change the expression profile of key genes essential for

physiological and pathologic processes (McKay and Mathers 2011). Epigenetic modifications contribute significantly in the modulation of gene transcription through DNA methylation, histone modifications and non-coding microRNAs (miRNA). DNA methylation is generally associated with the suppression of gene transcription, whereas acetylation of histones is predominantly associated with gene induction (Woo et al. 2017). RNA interference on other hand is an epigenomic process where genes are turned off posttranscriptionally by a group of small, noncoding, endogenous miRNA (Bhat and Kapila 2017). Although the area of epigenetics has come under tremendous contemplation recently but still scanty literature is available about association of host epigenome with probiotics and is thus least understood.

Under present investigation, the impact of probiotic bacteria *L. rhamnosus* (LR; MTCC 5897) and *L. fermentum* (LF; MTCC 5898) on the host epigenome viz. global H3 and H4 acetylation and DNA methylation were assessed in the presence of inflammatory stimulus of *E. coli*. Human colon

Fig. 2 Effect of probiotic lactobacilli (*L. rhamnosus*: LR, *L. fermentum*: LF and their mixture: RF) during challenge with *E. coli* on global H4 histone acetylation in intestinal Cao-2 cells. **a** Exclusion (Ex). **b**

Competition (Com). **c** Displacement (Dis). Values are expressed as mean $\pm$ SEM ($n = 3$ for each treatment) and values with different superscript letters are significantly different at $p < 0.01$

epithelial Caco-2 cells treated with probiotic strains LR, LF and their mixture RF individually for 6 h displayed significant reduction in the percent global histone H4 and H3 acetylation in contrast to remarkably high acetylation observed during *E. coli* exposure. The increased H3 and H4 acetylation of histone proteins by *E. coli* exposure might suggest the enhanced inflammatory response by host cells against inflammatory conditions in contrast to suppressed levels of H3 and H4 acetylation patterns indicating the healthy environment set in by probiotic lactobacilli strains. In context to this interpretation, we have earlier established that probiotic strains *L. rhamnosus* and *L. fermentum* were able to alleviate the host inflammation by suppressing the expression of inflammatory interleukin-4 and interleukin-10 in contrast to increased interferon- γ levels that might have been modulated by involving epigenetic machinery (Sharma et al. 2014a; Sharma et al. 2014b; Saliganti et al. 2015). Previously, Lee et al. (2017a) reported that the commensal probiotic lactobacilli strains *Lactobacillus gasseri* and *Lactobacillus reuteri* in a strain-

specific manner modulated the expression of DEFB1 (Defensin Beta 1) gene by changing the acetylation patterns at specific regions in vaginal VK2/E6E7 epithelial cells. On one hand, *L. gasseri* showed enhanced expression of DEFB1 due to enrichment of active histone marks such as AcH3 and H2A.Z whilst on the other treatment with *L. reuteri* decreased such active histone marks that subsequently resulted in reduced DEFB1 expression. Increased global H3 and H4 acetylation levels were found in human macrophage cells upon exposure to inflammatory agents such as ethanol and LPS that elevated the pro-inflammatory cytokine profile of host cells leading to chronic inflammation (Kendrick et al. 2010). Enhanced H4 acetylation were also seen when gastric epithelial cells were exposed to *H. pylori* emphasising that chromatin alterations might contribute to the pathogenic mechanisms underlying the development and progression of *H. pylori*-associated diseases (Xia et al. 2008). In similar context, Ghadimi et al. (2012) observed significant reduction in the global H3 acetylation levels when HT-29/B6 or T84 monolayers in 3D

Fig. 3 Effect of probiotic lactobacilli (*L. rhamnosus*: LR, *L. fermentum*: LF and their mixture: RF) during challenge with *E. coli* on global H3 histone acetylation in intestinal Cao-2 cells. **a** Exclusion (Ex). **b**

Competition (Com). **c** Displacement (Dis). Values are expressed as mean $\pm$ SEM ($n = 3$ for each treatment) and values with different superscript letters are significantly different at $p < 0.05$

culture systems were separately treated with probiotic *B. breve* or *L. rhamnosus*, respectively. The decreased acetylation levels were responsible for alleviating the inflammatory cascade set in inflammatory stimulus that subsequently downregulate the expression of the inflammatory bowel disease causing factors. Recent evidences have further consolidated the perception that commensal gut microflora has a critical role in gut homeostasis and alteration in their composition is majorly associated with the development of gut diseases through host cell epigenome modulation. Depletion of gut microbiota by broad-spectrum antibiotic treatment induced massive change in the global epigenetic signatures of small intestinal cells including the several histone modifications at specific enhancer and promoter sites (Gury-BenAri et al. 2016). In earlier study, the interdependence of commensal bacteria and histone deacetylases in the regulating the intestinal homeostasis was also demonstrated, which clearly suggested that histone acetylation of host genes might change in the presence of commensal gut microbes (Alenghat and Artis 2014). Microbe-

derived metabolites such as acetate and butyrate when supplemented under in vitro and in vivo conditions also have been established to modulate host epigenome by changing the acetylation patterns of cells (Bhat and Kapila 2017). The gut microbes and their fermentation products were very essential in the attenuation of colorectal tumorigenesis by changing the histone acetylation patterns and the expression of associated genes (Donohoe et al. 2012; Donohoe et al. 2014). Spermine secretion by gut microbes *B. thetaiotaomicron* and *Fusobacterium varium* have been found to modulate the histone acetylation, which subsequently showed beneficial effects on ageing and age-related memory deficits (Noack et al. 2000; Stilling et al. 2014). However, in contrast to present findings, Bourassa et al. (2016) demonstrated the increased acetylation patterns induced by butyrate derived from *Clostridium*, *Eubacterium* and *Butyrivibrio* that subsequently improved the memory impairment and learning power by increasing the expression of learning-associated genes in Alzheimer mice model. However, Rada-Iglesias et al. (2007)

Fig. 4 Global DNA methylation patterns in intestinal epithelial cells with probiotic lactobacilli (*L. rhamnosus*: LR, *L. fermentum*: LF and their mixture: RF) during challenge with *E. coli*. **a** Exclusion (Ex). **b**

Competition (Com). **c** Displacement (Dis). Values are expressed as mean $\pm$ SEM ($n = 3$ for each treatment) and values with different superscript letters are significantly different at $p < 0.05$

highlighted the role of butyrate derived from large bowel microbial fermentation in decreasing the histone acetylation at selected chromatin regions of hepatocarcinoma HepG2 and colon adenocarcinoma HT-29 cells.

Under present investigation, global DNA methylation which typically suppresses the gene activity appeared unaltered when intestinal epithelial Caco-2 cells were exposed to either of the probiotic strains or their mixture and live *E. coli* which is in accordance with previous finding where insignificant changes in global DNA methylation patterns were noticed upon exposure to commensal probiotics *B. breve* or *L. rhamnosus* under in vitro conditions (Ghadimi et al. 2012). Interestingly, they also reported insignificant changes in DNA methylation patterns of epithelial cells upon exposure to inflammatory agent LPS. However contrarily to present finding, Schmeck et al. (2005) reported the induction of DNA methylation changes in *H. pylori*-infected human

endothelial cells that were involved in the regulation of host inflammatory response. Probiotic bacteria *Lactobacillus acidophilus* were also reported to change DNA methylome of intestinal cell which subsequently elevated the expression tumour-suppressing genes such as *Icam 5*, *Clstn2*, *Ppm1e*, *Runx3*, *Timp3*, *Rgl1* and *Rassf1a* and thereby avert colorectal cancer (Lightfoot et al. 2013). Differential DNA modification patterns were seen in human foetal and adult intestinal epithelial cell lines (H4 and NCM460, respectively) upon interaction with probiotics *L. acidophilus* and *Bifidobacterium infantis* or pathogenic bacteria *Klebsiella* spp. (Cortese et al. 2016). In a dietary intervention study with postmenopausal women, altering plasma levels of microbial metabolites folate were found to directly influenced lymphocyte DNA methylation levels (Jacob et al. 1998). Commensal gut microbes have very essential role in maintaining the DNA methylation patterns of important host genes such as *TLR4* that are involved in

sensing the external environmental stimuli that subsequently modulate host immune response (Takahashi et al. 2011). By modulating the DNA methylation patterns of host cells at TLR4 locus, the human intestinal cells were found to retaliate very poorly to external LPS stimulus reflecting the critical role of epigenome in countering the host responses.

Under the current investigation, epigenetic signatures of intestinal epithelial cells changed considerably when probiotic lactobacilli treated cells were challenged with *E. coli*. The global H4 and H3 histone acetylation levels were suppressed dramatically when human intestinal Caco-2 cells were treated with LR, LF and their mixture RF during challenge with *E. coli* under exclusion, competition and displacement milieu compared to *E. coli* exposure alone. This could be due to reduced inflammation of epithelial Caco-2 cells as probiotic strains may have inhibited *E. coli* from inducing its inflammatory signalling cascades. Similar to our observation, co-incubation of transforming growth factor- β 1 (TGF- β 1) as an antiinflammatory agent with either non-pathogenic commensal enteric bacterial species *B. vulgates* or LPS on intestinal epithelial CMT93 cells reduced histone 3 hyper-acetylation which subsequently inhibited the expression of pro-inflammatory *IL-6* gene (Haller et al. 2003). In the same way, Ghadimi et al. 2012 delineated a reversal in the LPS induced hyper-acetylation when commensal probiotic *B. breve* or *L. rhamnosus* were concurrently added with 3D co-culturing system of HT-29/B6 or T84 with PBMC cells.

In case of global DNA methylation patterns, lactobacilli strains were also able to enhance the host cell nuclear methylation levels when human intestinal colon cells (Caco-2) were treated with probiotic (LR, LF and RF) during exclusion and competition mode of treatment. However, no such prominent changes were observed when *E. coli* was incubated with epithelial cells prior (displacement assay) to probiotic lactobacilli treatment. This indicated maintenance of DNA methylation patterns in presence of inflammatory conditions causing genome silencing parallel to control group which could not even be reversed by posttreatment with probiotic strains. However, increased global DNA methylation during exclusion and competition assays depicted presence of healthier conditions due to prophylactic effect of probiotic with less expression of inflammatory genes. These results are in consonance with previous studies where the global methylation status increased considerably in kidney tissue of C57BL/6 (H-2Ab) mice when administrated intraperitoneally with commensal bacteria *Bifidobacterium adolescentis* or *B. longum* before and during induction of ischemia and reperfusion injury (IRI), respectively (Andrade-Oliveira et al. 2015). Increased global DNA methylation levels were also observed when LPS and *B. breve* or *L. rhamnosus* were simultaneously added to HT-29/B6 and T84 cell monolayers (Ghadimi et al. 2012). Recently, increased methylation patterns of nitric oxide synthase (NOS) gene in Wistar rat burn injury model were

reported when provided with combined therapy of glutamine and probiotics compared to control animals and thus protected them against burn-induced oxidative stress and inflammation (Gong et al. 2017). These epigenetic modifications in response to probiotic bacteria or in presence of inflammatory agent could directly or indirectly activate or inhibit the critical components of epigenomic machinery by interfering in the activity of enzymes such as methyltransferases, deacetylases, acetyltransferases, nucleases and serine-threonine protein kinases, which are central in reprogramming and/or in maintaining the posttranslation modification of histone and other proteins.

In conclusion, it has been established that exposure of intestinal epithelial Caco-2 cells to probiotic bacteria *L. rhamnosus* and *L. fermentum* brought out considerable changes in the core epigenetic signatures viz. histone acetylation and DNA methylation of host cells during challenge with inflammatory agent *E. coli* that might be responsible for the efficacy of these probiotic strains as established previously during alleviation of allergic response in weanlings and in combating immunosenescence in aged mice. However, lot need to ponder upon the gene specific modifications and the epigenetic machinery that could be involved in bringing out such modifications.

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Compliance with ethical standards

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

Research involving human participants and/or animals N/A.

Informed consent N/A.

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