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Lactobacillus spp. Inhibits the Growth of HCT-116 and Reduces IL-8 Secretion by *Salmonella* typhimurium-Infected HCT-116 Colorectal Carcinoma Cells

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Abstract

Salmonella typhimurium causes symptoms resembling typhoid fever and gastroenteritis in humans. Its toxicity is due to an outer membrane consisting largely of lipopolysaccharides (LPS) which is responsible for the host immune response. The aim of this study is to evaluate the antimicrobial, anti-apoptotic ability of *Lactobacillus plantarum* and reduce *Salmonella*-induced pro-inflammatory cytokine IL-8 secretion. Adhesive tests were performed using lactobacilli co-cultured with the colon cancer cell line HCT-116 for 2 hours. The strains displaying the highest adhesion were selected for downstream 3- (4, 5- Dimethylthiazol -2-yl) -2, 5- diphenyltetrazolium bromide (MTT) tests to assess cytotoxicity. The supernatants of *Lactobacillus* cultured with HCT-116 cells for 24 and 48 h to evaluate the inhibitory effect. To determine Interleukin 8 (IL-8) secretion in colon cancer induced by *S.* typhimurium, we stimulated HCT-116 cells with *S.* typhimurium and co-cultured with lactobacilli for 24 h. Lactobacilli had the most significant inhibitory effects on cell growth, and their inhibitory effects were time-dependent. Strain No. 03-03-026 caused cancer cell deoxyribonucleic acid (DNA) fragmentation, and the anti-apoptosis protein (B-cell lymphoma 2) was reduced in the HCT-116 cells as determined. IL-8 production in colon cancer cells was significantly reduced by these lactobacilli. Our results suggested that lactobacilli maybe effectively reduce the numbers of *S.* typhimurium, IL-8 levels and the anti-apoptotic phosphorylated-p38 mitogen-activated protein kinase and B-cell lymphoma 2 proteins. *Lactobacillus* can be added to the diet as a food additive to prevent colorectal cancer and used to be the prophylactic agent against *S.* typhimurium.

Keywords: Colorectal cancer; *Lactobacillus*; Interleukin-8; Anti-apoptotic phosphorylated-p38; *Salmonella* typhimurium

1 Introduction

Salmonella, a Gram-negative bacterium, is widely present in the intestines of animals and humans and is excreted in the faeces, and it may cause enteritis. Transmission is via the faecal-oral route, spreading in water, food, and among organisms. *Salmonella* plays an important role in food hygiene and safety. Among potentially contaminated food sources, the key sources of human infection are animal feed and foods such as eggs and meat. Improper food handling may result in large-scale contamination and food poisoning cases (Schlosser et al., 2000). Some researches indicated *S.* typhimurium as the primary causative agent of food poisoning world-

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wide for the past two decades (Khakhria et al., 1997; Trémolières, 1996). Lactic acid bacteria (LAB) are specifically adsorbed by epithelial cells to establish and to outcompete other bacterial species in the gastrointestinal tract (Jiang et al., 2019). Lee and Salminen highlighted bacterial survival in the gastrointestinal tract and transient adsorption of intestinal epithelial cells (Lee & Salminen, 1995). In addition, Lee et al. proposed that probiotics with good adsorption capacity can prevent the adsorption of pathogenic bacteria such as *Salmonella* (Lee et al., 2000). Lee et al. also demonstrated that probiotics can invade intestinal cells and reduce gastrointestinal tract stimulation caused by pathogenic bacteria and infection (Lee et al., 2000). Intestinal mucus is an important site for the adsorption and colonization of probiotic strains, but it is difficult to perform probiotic biosorption tests in vivo (Mikelsaar et al., 1998). Therefore, the most commonly used method is an in vitro cell culture test. The cell lines commonly used include the human rectal epithelial cell Caco-2 (Hirano et al., 2003). The Caco-2 cell line is advantageous due to its ability to exhibit the type and functional differentiation in vitro, and this cell line possesses mature intestinal cells and functional microvilli (Hauri et al., 1985; Hidalgo et al., 1989; Jamyuang et al., 2019).

The pre-treatment of Caco-2 cells with the probiotic bacteria *L. plantarum* can reduce *Salmonella*-induced pro-inflammatory cytokine IL-8 secretion by nuclear factor κB (NF-κB) activation, which typically results in intestinal inflammation diseases (Lépine et al., 2018; Ren et al., 2013). A recent study corroborated these results in illustrating that pre-treatment with 1 $\times$ 10^6 CFU/mL *L. rhamnosus* GG for 12 h effectively suppressed IL-8 mRNA abundance, induced by TNF-α, in Caco-2 cells (Zhang et al., 2005). Roselli et al. utilized *Bifidobacterium animalis* MB5 and LGG to inhibit Caco-2-induced IL-8 expression in enterotoxigenic *E. coli* K88 (Roselli et al., 2006). IL-8 mRNA expression was significantly lower in Caco-2 cells co-cultured with *B. animalis* MB5 or LGG and *E. coli* than Caco-2 cells cultured with *E. coli* alone. A significant down-regulation of the anti-inflammatory cytokine TGF-β1 was also observed in the *E. coli*-only group.

At present, probiotics and their fermented products are beneficial to human health such as promoting anti-tumour growth, antimicrobial, antioxidant and intestinal health (Jang et al., 2019; Lee et al., 2000; Lee & Salminen, 1995). Probiotics have become popular in recent years and recommended as preventive strikes against rectal cancer (Zhang et al., 2005). However, different probiotic strains may yield differential anti-cancer or anti-tumour activity, and their mechanisms are unclear and require further study. Herein, we investigated plant-derived *Lactobacillus* using the colon cancer line HCT-116 to explore whether they can effectively reduce the anti-apoptotic phosphorylated-p38 mitogen-activated protein kinase (p-p38 MAPK) and B-cell lymphoma 2 (Bcl-2) proteins. Additionally, we investigated the impact of probiotics on IL-8 *S.* typhimurium dynamics in colon cancer lines HCT-116.

2 Methodology

2.1 Bacterial strains, cell lines, and culture conditions

Lactic acid bacteria (LAB) strains were isolated from fruit and fermented plant products, and the stock culture collection was maintained at -80 ^{o}C in 20 % glycerol. One hundred microliters of LAB cells were propagated twice in 5 mL Lactobacilli de Man, Rogosa and Sharpe (MRS) broth (Difco, Spark, MD, USA) containing 0.05 % L-cysteine for 18-20 h at 37 ^{o}C before experimental use. S. typhimurium (strain I50) was isolated from a patient with foodborne-induced diarrhoea obtained from the National Center for Disease Control, Taipei, Taiwan. For cultivation, one loopful of *Salmonella* cells was inoculated into 5 mL of tryptic soy broth and incubated at 37 ^{o}C for 12 h.

HCT-116 cell lines were obtained from the Bioresource Collection and Research Center (BCRC), Hsin-Chu, Taiwan. HCT-116 (BCRC 60349, human colorectal carcinoma) cells were grown in McCoy's 5A Medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) Fetal Bovine Serum (FBS), 1% Nonessential amino acids (NEAAs), and 1.1 g sodium bicarbon-

ate. Cryostats containing the respective cell lines were transported from liquid nitrogen to a 37 ^{o}C water bath for rapid thawing. Cell suspensions were thawed in a 75 cm^2 cell culture flask or cell culture dish while a sterile pipette was used to add the appropriate amount of cell culture medium. Cells were incubated at 37 ^{o}C at 5 % CO_2.

2.2 Adhesion of *Lactobacillus* to HCT-116 cell lines

The method described by Tsai et al. was used (Tsai et al., 2019). HCT-116 cells were cultured in 75 cm^2 plastic tissue culture flasks (GIBCO). Cells were washed twice with PBS, then transferred (4×10^5 cells/ mL) with 0.05 % trypsin to 24-well plates containing fresh tissue culture medium without penicillin-streptomycin. Cells were kept at 37 ^{o}C in 5 % CO_2 until monolayers were achieved in each well. Prior to the adhesion test, all bacterial strains were washed twice with PBS and centrifuged for 10 min at 8000 × g (Hettich EBA 12R, Tuttlingen, Germany). Bacterial cells were resuspended in 1 mL McCoy's 5A Medium; suspensions (100 μL) were transferred to the 24-well plates (1×10^8 CFU/mL) containing the HCT-116 cells and incubated for 2 h at 37 ^{o}C in 5 % CO_2. After incubation, cells were washed twice with PBS, fixed with 5 % formalin for 30 min, washed four times with PBS, and then stained with crystal violet for 5 min.

2.3 Cell viability analysis

To understand the effects of the strain 03-03-026 and strain 03-03-027 on cell growth, the two strains were co-cultured with HCT-116 colorectal cancer cells, and cell viability was observed. HCT-116 cells were cultured for 12 h with strain 03-03-026 and strain 03-03-027 under the following conditions: (1) LAB cells grown to 10^7 CFU/mL or more, (2) *Lactobacillus* supernatants (SCS), (3) LAB supernatants at pH = 7.2, (4) Heat-treated (100 ^{o}C, 30 min) LAB supernatants, (4) Heat-treated LAB, (5) MRS medium containing LAB, and (6) Heat-treated MRS medium containing LAB.
Method described by Watson et al. was used (Watson et al., 2000). Briefly, 3- (4, 5-dimethylthiazol- 2-yl) - 2, 5- diphenyl tetrazolium bromide (MTT), a colorless, transparent tetrazolium salt, is reduced to yield a purple formazan crystal by mitochondrial dehydrogenase in living cells. In total, 100 μL (5×10^4 cells/mL) of cells were seeded onto a 96-well plate, and the cells were cultured overnight at 37 ^{o}C in a 5 % CO_2 incubator. Cells were gently washed twice with 1× PBS, and after discarding PBS, 100 μL of *Lactobacillus* suspensions were added to the wells. The results were subsequently analysed after 12, 24, 36, and 48 h. For the analyses, media solutions were aspirated from the 96-well plate, and the cells were gently washed twice with 1× PBS, followed by removal of PBS via aspiration. Second, 100 μL of MTT solution were added to the cells. After 2-h incubation at 37 ^{o}C in a 5 % CO_2 incubator, supernatants were removed and 200 μL of dimethyl sulfoxide (DMSO) were added to the wells, which was followed by continuous shaking for 10 min to solubilize the purple formazan crystals. An enzyme-linked immunosorbent assay (ELISA) reader (Model 680, BIO-RAD, Hercules, CA, USA) was used to read the absorbance at 570 nm.

2.4 Detection the level of IL-8 by Enzyme-linked immunosorbent assay (ELISA)

S. typhimurium (10^8 CFU/mL) was centrifuged (8,230 × g, 10 minutes), washed twice with 1 × PBS, and resuspended in cell culture media. An ultrasonic grinder (HOYU Ultrasonic 250, Taiwan) was then used (output power amplitude: 40 %, time: 6-8 hours), and cells were then filtered with a 0.22 μM filter and stored at -20 ^{o}C for later use.
HCT-116 cells were cultured in a 24-well tissue plate and incubated in medium with or without *S.* typhimurium and with either LAB strain No. 03-303-026 or No. 03-03-027 for 37 ^{o}C, 24 h. Afterwards, supernatants were collected and ELISA performed to assay IL-8 secretion using the manufacturer's instructions (BD Biosciences, CA, USA). 96-well Immuno-Maxisorp plates (Nunc, Roskilde, Denmark) were coated

with monoclonal antibodies for IL-8, and placed in an incubating buffer overnight at 4 ^{o}C. The plates were blocked and washed 3 times. Samples were added to the plates and incubated for 2 hours at room temperature. The plates were washed 3 times again, and biotinylated anti-human IL-8, along with horseradish peroxidase (HRP)-conjugated streptavidin, were added for the detection of IL-8, and incubated 1 hour at room temperature. The reactions were developed using 3, 3´, 5, 5´-tetramethylbenzidine (TMB) substrate for 30 min at room temperature. The colour reactions were stopped using 2N H_2SO_4 and absorbance was measured at 450 nm.

2.5 Apoptotic proteins p-p38 and Bcl-2 in HCT-116 cells

Apoptosis-associated protein expression in HCT-116 cells was determined via Western blot analysis. HCT-116 cells (1.5 × 10^6 cells/mL) were added to a 10 cm plate and cultured overnight at 37 ^{o}C and 5 % CO_2 to encourage cell attachment, division, and proliferation. Various concentrations of LAB strains No. 03-03-026 and No. 03-03-027 (10^5, 10^6, or 10^7 CFU/mL) mixed with cell culture media were added to the plate, and the cells were cultured for 24 h at 37 ^{o}C and 5 % CO_2. Cells were collected and then lysed in radio immunoprecipitation assay buffer for 30 min on ice. The cells were then centrifuged at 24,000 × g (Hettich EBA 12R, Tuttlingen, Germany) for 5 min at 4 ^{o}C. Resulting supernatants (extracts of cellular proteins) were subjected to quantitative protein analysis using the Invitrogen Qubit® fluorimeter (Life Technologies, Waltham, MA, USA). Cellular protein extracts were mixed with 5X protein loading dye, heated at 95 ^{o}C for 5 min, and loaded in SDS-PAGE wells. Following electrophoresis, proteins on the gel were transferred to a nitrocellulose membrane for 1 h at 37 ^{o}C, with the addition of 5 % skim milk as the blocking buffer. Subsequently, the membrane was washed in TBST [20 mM Tris-HCl, 137 mM NaCl, containing 0.1% (v/v) Tween-20, pH 8] three times and incubated with the primary antibody (either p-p38 or Bcl-2 monoclonal antibody) overnight at 4 ^{o}C. After three washes with TBST, the secondary antibody was added and incubated for 1 h, which was followed by TBST washes. Gel images were captured using a luminometer, and bands were observed.

2.6 Genomic DNA preparation and PCR amplification

Total chromosomal DNA of LAB cells cultured overnight in MRS broth was extracted with the Blood & Tissue Genomic DNA Extraction Miniprep System (Viogene Laboratories, Taipei, Taiwan) according to the manufacturer's instructions. Eluted DNA was stored at -20 ^{o}C and 1 μL was used for downstream PCR amplification. PCR primers are shown in Table 1. One microliter of DNA was mixed with 24 μL PCR buffer containing 1× PCR buffer (10 mM Tris-HCl, pH 8.8; 1.5 mM MgCl2; 50 mM KCl; 0.1 % Triton X-100); 200 μM each of dATP, dGTP, dCTP, and dTTP (Boeheringer Mannheim, Mannheim, Germany); 0.2 μM each of the PCR primers; and 0.4 units of Prozyme (PROtech Technology, Taipei). The 25 μL PCR mixture was heated at 94 ^{o}C for 1 min using a thermal cycler (GeneAmp PCR System 9600; Perkin-Elmer), followed by 35 PCR cycles. For each PCR cycle, denaturation, annealing, and extension were carried out at 94 ^{o}C for 35 s, 58 ^{o}C (for *L. pentosus*) or 65 ^{o}C (for *L. plantarum*) for 35 s, and 72 ^{o}C for 40 s, respectively. Final extension was carried out at 72 ˚C for 4 min. To detect the amplified product, 3.5 μL of the PCR product was electrophoresed on a 2 % agarose gel in 0.5× TAE buffer (10× TAE: 40 mM Tris acetate, pH 7.6; 10 mM Na_2EDTA). The gel was visualized, and PCR products were identified by comparison with the molecular weight markers of a 100 bp DNA ladder (Promega Co., Madison, WI, USA), and target DNA PCR length of *L. pentosus* is 247 bp, as well as *L. plantarum* is 270 bp.

2.7 API 50 CHL system

Fermentation of carbohydrates was determined using API 50 CHL, a standardized system consisting of 50 biochemical tests for the study of carbohydrate metabolism by microorganisms. API 50 CHL was used for the identification

Table 1: PCR primers for the detection of *tuf* or *recA* genes in *Lactobacillus* spp

Target strain	Primer	Sequences	Accession No.a	Location	Length (bp)
L. pentosus	Lpen_rF	5'-AACAATTTCCAGCGGGTCAC-3'	AJ640079	*recA gene*	247
	Lpen_rR	5'-ATCTGGTTGTGAAAGTAACAAA-3'			
L. plantarum	Lpla_rF	5'-GTATATCGATGCCGAAAATGCAC-3'	AJ621666	*recA gene*	270
	Lpla_rR	5'-GTCCCTGATAACTTCCGGAGC-3'			

[a]The accession numbers of *tuf* gene were obtained from GenBank database

of *Lactobacillus* and related genera according to the manufacturer's instructions (BioMérieux, Marcy-l'Etoile, France).

2.8 Statistical analysis

Data were presented as means ± standard error (SEM) of the three replicates. The Statistical Package for the Social Sciences, version 12.0, and software package was used. One-way analysis of variance (one-way ANOVA) was used for each experimental group. The Duncan's new multiple range tests analysed the differences in the mean values among the experimental groups. $P < 0.05$ was considered statistically significant.

3 Results and Discussion

3.1 Absorptive colorectal cancer cells

In this study, strains were screened for their ability to colonize HCT-116 colorectal cancer cells. The average number of bacteria adsorbed on HCT-116 cells was 29.4 ± 7.1 for strain 03-03-026, whereas the average numbers of adsorbed bacteria were 43.1 ± 14.7 for strain 03-03-027 (Table 2).

Lactobacilli are natural colonizers of the human gastrointestinal tract and subdominant colonizers in the colon. *Lactobacillus* has certain characteristics to exert probiotic effect, such as bacterial adhesion and excluding enteric pathogens and their immunomodulatory effects (Kotzamanidis et al., 2010). Rinkinen et al. found that host-derived probiotics may also be effectively adsorbed on the intestinal mucosa of other hosts, and this adsorption was mainly based on the strain characteristics of the probiotics itself, not its host source (Rinkinen et al., 2003).

3.2 Cell viability analysis

After 12 h of HCT-116 cells treated with 03-03-026, significant inhibited the growth of HCT-116 cells compared to the control group (HCT-116 cells alone) (Fig. 1a). Strain 03-03-027 was co-cultured with HCT-116 cells for 12 and 24 h under the aforementioned conditions. Except when cell lines were co-cultured with 105 and 106 CFU/mL LAB, all other conditions significantly inhibited growth (Fig. 1b). In addition, when 03-03-026 and 03-03-027 were unadjusted, the pH values of the supernatants were 4.32 and 4.78 respectively, which may have reduced the cell survival rate. Therefore, the supernatant was adjusted to a pH value of 7.2, which significantly increased the cell survival rate.

O'Hara et al. used the MTT and Terminal deoxynucleotidyl transferase dUTP nick end abelling (TUNEL) assays to determine inhibition of cytokine-induced apoptosis in epithelial cells and confirmed that *L. plantarum* promotes the balance of intestinal epithelial cells and increases their survival (O'Hara et al., 2006). The adsorption capacity of LAB can hinder the absorption of many pathogenic and nonpathogenic bacteria and colonize the host's intestinal cells.

3.3 ELISAs to determine IL-8 secretion

We co-cultured *S.* typhimurium flagella-purified material with HCT-116 cells and found that the secretion of IL-8 was 1,876 ± 262 pg/mL (Fig. 2). After this experiment, we ultra sonicated

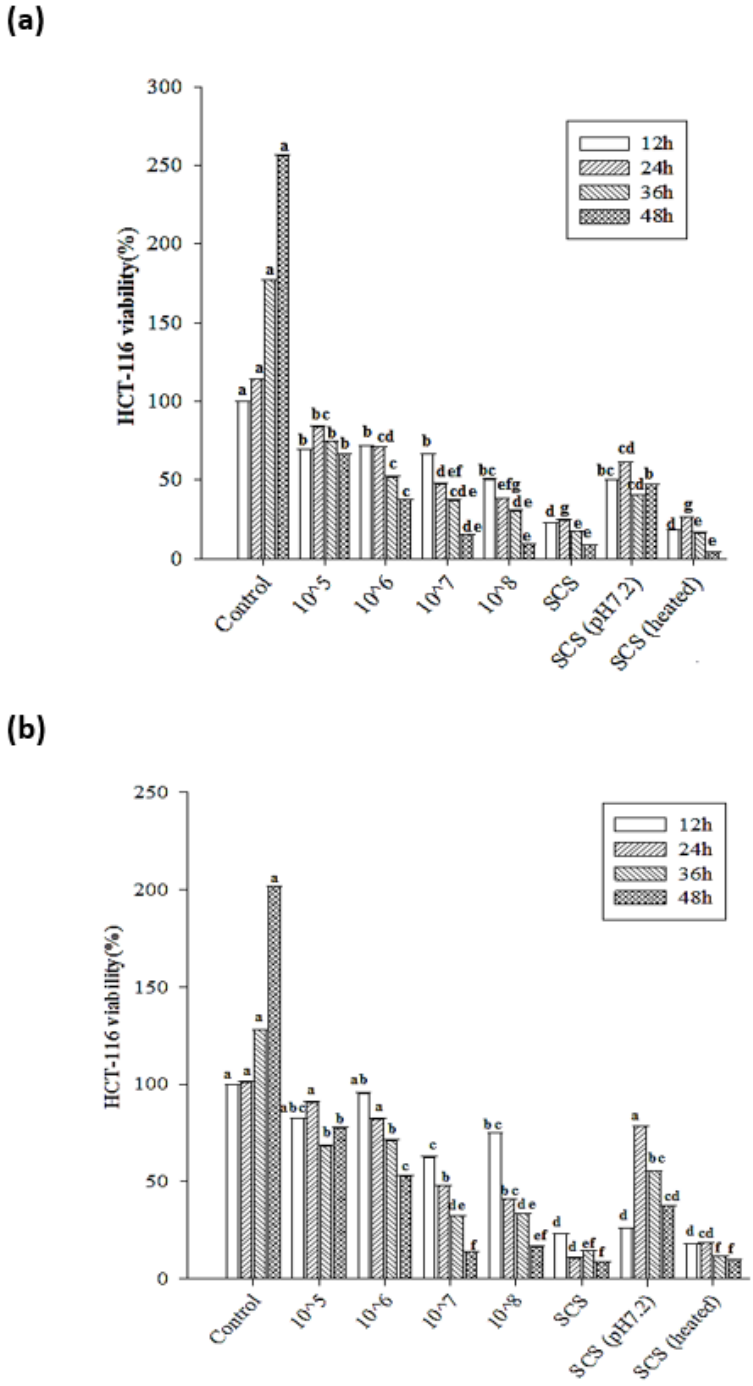


Figure 1: The effect of strain 03-03-026 on the viability of (a) HCT-116, and strain 03-03-027 on the viability of (b) HCT-116 for 12 h, 24 h, 36 h and 48 h. The viability of cell was determined by MTT assay. Each value is mean ± standard derivation of replicate analyses. Means with different superscript letters in the same bar are significant differences ($p<0.05$) using the Duncan's multiple rang test.

Table 2: Adhesion of various lactic acid bacteria strains onto the HCT-116 cell line.

Strain NO.	Adherence to the epithelial cells[a]
	HCT-116
03-03-026	29.4 ± 7.1
03-03-027	43.1 ± 14.7

Each adhesion assay was conducted in triplicate with cells from three successive passages. Adhesion assays were monitored after 2 h of incubation. The table presents mean numbers ± standard deviation of bacteria adhering per epithelial cells.

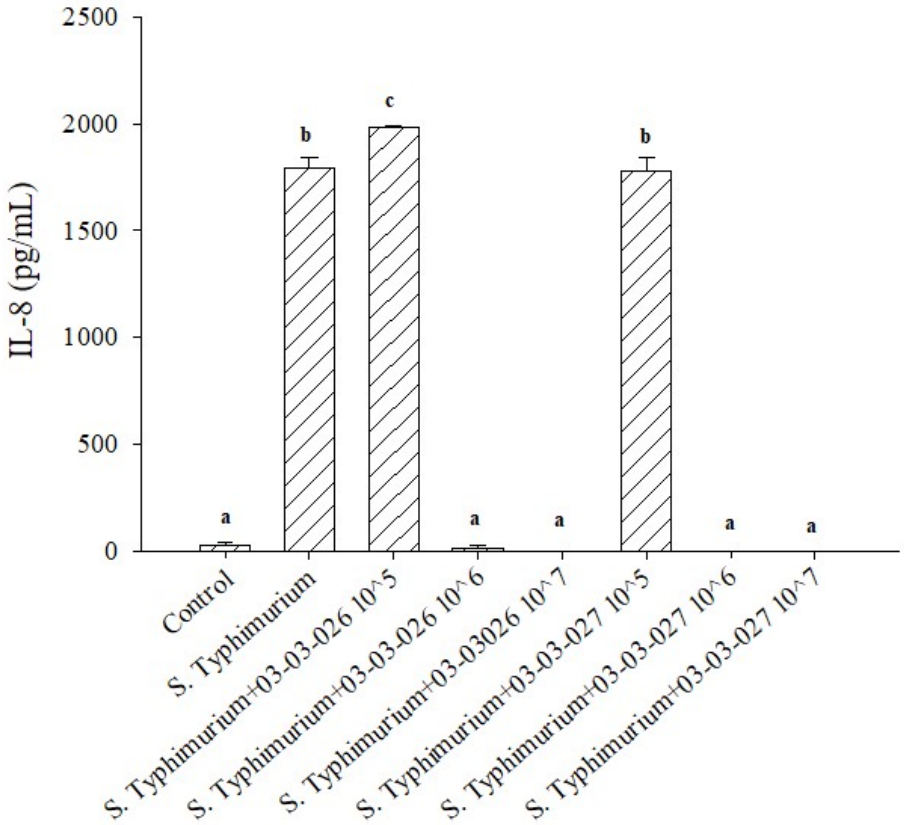


Figure 2: The effect of lactic acid bacteria on *S.* typhimurium I50 -induced IL-8 secretion by HCT-116 cells. Each value is mean ± standard derivation of replicate analyses. Means with different superscript letters in the same bar are significant differences ($p<0.05$) using the Duncan's multiple rang test.

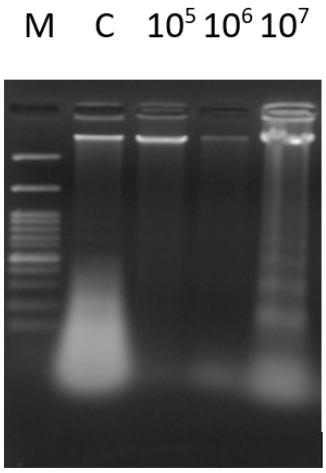


Figure 3: Effect of strain 03-03-026 on DNA fragmentation in HCT-116 cells for 48h.

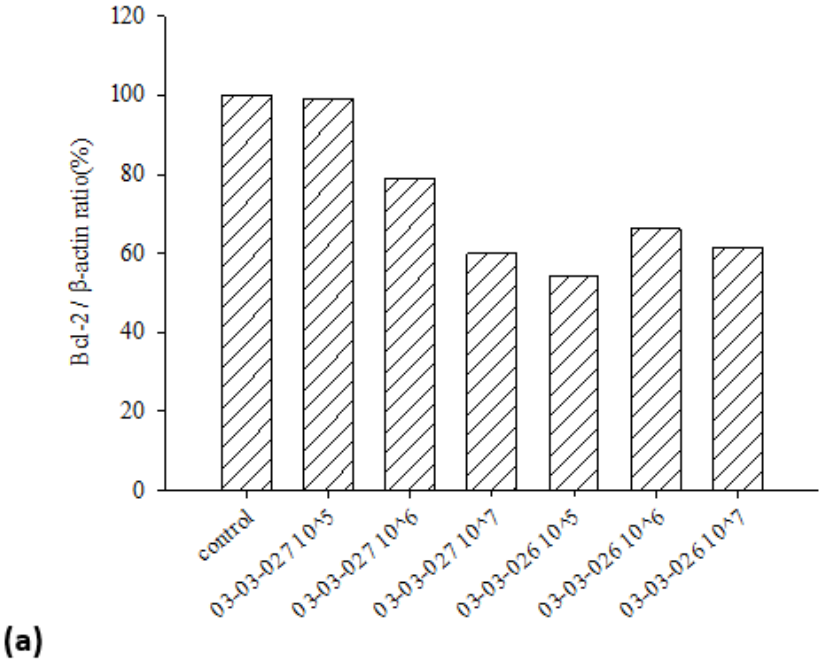
S. typhimurium and co-cultured with HCT-116 cells under the following conditions for 24 h to assess IL-8 secretion: (1) control group (only HCT-116 cells); (2) cells and ultra sonicated *S.* typhimurium only; (3) cells, ultra sonicated *S.* typhimurium, and 03-03-026 (10^5, 10^6, and 10^7 CFU/mL); (4) and cells, ultra sonicated *S.* typhimurium of different concentrations, and 03-03-027 (10^5, 10^6 and 10^7 CFU/mL). For co-cultures of HCT-116 cells and *S.* typhimurium, IL-8 secretion was 1,736.8 ± 15.7 pg/mL (Fig. 2). HCT-116 cell lines were significantly stimulated by *S.* typhimurium to secrete IL-8. When 03-03-026 and 03-03-027 reached concentrations of 10^6 CFU/mL and 10^7 CFU/mL, IL-8 secretion in HCT-116 cell lines were significantly inhibited (Fig. 2).

Xu et al. investigated the effects of LPS (1 μg/mL) on rat intestinal microvascular endothelial cells and found that adding lactate (7.5 μL/mL) to the medium decreased LPS-induced TNF-α and IL-6 mRNA expression (Xu et al., 2013). O'hara et al. showed that *S.* typhimurium UK1 rapidly induced IL-8 mRNA expression in HT-29 cells in 2 h, and IL-8 mRNA expression was regulated by NF-κB, which the authors determined was not activated due to *B. infantis* 35624 and *L. salivarius* UCC118 nor did these strains impact IL-8 production in HT-29 cells (O'Hara et al., 2006). In addition, the authors assessed *S.* typhimurium infection with *B. infantis* and *L. salivarius* in HT-29 cells for 2 h and observed that NF-κB significantly reduced binding activity. Since this result was only observed in confluent HT-29 cells, it suggests that the intact barrier function is important to normal epithelial immune activity.

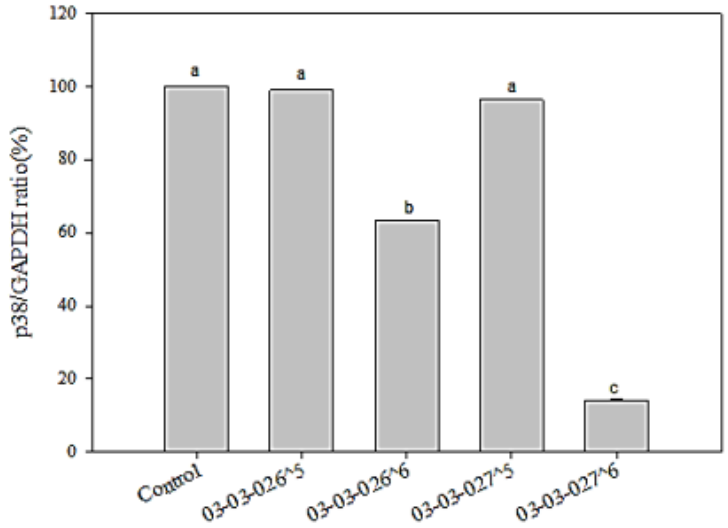
3.4 DNA fragmentation assay, protein expression of Bcl-2 and Phosphorylated-p38 (p-p38) dynamics

HCT-116 cells were co-cultured with 03-03-026 for 48 h to perform a DNA fragmentation assay. Fig. 3 shows that the strain 03-03-026 (10^7 CFU/mL) has DNA fragmentation, but not at 10^5 and 10^6 CFU/mL. Bcl-2 (B-cell lymphoma 2), encoded in humans by the BCL2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell death (apoptosis), by either inducing (pro-apoptotic) or inhibiting (anti-apoptotic) apoptosis. In addition, Bcl-2 expression was observed. Fig. 4(a) shows that Bcl-2 expression in HCT-116 cells decreased with co-cultivation of the two strains. The inhibitory effect was also apparent, indicating that the two lactic acid bacteria may induce apoptosis. Fig. 4(b) shows that when co-cultured with either of two strains, the expression of p38 kinase in HCT-116 cells significantly reduced; with the effect of 03-03-27 strain was significantly greater than that of 03-03-026. The increase in LAB concentration yielded an enhanced inhibitory effect. Additionally, we further found that phosphorylation of p38 in HCT-116 cells significantly increased. Our results indicated that the two LAB strains inhibited IL-8 secretion and may reduce intestinal cell inflammation via affecting p38 kinase.

Aggarwal et al. illustrated that DNA fragmentation is an indicator of apoptosis (Aggarwal et al., 2004). The Bcl-2 family proteins play different roles in regulating programed cell apoptosis in mitochondria by promoting or inhibiting the release of apoptotic molecules from the mitochondria into the cytoplasm (Marzo et al., 1998). This protein family regulates mitochondrial per-



(a)



(b)

Figure 4: The effects of lactic acid bacteria 03-03-026 and 03-03-027 on the expression of (a) Bcl-2 and (b) hosphorylated p38/ p38 kinase in HCT-116 cells after co-culture for 24 h

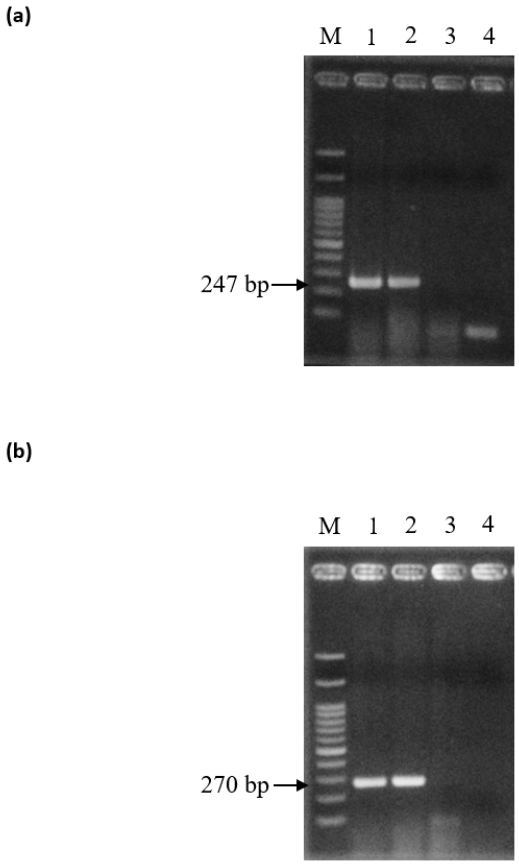


Figure 5: The PCR result of strain 03-030-026 and 03-030-027 using (a) *L. pentosus* and (b) *L. plantarum* species specific-primers of tuf gene. M: Marker (100 bp ladder); lane 1 (sample): (a) strain 03-030-026 and (b) strain 03-030-027; lane 2 (positive control): (a) *L. pentosus* BCRC 17972 and (b) *L. plantarum* BCRC 10069; lane 3 (negative control): (a) *L. plantarum* BCRC 10069 and (b) *L. pentosus* BCRC 17972; lane 4 (blank control): ddH2O.

meability by interacting with adenosine translocators and voltage-dependent ion channels for ADP/ATP exchange or interfering with oxidative phosphorylation during programed apoptosis (Marzo et al., 1998). The 3D structure of Bcl-2 was shown to have the ability to form channels on the mitochondrial membrane (Minn et al., 1997; Muchmore et al., 1996; Schendel et al., 1998). Bcl-2 regulates anti-apoptotic function of cells via post-transcriptional modifications (including phosphorylation, dimer formation, or proteolysis) (Cheng et al., 2001; Yin et al., 1994).

3.5 Biochemical and molecular identification of LAB strains

Fig. 5 demonstrates that strain 03-03-026 identified as *Lactobacillus* pentosus (93.6%). Strain 03-03-027 identified as *Lactobacillus* plantarum (99.2%) by API 50CHL. Molecular identification, via PCR, corroborated our biochemical findings, yielding the same results.

4 Conclusions

In this study, two strains, *Lactobacillus pentosus* 03-03-026 and *L. plantarum* 03-03-027, demonstrated good adsorption onto HCT-116 cells. When investigating these LAB strains in the context of inflammation, ultra-sonicated *S.* typhimurium co-cultured with the LAB strains in HCT-116 cells. We herein showed that LAB can inhibit IL-8 secretion. Assessing via Western blotting, the expression of phosphorylated p38 protein decreased after co-culturing of HCT-116 cells with *L. pentosus* 03-03-026 and *L. plantarum* 03-03-027, which also illustrated that these LAB strains can reduce inflammation. We also showed that co-culturing HCT-116 cells with *L. plantarum* 03-03-027 led to DNA fragmentation of the HCT-116 cells. In addition, after co-culturing HCT-116 cells with *L. pentasus* 03-03-026 and *L. plantarum* 03-03-027, Bcl-2 protein expression decreased, indicating that these LAB strains may promote apoptosis. Further studies are warranted to investigate the anti-tumour effects in the animal model.

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