

## Adhesion Ability and Cytotoxic Evaluation of *Lactobacillus* Strains Isolated from Malaysian Fermented Fish (Pekasam) on Ht-29 and Ccd-18Co Intestinal Cells

(Keupayaan Perlekatan dan Penilaian Sitotoksik Strain *Lactobacillus* Dipencilkan daripada Ikan Pekasam Malaysia ke atas Sel Usus Ht-29 dan Ccd-18Co)

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### ABSTRACT

*Bacterial adhesion to host cells is the most important probiotic character. However, the adhesion of probiotic should not affect the viability of the host cells. In this study, Lactobacillus plantarum strain L8, Lactobacillus plantarum strain L20 and Lactobacillus pentosus strain S1 were tested for their cytotoxic effects through MTT assay and their ability to adhere and colonize on HT-29 and CCD-18Co intestinal cells as detected microscopically using light microscopy and Scanning Electron Microscopy (SEM). No cytotoxicity effects were observed on both intestinal cells following 24 h treatment with all Lactobacillus strains. Additionally, all strains demonstrated strong adhesive activity where more than 100 bacteria adhered to both intestinal cells although differences in the adhesion scores observed among different strains. The adhesion as observed via SEM showed an autoaggregative pattern and adhered as clusters on the surface of both intestinal cells. In conclusion, all three Lactobacillus strains are non-cytotoxic to both cells with strong adhesion ability on intestinal cells and this study also proved that Malaysian fermented fish are good source of probiotic bacteria.*

*Keywords: Adhesion; Lactobacillus; probiotics; scanning electron microscopy (SEM)*

### ABSTRAK

*Perlekatan bakteria pada sel perumah merupakan kriteria probiotik yang paling penting. Walau bagaimanapun, perlekatan probiotik mestilah tidak menjejaskan keviabelan sel perumah. Dalam kajian ini, Lactobacillus plantarum strain L8, Lactobacillus plantarum strain L20 dan Lactobacillus pentosus strain S1 telah diuji kesan sitotoksik ke atas sel usus melalui ujian MTT dan keupayaan mereka untuk melekat dan mengkoloni sel usus HT-29 dan CCD-18Co seperti yang dikesan secara mikroskopik menggunakan mikroskop cahaya dan pengimbasan mikroskop elektron (SEM). Keputusan kajian menunjukkan kesan sitotoksik tidak terdapat pada kedua sel usus berikut rawatan 24 jam dengan semua strain Lactobacillus. Selain itu, semua strain menunjukkan aktiviti perlekatan yang kuat untuk kedua sel usus walaupun terdapat perbezaan skor perlekatan pada strain yang berbeza. Paten perlekatan di bawah SEM menunjukkan corak autoagregatif dan melekat secara kluster pada permukaan kedua-dua sel usus. Kesimpulannya, kesemua strain Lactobacillus yang dikaji adalah tidak sitotoksik pada kedua sel dengan mempamerkan keupayaan perlekatan yang kuat pada sel usus.*

*Kata kunci: Lactobacillus; pengimbasan mikroskop elektron (SEM); perlekatan; probiotik*

### INTRODUCTION

Interest in the discovery of new probiotics from natural and sustainable resources for health and civilization development is growing. The origins, non-pathogenicity characteristic and *in vitro* adherence ability to the intestinal cells of the strain are the most important criteria to be considered a probiotic (Coconier et al. 1992). *Lactobacillus* is one of the most characterized probiotic microorganisms (Holm 2003) because of its ability to provide therapeutic benefits by producing lactic acid and antibacterial substances to stimulate and modulate the host immune system (Liong 2008). Lactobacilli are Gram-positive anaerobic or facultative aerobic bacteria that can be isolated from different resources (Wang et al. 2009). They are non-pathogenic and non-spore-forming rods in morphology with the majority inhabiting the

intestinal tract cavity. Currently, the genus *Lactobacillus* has been widely used as a probiotic source (Pringsulaka et al. 2015) as it has several health-promoting effects and claims, such as improved gastrointestinal immune response (Christensen et al. 2002), prevention of diarrhea (Biller et al. 1995), treatment of colon cancer (Koop-Hoolithan 2001), attenuate hypertension (Yap et al. 2016) and many others. Probiotic is designated by FAO/WHO (2002) as a live microorganism that offers health benefits to host cells when administered in sufficient quantity. One of the important benefits of probiotics to humankind is their ability to colonize the gastrointestinal tract (Lee et al. 2017).

The excellent capacity to adhere to epithelial cells and mucosal surfaces has been suggested to be an important step of colonization as it could protect the gastrointestinal environment from pathogens by competing for the receptor

sites on the cell surface (Nader-Macias et al. 2007). Bacterial adhesion is a complex process of innate and adaptive interactions involving direct contact between the bacterial cell membrane and the receptor of host cells on the epithelial surface (An & Friedman 2000). Chang et al. (2004) showed that adhesion is one of the strategies used by bacteria to stabilize in different ecological niches by competing for nutrients with other bacteria. Other studies reported that the adhesion ability of probiotic bacteria to intestinal cells could prevent the attachment of pathogens, thus protecting the intestinal tract from infection (Lee et al. 2000). Probiotic bacteria can successfully inhibit the adhesion of pathogenic microorganisms, such as *Salmonella*, *Escherichia coli*, *Staphylococcus aureus* and *Clostridium difficile* (Collado et al. 2007). Ayeni et al. (2011) isolated *Lactobacillus paracasei* and *Lactobacillus brevis* from Nigerian traditional fermented dairy foods and they showed good adhesion ability to Caco-2 and HT-29 epithelial intestinal cells. Similarly, Ren et al. (2014) reported that the *Lactobacillus plantarum* strain CGMCC 1.557 isolated from vegetable showed the most adhesion and produced the highest quantity of exopolysaccharides among eight other *Lactobacillus* strains.

Currently, both health and industrial sectors are showing great interest in the search for new strains of probiotics with functional properties, especially from traditional fermented foods (Ayeni et al. 2011). Non-industrialized countries offer a variety of traditional fermented foods, which comprise a pool of new strains with excellent functional properties (Thapa et al. 2006). *In vitro* studies on cell lines have been used as an approach to evaluating various characteristics, such as anticancer activity (Hilde et al. 2003), stimulation of the immune system (Isolauri et al. 2001), antimicrobial activity to inhibit the growth of pathogenic bacteria (Ghani et al. 2018) and protection and stabilization of gut microflora (Gibson et al. 1997) by potentially probiotic bacteria. Evaluating the adherence activity of bacteria using human epithelial cell lines as an *in vitro* model has been a common procedure in the preliminary screening of potential probiotic strains. Scanning electron microscopy (SEM) is one of the important tools used to investigate membrane integrity. In the present study, the cytotoxicity potential and adherence capability of *L. plantarum* strain L8, *L. plantarum* strain L20 and *L. pentosus* strain S1 isolated from Malaysian fermented fish (pekasam) on HT-29 and CCD-18Co intestinal cells were evaluated. This study aims to provide beneficial information on the safety and potential probiotic activity of all strains isolated locally from Malaysian fermented fish.

## MATERIALS AND METHODS

### BACTERIAL STRAINS AND GROWTH CONDITIONS

Three *Lactobacillus* strains were used in this study; *L. plantarum* strain L8, *L. plantarum* strain L20 and *L.*

*pentosus* strain S1 were identified using GenBank with accession numbers KT591874, KT 591875 and KT920464 (Ida Muryany et al. 2016), respectively. All the strains were isolated from the Malaysian fermented fish (*from species Johnius Belangerii* and *Thynnichthys thynnoides*) known as *pekasam*. *Lactobacillus* strains were grown at 37°C for 48 h in MRS agar (deMan, Rogosa and Sharpe) (Oxoid, Australia) before being transferred to the MRS broth (Oxoid, Australia) for overnight incubation at 37°C.

### INTESTINAL CELL LINES AND CULTURE CONDITIONS

Human adenocarcinoma cell lines HT-29(ATCC® HTB38™) and human colonic fibroblast CCD-18Co (ATCC® CRL-1459™) were cultured in McCoy's 5A Medium (Sigma, USA) and Eagle's Minimum Essential Medium (Sigma, USA), respectively. Both media were supplemented with 10% (v/v) fetal bovine serum (FBS; Sigma, USA) and 100 µg mL<sup>-1</sup> of penicillin-streptomycin (Sigma, USA). Both cells were cultured in Nunc™ tissue culture flasks (Thermo Fisher Scientific, USA) at 37°C in a 5% CO<sub>2</sub> using a humidified incubator (Binder, USA). The cell culture medium was replaced with fresh medium every other day.

### CYTOTOXICITY ACTIVITY OF PROBIOTICS USING MTT ASSAY

The viability testing of the cells incubated with the bacteria was performed using the modification method by Mario et al. (2014) via the determination of metabolic activity of the mitochondrial lactate dehydrogenase enzyme (Ivec et al. 2007; Mosmann 1983). HT-29 and CCD-18Co cells were seeded separately at 10<sup>5</sup> cells per well using NUNC™ 96 flat bottom well plates (Thermo Scientific USA) and were incubated in a humidified incubator supplemented with 5% CO<sub>2</sub> atmosphere at 37°C for 24 h. Monolayer cells were washed twice with sterile PBS and added with 100 µL of bacteria suspension at 10<sup>9</sup>, 10<sup>8</sup>, 10<sup>7</sup>, 10<sup>6</sup>, 10<sup>5</sup> and 10<sup>4</sup> CFU mL<sup>-1</sup> concentrations in a medium without FBS and antibiotics (incomplete medium). *Lactobacillus* strains L8, L20, and S1 suspensions were each added in triplicate to separate wells and plates and incubated for 4 h in 5% CO<sub>2</sub> at 37°C to allow bacteria to attach to cell monolayers. The cells were washed three times with sterile PBS at 37°C after incubation to remove non-attached bacteria. After washing, 100 µL of fresh incomplete medium was added to the cells and plates and incubated for 24 h in a humidified incubator supplemented with 5% CO<sub>2</sub> at 37°C. The cells that were not treated with additional bacteria were used as negative controls, while cells treated with hydrogen peroxide was used as a positive control. The medium was discarded after incubation, and the wells with monolayer cells were washed twice with sterile PBS at 37 °C. Then, 200 µL of fresh incomplete medium and 20 µL of 0.5% (w/v) MTT solution (Sigma USA) were added to each well. After the addition of the MTT solution, the plates were incubated further for 4 h in the incubator supplemented with 5% CO<sub>2</sub> at 37°C. The medium with MTT solution was discarded and 200 µL of dimethyl sulfoxide (Sigma USA)

was added to each well to solubilize the formazan crystals formed, followed by incubation at room temperature for 30 min. The absorbance was measured spectrometrically after incubation at 570 nm using the microplate reader (Tecan Switzerland).

#### ADHESION DETERMINATION

The adhesion abilities of the *Lactobacillus* strains were identified according to the method described previously by Jacobsen et al. (1999). Both cell monolayers, HT-29 and CCD-18Co intestinal cells ( $10^5$  cells mL<sup>-1</sup> in 6 wells), were washed twice with PBS and 3 mL of incomplete medium was added to each well. The wells were incubated for 30 min before the inoculation of bacteria. Then, 100 µL of overnight culture of bacteria with  $10^8$  CFU mL<sup>-1</sup> was added to each well containing the monolayer cells. After incubation for 3 h at 37°C, all the wells were washed three times with PBS to release the unbound bacteria. The cells were then fixed with 3 mL of methanol (Merck Germany) and incubated for 10 min at room temperature. The cells were stained with 3 mL of Giemsa stain solution (1:50) (Merck Germany) after the removal of methanol and left for 30 min at room temperature. The wells were washed three times in the buffer solution to remove excess stain. The plates were air dried and examined under an inverted microscope (Leica Germany). The number of Lactobacilli that adhered was counted in 20 randomly picked microscopic fields for each intestinal cell. The strain was considered non-adhesive when the total number of bacteria in the 20 microscopic fields was less than 40 bacteria, adhesive when the number was between 41 and 100 and strongly adhesive when more than 100 (Jacobsen et al. 1999).

#### SCANNING ELECTRON MICROSCOPY

An observation was made by SEM for a qualitative examination of the adhesion. Coverslips (13 mm) (Sarstedt USA) were placed at the bottom of the 24-well tissue culture plates (Thermo Fisher Scientific USA). Both the HT-29 and CCD-18Co cells (about  $2 \times 10^5$  cells mL<sup>-1</sup>) were seeded and incubated at 37°C in 5% CO<sub>2</sub> until they reached 80% - 90% confluency. The cell monolayers were washed twice with PBS (pH7.2) before a 900 µL antibiotic-free medium was added. Overnight cultures of *Lactobacillus* strains in MRS broth were centrifuged for 10 min at  $1008 \times g$  and the pellets were re-suspended in an antibiotic-free medium to a final concentration of  $10^8$  CFU mL<sup>-1</sup>. The wells containing monolayer cells and 100 µL bacteria suspensions were incubated for 4 h at 37°C in a humidified incubator supplemented with 5% CO<sub>2</sub>. After incubation, the HT-29 and CCD-18Co cell monolayers (in a different well) were washed three times with 0.1 M phosphate buffer (pH7.2) to release any unbound bacteria. The cells were fixed with 2.5% (v/v) glutaraldehyde (Sigma Aldrich, USA) in 0.1 M phosphate buffer for 2 h at room temperature. Then, the cells were dehydrated in a graded ethanol series (50% v/v, 70% v/v, 80% v/v, 90% v/v and 95% v/v) for 15 min each

session, followed by the dehydration step (twice) in 100% ethanol for 30 min. The cover slips containing the cells were air dried at room temperature for 30 min, mounted on stubs and coated with gold for 15 s. The specimens were then examined through SEM (Live Stereoscopic VPSEM; Hitachi Japan) (Ida Muryany et al. 2016).

#### STATISTICAL ANALYSIS

SPSS version 21 was used for the statistical analyses of the MTT assay and adhesion scores. The data were evaluated using the one-way analysis of variance followed by the Tukey test with the significant value were set at  $P < 0.05$ .

## RESULTS AND DISCUSSION

#### CYTOTOXICITY ACTIVITY OF *LACTOBACILLUS* STRAINS ON INTESTINAL CELLS

Studying the safety of the isolates against intestinal cells before they are utilized as probiotics is important to ensure probiotics would not harm and affect health of consumers. The viability of the intestinal cells exposed to *Lactobacillus* strains; L8, L20 and S1, is shown in Figure 1. The results obtained indicated the viability of both the HT-29 and CCD-18Co cells incubated with all *Lactobacillus* strains compared with the control (Figure 1). No cytotoxic effects were observed on both cell lines following the 24 h incubation at  $10^9$ ,  $10^8$ ,  $10^7$ ,  $10^6$ ,  $10^5$  and  $10^4$  CFU mL<sup>-1</sup> concentrations of the *Lactobacillus* strains used. The viability of both cells treated with all probiotics at various concentrations showed no significant difference ( $P > 0.05$ ) from the untreated cells (control). Similarly, the statistical analysis of the different strains of *Lactobacillus* also showed no significant difference ( $P > 0.05$ ) in the viability of both intestinal cells. MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay has been widely used to screen the cytotoxic potential of various compounds of interest. MTT assay is reliable, non-time consuming, inexpensive and always used as the preliminary screening method for the measurement of cell viability and membrane integrity (Sylvester 2011). MTT assay is based on the ability of an active dehydrogenase enzyme to reduce the tetrazolium yellow substrate into formazan dark blue crystals. The reduction serves as an index of living, metabolically active cells (Mosmann 1983). Our results showed that no cytotoxic effects were observed in both intestinal cell lines following incubation with all *Lactobacillus* strains.

Similar results were also described by Rossella et al. (2009) following the incubation of Caco-2 cells with *L. plantarum*. No significant effect on the viability of the cells was observed after the 12, 24 and 48 h treatment, but an increase in the viability of intestinal epithelial cells was observed instead. Our results agreed with the earlier observation by Mario et al. (2014) that *L. plantarum* strain PCS20 and strain PCS26 had no toxic effects on the HIEC and HUIEC cell lines. By contrast, Ewaschuk et al.

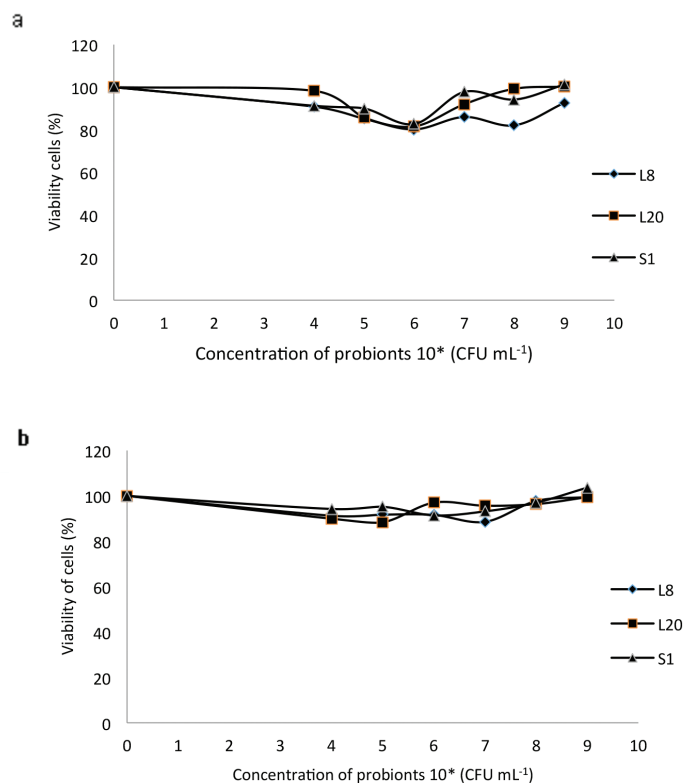


FIGURE 1. Cytotoxic effects of probiotics *Lactobacillus* strains on (a) HT-29 cells and (b) CCD-18Co cells treated with different concentrations of probiotics for 24 h. The cytotoxicity activity was determined using the MTT assay at an optical density of 570 nm. No significant different ( $P > 0.05$ ) was observed in the viability of both cells treated with all probiotics at various concentrations with untreated cells (control) and no significant difference ( $P > 0.05$ ) was also observed in the viability of both intestinal cells among different strains of *Lactobacillus*

‡L8, *L. plantarum* strain L8; L20, *L. plantarum* strain L20; S1, *L. pentosus* strain S1

(2006) reported that *L. plantarum*, *L. casei*, *B. longum*, *Streptococcus thermophilus* and *Bifidobacterium breve* reduced the viability and induced the apoptosis of HT-29 and Caco-2 cells following a 24 h treatment. According to Hojjat et al. (2014), the toxic effects and positive role of lactic acid bacteria are strain dependent and vary on intestinal cells, but the mechanism of these bacteria is still poorly understood. However, no study on the cytotoxic activities of *L. pentosus* has been found in previous literature.

#### ADHESION ABILITY OF *LACTOBACILLUS* STRAINS ON INTESTINAL CELLS USING ADHESION ASSAY

The quantitative binding of the *Lactobacillus* strains on the HT-29 and CCD-18Co cells was determined by direct microscopic examination using Giemsa staining (Figures 3 and 4). The results indicated that all the *Lactobacillus* strains tested were categorized as strongly adhesive because more than 100 bacteria were adhered on the cells in 20 randomly selected microscopic fields (Figure 2). The highest level of adherence was observed with L20, followed by L8 and then S1. Both the L20 and S1 strains presented higher adherence to CCD-18Co cells than to HT-29 cells. Figure 2 shows that the adhesion scores of both

cells treated with L8 were significantly different ( $P < 0.05$ ) from that of the untreated cells. No significant difference ( $P > 0.05$ ) was observed between the HT-29 and CCD-18Co cells. However, the adhesion scores of L20 and S1 on the CCD-18Co cells were significantly different ( $P < 0.05$ ) in all groups of treatment. The mucosal of the intestine absorbs essential nutrients from the lumen for the body and produces mucous and cytokines with protective and signaling characteristics (Artis 2008). Therefore, probiotics should adhere onto the mucosal surfaces and then interact with the host surroundings. Adhesion is a complex route with multistep processes involving both non-specific mechanisms and a specific ligand receptor (An & Friedman 2000). Our findings show that the adhesion scores of all bacteria tested indicates excellent abilities to adhere and colonize on both intestinal cells (Figures 3 and 4) according to the classification by Jacobsen et al. (1999). The average numbers of bacteria adhering to the intestinal cells were greater than 100 bacteria/20 microscopic fields. This finding is similar to that obtained by Raj et al. (2011) using the isolates from *L. plantarum* Lp91 and *L. plantarum* Lp9. Both strains were the most adhesive, whereas *Lb. delbrueckii* CH4 showed the least adhesion activity among the isolates based on the same classification suggested by Jacobsen et al. (1999) on HT-



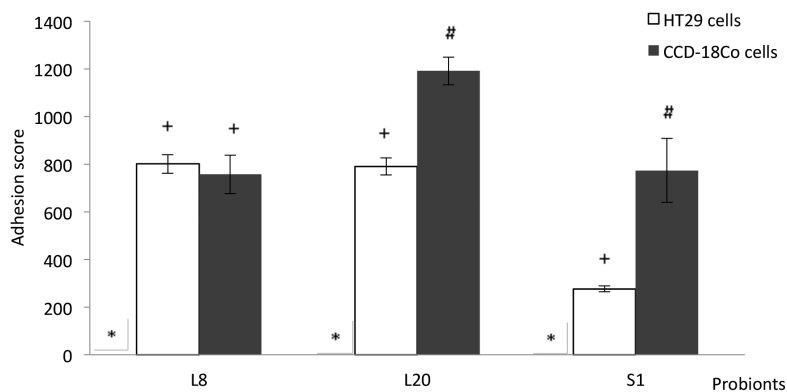


FIGURE 2. Adhesion scores of *Lactobacillus* strains isolated from Malaysian fermented fish to HT-29 and CCD-18Co cells. Different symbols on the bar indicate significant differences at  $P < 0.05$ . Symbol (\*) indicates untreated cells which means no adhesion score

‡L8, *L. plantarum* strain L8; L20, *L. plantarum* strain L20; S1, *L. pentosus* strain S1

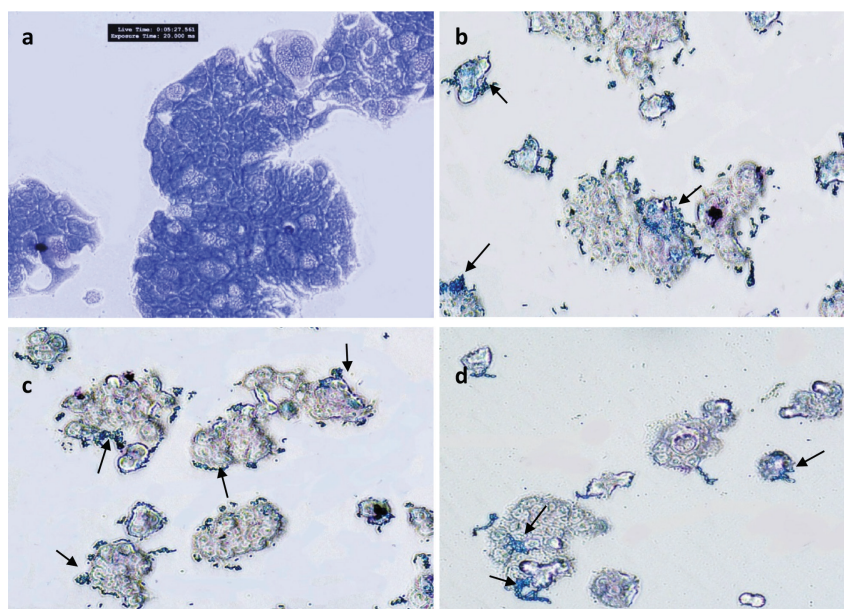


FIGURE 3. Arrows indicate the adhesion of *Lactobacillus* strains on HT-29 cell lines observed under an inverted microscope (40 $\times$ ) following staining with Giemsa stain (a) Untreated HT-29 cells, (b) *L. plantarum* strain L8, (c) *L. plantarum* strain L20 and (d) *L. pentosus* strain S1

29 and Caco2 cell lines. The previous study conducted by Pringsulaka et al. (2015) also showed that the *L. plantarum* strain P6 exhibited an excellent ability to adhere to COLO 205 cell lines as observed using light microscopy with Gram staining. In addition, Wang et al. (2009) reported that *L. plantarum* L2 was the most adhesive strain to adhere to IEC-6 and CaCo-2 cells.

Normal microbiota and pathogenic microorganisms have been shown to produce specific compounds that are also involved in their adhesion activity to epithelial host cells. According to a previous report by Otero and Nader-Macias (2007), various macromolecules called adhesins, which could cause an excellent ability to adhere to intestinal epithelium, were characterized in *Lactobacillus*. The

different amounts of adhesins produced by *Lactobacillus* generate different rates of adhesion activity for different strains. Chauviere et al. (1992) also reported that only some strains of *Lactobacillus* were able to adhere to intestinal epithelium and they varied within the same species. Our results also showed that *Lactobacillus* strains could also be regarded to have an excellent adhesion property to act as potential probiotics.

#### ADHESION PATTERN OF *LACTOBACILLUS* STRAINS ON CELL LINES OBSERVED USING SCANNING ELECTRON MICROSCOPY

The electron microphotographic images obtained from the SEM illustrated the adhesion of *Lactobacillus* strains on

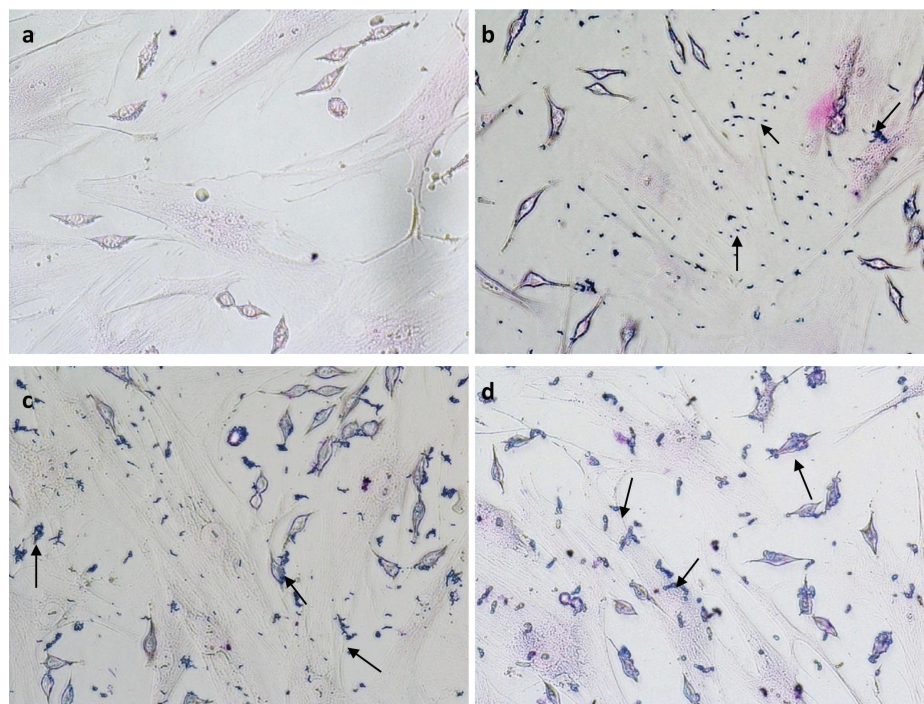


FIGURE 4. Arrows indicate the adhesion of *Lactobacillus* strains on CCD-18Co cell lines observed under an inverted microscope (40 $\times$ ) following staining with Giemsa stain (a) Untreated CCD-18Co cell, (b) *L. plantarum* strain L8, (c) *L. plantarum* strain L20 and (d) *L. pentosus* strain S1

the surfaces of HT-29 (Figure 5) and CCD-18Co intestinal cells (Figure 6). These three strains demonstrated an autoaggregative pattern and physically interacted with each other as a group on the surface and on the side of the epithelial cells. Photographs also showed that the attachment of *Lactobacillus* strains onto both intestinal cells did not cause any cell damage or epithelium loss. Both HT-29 and CCD-18Co intestinal cells remained intact with no morphological shrinkage observed. Many protruding structures and abundant cilia could be seen on the surface of the CCD-18Co cells. The bacteria showed good attachment on the surface of the cells, thus suggesting that they interacted well with the host (Pavelka & Roth 2015). Under the SEM observation, all *Lactobacillus* strains tested showed the ability to adhere to both HT-29 and CCD-18Co intestinal cells. All strains were bound to both cells through the formation of attachments, which were characterized by the association with the external protein S-layer (Frece et al. 2005) and production of lipoteichoic acid by adherent bacteria (Tannock 1990). All *Lactobacillus* strains manifested on both cell lines with the same adhesive qualities and remained stable *in situ*. Antonio et al. (2005) claimed that LAB including *Lactobacillus* could form a biofilm on the mucosal surfaces that could confer a physical barrier for non-desirable microorganisms. Furthermore, LAB also adhered to the epithelial layer covered by mucus on the intestinal mucosa (Collado et al. 2007).

In our microscopic observation, SEM images also showed an autoaggregative pattern of bacterial attachment. Autoaggregation is a reversible congregation and

accumulation of bacterial cells belonging to the same strain on the host cells (Gobin 2011). A previous study demonstrated that autoaggregation was strongly related to adhesion (Del Re et al. 2000) and that the adherence to intestinal mucosa was the first step to intestinal colonization and could enhance the protection of gastrointestinal tract (Alander et al. 1999). Our results were similar to those of Tamara et al. (2012), in which three *L. plantarum* strains (S1, A and B) showed high autoaggregation percentages of more than 80%. The attachment of the bacteria on the intestinal cells are very good to prevent their detachment which can be caused by strong forces. Therefore, the bacteria could remain adhered to the intestinal cells even after tough and long treatments during the preparation of the samples (Otero & Nader-Macias 2007). As conclusion, probiotic bacteria with strong adherence activity onto intestinal cells could have good interaction with the host then will show excellent immunomodulatory activity.

#### CONCLUSION

In the current study, the potential probiotic of *Lb. plantarum* strain L8, *Lb. plantarum* strain L20 and *Lb. pentosus* strain S1 had demonstrated no cytotoxic effects towards both HT-29 human adenocarcinoma cells and CCD-18Co human colon fibroblast cells. Moreover, these *Lactobacillus* strains have shown high ability to adhere to both intestinal cells as determined using the Giemsa staining and observed under inverted microscopy. Scanning electron microscopy also showed the pattern of adhesion of all *Lactobacillus*



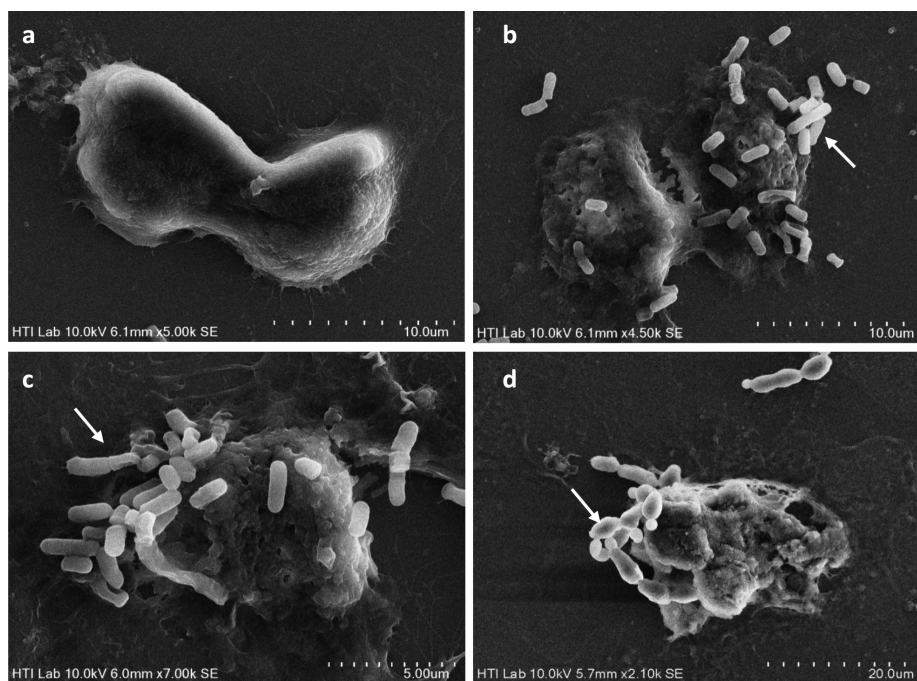


FIGURE 5. Adhesion patterns of *Lactobacillus* strains on the surface of HT-29 intestinal cells by SEM. *Lactobacillus* strain adheres and aggregates to the surface of cells (a) Untreated HT-29 cells, (b) *L. plantarum* strain L8, (c) *L. plantarum* strain L20, and (d) *L. pentosus* strain S1. Arrows indicate the attachment and autoaggregative pattern of *Lactobacillus* strains on HT-29 cells

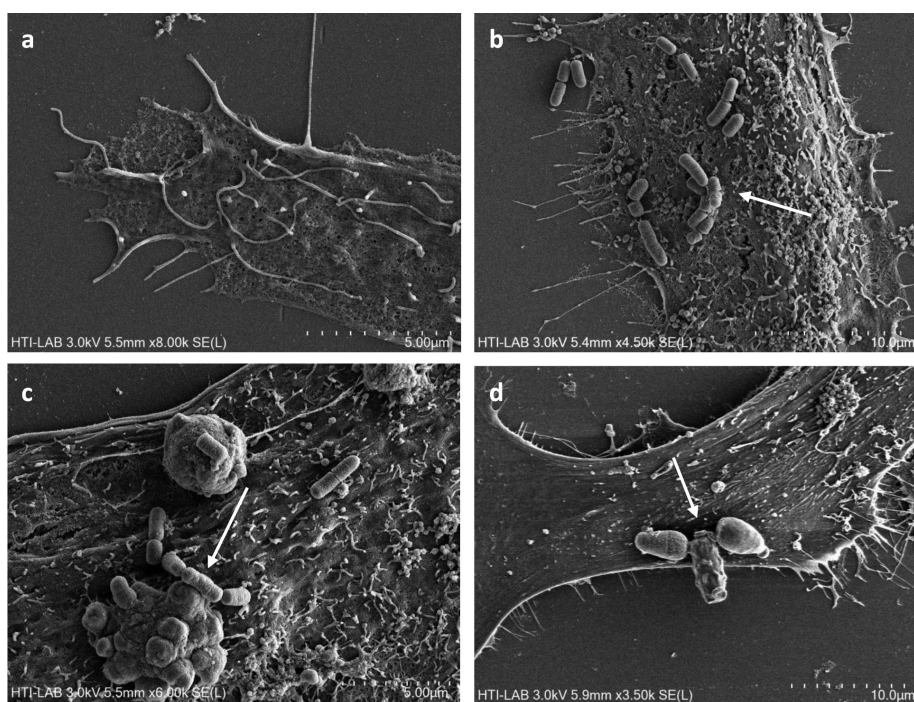


FIGURE 6. Adhesion patterns of *Lactobacillus* strains on the surface of CCD-18Co intestinal cells by SEM. *Lactobacillus* strain adhered and were aggregated to the surface of cells (a) Untreated CCD-18Co cells, (b) *L. plantarum* strain L8, (c) *L. plantarum* strain L20, and (d) *L. pentosus* strain S1. Arrows indicate the attachment and autoaggregative pattern of *Lactobacillus* strains on CCD-18Co cells

strains could also remain stable on the surface and sides of the cells. Further studies are still needed to elucidate the

mechanism behind the intimate attachment between the potential probiotic lactobacilli and intestinal cells.

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