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FULL PAPER

Antibacterial, simulated digestive system tolerance and colon cancer cell lines adhesion properties of selected lactic acid bacteria of food origin

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Abstract

Lactic acid bacteria (LAB) are widespread in nature and are one of the major microbial groups involved in the fermentation of different types of food. LAB possess a large number of metabolic properties that are responsible for the organoleptic characteristics of the final product including antioxidants, organic acids, antimicrobial compounds, modulating and improving microbial balance in the gut. Therefore, to determine the appropriate LAB for the development of functional food products, commercial LAB strain screening had been carried out. The antibacterial activity, tolerant to acids (pH 2, 3 and 4), bile salts (0.3%, 0.5% and 1.0%) and adhesion to colon cancer cell lines (HT29 and SW480) of several LABs (*Lactobacillus paracasei* UALpc-04™, *Lactobacillus plantarum* UALp-05™, *Lactobacillus acidophilus* DDS®-1, *Bifidobacterium bifidum* UABb-10™ and *Streptococcus thermophilus* UASt-09™) were performed. The studies indicated that *Lb. plantarum* UALp-05™ and *B. bifidum* UABb-10™ showed more than 10^6 CFU/mL viability when tested against acid and bile saline with higher adherence to cancer cell lines compared to other strains. The antibacterial activity showed that these two LAB strains also gave a higher inhibition zone of 13 to 17 mm against five strains of pathogens (*Escherichia coli* ATCC® 0157™, *Salmonella enterica* serovar Typhimurium ATCC® 53648™, *Salmonella enterica* serovar Enteritidis (MDC15), *Streptococcus bovis* ATCC® 9809™ and *Listeria monocytogenes* ATCC® 51772™). Our results indicated that *Lb. plantarum* UALp-05™ and *B. bifidum* UABb-10™ strains could be used as adjunct cultures for contributing to the health of gastrointestinal system and decreasing the risk factor of colon cancer.

1. Introduction

In recent times, cancer has been diagnosed as one of the diseases dramatically increasing around the world. According to Schliemann *et al.* (2020), in Malaysia, colon cancer is the third leading cause of death due to cancer and is more common in men than women. In addition to being caused by genetic factors, colon cancer is often associated with environmental factors such as lifestyle and diet (Imen *et al*., 2013). Diverse lifestyles and diets cause the intestinal environment and the composition of the intestinal microbiota to change. Conlon and Bird (2015) reviewed that an unhealthy lifestyle such as smoking and lack of exercise can potentially impact the microbiota in the large bowel to become a risk factor for colorectal cancer. Indeed, toxic particles from smoking will increase the composition of *Bacteroids* - *Prevotella* in the intestines causing inflammatory bowel disease. Meanwhile, a lack of exercise is usually associated with an obesity problem that might shift the populations of microbial by increasing *Firmicutes* and decreasing *Bacteroidetes*. This microbial shift due to high-fat content in the body will form taurine-conjugated bile acids, leading to increasing numbers of inflammatory gut microbes. On the other hand, Ramos and Martin (2020) mentioned that nutrients and bioactive compounds in one's diet will modulate the composition and functionality of gut microbiota. For instance, the relative abundance of bacteria genera linked to gastrointestinal diseases is increased in animal protein -based diets that consequently lowers the abundance of probiotics such as *Bifidobacterium.* In contrast, a plant

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protein-based diet promotes the growth of probiotics to stimulate the production of short-chain fatty acids as the main nutrition source for colon cells.

Although various modern equipment and medicines are available to treat the disease, the recovery process takes time and requires high expenses (Sears and Garrett, 2014). Currently, treatments for colon cancer include surgery, targeted therapy, radiation therapy and chemotherapy (Banerjee *et al.,* 2020; Mocan, 2021). Skelton IV *et al.* (2020) added that adjuvant therapies are suggested for six months after surgery to improve the survival rates of patients, followed by six months of care and every two months post-operative monitoring. Therefore, there is an urgent need to seek alternative solutions to prevent and treat these gastrointestinal disorders.

'Probiotic' is a term derived from Greek words of 'pro' and 'bios' that means 'of life.' It usually refers to a group of bacteria that are beneficial to human and animal health. The use of probiotics in the prevention of gastrointestinal cancer is a current research direction (Barnes and Yeh, 2015; Reis *et al*., 2017). Moghaddam (2011) reported that The United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) have defined probiotics as "living microorganisms when taken (referring to humans) and given in sufficient amounts will provide health benefits to the host". Previous research has shown that intake of beneficial microorganisms in the daily diet can improve health and reduce the risk of diseases such as irritable bowel syndrome, inflammatory bowel disease and Crohn's disease (Mary *et al*., 2019) by modulating microbial imbalances in the host's intestine, stimulating the growth of beneficial microorganisms, lowering the number of pathogens and eliminating carcinogens in the gastrointestinal system (Guarner and Malagelada, 2003; Chiang and Pan, 2012). In addition, it also enhances the immune function of the host (Isolauri *et al*., 2001), improves digestion (Jager *et al*., 2018), absorbs nutrition (Vivarelli *et al.,* 2019) and also reduces constipation problems (Miller *et al*., 2017). Most probiotic microorganisms are lactic acid bacteria (LAB) isolated from healthy intestinal microflora (Tsung-Yen *et al*., 2012) such as *Lactobacillus*, *Bifidobacterium* and *Streptococcus* are genera that are often widely used as probiotics (Argyri *et al*., 2013; Akoglu *et al*., 2015; Vemuri *et al*., 2017). LAB is characterized by producing lactic acid and metabolites including antioxidants, organic acids and antimicrobial compounds that are beneficial in modulating and improving microbial balance in the gut (Aswathy *et al*., 2008). Most of the metabolites will be absorbed into the circulation to either act directly on colon tissues as host or further be metabolized as bioactive compounds to positively affect the host metabolism (Fujisaka *et al.,* 2018). To provide health benefits, probiotics need to survive when passing through an extreme environment in the digestive tract in sufficient amounts (at least 10^6 CFU/mL) (Pairat and Sudthidol, 2016), are tolerant to acids, bile salts, have antimicrobial effects and ability to adhesion to colon cancer cells (Toscano *et al*., 2015). Meanwhile, the criteria of 'health beneficial in adequate amounts of probiotics to be used in dietary supplements and foods as proposed by the International Scientific Association for Probiotics and Prebiotics (ISAPP) is further clarified by Binda *et al.* (2020) by outlining four aspects, such as sufficient characterization of the probiotic strains, safe to use, at least one positive human clinical control is conducted to support its use and present as alive in the food product throughout consumption and shelf life.

LABs are a group of Gram-positive, spore-free bacteria that are phylogenetically placed under the phylum Firmicutes which are predominant in the order Lactobacillales. LAB is an example of the most popular microorganisms used for the production of fermented foods such as the fermentation of dairy products, meat grains and vegetables. Recently, research on LAB has been conducted intensively in the food industry, bioactive compounds, dairy products and probiotics (Farahani *et al*., 2017; Agung *et al*., 2018). Therefore, a screening study of five LAB strains, *Lactobacillus paracasei* UALpc-04™, *Lactobacillus plantarum* UALp -05™, *Lactobacillus acidophilus* DDS®-1, *Bifidobacterium bifidum* UABb-10™ and *Streptococcus thermophilus* UASt-09™ was conducted to determine LAB strains that have the potential to be applied as probiotic products. These bacteria strains were chosen as they are naturally present in human intestines and widely consumed as food products (yoghurt, cheese, pickles) as well as proven suitable for probiotic study (Saavedra *et al.,* 1994; Seddik *et al.,* 2017; Zielińska and Kołożyn-Krajewska, 2018).

2. Materials and methods

2.1 Bacterial strains and growth conditions

A total of five strains of LAB, *Lb. paracasei* UALpc -04™, *Lb. plantarum* UALp-05™, *Lb. acidophilus* DDS®-1, *B. bifidum* UABb-10™ and *S. thermophilus* UASt-09™ were obtained from UAS Laboratories, Edina, USA. As tested in other studies (Saeed and Salam, 2013), all these LAB strains were cultured using Mann-Rogassa-Sharpe agar (MRS) (Merck, Germany) with 20% glycerol and stored at -80°C before use. When required, a total of 100 µL of the frozen culture of LAB strain was added to 10 mL of MRS broth (Merck, Germany) and incubated at 37°C for 48 hrs.

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2.2 Tolerance to low pH

Studies on the survival of LAB strains in simulated gastric juice were conducted using the method of Argyri *et al*. (2013). The five LAB strains were inoculated into MRS broth and incubated at 37°C for 48 hrs. Next, the LAB strain with initial populations ranging from 7.0-9.0 log CFU mL^{-1} at turbidity of 600 nm OD was harvested by centrifugation (Eppendorf model 5810R, Germany) at 10,000 rpm for 5 mins. The pellets were then washed with 1.0 mL saline phosphate buffer (PBS) (Sigma-Aldrich, USA) pH 7.2 twice before being resuspended in PBS solutions pH 2, 3 and 4. Strain cultures were then incubated at 37°C for 0, 0.5, 1, 2 and 3 hrs. Each time sampling was performed, the cultures were washed and suspended along with the PBS volume. A series of dilutions and inoculations were performed into the MRS media. Cultures were then incubated at 37°C for 48 hrs. After 2 days, the cultures were analyzed for colony determination (CFU/mL).

2.3 Tolerance to bile salt

Survival of five LAB strains in simulated bile salts was conducted using Vinderola and Reinheimer (2003) method. Five LAB strains were inoculated into MRS broth and incubated at 37°C for 48 hrs. Next, the LAB strain at turbidity of 600 nm OD was harvested by centrifugation at 10,000 rpm for 5 mins. The pellets were then washed with 1.0 mL saline phosphate buffer (PBS) pH 7.2 twice. Then, the supernatant was removed and Oxgall solution (Sigma-Aldrich, USA) was added with concentrations of 0.3%, 0.5% and 1.0% of 1.0 mL for each sample prior to incubation in an incubator at 37°C. Samples were taken for analysis at every 0, 1, 2, 3 and 4 hrs. Each time sampling was performed, the cultures were washed and suspended along with the PBS volume. A series of dilutions and inoculations were performed into the MRS media. Cultures were then incubated at 37° C for 48 hrs. After 2 days, the cultures were analyzed for colony determination (CFU/mL).

2.4 Antibacterial assessment

The study of the antibacterial activity of LAB was according to the method of Andrews (2001) against five pathogenic bacteria consisting of *E. coli* ATCC®O157™, *S. enterica* ser. Typhimurium ATCC®53648™, *S. enterica* ser. Enteritidis MDC15, *S. bovis* ATCC®9809™ and *L. monocytogenes* ATCC®51772™. The antibacterial activity against these five LABs was performed using the good absorption assay method as suggested by Rima *et al.* (2013). All pathogenic bacteria were inoculated into Soy Tripticase broth (Difco, France) (30 g/L) and then incubated at 37° C for 18 hrs. The concentration of pathogenic bacteria was estimated based on the MacFarland Turbidity index of 0.5 (Thermo Fisher Scientific, USA) as a cell density standardization to calculate the estimated number of bacteria in the suspension (Zapata and Ramirez-Arcos, 2015). A total of five wells were prepared on each Muller Hinton agar (SRL, India) (21.0 g/L) that was filled with 100 µL of MRS LAB broth in three wells, one well for Ringer's solution and one well for antibiotics (1% penstrep) (Nacalai Tesque, Japan). Ringer's solution and antibiotics acted as positive and negative controls, respectively. After that, Muller Hinton was incubated at 37°C for 18 hrs. Determination of antimicrobial activity was performed by measuring the enlightenment zone surrounding each agar well. The formation of the enlightened area in the form of a clear, visible halo indicated effective antimicrobial activity (Abid *et al.,* 2021).

2.5 Adhesion determination

2.5.1 Preparation of HT29 and SW480 colon cancer cell seeds

Procedures involving colon cell seed preparation, LAB initiator culture and adhesion assay were determined via the method of Maragkoudakis *et al*. (2006). The frozen cells of HT29 and SW480 (ATCC, USA) were retrieved from the liquid nitrogen storage tank (Statebourne model Bio 34, England). The cryovial was thawed rapidly and the content was transferred into a culture flask containing 7.0 mL of Roswell Park Medium Institute 1640 media (Merck, Germany). The flask was incubated at 37°C incubators supplemented with 5% CO_2 (Memmert model INC 153, Germany). After 24 hrs of incubation, the media was removed and replaced with an equal volume of fresh RPMI media. The steps were repeated alternately every 2 days until the cell reached 70-80% confluency. When 70-80% cell confluency had been reached, the media was discarded and the cells were rinsed with 2.0 mL phosphate buffer saline (PBS). The PBS was discarded and 2.0 mL of trypsin (Sigma-Aldrich, USA) was added to detach the cells and the flask was incubated for 5 min at 37°C with 5% CO_2. After 5 mins of incubation, 1:2 ratios of media were added to deactivate trypsin activity in the presence of serum. The cells were then pipetted out into a sterile 50 mL tube and centrifuged (Eppendorf model Centrifuge 5418, USA) at 1,000 rpm for 5 mins. The supernatant was discarded and the cells were resuspended in fresh media. About 10 µL of the cell suspension was mixed with 10 µL of trypan blue dye (0.4%) (Sigma-Aldrich, USA). The viable cell counts were determined under a microscope using hemocytometer chamber (Olympus model CKX 41, USA). A series of appropriate dilution was prepared and seeded into 96 well plates of 6 well plates. The plates

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were incubated at 37°C with 5% CO_2. The degree of confluency was monitored after 24 hrs under a microscope.

2.5.2 Preparation of lactic acid bacteria starter culture

A total of five species of LAB which consists of *Lb. paracasei* UALpc-04™, *Lb. plantarum* UALp-05™, *Lb. acidophilus* DDS®-1, *B. bifidum* UABb-10™ and *S. thermophilus* UASt-09™ were analyzed in the determination of adhesion with HT29 and SW480 colon cancer cells. Single colonies of LAB that had been cultured on MRS agar were inoculated into MRS broth and subsequently incubated in an incubator at 37°C for 48 hrs. After 48 hrs of fermentation, LAB growth in MRS broth was determined at an optical density of 600 nm using a microplate reader (Molecular Devices model Versa max, USA). The MRS broth was centrifuged at 10,000 rpm for 5 mins. Thereafter, the supernatant was discarded and subsequently added with 20 mL of PBS pH 7.0 solution. The solution was swirled well and then centrifuged at a speed of 10,000 rpm for 5 mins. After centrifugation, the supernatant was again removed. A total of 20 mL of Dulbecco's Modified Eagle's Medium (DMEM) media (Sigma-Aldrich, USA) was added to the remaining pellets. Next, the turbidity value of the LAB strain was determined at an optical density of 600 nm by using a microplate reader and performed a series of dilutions to obtain the optical density at 0.6.

2.5.3 Assay adhesion

Plates of 6 wells containing HT29 and SW480 colon cancer cell seedlings were removed from a carbon dioxide incubator and cell growth was observed through a light microscope. Cancer cells that achieved 70 to 80% growth were used in adhesion assayers with selected LABs. The supernatant found in the 6 well plates was removed and then rinsed twice through 2.0 mL PBS pH 7. Then, the PBS supernatant was discarded and inserted with 1.0 mL of DMEM culture containing LAB on each 6 well plates. Plates of 6 wells containing a mixture of cells and LAB were incubated in a carbon dioxide incubator at 37°C for 2 hrs. After that, the 6 well plates were removed from the incubator and subsequently the supernatant was discarded. The cells and LAB were rinsed twice through 2.0 mL PBS pH 7 to discard the supernatant. Next, 1.0 mL 0.04% Tween 80 (Sigma-Aldrich, USA) was added into a 6-well plate containing cells and LAB to separate the adhesions between cells and LAB. Then, the 6 well plates were incubated in a carbon dioxide incubator at 37°C for 10 mins. After that, the microbial cultures found in the 6 well plates were scraped and inserted into the 96 well plates. A series of dilutions was performed on the microbial suspension using PBS pH 7 buffer solutions prior to culturing 5.0 µL of the microbial suspension into MRS media. Media cultures were incubated using an incubator at 37°C for 48 hrs. After 2 days, the cultures were analyzed for colony determination (CFU/mL).

2.6 Statistical analysis

This experiment was performed using Completely Randomized Design (CRD) in a factorial arrangement with three replications. In addition, all data were analyzed using the method of analysis of variance (ANOVA). The Duncan test was performed to determine the extent of significant differences for each data that gave significant results at the level of $p<0.05$ by using a statistical analysis system (SAS Institute, 1985).

3. Results and discussion

A total of five LAB strains, *Lb. paracasei* UALpc-04™, *Lb. plantarum* UALp-05™, *Lb. acidophilus* DDS®-1, *B. bifidum* UABb-10™ and *S. thermophilus* UASt-09™ were studied for tolerance testing to low-pH gastric juice (4, 3 and 2) as shown in Figures 1, 2 and 3. Meanwhile, Figure 4 shows the survival of *Lb. plantarum* UALp-05™ against various pH of gastric juice simulated.

Most strains showed higher tolerance at pH 3 and pH 4 compared to pH 2 during the 3 hrs of incubation performed. Tolerance studies to low pH environments were found to vary between the five strains. The overall trend for the survival rate of LAB in this study against acidic environment was *Lb. plantarum* UALp-05™ > *Lb. paracasei* UALpc-04™ > *B. bifidum* UABb-10™ > *Lb. acidophilus* DDS®-1 > *S. thermophilus* UASt-09™. It was found that the *Lb. plantarum* UALp-05™, *B. bifidum* UABb-10™ and *Lb. acidophilus* DDS®-1 strains displayed higher tolerance in pH 2 gastric acid than the other two strains. The pH environment of the human stomach is between 1 (on an empty stomach) to 4.5 (after food intake) and the normal digestive process can run for up to 3 hrs (Wang *et al*. 2009). This low pH environment will kill most microorganisms. All LABs studied were found to be tolerant to pH 3 but were unable to maintain most of their viability after incubation for 3 hrs at pH 2. According to Sahadeva *et al*. (2011), exposure of LAB to gastric acid with pH ≤ 2 for 3 hrs will result in an intensive reduction of LAB viability. Moreover, LABs with probiotic properties have shown decreased tolerance at pH 2 and no viability was shown at pH 1 after 1 hr of *in vitro* exposure was carried out (Angmo *et al*., 2016). However, previous studies have shown that good probiotic strains should be able to tolerate gastric acid with a pH of 3 (Song *et al*., 2015; Ioanna *et al*., 2019).

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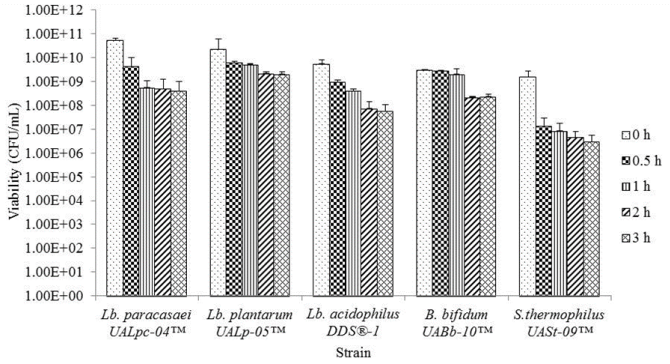

Figure 1. Survival of LAB against gastric juice at pH 4

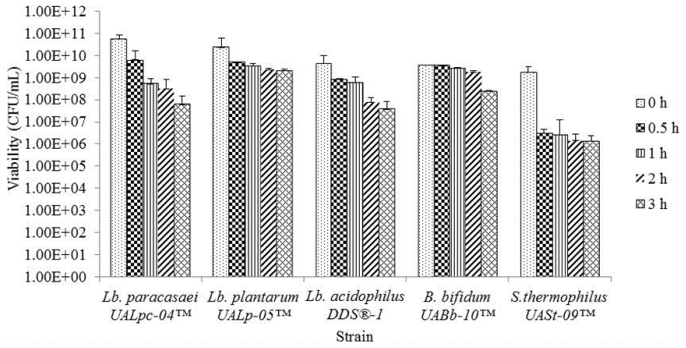

Figure 2. Survival of LAB against gastric juice at pH 3

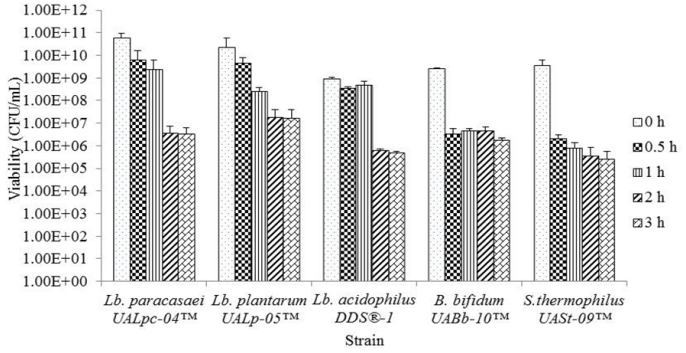

Figure 3. Survival of LAB against gastric juice at pH 2

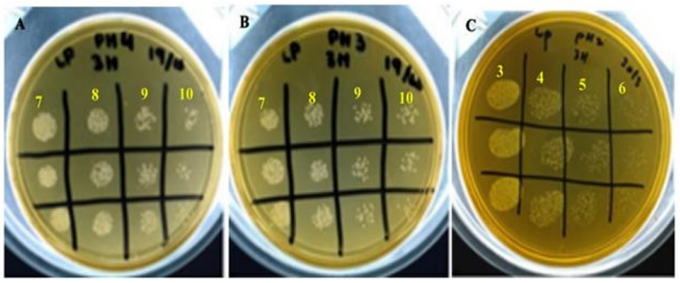

Figure 4. Bacterial survival *Lb. plantarum* UALp-05™ against simulated gastric juice at (A) pH 4; (B) pH 3 and (C) pH 2 after 3 hours of incubation

Resistance to bile salts is considered to be one of the important parameters for selecting potential BAL strains. Tolerance to bile salt concentrations of 0.15% to 0.30% found in the human digestive system has been recommended as an appropriate bile salt concentration for LAB selection (Fernandez *et al*., 2003; Song *et al*., 2015). Therefore, studies on five strains of lactic acid bacteria on three different concentrations of bile salts namely 0.3%, 0.5% and 1.0% were conducted as can be seen in Figure 5, Figure 6 and Figure 7, respectively.

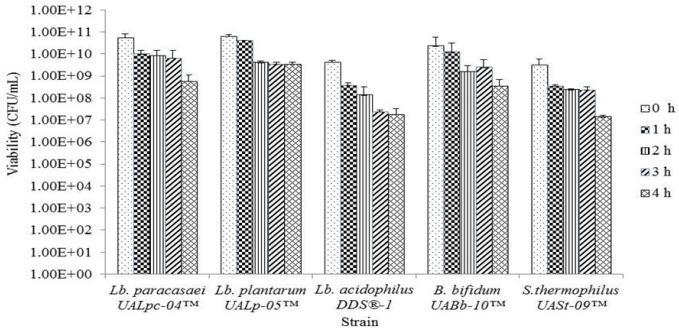

Figure 5. Survival of LAB against bile salts at a concentration of 0.3%

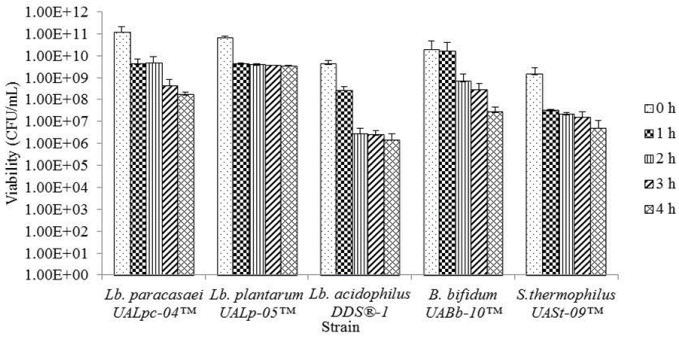

Figure 6. Survival of LAB against bile salts at a concentration of 0.5%

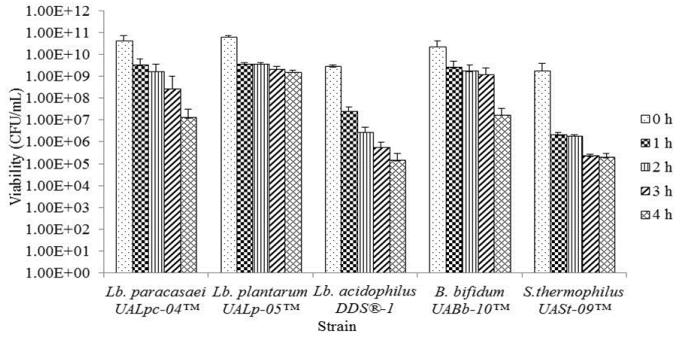

Figure 7. Survival of LAB against bile salts at a concentration of 1.0%

Almost most of the LAB strains studied showed higher tolerance to bile salts at concentrations of 0.3% and 0.5% compared to concentrations of 1.0% during the 4 h of incubation. Similar to acid tolerance, the trend for the survival rate of LAB against bile salts in this study was *Lb. plantarum* UALp-05™ > *Lb. paracasei* UALpc-04™ > *B. bifidum* UABb-10™ > *Lb. acidophilus* DDS® -1 > *S. thermophiles* UASt-09™. Bile salts are synthesized in the liver from cholesterol and also play an important role in the digestion and absorption of fats. The average bile salt concentration is about 0.5% (weight/volume) and food is in the small intestine for about 4 to 6 hrs (Ioanna *et al*., 2019). To maintain growth in the small intestinal environment, microorganisms should be able to tolerate extreme environments in the gastrointestinal tract especially in the presence of bile salts. Bile salts in the small intestine have the ability to reduce the viability rate of bacteria due to cell permeability modifications (Succi *et al*.,

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2005) and in turn cause oxidative stress and disruption to repair mechanisms in its DNA cells (Ruiz *et al*., 2013). Bile salts are a major component of the bile organs. These salts are produced and conjugated with amino acids (glycine or taurine) in the liver and stored in the gallbladder before being secreted into the duodenum during digestion to facilitate emulsification and dissolution of fats. The presence of food can also influence the activity of bile salts as they can bind to the food matrix thus preventing it from exerting toxic effects on microorganisms (Begley *et al*., 2005).

Most of the microorganisms that exhibit probiotic properties are in the LAB and bifidobacteria groups. Antibacterial activity can be defined as the ability of a bacterium to inhibit the growth of pathogens in the intestinal tract and it is one of the important perimeters in the selection of potential LAB. Therefore, studies of the antibacterial activity of the five LAB strains against pathogenic bacteria such as *E. coli* ATCC®O157™, *S. enterica* ser. Typhimurium ATCC®53648™, *S. enterica* ser. Enteritidis MDC15, *S. bovis* ATCC®9809™ and *L. monocytogenes* ATCC®51772™ were conducted.

Studies of the antibacterial activity of LAB against pathogenic bacteria showed a variety of inhibition zones as shown in Table 1. Among the five LAB strains studied, the *Lb. plantarum* UALp-05™ strain showed superior antibacterial activity against all Gram-positive and Gram-negative pathogen strains compared to the other four LAB strains as shown in Figure 9. The trends for antibacterial activity against *L. monocytogenes, S. enterica* ser. Typhimurium and *S. enterica* ser. *Enteritidis* were *Lb. plantarum* UALp-05™ > *B. bifidum* UABb-10™ > *S. thermophilus* UASt-09™ > *Lb. paracasei* UALpc-04™ > *Lb. acidophilus* DDS®-1. On the other hand, the antibacterial activity trend against *E. coli* was *Lb. plantarum* UALp-05™ > *S. thermophilus* UASt-09™ > *B. bifidum* UABb-10™ > *Lb. paracasei* UALpc-04™ > *Lb. acidophilus* DDS®-1 while the trend against *S. liboris* was *Lb. plantarum* UALp-05™ > *Lb. paracasei* UALpc-04™ > *S. thermophilus* UASt-09™ > *B. bifidum* UABb-10™ > *Lb. acidophilus* DDS®-1.

All LAB strains showed different inhibition zones against both Gram-positive and Gram-negative pathogenic strains. Antimicrobial activity is related to the fusion between LAB and pathogenic bacteria where during the fusion process occurs, antimicrobial substances derived from LAB inhibit the growth of pathogenic bacteria (Arena *et al*., 2018). According to Tambekar *et al*. (2009), antibacterial activity occurs due to the production of acetic acid and lactic acid that lower the pH of the medium, competition in obtaining nutrients, the production of bacteriocin or antibacterial compounds. In the context of intestinal health, beneficial microorganisms will contribute to health by enhancing the defence function of the intestinal system and exhibiting antimicrobial properties through the production of specific vitamins and short-chain fatty acids such as acetate, propionate and butyrate (Hemarajata and Versalovic, 2013). However, some previous researchers reported that the inhibitory action of LAB is not limited to lactic acid and acetic acid only but various other metabolites released into the growth medium also have antagonistic properties against some bacterial strains. Among such metabolites are formic acid, free fatty acids, ammonia, ethanol, hydrogen peroxide, diacetyl, acetone, 2,3-butanediol, acetaldehyde, benzoate, bacteriolytic enzymes and bacteriocin as well as several other inhibitors (Cizeikiene *et al*., 2013; Rocha and Malcata, 2016; Bartkiene *et al*., 2020). In addition, the effect of antibacterial activity exhibited by a particular LAB strain is also significantly influenced by many physical, chemical and nutritional environmental factors (Elayaraja *et al*., 2014; Li *et al*., 2017). Previous studies have also shown that certain LAB strains have anti-infection against pathogenic microbes in the gut (Arques *et al*., 2015; Campana *et al*., 2017). Therefore,

Table 1. Resistance of LAB strains to pathogenic strains

Strain	Inhibition zones (mm)±SD				
	E. coli ATCC®O157™	*L. monocytogenes* ATCC®51772™	*S. enterica serovar* Typhimurium ATCC®53648™	*S. enterica serovar* Enteritidis MDC15	*S. bovis* ATCC®9809™
Lb. paracasei UALpc-04™	13.444±1.347^{cd}	13.556±0.962^{b}	12.333±1.202^{c}	12.0±1.453^{b}	14.778±1.347^{ab}
Lb. plantarum UALp-05™	17.557±1.644^{b}	15.111±1.347^{b}	15.333±0.882^{b}	14.0±2.082^{b}	16.667±1.0^{a}
Lb. acidophilus DDS®-1	12.444±1.171^{d}	11.111±0.385^{c}	10.667±0.333^{c}	11.667±1.155^{b}	13.333±1.764^{b}
B. bifidum UABb-10™	14.222±1.83^{cd}	15.0±0.577^{b}	13.0±1.856^{c}	13.556±1.678^{b}	14.222±1.895^{ab}
S. thermophilus UASt-09™	15.111±0.385^{c}	14.556±1.347^{b}	12.778±1.895^{c}	12.556±1.836^{b}	14.667±1.453^{ab}
Penstrep (1%)	28.667±0.471^{a}	28.0±1.732^{a}	28.667±0.577^{a}	28.333±1.155^{a}	0^{c}
Ringers	0^{e}	0^{d}	0^{d}	0^{c}	0^{c}

Values are presented as mean±SD of triplicates. Values with different superscripts within the same column are significantly different ($p<0.05$).

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probiotics have the potential to be used as part of therapeutic therapy to restore the composition of the intestinal microbiota and the functioning of a healthy microbiota.

The selection of potential LAB is not only based on their tolerance to the pH of gastric juice and various percentages of bile salts as well as has antimicrobial effects, but also their ability to adhesion to colon cancer cells. Cell adhesion is a complex process that involves the contact between bacterial cell membranes and surfaces interacting with cancer cells. Therefore, the next study was to look at the adhesion ability of the five LAB strains to two human colon cancer cells involving HT29 and SW480 cells. The ability to attach to these cancer cells is one of the important elements of a LAB strain in order to provide more benefits to the human gastrointestinal system.

Notably, all LABs had adhesion ability to HT29 cells and SW480 cells as shown in Figure 8 and Figure 9, respectively. The *B. bifidum* UABb-10™ strain showed the highest cell adhesion percentage, followed by *Lb. plantarum* UALp-05™, *S. thermophiles* UASt-09™, *Lb. paracasei* UALpc-04™ and *Lb. acidophilus* DDS®-1. For HT29 cells adhesion, *B. bifidum* UABb-10™ and *Lb. plantarum* UALp-05™ did not show significant differences ($p>0.05$) from each other in terms of cell viability which was around 1.022×10^7 and 1.244×10^7 CFU/mL. Meanwhile, the *B. bifidum* UABb-10™ strain showed a significant difference ($p>0.05$) against *Lb. plantarum* UALp-05™, yet both strains still maintained their cell viability at around 10^6 to 10^7 CFU/mL.

In general, the percentages of cell adhesion of all LAB strains to SW480 cells (0.01-2.33%) were found to be relatively lower than that of HT29 cells (0.02-3.63%). The adhesion ability of colon cancer cells also plays a role in the selection of probiotic bacteria (Belguesmia *et al*., 2016). The adhesion ability of probiotics involves various biophysical and biochemical properties of probiotics and epithelial cells including electrostatic, hydrophobic, steric forces, passive forces and also involves specific cellular structures (Ranadheera *et al*., 2014). In addition, the adhesion ability is also influenced by the cell surface components and specific adhesion proteins released on the cell surface that can provide various levels of adhesion properties (Wang *et al*., 2018). According to Maragkoudakis *et al*. (2006) and Raj *et al*. (2011), cell adhesion between LAB strains with colon cancer cells was at around 0.4 to 12.5% depending on genus and strain.

Nevertheless, this establishes the potential for anticancer properties of LAB used in this study against colon cancer. Sharifi *et al.* (2017) highlighted that probiotics from fermented dairy products contain a high amount of antioxidants such as acetic acid and lactic acid to reduce the DNA damage that can cause cancer. They reviewed kefir, a fermented milk drink that has the ability to reduce expression of TGF-B1 and TGF-a in HT29 cells, thanks to the presence of LAB such as *Lactobacillus paracasei*, *Lactobacillus kefiri*, *Lactobacillus parabuchneri* and *Acetobacter lovaniensis*. Besides, Slizewska *et al*. (2021) explained that LAB is capable to inhibit cancer cells proliferation and induce apoptosis to lessen the cancer growth progress.

4. Conclusion

Tolerance to low pH and bile salts environment of LAB strains used in this study recorded similar survival rate trends, i.e. *Lb. plantarum* UALp-05™ > *Lb. paracasei* UALpc-04™ > *B. bifidum* UABb-10™ > *Lb. acidophilus* DDS®-1 > *S. thermophilus* UASt-09™. Most strains showed higher tolerance at pH 3 and pH 4

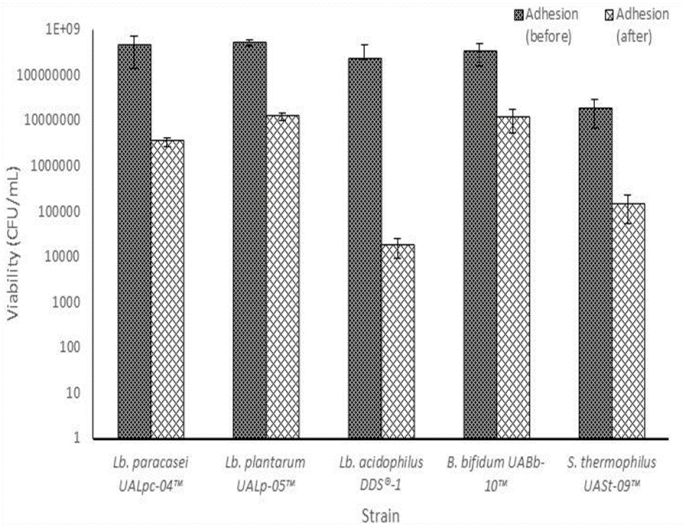

Figure 8. Adhesion of LAB strains to HT29 cells. Cell viability was measured against the adhesion ability of LAB strains to HT29 cells

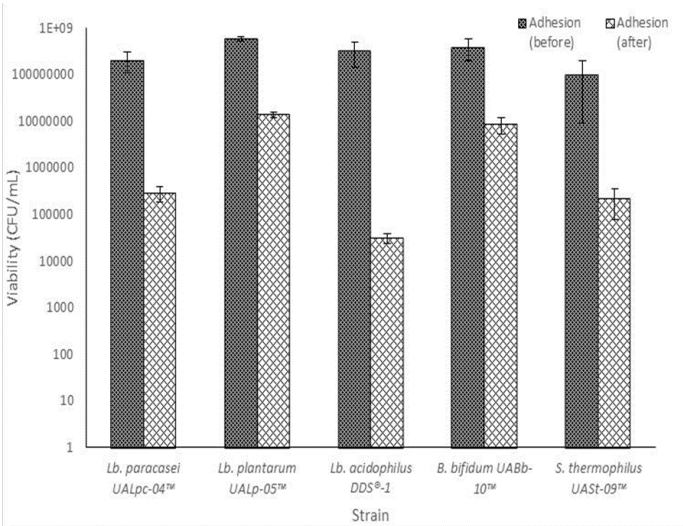

Figure 9. Adhesion of LAB strains to SE480 cells. Cell viability was measured against the adhesion ability of LAB strains to SW480 cells

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compared to pH 2 during the 3 hrs of incubation performed. Meanwhile, almost most of the LAB strains studied showed higher tolerance to bile salts at concentrations of 0.3% and 0.5% compared to concentrations of 1.0% during the 4 hrs of incubation. Studies of the antibacterial activity of LAB against pathogenic bacteria showed a variety of inhibition zones with *Lb. plantarum* UALp-05™ strain exhibiting superior antibacterial activity against all pathogen strains. For the anticancer study, all LABs possessed adhesion ability to HT29 cells and SW480 cells in the range of 0.01-2.33% and 0.02-3.63%, respectively. Out of five LAB used in this study, only *B. bifidum* UABb-10™ *and Lb. plantarum* UALp-05™ strains displayed viability at around 10^6 to 10^7 CFU/mL to demonstrate their survival of passing through the gastrointestinal tract as probiotic strains that provide additional health benefits to host cells.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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