LWT - Food Science and Technology 118 (2020) 108864

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Inhibitory effects of probiotic potential lactic acid bacteria isolated from kimchi against *Listeria monocytogenes* biofilm on lettuce, stainless-steel surfaces, and MBEC™ biofilm device

Md Iqbal Hossain[a], Md Furkanur Rahaman Mizan[a], Md Ashrafudoulla[a], Shamsun Nahar[a], Hyun-Jung Joo[a], Iqbal Kabir Jahid[b], Si Hong Park[c], Keun-Sung Kim[d], Sang-Do Ha[a,*]

[a] Department of Food Science and Technology, Advanced Food Safety Research Group, Brain Korea 21 Plus, Chung-Ang University, 72-1 Nae-Ri, Daedeok-Myun, Anseong, Gyunggido, 456-756, South Korea
[b] Department of Microbiology, Jashore University of Science and Technology, Bangladesh
[c] Department of Food Science and Technology, Oregon State University, 3051 SW Campus Way, Corvallis, OR, 97331, USA
[d] Department of Food Science and Technology, Food Microbiology Lab, Chung-Ang University, 72-1 Nae-Ri, Daedeok-Myun, Anseong, Gyunggido, 456-756, South Korea

ARTICLE INFO

Keywords:
Lactic acid bacteria (LAB)
Probiotics
Listeria monocytogenes
Biofilm

ABSTRACT

Probiotics show great promise as alternative and environmentally friendly candidates to control microbial pathogens. Here, six isolated lactic acid bacteria (LAB) were chosen based on their anti-listerial activity. Anti-listerial LAB isolates were identified by 16S rRNA gene sequencing. The anti-listerial activities of these isolates were evaluated by *Listeria monocytogenes* biofilm inhibition assays on stainless-steel coupons (SS), lettuce, and a minimum biofilm eradication concentration (MBEC™) biofilm device. Results revealed that following co-culture with LAB for 24 h, *L. monocytogenes* biofilm cells were inhibited by up to 2.17 log CFU/cm^2, 1.62 log CFU/cm^2, and 1.09 log CFU/peg on SS, lettuce, and MBEC™, respectively. Although these LAB bacteria suppressed *L. monocytogenes* biofilm formation on both surfaces, the inhibitory effect on lettuce surfaces was lower than that on SS. These results support the potential use of LAB strains to inhibit biofilm formation by pathogenic bacteria on vegetable products and in the food industry, without associated risk to consumers.

1. Introduction

Probiotics are defined as non-pathogenic microbes, which, when administered in sufficient amounts, provide health benefits to the host (FAO/WHO, 2002). Lactic acid bacteria (LAB) are well-known probiotics with the most common genera being *Lactobacillus* (Argyri et al., 2013). Due to a long history of application in the food industry, LAB have been given Generally Recognized as Safe (GRAS) status by the US Food and Drug Administration (FDA), and received the "Qualified Presumption of Safety (QPS)" status by the European Food Safety Authority (EFSA) (Leuschner et al., 2010). Probiotic microorganisms must overcome the hostile conditions of and survive passage through the human gastrointestinal tract, which comprises the oral cavity, stomach, and small intestine (Bove et al., 2012). They must also function by exhibiting resistance to acid, bile, and lysozyme, adhering to intestinal epithelial cells, and antagonizing pathogen activity (Peres et al., 2014), one of the most important requirements recommended by the FAO/WHO (2002). Kimchi is a traditional Korean fermented food, and an excellent source of probiotic LAB. To our knowledge, LAB isolates with potential probiotic characteristics have been isolated from kimchi by several researchers (Khan & Kang, 2016; Wen, Philip, & Ajam, 2016). Moreover, 16S rRNA gene sequencing analysis is routinely used to identify these species.

Listeria monocytogenes is an important foodborne pathogen and the causative agent of listeriosis, which has a mortality rate of 20–30% in at-risk humans (David & Cossart, 2017). Refrigerated ready-to-eat (RTE) foods have been proposed as a contingency measure for listeriosis (Sillankorva, Oliveira, & Azeredo, 2012). The FoodNet database identified 127 listeriosis cases in 2016 in the USA (Marder et al., 2017). Biofilms are an assemblage of living microorganisms that can attach to and grow on any surfaces (biotic and abiotic) (Srey, Jahid, & Ha, 2013). *L. monocytogenes* can form biofilm on 17 various types of surface (Beresford, Andrew, & Shama, 2001). Notably, this organism can persist and attach to surfaces of food (biotic) and food processing appliances

* Corresponding author. School of Food Science and Technology, Chung-Ang University, 72–1 Nae-Ri, Daedeok-Myun, Anseong-Si, Gyunggido, 456-756, South Korea.
E-mail address: sangdoha@cau.ac.kr (S.-D. Ha).

https://doi.org/10.1016/j.lwt.2019.108864
Received 30 June 2019; Received in revised form 2 November 2019; Accepted 19 November 2019
Available online 20 November 2019
0023-6438/

(abiotic), where it can grow as biofilm (Mizan, Jahid, & Ha, 2015). Biofilm formation can lead to food contamination, food product deterioration, shortened shelf-life of products, and foodborne outbreaks (Bridier et al., 2015; Simoes, Simoes, & Vieira, 2010), which are responsible for economic losses in the food industry (Simoes et al., 2010).

Several biological strategies have been examined to inhibit foodborne pathogenic microbial biofilms in the food industry, without inducing sensorial effects on food or corrosion problems with metals. Stainless-steel (SS) is widely applied as a food contact surface due to its mechanical strength, corrosion resistance, and longevity (Marques et al., 2007). Several studies have focused on the inactivation of *L. monocytogenes* by LAB (Ibarreche, Castellano, & Vignolo, 2014; Zhao et al., 2013). The production of bacteriocins by LAB can increase their capability to control foodborne pathogens and food spoilage microorganisms on food products (Dal Bello et al., 2012). This strategy is based on a competitive exclusion approach, wherein microorganisms that have antagonistic activity toward pathogenic microorganisms can control or inactivate them, depending on the competition for nutrients and sites of attachment, as well as the production of antimicrobial compounds (Ukuku, Latiful, Kassama, Mukhopadhyay, & Olanya, 2015). Competition between bacterial species can result in the suppression of competitors through the development of species-specific mechanisms to obtain fixed, available resources (Hibbing, Fuqua, Parsek, & Peterson, 2009).

However, few studies have examined the inhibitory effect of LAB against foodborne pathogens. Li, Jia, Zhou, Fang, and Chen (2017) evaluated the protective inhibitory effect of *Lactobacillus fermentum* R6 against *Clostridium perfringens*, and noted that the growth, as well as germination and outgrowth of *C. perfringens* spores, *in vitro* and in chicken breast meat was effectively inhibited by *L. fermentum* R6 under temperature abuse conditions. In response to the recent increased interest in research to control foodborne pathogens by LAB, the application of potential probiotic LAB as an anti-biofilm strategy is a promising option for food safety. Therefore, the main aims of this research were as follows to perform a thorough screening of anti-listerial LAB isolated from kimchi and investigated the cell surface properties (auto-aggregation and hydrophobicity) and evaluate the inhibitory effect of the anti-listerial LAB isolates against *L. monocytogenes* biofilm formation on fresh produce (lettuce), a food-processing surface (SS), and the MBEC™ biofilm device.

2. Material and methods

2.1. Cultivation of LAB and screening of anti-listerial isolates

Thirty-four LAB strains isolated from kimchi were utilized in this study. Bacterial isolates were collected from the Food Microbiology Laboratory, Chung-Ang University.

(South Korea). LAB were grown in MRS (de Man, Rogosa and Sharpe) broth at 30 °C for 24 h and single colonies were picked from MRS agar plates incubated at 30 °C for 48 h. For long-term storage, isolates were maintained at −80 °C in MRS broth containing 15% glycerol. Two probiotic strains, *Lactobacillus acidophilus* KACC12419 (Korean Agricultural Culture Collection) and *Lactobacillus paracasei* KACC12427, were used in this study.

A spot-on-lawn method was implemented to evaluate the inhibitory activity of LAB on pathogenic *L. monocytogenes* (ATCC19113, ATCC19117, ATCC15313) strains. Briefly, the *L. monocytogenes* indicator strain was grown in TSB (tryptic soy broth) and 100 μL of an overnight culture (10^8 CFU/mL) was plated on BHI (brain heart infusion) agar. Test LAB cultures were spotted (10 μL) on the agar plate surface and incubated overnight at 30 °C to assess inhibition. After incubation, inhibition zones were determined. A clear halo around the spot was scored as positive.

2.2. Identification of selected anti-listerial LAB isolates

Identification of the anti-listerial LAB isolates was confirmed by 16S rRNA gene sequencing analysis using the universal primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTA CGACTT-3′) (Bioneer Corporation, Daejeon, Korea). DNA sequencing of the amplified fragments was carried out by SolGent sequencing services company (Daejon, South Korea). The obtained sequences were compared with those available in the GenBank database, using the Basic Local Alignment Search Tool (BLAST) at the National Center of Biotechnology Information website (http://www.ncbi.nlm.nih.gov/).

2.3. Aggregation activity

2.3.1. Cell auto-aggregation

Cell auto-aggregation abilities were assessed according to Collado, Meriluoto, and Salminen (2008) with modifications. LAB strains were grown in MRS broth overnight. After centrifugation (5000 × *g*, 4 °C, 15 min); the harvested cells were washed twice with phosphate-buffered saline (PBS; pH 7.4) and resuspended in PBS at an absorbance of 0.25 ± 0.05 at 600 nm ($OD_{600\ nm}$) to determine the number of viable bacteria (10^8 CFU/mL). Four milliliters of LAB bacterial suspensions was vortexed for 10 s and incubated at 30 °C. At different intervals (3 and 24 h), the upper suspension was cautiously obtained without vortexing, and the $OD_{600\ nm}$ was determined. The cell auto-aggregation percentage of LAB bacteria was expressed as $[1 - A_t/A_0] \times 100$, where A_t represents the absorbance at time t = 3 or 24 h, and A_0 is the absorbance at t = 0 h.

2.3.2. Cell surface hydrophobicity

Three different solvents, including two nonpolar solvents (xylene and *n*-hexadecane), and one monopolar and basic solvent (ethyl acetate), were used for this test, which was performed as detailed by Ibarreche et al. (2014) and Mizan et al. (2016), with modifications. LAB isolates were cultivated in MRS broth at 30 °C for 24 h. Pellets were collected by centrifugation, washed twice with 150 mM potassium phosphate buffer (pH 7.0), and resuspended in the same buffer to obtain an $OD_{600\ nm}$ (A_0) range of 0.25–0.60. Then, 3 mL of cell suspension was mixed individually with 1 mL of hydrocarbons (xylene, hexadecane, and ethyl acetate). After 10 min at room temperature, the two-phase solution was vigorously vortexed for 2 min and maintained in an incubator at 30 °C for 1 h to separate the aqueous and organic phases. The aqueous phase was carefully removed to determine the absorbance at 600 nm (A_1). The percentage of cell surface hydrophobicity was calculated based on the following formula:

$$\text{Hydrophobicity (\%)} = (1 - A_1/A_0) \times 100$$

2.4. Inhibition of Listeria monocytogenes biofilm activity by LAB

2.4.1. Standardized culture for biofilm experiments

LAB isolates and *L. monocytogenes* ATCC19113 were stored at −80 °C. Each LAB isolate (100 μL) was activated by two subcultures in MRS broth, whereas *L. monocytogenes* was cultured in TSB. Individual cultures were centrifuged (5000 × *g*, 4 °C, 15 min) and washed twice with PBS. Pellets were resuspended in PBS, and bacterial concentrations were determined by plating on MRS agar plates for LAB and PALCAM agar for *L. monocytogenes*.

2.4.2. Effect of LAB inoculation against Listeria monocytogenes biofilm formation on stainless-steel (SS) coupons

Stainless-steel coupons (2 × 2 × 0.1 cm, type: 304) were prepared as described (Shen et al., 2012). Each SS coupon was completely submerged into 10 mL of TSB in 50-mL Falcon tubes, and 100 μL of 10^8 CFU/mL LAB culture and 100 μL of 10^5 CFU/mL of *L.*

monocytogenes culture were added simultaneously. A sample containing only *L. monocytogenes* was considered the control. All samples were incubated without shaking at 30 °C for 24 h for biofilm formation. After incubation, each SS coupon was removed from the bacterial suspension and gently washed twice in a sterile beaker containing sterile distilled water to remove unattached or free cells. After washing, each SS coupon was transferred to a small Petri dish (55 × 12 mm) containing 2 mL of 0.1% peptone water (PW; Oxoid, UK), scrubbed, transferred to a test tube, and vortexed for 2 min to disperse the biofilm. Enumeration of *L. monocytogenes* biofilm cells was performed by serial dilutions in PW and spreading onto PALCAM agar, which was incubated at 30 °C for 48 h.

2.4.3. Effect of LAB inoculation against Listeria monocytogenes biofilm formation on lettuce

Bacterial growth and the inoculation of lettuce leaves were performed as described by Patel and Sharma (2010), with modification. Iceberg lettuce was purchased from a local grocery market in Anseong, South Korea, and stored at 4 °C until use. The outermost leaf layers were discarded, and the inner leaves were cut into 3 × 3 cm pieces with a sterile knife. Lettuce pieces were exposed to UV light in a biosafety cabinet for 30 min on each side to kill background microbiota. Each lettuce piece was aseptically immersed for 120 s in a 50-mL tube containing 10 mL of sterile water, and 100 μL of 10^8 CFU/mL LAB and 100 μL of 10^5 CFU/mL *L. monocytogenes* were added simultaneously. A sample with only *L. monocytogenes* was considered the control. After submersion, samples were removed to a sterile Petri dish and air-dried under laminar airflow for 20 min each side at room temperature. Individual samples were incubated for 24 h at 10 and 25 °C on filter paper soaked with sterile water and sealed in a Petri dish for biofilm formation. After incubation, each lettuce piece was removed from the petri dishes and washed twice in a sterile beaker containing sterile distilled water to remove unattached cells. After washing, each piece was submerged in 50 mL of 0.1% PW (Oxoid, UK) in a sterile stomacher bag (Nasco Whirl-Pak, USA) and processed using a stomacher at the highest speed (No. 4) for 4 min to detach biofilm-forming bacteria. The enumeration of *L. monocytogenes* biofilm cells was performed by serial dilutions and spreading onto PALCAM agar, which was incubated at 30 °C for 48 h.

2.4.4. Effect of LAB inoculation against Listeria monocytogenes biofilm formation on MBEC™ biofilm device

The inhibitory effect of LAB against *L. monocytogenes* biofilm was evaluated on MBEC™ (Innovotech Inc., Edmonton, Canada) biofilm inoculator with 96-well base pegs. Biofilms were established on the pegs (108.9 mm^2/peg) of the MBEC™ by following the manufacturer's instructions. In brief, the standardized cultures were diluted with fresh TSB medium, then 100 μL each of diluted LAB strain inoculum (10^8 CFU/mL) and *L. monocytogenes* culture (10^5 CFU/mL) were transferred simultaneously to each well of the 96-well plate. TSB and only diluted *L. monocytogenes* culture (100 μL each) were added to a set of wells as positive controls. A broth-only wells (i.e., uninoculated medium) was used as a negative control. The peg lid was placed on the microtiter base. The device was placed on the platform shaker set at 110 rpm in a humidified incubator at 30 °C. The plate was incubated for 24 h. After biofilm formation, the lid was placed into another 96-well plate containing 250 μL of saline solution per well for 10 s to remove the planktonic bacteria attached to the pegs. The biofilm cells were recovered from the treated and control pegs by sonication, followed by vortexing. Enumeration of *L. monocytogenes* biofilm cells was performed by serial dilutions in PW and spreading onto PALCAM agar, which was incubated at 30 °C for 48 h.

2.5. Characterization of biofilm formation using field emission scanning electron microscopy (FE-SEM)

Biofilm formation on SS and lettuce by *L. monocytogenes* and LAB was visualized by FE-SEM, as described by Jahid, Lee, Kim, and Ha (2013), with some modifications. Samples were fixed in 2.5% glutaraldehyde in PBS at room temperature for 4 h. The samples were then serially treated with ethanol (50, 60, 70, 80, and 90% for 15 min each, and 100% two times for 15 min each), and successively dehydrated by soaking in 33, 50, 66, and 100% hexamethyldisilazane in ethanol (15 min per solution). The dehydrated samples were sputter-coated with platinum and visualized under an FE-SEM (Hitachi/Baltec, S-4700).

2.6. Confocal laser scanning microscopy (CLSM)

The Film Tracer™ Live/Dead biofilm viability kit (Molecular Probes, Inc., Eugene, OR, USA) was used to differentiate live and dead bacteria in the biofilm, according to the manufacturer's instructions. Biofilm samples were imaged under a confocal laser microscope (Carl Zeiss LSM 710) using an Argon laser at 488 nm for excitation (emission 500–550 nm) and a 40 × water-immersion objective lens. The Live/Dead BacLight™ bacterial viability kit contains a mixture of SYTO9 green-fluorescent nucleic acid stain (specific for intact live bacteria) and propidium iodide red fluorescent nucleic acid stain (specific for membrane damaged or non-viable bacteria). Both categories of the cells were observed. For each experiment, multiple CLSM images were chosen from each biofilm sample for microscopic analysis.

2.7. Statistical analysis

Each experiment was conducted in at least triplicate. Data are provided as mean ± standard error of the mean (SEM). Statistical analysis involved analysis of variance (ANOVA) and Duncan's new multiple test at $p < 0.05$, using SAS 9.2 version (SAS Institute Inc., Cary, NC, USA). Graph Pad Prism 5.03 for Windows (Graph Pad Software, Inc., La Jolla, CA, USA) was also used.

3. Results and discussion

3.1. Cultivation, screening, and identification of selected anti-listerial LAB isolates

Among 34 LAB isolates, six showed anti-listerial activity and were successfully identified by 16S rRNA sequencing. Sequence analysis showed that six of the isolated anti-listerial LAB were classified into two major groups of LAB, namely *Lactobacillus* and *Leuconostoc*. 16S rRNA sequence identification confirmed that the three isolates belonged to *Lactobacillus plantarum* (isolates I.60, M.2, and M.21) and the other three isolates belonged to *Lactobacillus curvatus* (isolate B.67), *Lactobacillus sakei* (isolate D.7), and *Leuconostoc mesenteroides* (isolate J.27), respectively. According to Lee et al. (2015), *Lactobacillus plantarum* strain is the dominant microorganism responsible for most of the kimchi fermentation in the middle and late stages. The GenBank accession numbers for each isolate are presented in Table 1. Previously, many new LAB isolates have been isolated from kimchi and identified by using 16S rRNA gene sequencing (Khan & Kang, 2016; Wen et al., 2016; Yang et al., 2019).

3.2. Aggregation activity (auto-aggregation and hydrophobicity)

Interactions among cell surface components such as carbohydrates, proteins, and lipoteichoic acid are associated with cell aggregation. The percentage of auto-aggregation for selected isolates ranged from 10.71 to 15.49% and 53.61–71.06% after 3 and 24 h of incubation, respectively (Table 2). All LAB isolates exhibited increased auto-aggregation

Table 1
Identified LAB isolates by 16S rRNA gene sequencing and their GenBank accession number.

Isolates	Species	NCBI accession No
B.67	*Lactobacillus curvatus*	MH304289
D.7	*Lactobacillus sakei*	MH304290
I.60	*Lactobacillus plantarum*	MH304291
J.27	*Leuconostoc mesenteroides*	MH304292
M.2	*Lactobacillus plantarum*	MH304293
M.21	*Lactobacillus plantarum*	MH304294

after 24 h, compared with that after 3 h, in a strain-dependent manner, and this difference was significant ($p < 0.05$; Table 2). After 24 h, isolate M.21 (71.06%) exhibited the highest auto-aggregation potential, whereas isolates M.2 (69.63%), D.7 (68.15%), J.27 (66.68%), and KACC12427(66.25%) also showed higher auto-aggregation than the other strains. In contrast, the lowest auto-aggregation was exhibited by isolate I.60 (53.60%). Same species of M.2 and M.21 isolates did not show a significant difference ($p < 0.05$) in auto-aggregation, while significance difference was observed with another isolate I.60. However, M.2, M.21, and I.60 isolates belongs to *Lactobacillus plantarum* (Table 2). The percentage of auto-aggregation of *Lactobacillus* strains increases with incubation time until it becomes constant (Dias, Duarte, & Schwan, 2013). So far, Goh and Klaenhammer (2010) explained that self-aggregation ability is enhanced by aggregation boosting factors with the increase in time of incubation. Das, Khowala, and Biswas (2016) had studied auto-aggregation percentages for LAB isolates. Moreover, Gómez, Ramiro, Quecan, and de Melo Franco (2016) have also been noted auto-aggregation for LAB isolates. Similar results were demonstrated by Angmo, Kumari, and Bhalla (2016) and Abushelaibi, Al-Mahadin, El-Tarabily, Shah, and Ayyash (2017), that auto-aggregation of LAB strains increases with incubation time.

In the current study, LAB isolates exhibited remarkable hydrophobicity against three hydrocarbons, namely xylene, hexadecane, and ethyl acetate (Table 2). The percentages of cell surface hydrophobicity ranged from 7.61 to 71.28%, 10.86–50.82%, and 21.15–43.16% for xylene, hexadecane, and ethyl acetate, respectively (Table 2). Isolates B.67, M.2, and KACC12427 exhibited higher cell surface hydrophobicity towards xylene, hexadecane, and ethyl acetate than other examined isolates. The highest hydrophobic isolate was M.2 with xylene (71.27 ± 0.93%, $p < 0.05$, Table 2) and with n-hexadecane (50.81 ± 1.39%). While, M.2 displayed moderate hydrophobicity (29.86 ± 0.90%) with ethyl acetate. For the similar LAB strains, the three solvents (xylene, n-hexadecane and ethyl acetate) impact significantly with their cell surface hydrophobicity. Angmo et al. (2016) observed < 5%–47% hydrophobicity for LAB strains against hexadecane but did not test any other hydrocarbons, while Das et al. (2016) reported percentages of 22.2–25.0% for three bacteriocinogenic *L. casei* isolates. Abushelaibi et al. (2017) noted hydrophobicity percentages of 0.6–16.2%, 1.6–57.9%, and 2.7–67.0%, for LAB strains against hexadecane, xylene, and octane, respectively. The finding of the present study in term of the hydrophobicity values are in accordance with previous findings of Riaz Rajoka et al. (2017) for *L. rhamnosus* isolated from human milk.

Auto-aggregation and surface hydrophobicity provide colonization advantages for microorganisms within the intestinal tract. Colonization of probiotics in the intestinal tract creates a valuable host defense, preventing the entry of external pathogens, which suggests that these factors might be responsible for interactions with the host and beneficial effects (García-Cayuela et al., 2014). Theoretically, aggregation abilities among bacteria could be important factors that potentially inhibit adherence of pathogenic bacteria to receptors on the epithelial surface, preventing surface colonization by pathogens (García-Cayuela et al., 2014). Collado et al. (2008) suggested that competitive exclusion is the most potential mechanism to inhibit pathogen adhesion. Bacterial attachment, colonization, and biofilm formation correlate with cell surface hydrophobicity. However, aggregation can also increase the secretion of inhibitory compounds (Kaewnopparat et al., 2013).

3.3. Biofilm inhibition activity by LAB

Anti-adhesive and anti-biofilm-forming attributes of LAB have been evaluated under different circumstances. Importantly, there is evidence that pathogenic microbial colonization can be prevented by LAB (Argyri et al., 2013; Vijayakumar et al., 2015). Moreover, Schuenzel and Harrison (2002) demonstrated that a culture with inhibitory capacity could control vegetable contamination, thereby increasing the product shelf-life and promoting safety. Therefore, the application of probiotic microorganisms could improve quality, prolong food shelf-life, and control food contamination (Wei, Wolf, & Hammes, 2006). However, Hossain, Sadekuzzaman, and Ha (2017) reviewed that the use of various LAB strains with probiotic potential might inhibit the growth of and biofilm formation by foodborne pathogens on food and food contact surfaces.

In this study, the *L. monocytogenes* population was affected significantly ($p < 0.05$) by co-inoculation with LAB isolates on SS coupons, resulting in an inhibition of approximately 2.17 log CFU/cm^2 at 30 °C for 24 h when compared with *L. monocytogenes* alone (Figs. 1 and 3). The highest number of biofilm cells were inhibited by isolate B.67 (2.17 log CFU/cm^2) and the lowest inhibition was observed for isolates M.21 (approximately 1.45 log CFU/cm^2). The effectiveness of LAB microorganisms as bioprotective agents has been documented in several studies. Zhao, Doyle, and Zhao (2004) demonstrated that *L. monocytogenes* populations adherence to SS coupons were reduced more than 5 log CFU/cm^2 with *Lactococcus lactis* and *Enterococcus durans*. In another study, *L. lactis* UQ2 inhibited planktonic *L. monocytogenes* and biofilm formation by more than 5 log cycles on SS coupons (García-Almendárez, Cann, Martin, Guerrero-Legarreta, & Regalado, 2008). Minei, Gomes, Ratti, D'angelis, and De Martinis (2008) reported *L.*

Table 2
Auto-aggregation and hydrophobicity assay for LAB isolates.

LAB Isolates	Autoaggregation %		Hydrophobicity %		
	3 h	24 H	Xylene	n-Hexadecane	Ethyl acetate
B.67	15.002 ± 0.67^{a}	62.463 ± 1.46^{c}	21.171 ± 0.95^{d}	46.597 ± 1.05^{b}	43.154 ± 0.74^{a}
D.7	11.025 ± 0.67^{b}	68.148 ± 1.58^{ab}	7.607 ± 0.70^{e}	11.057 ± 1.05^{d}	22.819 ± 0.81^{cd}
I.60	10.706 ± 0.69^{b}	53.606 ± 1.10^{e}	8.717 ± 0.82^{e}	11.177 ± 1.17^{d}	24.297 ± 1.10^{c}
J.27	11.188 ± 0.92^{b}	66.677 ± 0.95^{b}	10.337 ± 0.95^{e}	10.860 ± 0.85^{d}	21.151 ± 0.76^{d}
M.2	15.490 ± 0.49^{a}	69.632 ± 1.36^{ab}	71.274 ± 0.93^{a}	50.817 ± 1.39^{a}	29.863 ± 0.90^{b}
M.21	12.624 ± 0.68^{b}	71.062 ± 1.31^{a}	9.373 ± 0.83^{e}	12.630 ± 1.21^{d}	24.890 ± 0.83^{c}
KACC12419	10.721 ± 0.78^{b}	57.729 ± 0.86^{d}	24.881 ± 0.82^{c}	12.573 ± 1.22^{d}	23.997 ± 0.94^{c}
KACC12427	10.934 ± 0.98^{b}	66.253 ± 1.34^{b}	54.690 ± 1.44^{b}	41.891 ± 1.72^{c}	24.981 ± 0.70^{c}

Values represented as mean ± SEM (n = 3); for each column, different subscripts lowercase letters indicate significantly different according Duncan's multiple-range test ($p < 0.05$) between different strains.

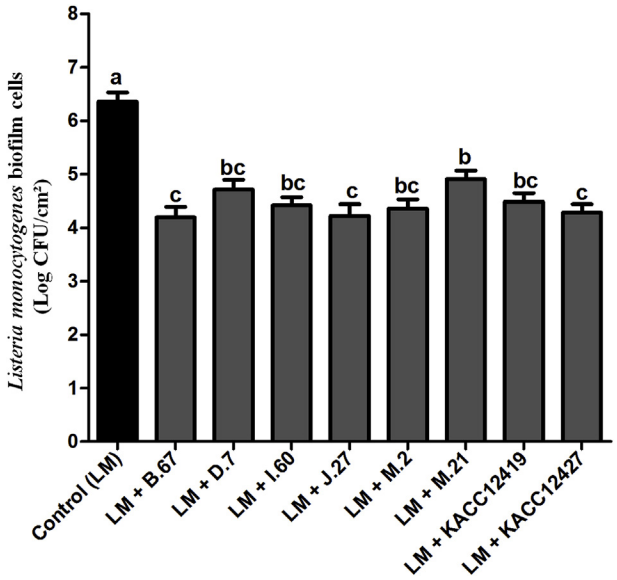

Fig. 1. Inhibition of *Listeria monocytogenes* biofilm (24 h) formation on stainless-steel (SS) at 30 °C after co-culture with lactic acid bacteria (LAB) isolates. After treatment, biofilm bacteria were enumerated by serial dilutions and plate counts. Data represent mean ± SEM of three independent experiments. Within each treatment, values marked with the different letters differ significantly different based on Duncan's multiple range test ($p < 0.05$).

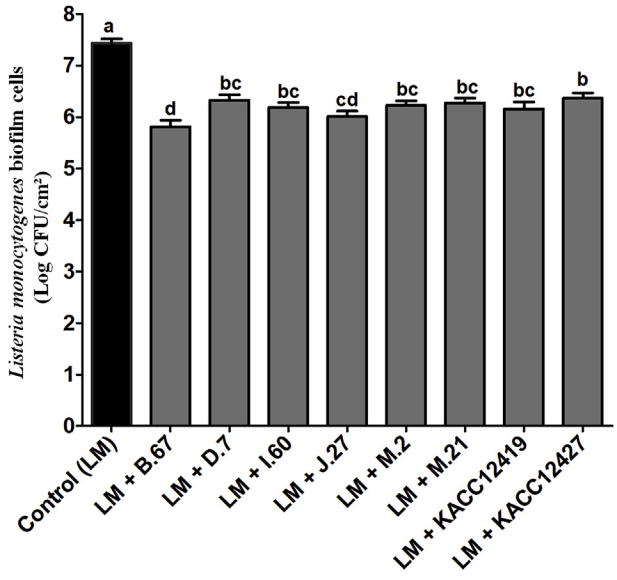

Fig. 2. Inhibition of *Listeria monocytogenes* biofilm (24 h) formation on lettuce at 25 °C after co-culture with lactic acid bacteria (LAB) isolates. After treatment, biofilm bacteria were enumerated by serial dilutions and plate counts. Data represent mean ± SEM of three independent experiments. Within each treatment, values marked with the different letters differ significantly different based on Duncan's multiple range test ($p < 0.05$).

monocytogenes biofilm formation was not encountered up to 48 h in co-culture with *Enterococcus faecium* strain on SS coupons. Similarly, co-culture studies with *L. monocytogenes* and *Lactobacillus sakei* 1 have been carried out by Winkelströter, Gomes, Thomaz, Souza, and De Martinis (2011), and they noted that *L. monocytogenes* populations on SS coupons were reduced by less than 3 log CFU/cm^2 with *L. sakei* 1. Moreover, Winkelströter, Tulini, and De Martinis (2015) showed that co-culture of *L. monocytogenes* with *Lactobacillus paraplantarum* FT259 significantly inhibited *L. monocytogenes* adherence to SS coupons by approximately 2.4 log cycles at 24 and 48 h and 1.86 log cycles at 72 h.

In this study, the biofilm inhibitory potential of selected LAB was also tested against *L. monocytogenes* on lettuce leaves stored at 25 °C for 24 h, and *L. monocytogenes* biofilm was inhibited by approximately 1.07–1.62 log CFU/cm^2 (Figs. 2 and 3), as compared with *L. monocytogenes* alone. Among the tested LAB, isolate B.67 exerted the best effect, and inhibited *L. monocytogenes* biofilm formation by approximately 1.62 log CFU/cm^2, whereas KACC12427 exhibited the least inhibition of *L. monocytogenes* biofilm (1.07 log CFU/cm^2). In a study, co-culture studies with *L. monocytogenes* and *L. lactis* have been carried out by Palmai and Buchanan (2002), and they noticed that *L. monocytogenes* levels onto seeds were reduced by approximately 1 log. Trias, Bañeras, Badosa, and Montesinos (2008), it was found that five LAB strains were effective at inhibiting *L. monocytogenes* on lettuce cuts and golden delicious apple wounds stored at 25 °C for 96 h. Moreover, Siroli et al. (2015) showed the efficacy of the two *Lactobacillus plantarum* strains to control *L. monocytogenes* on lettuce and apples. Inhibition of *L. monocytogenes* biofilm, by approximately 0.5–0.8 log CFU/cm^2, was observed upon co-inoculation with LAB isolates on lettuce leaves at 10 °C (data not shown), but this effect was not considered sufficient. The inhibitory effect of this study was comparatively greater at 25 °C than at 10 °C, and the growth of the biocontrol agents LAB and pathogens was raised by higher temperatures than at low temperatures (Leverentz et al., 2006).

Here, LAB efficacy against *L. monocytogenes* biofilm formation on lettuce was examined at two different temperatures (10 and 25 °C) for 24 h. The lower assay temperature (10 °C) was chosen to reduce the effect of bacterial growth (Garrood, Wilson, & Brocklehurst, 2004) and because it is a common temperature employed during the various food processing stages (Else, Pantle, & Amy, 2003). The room temperature (25 °C) was selected to simulate the supply chain conditions that lettuce is typically exposed during a hot summer condition from farm to table, as described in a previous study (Koseki & Isobe, 2005). Moreover, Moore-Neibel, Gerber, Patel, Friedman, and Ravishankar (2012) noted that foodborne pathogens are known to survive for long periods at different temperatures on lettuce and other produce. Under all circumstances examined in this study, biofilm formation was improved by higher temperatures than at low temperatures ($p < 0.05$), which was consistent with the results on lettuce observed by Sadekuzzaman, Yang, Mizan, Kim, and Ha (2017). However, it was apparent that LAB was comparatively less potent against biofilm formation on lettuce surface than on stainless steel, which could be because of different factors, including the food surface topography, the physiochemical properties of food and the interaction of LAB with organic food ingredients, and the resistance mechanisms of the pathogenic bacteria against the LAB. The leaf surface is mostly approbative for bacterial attachment and biofilm formation, which could be the main factor for this effect. Other factors, such as dense exopolysaccharide-like material that surrounded the biofilm, shifting the bacterial physiology and the leaf surface topography, might provide an extra level of protection against LAB. Moreover, surface characteristics, the carbohydrate content, salt content, and antioxidants present in lettuce might have reduced the effectiveness of the LAB (Sadekuzzaman et al., 2017).

The efficacy of LAB against *L. monocytogenes* biofilms was also evaluated on an MBEC™ biofilm device. The MBEC™ microplate device, formerly known as the Calgary device, facilitates biofilm formation and is used to evaluate the efficacy of antimicrobials against biofilm cells. Here, we developed biofilm on the pegs of the MBEC™ plate and evaluated the inhibitory efficacy of LAB to *L. monocytogenes* biofilms, which, as shown in Fig. 4, was approximately 0.60–1.09 log CFU/peg when compared with *L. monocytogenes* alone. The highest number of biofilm cells were inhibited by isolate B.67 (1.09 log CFU/peg) and the lowest inhibition was observed for strain KACC12427 (approximately 0.60 log CFU/peg). To our knowledge, no studies have been reported about the inhibitory effect of probiotic LAB on biofilm developed by foodborne pathogens on MBEC™ device.

Probiotic LAB have diverse inhibitory mechanisms that can disrupt

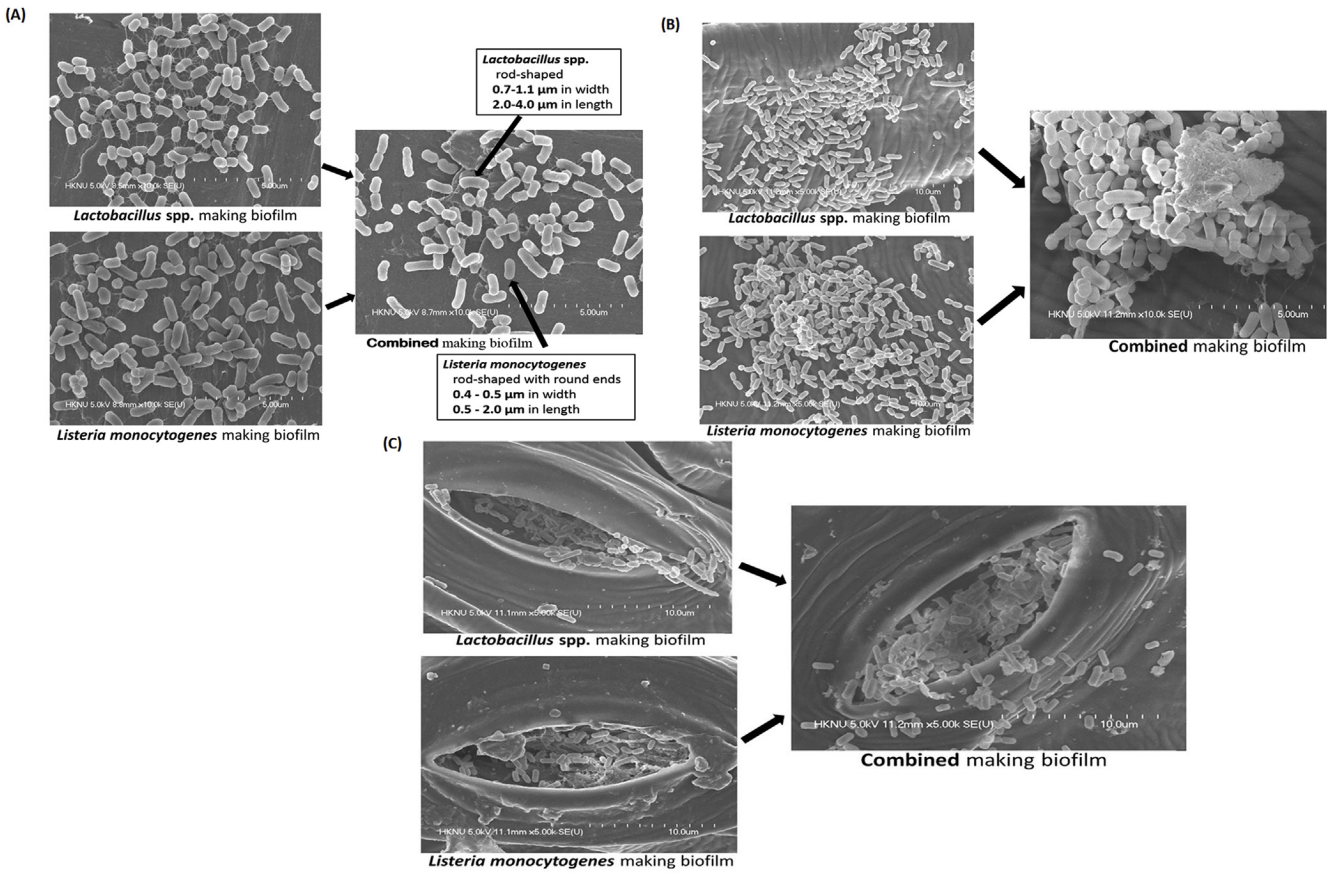

Fig. 3. Field emission scanning electron micrograph of a 24-h biofilm on (A) stainless-steel (SS), and (B) lettuce leaf surface and (C) stomata.

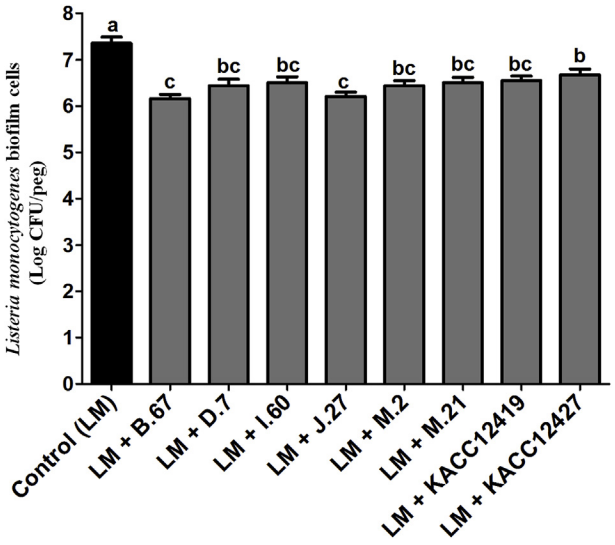

Fig. 4. Inhibition of *Listeria monocytogenes* biofilm (24 h) formation on MBEC™ biofilm device at 30 °C after co-culture with lactic acid bacteria (LAB) isolates. After treatment, biofilm bacteria were enumerated by serial dilutions and plate counts. Data represent mean ± SEM of three independent experiments. Within each treatment, values marked with the different letters differ significantly different based on Duncan's multiple range test ($p < 0.05$).

the formation of pathogenic microbial biofilms. LAB can inhibit the growth of pathogens by releasing antimicrobial metabolites (organic acids, hydrogen peroxide, and bacteriocins) and inhibitory exopolysaccharides (EPS) surrounding the pathogenic microorganisms (Chlebowska-Smigiel Gniewosz, Kieliszek, & Bzducha-Wrobel, 2017). Bacteriocin initiates the formation of pores in the bacterial cell membrane, which results in cell content leakage loss of essential molecules and ions causes ultimate cell death; can also interference with targeted bacterial DNA replication to causes cell death (Cotter, Ross, & Hill, 2013; Ennahar, Sashihara, Sonomoto, & Ishizaki, 2000). Additionally, organic acids (e.g., lactic and acetic acids) provide an unfavorable acidic condition that can inhibit the pathogens whereas LAB are well-suited to high acidic conditions (Servin, 2004). However, the competition for nutrients, growth factors, and adhesion sites could also interfere with biofilm formation in pathogenic microorganisms (Hossain et al., 2017; Silva, Silva, & Ribeiro, 2018). Fig. 5 illustrates the hypothetical mechanisms of probiotic action against biofilm development by *L. monocytogenes* pathogen. Micrographs of CLSM in Fig. 6 indicated the biofilm formed by *L. monocytogenes* alone and in co-culture experiments with LAB strains. From the visualization analysis of cell viability (live or dead), the biofilm developed by *L. monocytogenes* was homogenous with more live cells (green; Fig. 6A), whereas biofilm was significantly damaged with dead cells (Fig. 6B and C) when *L. monocytogenes* was co-cultured with LAB strains. These images provided further support that the ability of *L. monocytogenes* to form biofilm was hampered in the presence of LAB strains. While the exact mechanism is not yet known, it can be inferred that strains of LAB with probiotic potential halt the biofilm formation either through exclusion mechanisms or production of anti-microbial compounds (organic acids, hydrogen peroxide, and bacteriocins) that interact with the pathogen or components of the biofilm matrix.

Screening and selection of the anti-listerial LAB strains are vital issue when using LAB as a biocontrol agent against *L. monocytogenes* because not all LAB have anti-listerial capability. These LAB, which were selected for this study due to their effectiveness against *L. monocytogenes*, make promising candidates to evaluate their potential to inhibit *L. monocytogenes* biofilms. In food industry, the LAB culture could

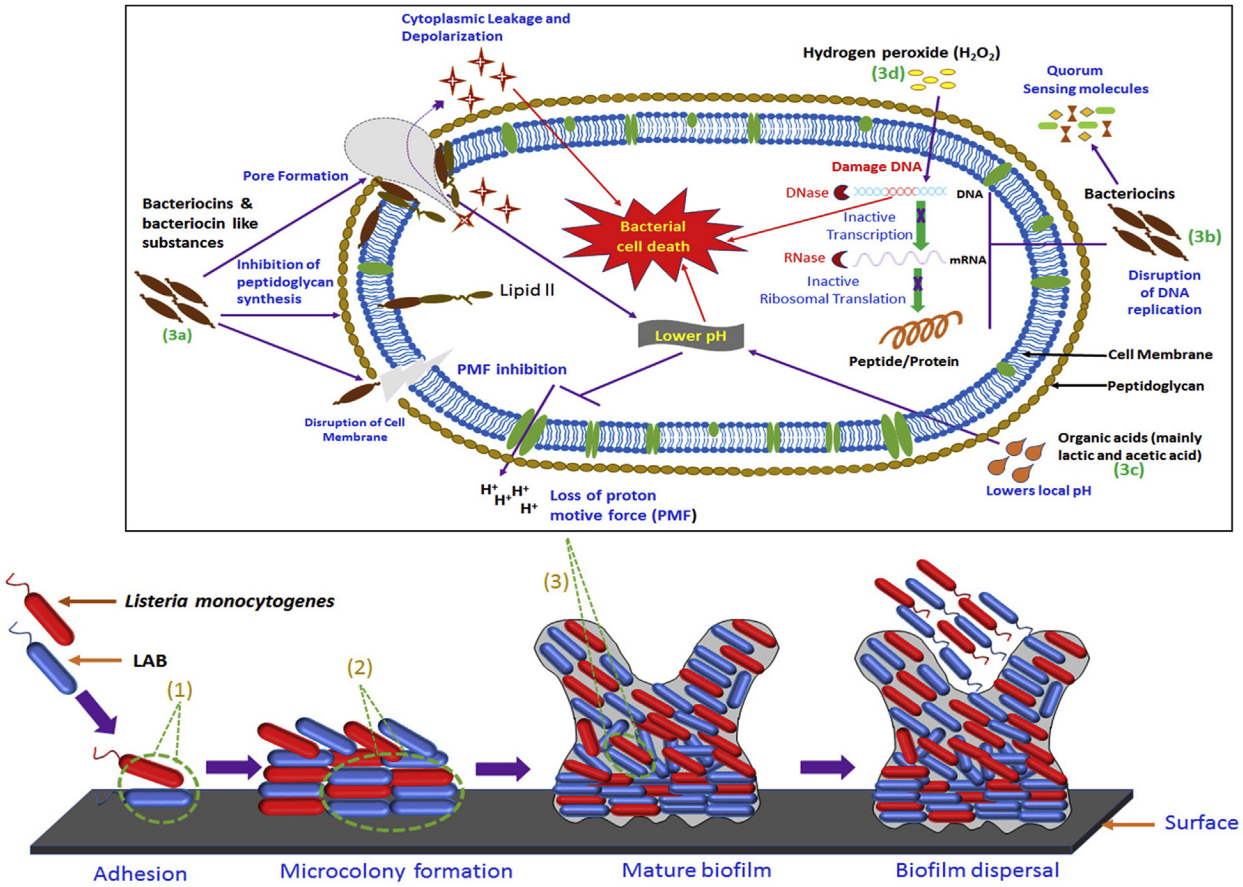

Fig. 5. Hypothetic mechanisms of probiotic action against biofilm development by *Listeria monocytogenes* pathogen. (1). Inhibition of *L. monocytogenes* pathogen adhesion; (2). Competitive exclusion approach toward *L. monocytogenes* pathogenic microorganisms; (3). Production of anti-microbial substances against *L. monocytogenes*: (3a). Bacteriocins interact with lipid-II cell wall component (serves as a recognition 'anchor') and working as cytoplasmic membrane perturbators to promote the dissociation of lipid-II molecules. These actions inhibit the normal cell cycle and cell wall synthesis of targeted cell, and also cause the formation of pores in bacterial cytoplasmic membrane, leading to the cell death via dissipation of proton motive force of the bacterial system; (3b). Bacteriocins inhibit the DNA replication and cause DNA damage, ultimately resulting in cell death by inhibition of DNA gyrase, RNA polymerase, and Aspartyl-tRNA synthetase; (3c). Organic acids provide an unfavorable acidic condition that can inhibit the pathogen growth, proceeding to the cell death via dissipation of proton motive force of the bacterial system; (3d). Hydrogen peroxide (H_2O_2) interferes with targeted bacterial DNA, RNA, and protein metabolism, which results in cell death via DNA damages.

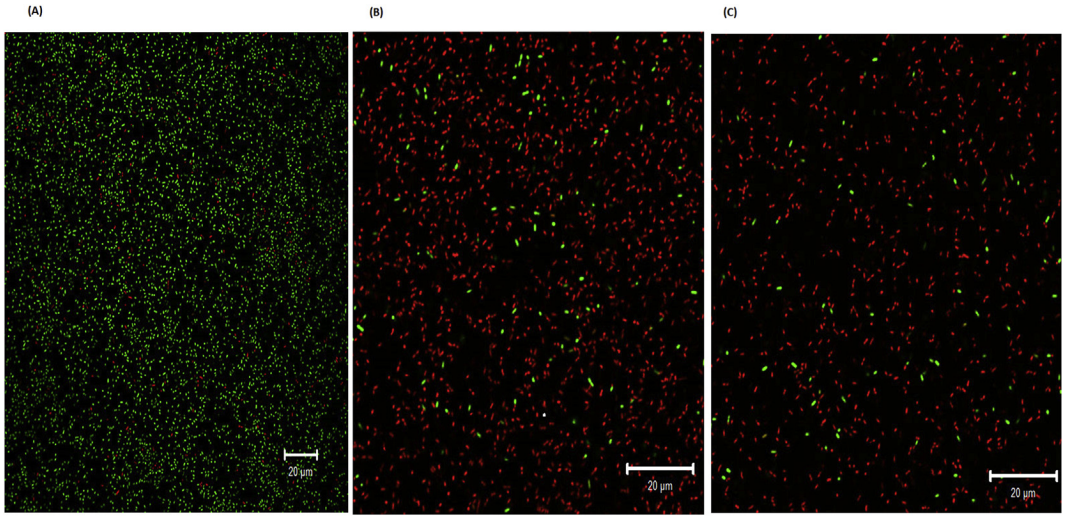

Fig. 6. Confocal Laser Scanning Microscopic (CLSM) images of *Listeria monocytogenes* biofilm cells viability by live/dead assay with or without co-culture of lactic acid bacteria (LAB) isolates. Green represents live cells and red represents dead cells. (A) *Listeria monocytogenes* biofilm (control). (B) Co-culture with isolate J.27. (C) Co-culture with isolate B.67. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

be applied on food surfaces by spraying or via a cloth or sponge to avoid environmental impacts, so that the target surface is completely covered. Based on bio-protective approaches by using GRAS microorganisms and their metabolites are considered as suitable for consumers health and could also diminish the bad effects of nutritional and sensorial properties on food (Oliveira, Abadias, Colás-Medà, Usall, & Viñas, 2015). The buildup of protective biofilms with probiotic LAB in food could help to prevent the food contamination problems into the food chain. Thus, the finding of the present study support the potential use of probiotic LAB strains as natural barriers and bio-preservatives to control *L. monocytogenes* biofilm formation, or that by other pathogenic bacteria, on vegetable products and in the food industry, without posing a risk to consumers; however, the actual mechanism of this inhibition still now unnoted and will be scanned in the future.

4. Conclusions

In this study, five *Lactobacillus* LAB isolates and one *Leuconostoc* LAB isolate showed anti-listerial capability. Overall, all the studied isolates exhibited remarkable auto-aggregation and hydrophobicity potential. In addition, our results demonstrated that the LAB strains exerted an inhibitory effect against *L. monocytogenes* biofilms formation on fresh produce (lettuce), SS, and on the MBEC™ biofilm device. Our current study are very encouraging in the fact that these LAB strains could be used as an alternative for the prevention of *L. monocytogenes* biofilm formation on food and food manufacturing equipment's without any adverse effects. The inhibitory effect of LAB against pathogens biofilms on food contact surfaces should be investigated on an industrial scale. More studies must be conducted to scale-up this protective strategy for its industrial application to ensure microbial safety of food. While future studies will be required to identify the active components responsible for the anti-listerial as well as antibiofilm activity within these LAB strains, and to elucidate the molecular mechanism of action of these components. In addition, further studies are required to examine the interactions between the tested LAB isolates and fresh produce surfaces to control the contamination of *L. monocytogenes*. Moreover, advance research with real environment biofilms consisting of mixed species of *L. monocytogenes* is required to fully realize the inhibitory potential of the LAB strains. However, the current research might contribute toward the future advancement of potential LAB-based biofilm control strategies.

Declaration of competing interest

No potential conflict of interest was reported by the authors.

Acknowledgement

This research was supported by a Chung-Ang University Research Scholarship Grant in 2017 and Ministry of Food and Drug Safety in 2017 (grant number 17162MFDS036).

References

Abushelaibi, A., Al-Mahadin, S., El-Tarabily, K., Shah, N. P., & Ayyash, M. (2017). Characterization of potential probiotic lactic acid bacteria isolated from camel milk. *LWT-Food Science and Technology, 79*, 316–325.

Angmo, K., Kumari, A., & Bhalla, T. C. (2016). Probiotic characterization of lactic acid bacteria isolated from fermented foods and beverage of Ladakh. *LWT-Food Science and Technology, 66*, 428–435.

Argyri, A. A., Zoumpopoulou, G., Karatzas, K.-A. G., Tsakalidou, E., Nychas, G.-J. E., Panagou, E. Z., et al. (2013). Selection of potential probiotic lactic acid bacteria from fermented olives by *in vitro* tests. *Food Microbiology, 33*(2), 282–291.

Beresford, M. R., Andrew, P. W., & Shama, G. (2001). *Listeria monocytogenes* adheres to many materials found in food-processing environments. *Journal of Applied Microbiology, 90*(6), 1000–1005.

Bove, P., Gallone, A., Russo, P., Capozzi, V., Albenzio, M., Spano, G., et al. (2012). Probiotic features of *Lactobacillus plantarum* mutant strains. *Applied Microbiology and Biotechnology, 96*(2), 431–441.

Bridier, A., Sanchez-Vizuete, P., Guilbaud, M., Piard, J.-C., Naïtali, M., & Briandet, R. (2015). Biofilm-associated persistence of food-borne pathogens. *Food Microbiology, 45*, 167–178.

Chlebowska-Smigiel, A., Gniewosz, M., Kieliszek, M., & Bzducha-Wrobel, A. (2017). The effect of pullulan on the growth and acidifying activity of selected stool microflora of human. *Current Pharmaceutical Biotechnology, 18*(2), 121–126.

Collado, M. C., Meriluoto, J., & Salminen, S. (2008). Adhesion and aggregation properties of probiotic and pathogen strains. *European Food Research and Technology, 226*(5), 1065–1073.

Cotter, P. D., Ross, R. P., & Hill, C. (2013). Bacteriocins—a viable alternative to antibiotics? *Nature Reviews Microbiology, 11*(2), 95.

Dal Bello, B., Cocolin, L., Zeppa, G., Field, D., Cotter, P. D., & Hill, C. (2012). Technological characterization of bacteriocin producing *Lactococcus lactis* strains employed to control *Listeria monocytogenes* in cottage cheese. *International Journal of Food Microbiology, 153*(1–2), 58–65.

Das, P., Khowala, S., & Biswas, S. (2016). *In vitro* probiotic characterization of *Lactobacillus casei* isolated from marine samples. *LWT-Food Science and Technology, 73*, 383–390.

David, D. J., & Cossart, P. (2017). Recent advances in understanding *Listeria monocytogenes* infection: The importance of subcellular and physiological context. *F1000Research, 6*, 1126.

Dias, F. S., Duarte, W. F., & Schwan, R. F. (2013). Evaluation of adhesive properties of presumptive probiotic *Lactobacillus plantarum* strains. *Bioscience Journal, 29*(5).

Else, T. A., Pantle, C. R., & Amy, P. S. (2003). Boundaries for biofilm formation: Humidity and temperature. *Applied and Environmental Microbiology, 69*(8), 5006–5010.

Ennahar, S., Sashihara, T., Sonomoto, K., & Ishizaki, A. (2000). Class IIa bacteriocins: Biosynthesis, structure and activity. *FEMS Microbiology Reviews, 24*(1), 85–106.

FAO/WHO (2002). *Probiotics in food. Health and nutritional properties and guidelines for evaluation.* Rome: Food and agriculture organization of the United Nations and World Health Organization FAO food and nutrition paper no. 85.

García-Almendárez, B. E., Cann, I. K., Martin, S. E., Guerrero-Legarreta, I., & Regalado, C. (2008). Effect of *Lactococcus lactis* UQ2 and its bacteriocin on *Listeria monocytogenes* biofilms. *Food Control, 19*, 670–680.

García-Cayuela, T., Korany, A. M., Bustos, I., de Cadiñanos, L. P. G., Requena, T., Peláez, C., et al. (2014). Adhesion abilities of dairy *Lactobacillus plantarum* strains showing an aggregation phenotype. *Food Research International, 57*, 44–50.

Garrood, M. J., Wilson, P. D. G., & Brocklehurst, T. F. (2004). Modelling the rate and strength of attachment of *Listeria monocytogenes, Pantoea agglomerans* and *Pseudomonas fluorescens* to vegetable tissue at 10 C. *Applied and Environmental Microbiology, 70*, 3358–3565.

Goh, Y. J., & Klaenhammer, T. R. (2010). Functional roles of aggregation-promoting-like factor in stress tolerance and adherence of *Lactobacillus acidophilus* NCFM. *Applied and Environmental Microbioogy, 76*(15), 5005–5012.

Gómez, N. C., Ramiro, J. M., Quecan, B. X., & de Melo Franco, B. D. (2016). Use of potential probiotic lactic acid bacteria (LAB) biofilms for the control of *Listeria monocytogenes, Salmonella* typhimurium, and *Escherichia coli* O157: H7 biofilms formation. *Frontiers in Microbiology, 7*, 863.

Hibbing, M. E., Fuqua, C., Parsek, M. R., & Peterson, S. B. (2009). Bacterial competition: Surviving and thriving in the microbial jungle. *Nature Reviews Microbiology, 8*, 15–25.

Hossain, M. I., Sadekuzzaman, M., & Ha, S.-D. (2017). Probiotics as potential alternative biocontrol agents in the agriculture and food industries: A review. *Food Research International, 100*, 63–73.

Ibarreche, M. P., Castellano, P., & Vignolo, G. (2014). Evaluation of anti-*Listeria* meat borne *Lactobacillus* for biofilm formation on selected abiotic surfaces. *Meat Science, 96*(1), 295–303.

Jahid, I. K., Lee, N.-Y., Kim, A., & Ha, S.-D. (2013). Influence of glucose concentrations on biofilm formation, motility, exoprotease production, and quorum sensing in *Aeromonas hydrophila*. *Journal of Food Protection, 76*(2), 239–247.

Kaewnopparat, S., Dangmanee, N., Kaewnopparat, N., Srichana, T., Chulasiri, M., & Settharaksa, S. (2013). *In vitro* probiotic properties of *Lactobacillus fermentum* SK5 isolated from vagina of a healthy woman. *Anaerobe, 22*, 6–13.

Khan, I., & Kang, S. C. (2016). Probiotic potential of nutritionally improved *Lactobacillus plantarum* DGK-17 isolated from Kimchi–A traditional Korean fermented food. *Food Control, 60*, 88–94.

Koseki, S., & Isobe, S. (2005). Prediction of pathogen growth on iceberg lettuce under real temperature history during distribution from farm to table. *International Journal of Food Microbiology, 104*(3), 239–248.

Lee, M. E., Jang, J. Y., Lee, J. H., Park, H. W., Choi, H. J., & Kim, T. W. (2015). Starter cultures for kimchi fermentation. *Journal of Microbiology and Biotechnology, 25*(5), 559–568.

Leuschner, R. G., Robinson, T. P., Hugas, M., Cocconcelli, P. S., Richard-Forget, F., Klein, G., ... Suarez, J. E. (2010). Qualified presumption of safety (QPS): A generic risk assessment approach for biological agents notified to the European food safety authority (EFSA). *Trends in Food Science & Technology, 21*(9), 425–435.

Leverentz, B., Conway, W. S., Janisiewicz, W., Abadias, M., Kurtzman, C. P., & Camp, M. J. (2006). Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. *Applied and Environmental Microbiology, 72*, 1135–1140.

Li, P., Jia, S., Zhou, C., Fang, H., & Chen, C. (2017). Protective role of *Lactobacillus fermentum* R6 against *Clostridium perfringens in vitro* and in chicken breast meat under temperature abuse conditions. *Innovative Food Science & Emerging Technologies, 41*, 117–123.

Marder, E. P., Cieslak, P. R., Cronquist, A. B., Dunn, J., Lathrop, S., Rabatsky-Ehr, T., ... Zansky, S. (2017). Incidence and trends of infections with pathogens transmitted commonly through food and the effect of increasing use of culture-independent diagnostic tests on surveillance—foodborne Diseases Active Surveillance Network, 10

US Sites, 2013–2016. *MMWR. Morbidity and Mortality Weekly Report, 66*(15), 397.
Marques, S. C., Rezende, J. D. G. O. S., Alves, L. A. D. F., Silva, B. C., Alves, E., Abreu, L. R. D., et al. (2007). Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. *Brazilian Journal of Microbiology, 38*(3), 538–543.
Minei, C. C., Gomes, B. C., Ratti, R. P., D'angelis, C. E., & De Martinis, E. C. (2008). Influence of peroxyacetic acid and nisin and coculture with *Enterococcus faecium* on *Listeria monocytogenes* biofilm formation. *Journal of Food Protection, 71*(3), 634–638.
Mizan, M. F. R., Jahid, I. K., & Ha, S.-D. (2015). Microbial biofilms in seafood: A food-hygiene challenge. *Food Microbiology, 49*, 41–55.
Mizan, M. F. R., Jahid, I. K., Kim, M., Lee, K.-H., Kim, T. J., & Ha, S.-D. (2016). Variability in biofilm formation correlates with hydrophobicity and quorum sensing among *Vibrio parahaemolyticus* isolates from food contact surfaces and the distribution of the genes involved in biofilm formation. *Biofouling, 32*(4), 497–509.
Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M., & Ravishankar, S. (2012). Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. *Journal of Applied Microbiology, 112*(3), 485–492.
Oliveira, M., Abadias, M., Colás-Medà, P., Usall, J., & Viñas, I. (2015). Biopreservative methods to control the growth of foodborne pathogens on fresh-cut lettuce. *International Journal of Food Microbiology, 214*, 4–11.
Palmai, M., & Buchanan, R. L. (2002). Growth of *Listeria monocytogenes* during germination of alfalfa sprouts. *Food Microbiology, 19*, 195–200.
Patel, J., & Sharma, M. (2010). Differences in attachment of *Salmonella enterica* serovars to cabbage and lettuce leaves. *International Journal of Food Microbiology, 139*(1–2), 41–47.
Peres, C. M., Alves, M., Hernandez-Mendoza, A., Moreira, L., Silva, S., Bronze, M. R., ... Malcata, F. X. (2014). Novel isolates of lactobacilli from fermented Portuguese olive as potential probiotics. *LWT-Food Science and Technology, 59*(1), 234–246.
Riaz Rajoka, M. S., Mehwish, H. M., Siddiq, M., Haobin, Z., Zhu, J., Yan, L., ... Shi, J. (2017). Identification, characterization, and probiotic potential of *Lactobacillus rhamnosus* isolated from human milk. *LWT-Food Science and Technology, 84*, 271–280.
Sadekuzzaman, M., Yang, S., Mizan, M. F. R., Kim, H.-S., & Ha, S.-D. (2017). Effectiveness of a phage cocktail as a biocontrol agent against *L. monocytogenes* biofilms. *Food Control, 78*, 256–263.
Schuenzel, K. M., & Harrison, M. A. (2002). Microbial antagonists of foodborne pathogens on fresh, minimally processed vegetables. *Journal of Food Protection, 65*(12), 1909–1915.
Servin, A. L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiology Reviews, 28*(4), 405–440.
Shen, C., Luo, Y., Nou, X., Bauchan, G., Zhou, B., Wang, Q., et al. (2012). Enhanced inactivation of *Salmonella* and *Pseudomonas* biofilms on stainless steel by use of T-128, a fresh-produce washing aid, in chlorinated wash solutions. *Applied and Environmental Microbiology, 78*(19), 6789–6798.
Sillankorva, S. M., Oliveira, H., & Azeredo, J. (2012). Bacteriophages and their role in food safety. *International Journal of Microbiology, 2012*.
Silva, C. C. G., Silva, S. P. M., & Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in Microbiology, 9*, 594.
Simoes, M., Simoes, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT-Food Science and Technology, 43*(4), 573–583.
Siroli, L., Patrignani, F., Serrazanetti, D. I., Tabanelli, G., Montanari, C., Gardini, F., et al. (2015). Lactic acid bacteria and natural antimicrobials to improve the safety and shelf-life of minimally processed sliced apples and lamb's lettuce. *Food Microbiology, 47*, 74–84.
Srey, S., Jahid, I. K., & Ha, S. D. (2013). biofilm formation in food industries: A food safety concern. *Food Control, 31*(2), 572–585.
Trias, R., Bañeras, L., Badosa, E., & Montesinos, E. (2008). Bioprotection of golden delicious apples and iceberg lettuce against foodborne bacterial pathogens by lactic acid bacteria. *International Journal of Food Microbiology, 123*(1–2), 50–60.
Ukuku, D. O., Latiful, B., Kassama, L. S., Mukhopadhyay, S., & Olanya, M. (2015). Survival, injury and inactivation of human bacterial pathogens in foods: Effect of non-thermal treatments. *Foodborne pathogens and food safety (food biology series)* (pp. 82–96). Boca Raton CRC Press.
Vijayakumar, M., Ilavenil, S., Kim, D. H., Arasu, M. V., Priya, K., & Choi, K. C. (2015). *In-vitro* assessment of the probiotic potential of *Lactobacillus plantarum* KCC-24 isolated from Italian rye-grass *(Lolium multiflorum*) forage. *Anaerobe, 32*, 90–97.
Wei, H., Wolf, G., & Hammes, W. P. (2006). Indigenous microorganisms from iceberg lettuce with adherence and antagonistic potential for use as protective culture. *Innovative Food Science & Emerging Technologies, 7*(4), 294–301.
Wen, L. S., Philip, K., & Ajam, N. (2016). Purification, characterization and mode of action of plantaricin K25 produced by *Lactobacillus plantarum*. *Food Control, 60*, 430–439.
Winkelströter, L. K., Gomes, B. C., Thomaz, M. R., Souza, V. M., & De Martinis, E. C. (2011). *Lactobacillus sakei* 1 and its bacteriocin influence adhesion of *Listeria monocytogenes* on stainless steel surface. *Food Control, 22*(8), 1404–1407.
Winkelströter, L. K., Tulini, F. L., & De Martinis, E. C. (2015). Identification of the bacteriocin produced by cheese isolate *Lactobacillus paraplantarum* FT259 and its potential influence on *Listeria monocytogenes* biofilm formation. *LWT-Food Science and Technology, 64*(2), 586–592.
Yang, S. J., Lee, J. E., Lim, S. M., Kim, Y. J., Lee, N. K., & Paik, H. D. (2019). Antioxidant and immune-enhancing effects of probiotic *Lactobacillus plantarum* 200655 isolated from kimchi. *Food Science and Biotechnology, 28*(2), 491–499.
Zhao, T., Doyle, M. P., & Zhao, P. (2004). Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. *Applied and Environmental Microbiology, 70*(7), 3996–4003.
Zhao, T., Podtburg, T. C., Zhao, P., Chen, D., Baker, D. A., Cords, B., et al. (2013). Reduction by competitive bacteria of *Listeria monocytogenes* in biofilms and *Listeria* bacteria in floor drains in a ready-to-eat poultry processing plant. *Journal of Food Protection, 76*(4), 601–607.