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Growth Properties and Cholesterol Removal Ability of Electroporated *Lactobacillus acidophilus* BT 1088

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This study aimed to evaluate the effects of electroporation on the cell growth, cholesterol removal, and adherence abilities of *L. acidophilus* BT 1088 and their subsequent passages. The growth of electroporated parent cells increased (P<0.05) by 4.49–21.25% compared with that of the control. This may be attributed to the alteration of cellular membrane. However, growth of first, second, and third passages of treated cells was comparable with that of the control, which may be attributed to the resealing of transient pores on the cellular membrane. Electroporation also increased (P<0.05) assimilation of cholesterol by treated parent cells (>185.40%) and first passage (>21.72%) compared with that of the control. Meanwhile, incorporation of cholesterol into the cellular membrane was also increased (P<0.05) in the treated parent cells (>108.33%) and first passage (>26.67%), accompanied by increased ratio of cholesterol:phospholipids (C:P) in these passages. Such increased ratio was also supported by increased enrichment of cholesterol in the hydrophilic heads, hydrophobic tails, and the interface regions of the membrane phospholipids of both parent and first passage cells compared with that of the control. However, such traits were not inherited by the subsequent second and third passages. Parent cells also showed decreased intestinal adherence ability (P<0.05; decreased by 1.45%) compared with that of the control, without inheritance by subsequent passages of treated cells. Our data suggest that electoporation could be a potential physical treatment to enhance the cholesterol removal ability of lactobacilli that was inherited by the first passage of treated cells without affecting their intestinal adherence ability.

Keywords: Electroporation, lactobacilli, cholesterol, membrane, incorporation, passage

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Lactobacilli are the most common type of bacteria that exert probiotic properties. Probiotics have been defined by the FAO/WHO as "viable microorganisms which when administered in adequate amounts confer a health benefit to the host" [8]. They have been reported to exhibit several health beneficial effects such as improvement of intestinal microbial balance, prevention/reduction of diarrhea, stimulation of immune system, and prevention of gastrointestinal infection. In addition, a lowering effect on serum cholesterol by lactobacilli was also observed when minipigs were fed with meal supplemented with lactobacilli cells [6]. Xie *et al.* [26] also reported that the serum total cholesterol, low-density lipoprotein cholesterol, and triglycerides levels in rats were decreased upon consumption of lactobacilli. We have also previously found that the cholesterol removal ability of lactobacilli was mainly due to assimilation of cholesterol and incorporation of cholesterol into the cellular membrane [16, 17]. This reduction of cholesterol *in vivo* and removal of cholesterol *in vitro* by lactobacilli could play an important role in modulating the human cardiovascular disease, where a 1% reduction in serum cholesterol could reduce the risk of coronary heart disease by 2–3% [20].

Electroporation induces permeabilization of cell membrane *via* changing membrane dielectric properties [23]. This treatment is a three-step process that involves the creation, expansion, and resealing of transient pores [10]. The formation of pores as a response to electric field lasts for a few microseconds, followed by the expansion of pore size in a time range of 100 μs, and resealing of pores in the minute range. This enables the transportation of molecules from medium into the cells within the range of millisecond and minute [13]. An electric field is also known to affect mainly the cellular membranes and cause leakage of cell components *via* pore formation that subsequently leads to cell death. The reversible or irreversible pore formations are dependent on treatment conditions. Uptake of macromolecules without loss of viability can be observed during formation of reversible pores upon low and sublethal field strengths

[24]. We have previously reported that cell growth and removal of cholesterol of *Lactobacillus* (*L.*) *acidophilus* BT 1088, *L. acidophilus* FTCC 0291, *L. bulgaricus* FTCC 0411, *L. bulgaricus* FTDC 1311, and *L. casei* BT 1286 *via* assimilation of cholesterol and incorporation of cholesterol into the cellular membrane were increased upon electroporation, mainly as an effect of altered cellular membrane [15]. We postulate that the effect of electroporation on cholesterol removal ability of lactobacilli may be inherited by treated cells of subsequent passages. However, to date, there has been no information on this aspect.

In our previous study, *L. acidophilus* BT 1088 treated at 7.5 kV/cm for 3.5 ms showed higher cholesterol removal compared with other strains [15]. Thus, the objectives of this study were to determine the effect of electroporation on cell growth, cholesterol removal, ratio of cellular cholesterol: phospholipids, and also the possible location of cholesterol enrichment for *L. acidophilus* BT 1088 in the parent and subsequent passages of treated cells. Additionally, the effect of electroporation on the adhesion ability of *L. acidophilus* BT 1088 was evaluated.

Materials and Methods

Bacterial Cultures

L. acidophilus BT 1088 was obtained from the culture collection of Bioprocess Division, School of Industrial Technology, Universiti Sains Malaysia (Penang, Malaysia). Stock cultures were stored in sterile 40% (v/v) glycerol at −20°C. Prior to experimental use, lactobacilli were propagated in sterile de Mann−Rogosa−Sharpe (MRS) broth (Hi-Media, Mumbai, India) supplemented with 0.15% (w/v) L-cysteine-hydrochloride (Hi-Media, Mumbai, India) for three successive times and incubated at 37°C for 24 h.

Electroporation

The activated strain of *L. acidophilus* BT 1088 [10% (v/v); approximately 7.0 Log_{10} CFU/ml] was washed twice with 1 mM sucrose (R & M, Essex, UK) and resuspended into the same sucrose solution. The cell suspension in a 0.1 cm gap cuvette was then electroporated (MicroPulser; Bio-Rad Laboratories, Hercules, CA, USA) with a capacitance of 25 μF and resistance of 200 Ω and constant cooling at 4°C. The field strength was adjusted to 7.5 kV/cm and treated for 3.5 ms. The electroporated cells were immediately added into sterile MRS broth containing 100 μg/ml cholesterol (Hi-Media, Mumbai, India), 0.15% (w/v) L-cysteine-hydrochloride (Hi-Media), 0.3% (w/v) oxgall (Sigma-Aldrich, St. Louis, MO, USA), and 0.1% (w/v) pancreatin (Sigma-Aldrich, St. Louis, MO, USA). The mixture was adjusted to pH 8.0 in order to mimic the human small intestinal condition, followed by incubation at 37°C for 24 h. This was recorded as parent cells. Upon fermentation of 24 h, the treated parent cells [10% (v/v); approximately 7.0 Log_{10} CFU/ml] were transferred into new sterile MRS medium containing the same concentration of cholesterol, L-cysteine-hydrochloride, oxgall, and pancreatin, and incubated at 37°C for another 24 h. This was recorded as first passage. This step was repeated until the third passage. Untreated cells were used as a control. Untreated and treated cells of each passage were harvested upon fermentation for subsequent analyses.

Growth

The growth of *L. acidophilus* BT 1088 upon electroporation and subsequent passages of treated cells was determined *via* the pour plate method using MRS agar supplemented with 0.15% (v/v) L-cysteine-hydrochloride (Hi-Media). Plates were incubated at 37°C for 48 h.

Removal of Cholesterol

Electroporated cells were inoculated into sterile MRS broth containing 100 μg/ml cholesterol (Hi-Media), 0.15% (w/v) L-cysteine-hydrochloride (Hi-Media), 0.3% (w/v) oxgall (Sigma-Aldrich), and 0.1% (w/v) pancreatin (Sigma-Aldrich). Untreated cells were used as a control. The mixture was adjusted to pH 8.0 in order to mimic the human small intestinal condition. Then, the resulting mixture was incubated at 37°C for 24 h and centrifuged at 12,000 ×*g*, 4°C for 15 min to separate the supernatant and cells pellet. The supernatant was then collected for the determination of cholesterol assimilation as previously described [16].

Incorporation of Cholesterol into Cellular Membrane

Incorporation of cholesterol into cellular membrane was determined based on the determination of membrane cholesterol and phospholipids contents, and also membrane fluidity as detected by fluorescence anisotropy (FAn). The cholesterol and phospholipids contents were extracted from the cellular membrane as previously described [15]. Briefly, the cell pellet was mixed with 1 ml of methanol:chloroform [2:1 (v/v)] and vortexed at 25°C for 2 min. The mixture was left to stand for 24 h at 4°C and centrifuged (10,000 ×*g*, 4°C, 10 min) to collect the supernatant. The cell pellet was then washed twice with 1 ml of methanol:chloroform:distilled water [2:1:0.8 (v/v/v)] and all three supernatants were pooled and vortexed for 1 min. Subsequently, distilled water (1 ml) and chloroform (1 ml) were added and the mixture was vortexed for 2 min. The mixture was then allowed to separate and the chloroform layer (bottom) (1 ml) was collected and evaporated. The lipid residue was collected to analyze the total cholesterol and phospholipids content using commercial enzymatic kits (bioMerieux Corporation, Marcy l'Etoile, Rhone-Alpes, France). FAn of the cellular membrane was determined using fluorescent probes such as 1,6-diphenyl-1,3,5-hexatriene (DPH; Sigma-Aldrich, St. Louis, MO, USA), 1-(4-trimethylammonium)-6-phenyl-1,3,5-hexatriene (TMA-DPH; Sigma-Aldrich, St. Louis, MO, USA) and 8-anilino-1-napthalenesulfonic acid (ANS; Sigma-Aldrich, St. Louis, MO, USA), as previously described [15]. Briefly, cellular membranes of untreated and treated cells (OD_{600} of 0.3) were labeled with working probe solutions at a ratio of 3:1 by incubation at 37°C. The incubation time for DPH and TMA-DPH was 60 min whereas that for ANS was 90 min. A blank was prepared by replacing the working probe solutions with 0.155 mol/l sodium chloride. FAn was measured using a fluorescence spectrophotometer (Cary Eclipse). The excitation wavelength for DPH and TMA-DPH was 365 nm and for ANS was 390 nm. Emission was determined at 445 nm for DPH and TMA-DPH, and 490 nm for ANS. FAn was calculated according to the equation below:

$$Ar = (I_{vv} - GI_{vh})/(I_{vv} + 2GIvh) \quad (1)$$

where I_{vv} and I_{vh} are the fluorescence intensities obtained from a vertical polarizer, and a vertical and horizontal analyzer, respectively, and G is the instrumental grating factor. $G = I_{hv}/I_{vh}$, where I_{hv} is the intensity measured from a horizontal polarizer and a vertical analyzer.

Adhesion to HT-29

The adherence of *L. acidophilus* BT 1088 to human intestinal epithelial cells (HT-29) was performed according to Cammarota *et al.* [3]. HT-29 cells were grown in McCoy's 5A medium (Invitrogen, Milan, Italy) supplemented with 10% (v/v) fetal bovine serum (Invitrogen, Milan, Italy) and a total of 1% (w/v) mixture of antibiotics (1% of streptomycin and 0.61% of penicillin; Hi-Media, Mumbai, India), followed by incubation in a 95% air−5% CO_2 atmosphere. HT-29 cells were harvested when cells reached 70−80% confluence, with a change of medium every 2 days. The HT-29 cells were then washed three times with phosphate buffer saline (PBS; pH 7.4). The washed cells were trypsinized and resuspended in 1 ml of medium. The cell suspension was transferred to 6-well plates and incubated at 37°C for 24 h for cell differentiation and attachment. The monolayers were then washed three times with PBS. One milliliter of electroporated cell suspension and 1 ml of growth medium were added into the monolayers and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 3 h. Upon incubation, the monolayers were washed three times with PBS to remove unattached bacterial cells. The monolayers were then trypsinized to detach the attached bacteria. One milliliter of cell suspension was diluted in PBS and plated on MRS agar to measure the concentration of attached bacteria. Untreated lactobacilli cells were used as a control. The percentage of attached bacteria was calculated as follows:

$$\% \text{ Attached bacterial cells} = B/A \times 100\% \quad (2)$$

where A is the initial cells count of *L. acidophilus* BT 1088 added to the monolayer, and B is the attached lactobacilli cells count upon 3 h incubation.

Statistical Analysis

Data analysis was performed statistically with SPSS Inc. software (ver. 11.5) (Chicago, USA). Independent T-test was used to evaluate the significant differences between sample means, with significance level at $\alpha = 0.05$. All data presented were mean values of three separate runs (n = 3), unless stated otherwise.

RESULTS AND DISCUSSION

Growth of *L. acidophilus* BT 1088 Upon Electroporation

The growth of *L. acidophilus* BT 1088 increased over fermentation time for all passages studied (Fig. 1A). Electroporation increased ($P<0.05$) the growth of treated parent cells by 4.49−21.25% compared with that of the control ($P<0.05$). It has been reported that the cell membrane acts as a selective barrier that restricts uptake of molecules into the cells and excretion of wastes out of cells [25]. Electric fields induce an electric potential difference across the cellular membrane [13], causing formation of pores on the cellular membrane. These not only increase the permeability to molecules and ions [23], but also the uptake of nutrients

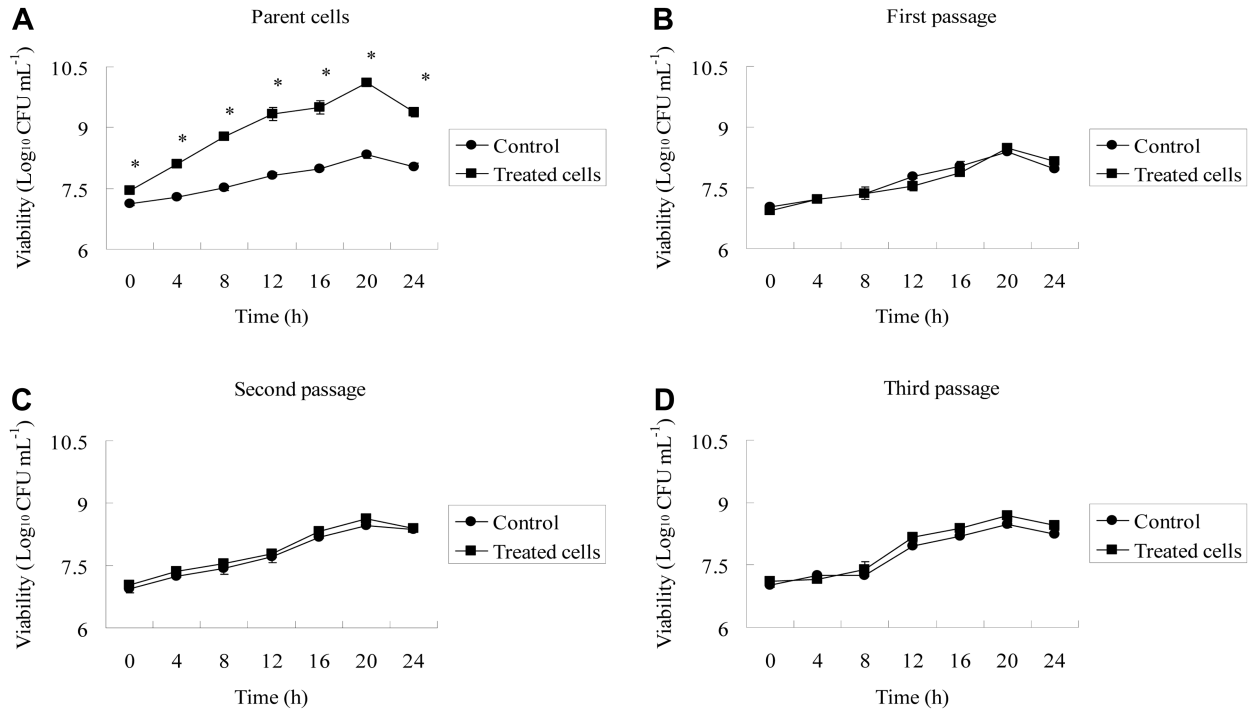

Fig. 1. Growth of control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.

Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *P<0.05 *via* independent T-test comparing between control and treated cells.

from the growth medium and excretion of wastes from the cell [13] that could subsequently increase cellular growth.

It has also been reported that pulsed electric fields are highly dependent on the type of microorganism and cell shape. Gram-positive bacteria have been found to be more resistant compared with Gram-negative bacteria when treated at intense electric field strength. Additionally, rod-shaped cells have also been reported to be inactivated only at a higher electric field strength that is more than five times of that used to inactivate the spherical cells in the same characteristic dimension. This could be due to Gram-positive bacteria having a thicker and more rigid cell membrane/wall that are resistant towards lysis by higher electric field strength [9]. Noci *et al.* [19] reported only a reduction of 1.1 log cycles in viability of *Listeria innocua* when cells were treated at a higher electric field of 30 kV/cm. Additionally, our data also showed that the growth of parent cells was increased ($P<0.05$) immediately upon treatment (0 h). Lactobacilli cells have been found to possess an aggregation-promoting factor, which contributed to the formation of cellular aggregates and chains [7]. Meanwhile, electroporation has been reported to declump aggregates [2]. Therefore, we postulate that the increased growth of lactobacilli cells immediately upon treatment may be attributed to the fragmentation of clumped cells.

However, the growth of the first (Fig. 1B), second (Fig. 1C), and third (Fig. 1D) passages of treated cells was comparable to that of the control, suggesting the resealing of temporary pores and recovery of cells from injuries. Ulmer *et al.* [24] also reported that cells were inactivated only at an electric field strength above 13 kV/cm, and below this field strength, the cell damage was reversible. Thus, treated parent cells were only subjected to sublethal effect at 7.5 kV/cm instead of serious injury and/or damage. This allows electropermeabilization to be completely reversible and cells remain viable and regain their ability to metabolize substrates and activities [21].

Removal of Cholesterol

An increase in viability of electroporated cells was closely related to their ability to remove cholesterol from the medium. The removal of cholesterol by *L. acidophilus* BT 1088 increased significantly ($P<0.05$) upon treatment (Fig. 2A). The parent cells removed 185.40–206.93% more cholesterol compared with that of the control ($P<0.05$). This result was in tandem with the growth of treated parent cells. During the applied electric field, a mechanical shock may be produced that could disrupt the structure of the cellular membrane at a local area [4]. It has been hypothesized that pore initiation may occur in the lipid domains and/or at protein channels. This may subsequently denature or modify the proteins [1], which could provide a path for the transmembrane transport of molecules in both directions [23]. Thus, this phenomenon may facilitate and enhance

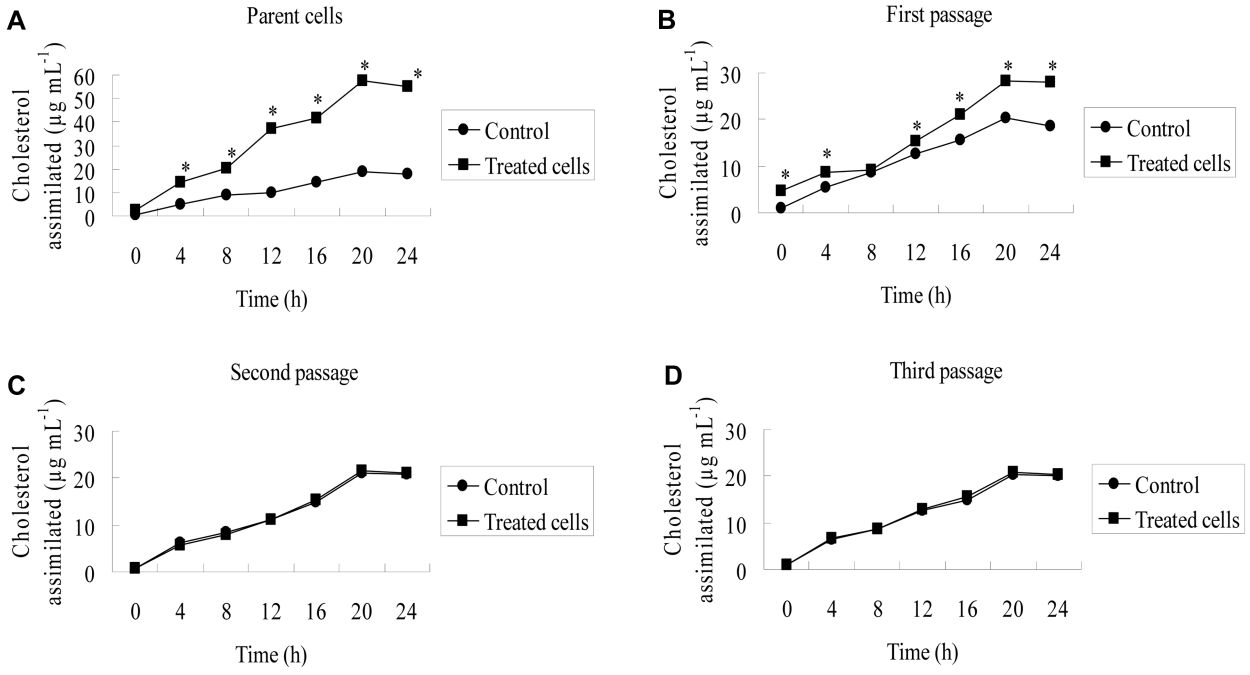

Fig. 2. Removal of cholesterol by control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.

Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *$P<0.05$ *via* independent T-test comparing between control and treated cells.

the uptake of molecules that subsequently increase the removal of cholesterol by electroporated cells. We had also previously reported the increase in membrane permeability upon electroporation for *L. acidophilus* BT 1088 treated at 7 kV/cm for 3.5 ms [15].

Such a trait was also inherited by the first passage of treated cells, where there was an increase in cholesterol removal exceeding 21.72% compared with that of the control ($P<0.05$; Fig. 2B). Cell membrane thickness has been found to be reduced upon electroporation owing to pores formation [24]. The resealing time of smaller electropores could be longer than larger pores, which may require recovery time from minutes to hours [4]. Hofmann *et al.* [11] also reported that electroporated cells require time (minutes up to hours) to recover to their original plasma membrane. In addition, Mehier-Humbert *et al.* [18] also reported that the opening of pores could last for almost 24 h. Thus, we believe that the first passage of electroporated cells may have experienced a delay in resealing of pores and thus maintained the membrane permeability needed for uptake and incorporation of impermeable small and large molecules such as cholesterol into the cellular membrane [11]. However, in the subsequent second (Fig. 2C) and third (Fig. 2D) passages of treated cells, the amounts of cholesterol removed were similar to that of the control, indicating possible complete resealing of pores.

Incorporation of Cholesterol into Cellular Membrane

The ratio of C:P in the cellular membrane of *L. acidophilus* BT 1088 was significantly increased ($P<0.05$) upon electroporation (Fig. 3A). In the parent cells, an increase exceeding 108.33% in the ratio of membrane C:P was observed compared with that of the control. In our previous study, we have reported that the incorporation of cholesterol into the cellular membrane upon electroporation led to an increased ratio of membrane C:P [15]. It has also been reported that diffusion of lipid molecules into the disrupted membrane regions is needed as a membrane generation mechanism of the cell to reseal pores formed [4]. On the other hand, we had also previously found that the membrane permeability of electroporated cells was due to lipid peroxidation [15]. A decrease in membrane phospholipids due to lipid peroxidation could also result in an increased ratio of cellular C:P.

In addition, electroporated cells of the first passage also showed a higher membrane C:P (by 26.67–75.00%) compared with that of the control ($P<0.05$; Fig. 3B). This result was in tandem with cholesterol removal by treated cells of the first passage. We believe that cholesterol continued to be incorporated into the membrane of the first passage of treated cells, as a result of unsealed pores, which subsequently increased the ratio of membrane C:P. However, this effect was not observed in the second (Fig. 3C) and

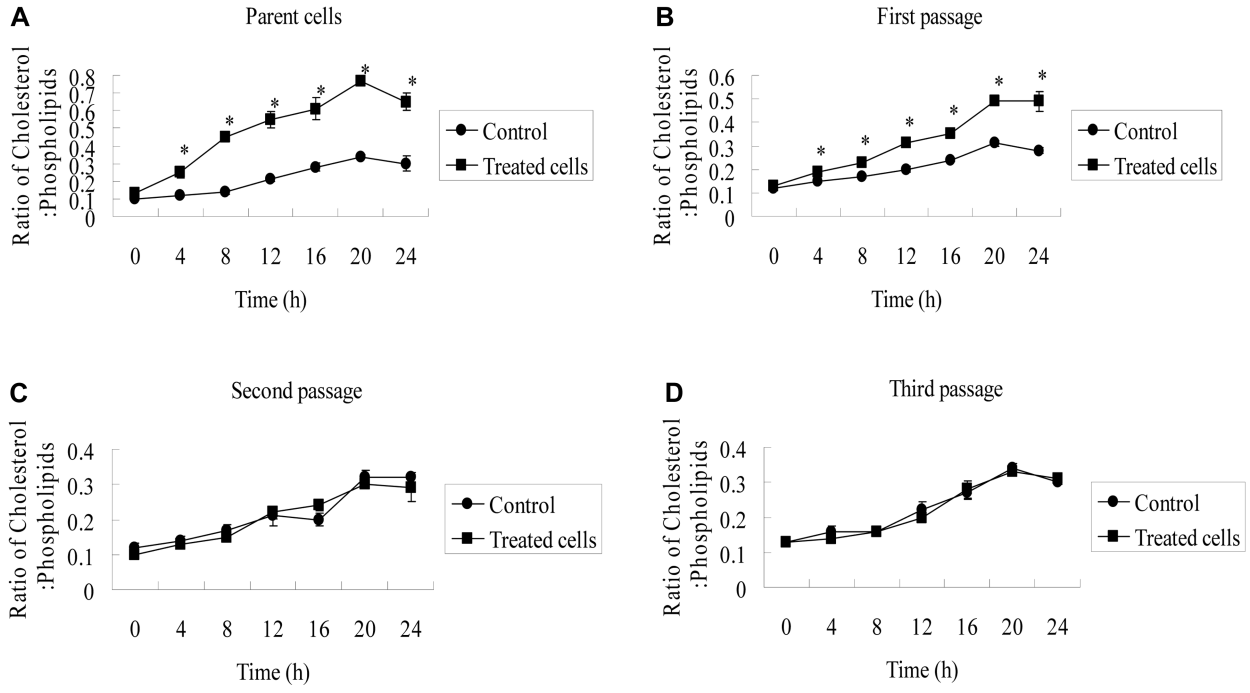

Fig. 3. Ratio of cholesterol to phospholipids of control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.

Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *P<0.05 *via* independent T-test comparing between control and treated cells.

third (Fig. 3D) passages of treated cells, indicating resealing of pores and insignificant incorporation of cholesterol.

We have also previously found that the cholesterol removed by treated cells could be incorporated in different regions in the lipid membrane bilayer [15]. In order to evaluate the possible location of such enrichment in the parent cells and their subsequent passages, different fluorescent probes were used to detect their fluorescence anisotropy (FAn). ANS is an anionic fluorescence probe that binds to the interface of polar and apolar regions of phospholipids. DPH is a hydrophobic fluorophore mainly distributed in the apolar region of membrane phospholipids bilayer, and TMA-DPH is an amphiphilic probe with a charged TMA group that binds to the polar head whereas DPH binds to the apolar region of the phospholipids [15].

There was an increase ($P<0.05$) in the membrane FAn of ANS (Fig. 4A), DPH (Fig. 5A), and TMA-DPH (Fig. 6A) in electroporated parent cells, with an increase of 79.17%, 140.00%, and 147.04%, respectively, compared with that of the control ($P<0.05$). This may be due to the changes of membrane dielectric properties upon electroporation that enhanced membrane permeability to molecules such as cholesterol from the medium into the cells and incorporated into different regions of the membrane bilayer [23]. In addition, the applied electric field could force the charged group of the cellular membrane such as the phosphate head groups of lipid molecules and ions absorbed into the membrane to move to the direction of the electric field [4]. This may subsequently increase the incorporation of cholesterol into the cellular membrane, as the cholesterol molecules could bind to the phosphate head groups of the lipid molecules *via* hydrogen bonds [27]. On the other hand, an increase in the surface hydrophobicity has been reported when membrane fusion occurs [22]. This could also result in increased incorporation of cholesterol into different regions of the membrane phospholipids bilayer.

A significant increase ($P<0.05$) in the FAn of DPH (Fig. 5B) and TMA-DPH (Fig. 6B) for electroporated cells of the first passage was also observed upon early fermentation up to the later stage of fermentation (0–24 h), whereas FAn of ANS increased during the first 4 h only. This result was in tandem with the ratio of C:P in the cellular membrane of treated cells of the first passage. During electroporation, local heating could be generated and this may lead to denaturation of protein and enzyme. This condition could affect the transport function of cells and impair cell survivability. However, such reversible damages can be repaired by the cells over a period of time [24]. We believe that electroporated parent cells may not have fully recovered from the injury within 24 h, and some membrane damages such as small pores, disrupted protein channels, and lack of phospholipids tails due to lipid peroxidation may be inherited by the treated cells of the first passage. Thus, the incorporation of cholesterol into the pores and

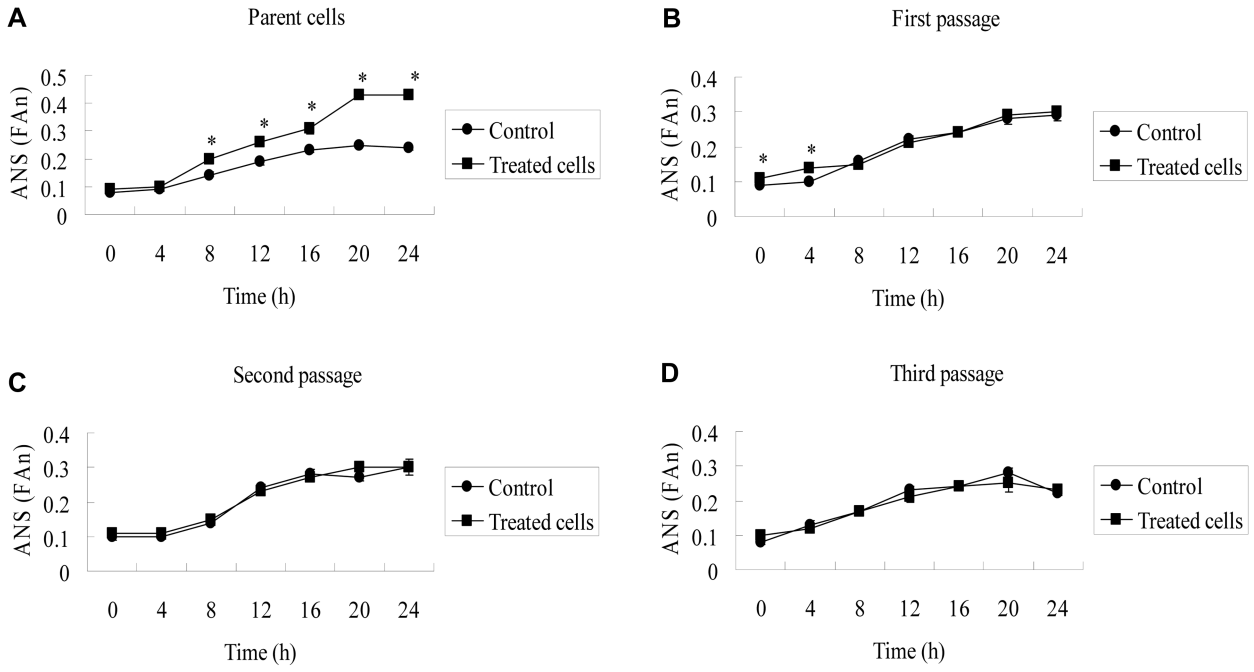

Fig. 4. Fluorescence anisotropy (ANS) of control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.
Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *$P<0.05$ *via* independent T-test comparing between control and treated cells.

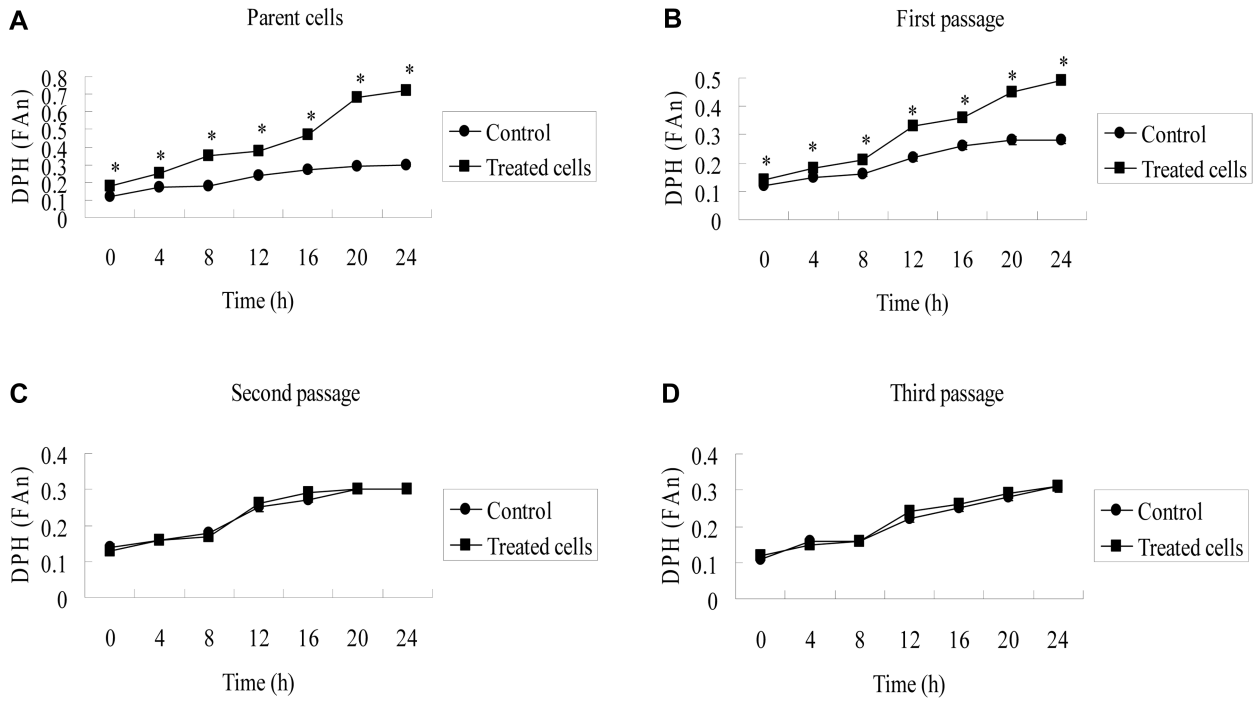

Fig. 5. Fluorescence anisotropy (DPH) of control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.
Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *P<0.05 *via* independent T-test comparing between control and treated cells.

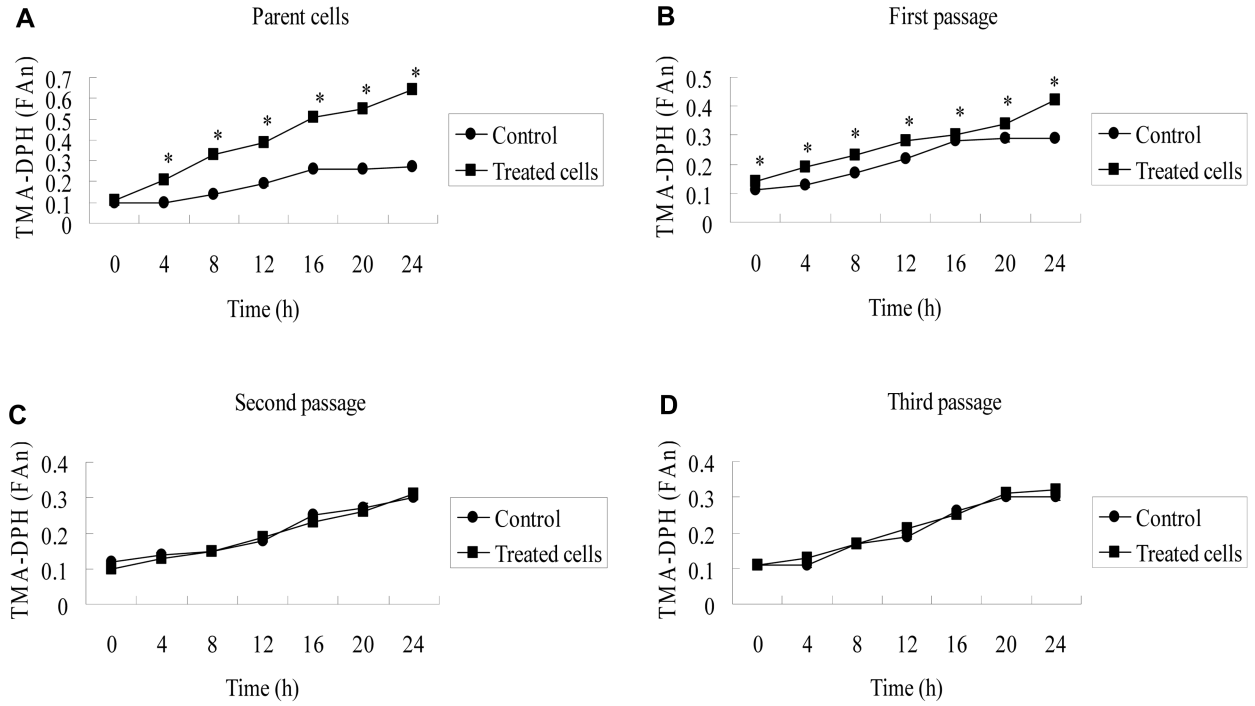

Fig. 6. Fluorescence anisotropy (TMA-DPH) of control and electroporated cells after fermentation at 37°C over 24 h for (**A**) parent cells, (**B**) first passage, (**C**) second passage, and (**D**) third passage.
Control: untreated cells; each subsequent passage corresponds to individual controls. Error bars represent standard error of means; n = 3. *P<0.05 *via* independent T-test comparing between control and treated cells.

damaged part of the membrane could occur as a membrane recovery process in the first passage of treated cells [4]. Therefore, an increase in FAn of DPH and TMA-DHP for treated cells of first passage was observed upon 0–24 h of fermentation.

Nevertheless, a higher increase in FAn of ANS was only observed upon fermentation for 4 h (Fig. 4B). It has been reported that the disrupted protein channels could reside on the membrane surface owing to conformational unfolding of proteins resulting in neutralization of charges [12]. Considering that ANS is a negative charge probe, the neutral charge (zero charge) in the interface of polar and apolar regions might not be favorable for the binding of ANS. Thus, binding of ANS occurred only upon early fermentation. However, the membrane FAn values of ANS (Fig. 4C and 4D), DPH (Fig. 5C and 5D), and TMA-DPH (Fig. 6C and 6D) for treated cells of the second and third passages were comparable to that of the control. This may be due to the complete resealing of pores that did not allow binding of cholesterol into the cellular membrane of treated cells of the second and third passages.

Adhesion to HT-29 Cells

It is important for lactobacilli cells to be able to adhere to intestinal cells and mucus, for better survival within the gastrointestinal tract prior to colonization and exertion of health benefits to the hosts [5]. A lower ($P<0.05$) intestinal adherence ability was observed for electroporated *L. acidophilus* BT 1088 cells, with a decrease of 1.45% ($P<0.05$) compared with that of the control (Fig. 7). The cell wall of Gram-positive bacteria comprises several structures such as a multilayered peptidoglycan sacculus decorated with proteins, teichoic acids, and polysaccharides, whereas some species may be surrounded by an outer shell of proteins packed in a paracrystalline (S-layer). This S-layer contains proteins (S-layer proteins) that mediate the interactions of cells with the environment [14]. Local heating generated upon electroporation near the cellular membrane could lead to the denaturation/modification of S-layer proteins that may subsequently reduce the attachment of cells to HT-29 [1]. However, the intestinal adherence ability of subsequent passages of treated cells was comparable to that of the control (Fig. 7). This may due to the production of new proteins *via* a repair system to replace the damaged proteins. We believe this has led to the improved intestinal adherence ability of the subsequent first, second, and third passages of treated cells.

In conclusion, electroporation increased the growth of *L. acidophilus* BT 1088, removal of cholesterol by *L. acidophilus* BT 1088, and the ratio of membrane C:P *via* incorporation of cholesterol into the cellular membrane. This may be due to the increased membrane permeability that subsequently increased assimilation of cholesterol and incorporation of cholesterol into the cellular membrane. Meanwhile, the saturation of cholesterol was found in different regions of the membrane bilayer including the hydrophilic heads, hydrophobic tails, upper regions of phospholipids, and the interface of apolar and polar regions of membrane phospholipids. Such traits were also observed for electroporated cells of the first passage, and disappeared in the subsequent second and third passages. Additionally, intestinal adherence of *L. acidophilus* BT 1088 to epithelial cells was also affected by electroporation, which showed a decrease in adherence ability upon treatment for parent cells. However, the intestinal adherence ability improved for the subsequent first, second, and third passages of treated cells. Data from our present study show that electroporation could be used to produce one passage of *L. acidophilus* BT 1088 with enhanced cholesterol removal ability.

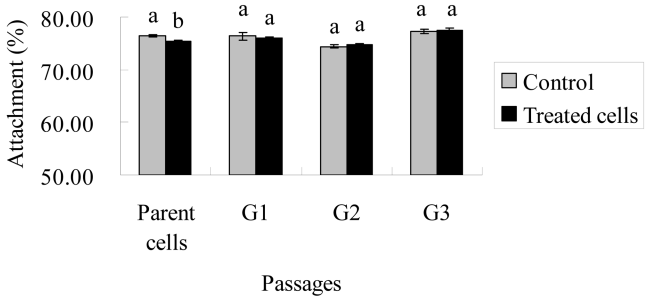

Fig. 7. Adhesion of control and electroporated lactobacilli cells to HT-29 after fermentation at 37°C at 24 h for parent cells and three subsequent passages.

[ab]Attachment with different lowercase superscripts are significantly different ($P<0.05$) within parent cells and subsequent passages. Control: untreated cells; each subsequent passage corresponds to individual controls. G, First passage; G2, Second passage; G3, Third passage. Error bars represent standard error of means; n = 3. * $P<0.05$ *via* independent T-test comparing between control and treated cells.

Acknowledgments

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References

1. Angersbach, A., V. Heinz, and D. Knorr. 2000. Effects of pulsed electric fields on cell membranes in real food systems. *Innovat. Food Sci. Emerg. Tech.* **1:** 135–149.
2. Bringel, F. and J. C. Hubert. 1990. Optimized transformation by electroporation of *Lactobacillus plantarum* strains with plasmid vectors. *Appl. Microbiol. Biotechnol.* **33:** 664–670.
3. Cammarota, M., M. De Rosa, A. Stellavato, M. Lamberti, I. Marzaioli, and M. Giuliano. 2009. *In vitro* evaluation of

Lactobacillus plantarum DSMZ 12028 as a probiotic: Emphasis on innate immunity. *Int. J. Food Microbiol.* **135:** 90–98.
4. Chang, D. C. 1989. Cell poration and cell fusion using an oscillating electric field. *Biophys. J.* **56:** 641–652.
5. Coconnier, M. H., T. R. Klaenhammer, S. Kerneis, M. F. Bernet, and S. L. Servin. 1992. Protein-mediated adhesion of *Lactobacillus acidophilus* BG2FO4 on human enterocyte and muscus-secreting cell lines in culture. *Appl. Environ. Microbiol.* **58:** 2034–2039.
6. du Toit, M., M. A. P. Franz, L. M. T. Dicks, U. Schillinger, P. Haberer, B. Warlies, *et al*. 1998. Characterization and selection of probiotic lactobacilli for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *Int. J. Food Microbiol.* **40:** 93–104.
7. Ewe, J. A., W. N. Wan Abdullah, R. Bhat, A. A. Karim, and M. T. Liong. 2012. Enhanced growth of lactobacilli and bioconversion of isoflavones in biotin-supplemented soymilk upon ultrasound-treatment. *Ultrason. Sonochem.* **19:** 160–173.
8. FAO/WHO. 2001. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Joint Food and Agricultural Organization of the United Nations and World Health Organization Expert Consultation Report, Córdoba, Argentina. Available from: http://www.who.int/foodsafety/publications/fs_management/probiotics/en/index.html. [Accessed on 7th August 2011].
9. Garcia, D., N. Gomez, J. Raso, and R. Pagan. 2005. Bacterial resistance after pulsed electric fields depending on the treatment medium pH. *Innovat. Food Sci. Emerg. Tech.* **6:** 388–395.
10. Golzio, M., J. Teissie, and M. P. Rols. 2000. Control by membrane order of voltage induced permeabilization, loading and gene transfer in mammalian cells. *Bioelectrochemistry* **53:** 25–34.
11. Hofmann, F., H. Ohnimus, C. Scheller, W. Strupp, U. Zimmermann, and C. Jassoy. 1999. Electric field pulses can induce apoptosis. *J. Membrane Biol.* **169:** 103–109.
12. Kalnina, I., T. Zvagula, N. Gabruseva, J. Kirilova, N. Kurjane, R. Bruvere, *et al*. 2007. Structural changes in lymphocytes membrane of Chernobyl clean-up workers from Latvia. *J. Fluoresc.* **17:** 633–638.
13. Kanduser, M., M. Sentjurc, and D. Miklavcic. 2006. Cell membrane fluidity related to electroporation and resealing. *Eur. Biophys. J.* **35:** 196–204.
14. Lebeer, S., J. Vanderleyden, and S. C. J. De Keersmaecker. 2008. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol. Mol. Biol. Rev.* **72:** 728–764.
15. Lye, H. S., A. A. Karim, G. Rusul, and M. T. Liong. 2011. Electroporation enhances the ability of lactobacilli to remove cholesterol. *J. Dairy Sci.* **94:** 4820–4830.
16. Lye, H. S., G. Rusul, and M. T. Liong. 2010. Mechanisms of cholesterol removal by lactobacilli under conditions that mimic the human gastrointestinal tract. *Int. Dairy J.* **20:** 169–175.
17. Lye, H. S., G. Rusul, and M. T. Liong. 2010. Removal of cholesterol by lactobacilli *via* incorporation and conversion to coprostanol. *J. Dairy Sci.* **93:** 1383–1392.
18. Mehier-Humbert, S., T. Bettinger, F. Yan, and R. H. Guy. 2005. Ultrasound-mediated gene delivery: Kinetics of plasmid internalization and gene expression. *J. Control. Release* **104:** 203–211.
19. Noci, F., M. Walkling-Ribeiro, D. A. Cronin, D. J. Morgan, and J. G. Lyng. 2009. Effect of thermosonication, pulsed electric field and their combination on inactivation of *Listeria innocua* in milk. *Int. Dairy J.* **19:** 30–35.
20. Ooi, L. G. and M. T. Liong. 2010. Cholesterol lowering effects of probiotics and prebiotics: A review of *in vivo* and *in vitro* findings. *Int. J. Mol. Sci.* **11:** 2499–2522.
21. Saulis, G., S. Satkauskas, and R. Praneviciute. 2007. Determination of cell electroporation from the release of intracellular potassium ions. *Anal. Biochem.* **360:** 273–281.
22. Teissie, J. and C. Ramos. 1998. Correlation between electric field pulse induced long-lived permeabilization and fusogenicity in cell membranes. *Biophys. J.* **74:** 1889–1898.
23. Tryfona, T. and M. T. Bustard. 2008. Impact of pulsed electric fields on *Corynebacterium glutamicum* cell membrane permeabilization. *J. Biosci. Bioeng.* **105:** 375–382.
24. Ulmer, H. M., V. Heinz, M. G. Ganzle, D. Knorr, and R. F. Vogel. 2002. Effects of pulsed electric fields on inactivation and metabolic activity of *Lactobacillus plantarum* in model beer. *J. Appl. Microbiol.* **93:** 326–335.
25. Wasungu, L., J. M. Escoffre, A. Valette, J. Teissie, and M. P. Rols. 2009. A 3D *in vitro* spheroid model as a way to study the mechanisms of electroporation. *Int. J. Pharm.* **379:** 278–284.
26. Xie, N., Y. Cui, Y. N. Yin, X. Zhao, J. W. Yang, Z. G. Wang, *et al*. 2011. Effects of two *Lactobacillus* strains on lipid metabolism and intestinal microflora in rats fed a high cholesterol diet. *BMC Complement. Altern. Med.* **11:** 53.
27. Ziarno, M. 2007. The influence of cholesterol and biomass concentration on the uptake of cholesterol by *Lactobacillus* from MRS broth. *Acta Sci. Pol. Technol. Aliment.* **6:** 29–40.