The Influence of Mild Pulsed Electric Field (PEF) on Acid Tolerance, Bile Tolerance, Growth and Protease Activity of the Dairy Culture Bacteria *Lactobacillus acidophilus* LA-K.

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Summary

The objective of this study was to determine the influence of mild PEF conditions on acid tolerance, growth, bile tolerance and protease activity of *L. acidophilus LA-K*. The treatments were positive square unipolar pulse width of 3 µs, pulse period of 0.5 second, voltage of 1 kV/cm, delay time of 20 µs and flow rate of 60 ml/min at 40.5°C PEF treatment temperature. The control was passed through the PEF system at the same flow rate (60 ml/min) without receiving any pulsed electric field condition. The acid tolerance was determined every 30 minutes for 120 minutes of incubation in acidified MRS broth at pH 2. Growth of the culture was determined hourly for 32 hours of incubation at 37°C in MRS broth. The bile tolerance was determined hourly for 16 hours of incubation in MRS-Thio broth supplemented with 0.3% (w/v) Oxgall (bovine bile) and 0.2% sodium thioglycolate. Samples were plated in duplicates using Lactobacilli MRS agar. The petriplates were incubated anaerobically at 37°C for 48 hours. Protease activity was determined by o-phthaldialdehyde (OPA) spectrophotometric assay at 0, 12, 24, 36 and 48 hours of incubation of inoculated skim milk at 40°C three replications were conducted. The experimental design was repeated measurements on complete randomized block, replications were the blocks. *Lactobacillus acidophilus* subjected to mild PEF conditions as well as the control were acid tolerant until the end of the 120 minutes of incubation but there was a significant ($P<0.0001$) decrease in the viable bacterial counts after each incubation time of 30 minutes. Mild PEF conditions studied significantly improved acid tolerance of the bacterium. The mild PEF treated culture reached the logarithmic phase of the growth an hour earlier than the control. *Lactobacillus acidophilus* LA-K exhibited tolerance to the bile conditions and exhibited similar growth patterns in the presence or absence of bile acids but the bacterium reached the stationary phase after 12 hours of incubation in bile conditions compared to 16 hours of incubation in the absence of bile conditions. Mild Pulsed Electric Field conditions had insignificant effect on bile tolerance of *L. acidophilus* LA-K. Mild Pulsed Electric Field treatment significantly ($P<0.0001$) enhanced the protease activity of the bacterium compared to the control.

Keywords: pulsed, electric field, *Lactobacilus acidophilus*, protease, bile, tolerance.

Introduction

High-intensity pulsed electric field (HIPEF) has been proven to inactivate microorganisms and have the potential to replace thermal processing for liquid foods (1) with minimal impact on nutritional and organoleptic qualities (2). Mild electric field intensity conditions form reversible pores in the cellular membrane whereas, drastic electric field intensity conditions lead to cellular death due to the irreversibility (3). It is well known that most if not all microorganisms are practically unaffected by electric fields of less than about 4-8 kV/cm (4). It was reported that electrical treatments could affect cell physiology (5). Other researchers (6) have shown that the presence of a moderate electric field (MEF) (1 V/cm) had interesting effects on biological materials. In addition to that (7) concluded that MEF accelerates growth in the early stage, but inhibits growth at the late stage of the fermentation of *Lactobacillus acidophilus*. The application of MEF (1V/cm) at frequency of 60 Hz has been shown to alter the metabolic activity of the microbial cells (8). *L.*

acidophilus is a probiotic bacterium widely used as an adjunct culture in approximately 80% of the yogurts in USA (9). Several studies reported yogurt cultures and *Lactobacillus acidophilus* as probiotics (10, 11, 12 and 13). To provide health benefits, probiotics must overcome physical and chemical barriers such as acid and bile in the gastrointestinal tract (14). Cellular stress begins in the stomach, which has a pH as low as 1.5-2 (15).

Once the cells have survived these hurdles, they can colonize and grow to enough numbers to produce the beneficial effect to the host. The time from entrance to release from the stomach was reported to be 90 minutes (16). *L. acidophilus* is a probiotic bacterium with several health benefits, including: Enhancement of the immune system in the immuno compromised people (17), reduction of various types of diarrhea in human (18), replace various pathogenic organisms in the intestine such as *S. aureus* and *E. coli* leading to a healthy microbiological balance (19), reduce serum cholesterol levels in human (18), improve symptoms of lactose intolerance (20), balancing of intestinal microflora (18), reduce the incidence rate of colon cancer (20), has anti-hyperglycemic and anti-hypertensive effects (11), treatment of urogenital infections and vaginitis in women (21) and alleviation of Crohn's disease (18).

Materials and Methods

The control and Pulsed Electric Field (PEF) treated samples were prepared by inoculating 10 ml of freshly thawed pure frozen concentrated culture of *Lactobacillus acidophilus* LA-K (Chr. Hansen's Laboratory, WI, USA) into 990 ml of sterile 0.1% peptone water that make it 1% (v/v) and treated in a pilot plant PEF system (OSU-4M). The mild PEF treatment conditions were positive square unipolar pulse widths of 3 µs, pulse periods of 0.5 sec., voltage of 1kV/cm, the delay time of 20 µs, the flow rate of 60 ml/min with 40.5^{0}C PEF treatment temperature. The control was passed through the PEF equipment at 60ml/min without receiving any pulsed electric field treatment. Prior to testing the PEF equipment was cleaned and sanitized with 5% Sodium hypochlorite solution and then rinsed thoroughly with sterile distilled water . The control and the PEF treated samples were tested for acid tolerance, growth, bile tolerance and protease activity. Three replications were conducted. The experimental design was repeated measurements on complete randomized block, Replications were the blocks. Data were analyzed using Proc Mixed model of Statistical Analysis System (SAS).

The acid tolerance of the *Lactobacillus acidophilus* LA-K was determined by the method proposed by Pereira and Gibson, (22) with slight modifications. The control and PEF treated samples were inoculated 10% (v/v) in acidified MRS broth previously adjusted to pH 2 using 1N HCl. The inoculated acidified MRS broth were incubated at 37°C and plating for every 30 minutes up to 120 minutes. Growth was determined by the method proposed by Lin and Young, (23) with slight modifications. Control and PEF treated samples were inoculated 10% (v/v) separately into MRS broth. Growth was determined hourly for 32 hours of incubation at 37°C. The bile tolerance was determined according to method proposed by Pereira and Gibson, (22) with slight modifications. The bile tolerance of the culture was analyzed in MRS-THIO broth supplemented with 0.3% (w/v) Oxgall (bovine bile) and 0.2 % (w/v) sodium thioglycolate. Control and PEF treated samples were inoculated 10% (v/v) separately in MRS-THIO broth and incubated at 37°C for 16 hours. 1 ml of the inoculated broth was serially diluted in peptone water (0.1% w/v) and plated in duplicates using using Lactobacilli MRS agar. The petriplates were incubated anaerobically at 37°C for 48 hours befor enumeration. The protease activity of the culture was determined by o-phthaldialdehyde (OPA) spectrophotometric method (24) with slight modification. The control and the PEF treated samples were inoculated 10% (v/v) separately into sterile skim milk and incubated at 40°C for 0, 12, 24, 36 and 48 hours.

Results and Discussion

The viability of the bacterium subjected to positive square unipolar pulse width of 3 µs for pulse period of 0.5 sec. using voltage of 1 kV/cm at 40.5°C PEF treatment temperature when incubated in acid condition (pH 2) over the five time points of 0, 30, 60, 90 and 120 minutes are shown in (Figure,1).

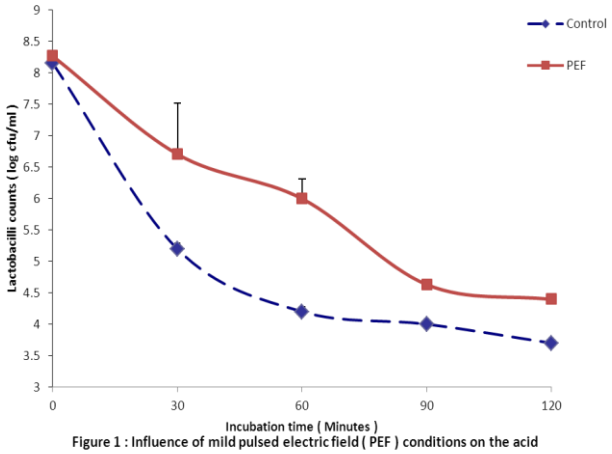


Figure 1 : Influence of mild pulsed electric field (PEF) conditions on the acid tolerance of *Lactobacillus acidophilus LA-K* .

Culture subjected to mild PEF conditions as well as the control were acid tolerant until the end of the 120 minutes of incubation. The time effect was significant (table,1). There was a significant (P<0.0001) decrease in the viable counts after each incubation time of 30 minutes. The PEF treatment effect was also significant (P<0.0001) (Table 1).

Table, 1: Mean square (MS) and Pr > F of mild pulsed electric field, minute and their interaction for acid tolerance of *Lactobacillus acidophilus* LA-K.

Source	Acid tolerance	
	MS	Pr > F
Pulsed Electric Field (PEF)	**6.8190**	**< 0.0001**
Minute	**16.8445**	**< 0.0001**
Pulsed Electric Field (PEF)* Minute	**0.7131**	**0.0002**
Error	**0.0778**	

From minutes 30 to 120 the acid tolerance of *Lactobacillus acidophilus* LA-K subjected to mild PEF conditions was significantly (P<0.001) higher than the control (Table,2).

Table, 2: Least square means for acid tolerance of *Lactobacillus acidophilus* LA-K as influenced by mild pulsed electric field (PEF).

Treatment	Acid tolerance LS Mean
Pulsed Electric Field (PEF)	**A 6.0039**
Control	**B 5.0503**

LS Means with different letters are significantly different (P<0.05).

Lactobacillus acidophilus was more acid resistant at pH 1.2 and 2.5 for 30 minutes, compared to *Bifidobacterium bifidum* which did not tolerate the acid conditions after 5 minutes (25). On the other hand, else researchers (26) observed that *Lactobacillus acidophilus* exhibited more acid tolerance than *L. casei.* at pH 2. Mild electrical stimulation treatment that alters the synthesis of molecular chaperones may affect bacterial resistance to acid stress. Exposure to acid stress induced de novo synthesis of several heat shock-like proteins by *Lactobacillus paracasei* F19 and 50:1 *and Lactobacillus plantarum* 2592, F5 and F26 strains which cross-reacted with stress proteins, this may protect other surface proteins and adhesions during transport through the gastrointestinal tract.

The growth of the bacterium expressed as log cfu/ml that subjected to positive square unipolar pulse width of 3 µs for pulse period of 0.5 sec. and voltage of 1kV/cm at 40.5°C PEF treatment temperature over the growth periods of 32 hours is shown in figure,2. The mild PEF treatment had a significant (P<0.0001) influence on the growth of *Lactobacillus acidophilus* LA-K (table, 3). The mild PEF treated culture reached the logarithmic phase of the growth an hour earlier than the control.

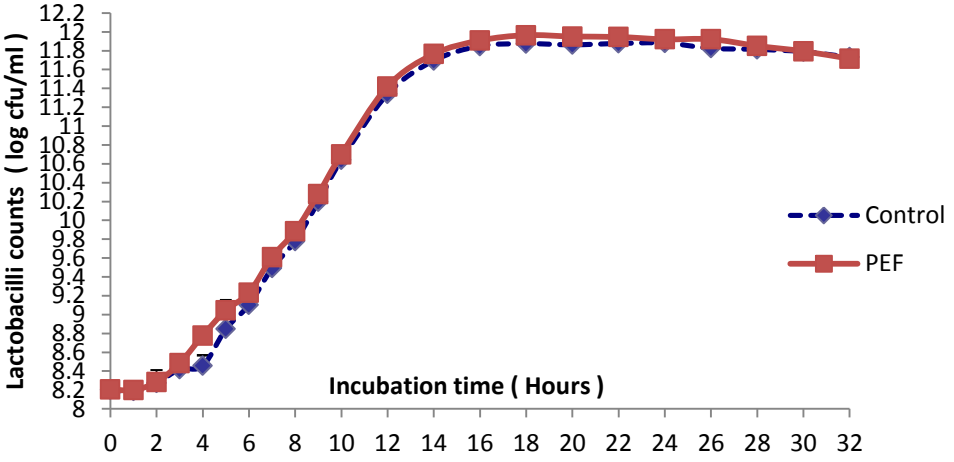
Growth of *Lactobacillus acidophilus* LA-K when subjected to the mild PEF treatment was significantly higher than the control (table,4). The exponential phase of the growth curve of the control was between hours 4 and 16 while that of the bacterium subjected to mild PEF treatment was between hours 3 and 16 (Figure,3). The stationary phase of the growth curve of the bacterium subjected to mild PEF treatment was between hours 16 and 26 while that of the control was between hours 16 and 24.

The mild PEF conditions significantly (P<0.05) enhanced the growth of *Lactobacillus acidophilus* LA-K. Liong and Shah (26) studied cholesterol removal ability of Lactobacilli strains and also observed growth of the bacterium *Lactobacillus acidophilus* to be predominant in the first 9-15 hours after which it reached a stationary phase. Electrical treatment could affect cell physiology (5). The application of moderate electric field (MEF) (1 V/cm) produced a shorter lag phase than conventional (control) fermentation of *Lactobacillus acidophilus* OSU at the same temperature (27). However, other studies under controlled temperature conditions have shown that MEF accelerate growth in the early stage, but inhibits growth at the late stage of fermentation of *Lactobacillus acidophilus* (7).

The bile tolerance expressed as log cfu/ml for the bacterium subjected to positive square unipolar pulse width of 3 µs for pulse period of 0.5 sec. and voltage of 1kV/cm at 40.5°C PEF treatment temperature over the bile tolerance periods of 16 hours is shown in Figure,3. The mild PEF treatment had non-significant (p=0.7458) effect on bile tolerance of the bacterium (table,3). *Lactobacillus acidophilus* LA-K exhibited good tolerance to the bile conditions with a significant (P<0.0001) increase in the viable bacterial counts (log cfu/ml) in both the control and the mild PEF treated cultures during the 16 hours of incubation in bile conditions (figure,3). The mild PEF treated cultures did not have significantly (P>0.05) different bile tolerance compared to the control (table,4). In the present study, we found that *Lactobacillus acidophilus* LA-K exhibited similar growth patterns in the presence or absence of bile acids (figures, 2 and 3) respectively, but the bacterium reached the stationary phase after 12 hours of incubation in bile conditions (figure,3) whereas, the bacterium reached the stationary phase after 16 hours of incubation in the absence of bile conditions (figure,2). Similar findings were reported by workers (26) studied the bile tolerance of different strains of Lactobacillus species and found that *Lactobacillus acidophilus* exhibited similar growth pattern in the presence or absence of bile acid (Oxgall). Shah and Jelen (28) attributed increased bile tolerance of *Lactobacillus acidophilus* to its rigid cell wall. Other researchers (29) strongly support the hypothesis that microbial bile salt hydrolase (BSH) function in the detoxification of bile salts and in doing so, increase the intestinal survival and persistence of producing strains in the hostile environment of the gastrointestinal tract.

The Optical Density (OD) (Absorbance) values of the protease activity of the bacterium subjected to the positive square unipolar pulse width of 3 µs for pulse period of 0.5 sec. and voltage of 1kV/cm at 40.5°C PEF treatment temperature over the five time points of 0, 12, 24, 36 and 48 hours are shown in figure,4. The treatment effect was significant (table,3). The protease activity of the bacterium subjected to mild PEF treatment were significantly (P<0.05) higher than the control (table,4). The hour effect was significant (table,3). A significant (P<0.0001) increase in the protease activities were found between12, 24, 36 and 48 hours of incubation at 40°C in the control samples. Also there were a significant (P<0.0001) increase in the protease activities of the mild PEF treated culture between 0, 12, 24, 36 and 48 hours of incubation at 40°C.

Mild PEF treatment significantly (P<0.0001) enhanced the protease activity of Lactobacillus acidophilus LA-K (table,4). Lactobacillus acidophilus LA-K subjected to mild PEF conditions exhibited significantly (P<0.0001) the highest protease activity at 36 hours of incubation at 40°C compared to the control (figure,4). In general, PEF processing affects protease activity and an enhancement in the initial activity was detected (30). Researchers (31) suggested that the PEF could originate small conformational changes leading to enhanced proteolytic activity.



Figure, 2: The influence of mild pulsed electric field conditions on the growth characteristics of *Lactobacillus acidophilus LA-K.*

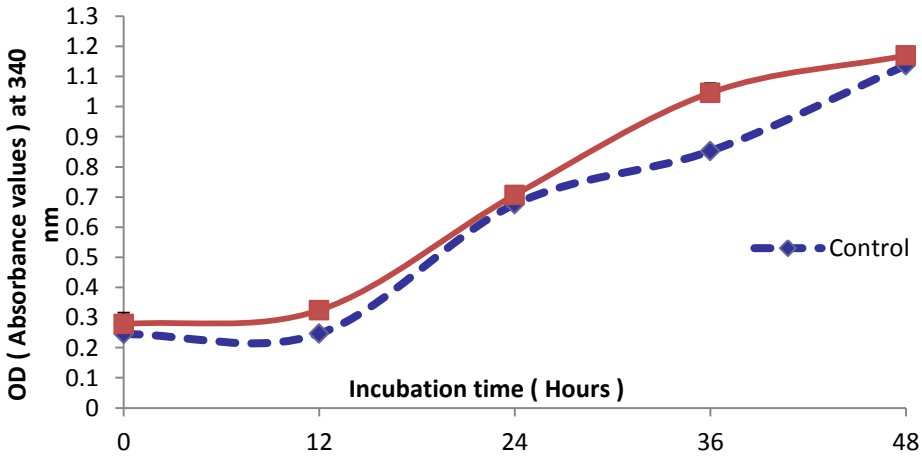
Table, 3: Mean square (MS) and Pr > F of mild PEF treatment, hour, and their interaction for growth, bile tolerance and protease activity of *Lactobacillus acidophilus* LA-K.

Source	Growth		Bile tolerance		Protease activity	
	MS	Pr > F	MS	Pr > F	MS	Pr > F
PEF	0.1558	<0.0001	0.0019	0.7458	0.0405	<0.0001
Hour	13.6507	<0.0001	10.2905	<0.0001	0.9395	<0.0001
PEF * hour	0.0035	0.1679	0.0039	0.9982	0.0073	<0.0001
Error	0.00258		0.0184		0.00027	

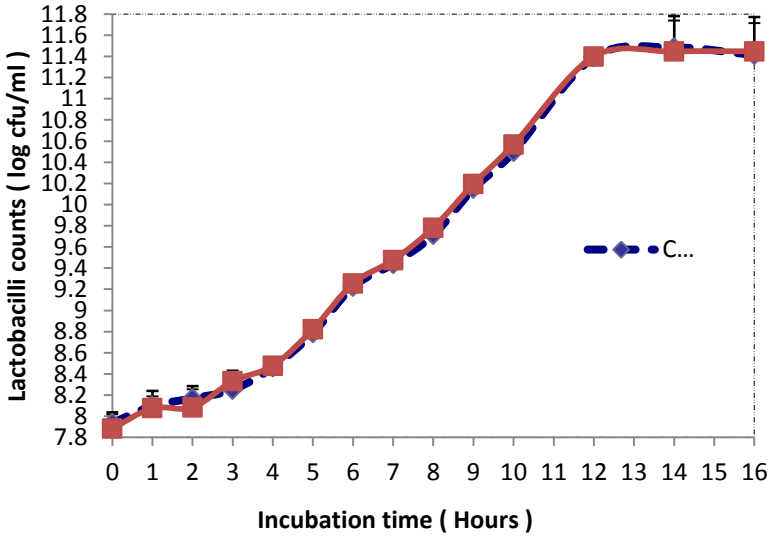
Table, 4: Least square means for growth, bile tolerance and protease activity of *Lactobacillus acidophilus* LA-K as influenced by mild pulsed electric field.

Treatment	Growth	Bile tolerance	Protease activity
	LSMean	LS Mean	LSMean
PEF	10.4891 A	9.5109 A	0.7048 A
Control	10.4204 B	9.5013 A	0.6313 B

LS Means with the different litters are significantly different (P<0.05).



Figure, 3: Influence of mild pulsed electric field conditions on the bile tolerance of *Lactobacillus acidophilus LA-K.*



Figure, 4: The influence of mild pulsed electric field conditions on the protease activity of *Lactobacillus acidophilus LA-K.*

References

1. Qin, B. L.; Pothakamury, U.R.; Vega, H.; Martin, O.; Barbosa-Canovas, G.V. and Swanson, B.G. (1995). Food pasteurization using high intensity pulsed electric fields. Food Technol., 49:55-60.
2. Sampedro, F.A.; Rodrigo, A.; Martinez, A. and Rodrigo, D. (2005). Quality and safety aspects of PEF application in milk and milk products. Crit. Rev. Food Sci. Nutr., 45:25-47.
3. Jayaram, S.H. (2000). Sterilization of liquid foods by pulsed electric fields. IEEE. Electr. Insul. Mag., 16:17-25.
4. Castro, A. J.; Barbosa-Canovas G.V. and Swanson, B.G. (1993). Microbial inactivation of foods by pulsed electric fields. J. Food Pro. Pres., 17:47-73.
5. Aronsson, K; Lindgren, M.; Johansson, B.R. and Ronner, U. (2001). Inactivation of microorganisms using pulsed electric fields: The influence of process parameters on *E. coli, L. innocua, L. mesentreroides and S. Cerevisiae*. Innonative Food Sci. and Emerging Techn., 2:41-54.
6. Wang, W.C. and Sastry, S. K. (1998). Study of electro-thermal effects on juice yield from cellular tissues. Paper presented at the IFT Annual Meeting, June 20-24. Atlanta, GA. USA.
7. Cho, H.Y.; Yousef, J.K. and Sastry, S. (1996). Growth kinetics of *Lactobacillus acidophilus* under ohmic heating. Biotechnol Bioeng., 49:334-340.
8. Loghavi, L.; Sastry, S.K. and Yousef, A.E. (2007). Effect of Moderate Electric Field on the metabolic activity and growth kinetics of *Lactobacillus acidophilus*. Biotechnol. Bioeng., 98:872-881.
9. Hutkins, R. (2006). Cultured dairy products. In: Microbiology and Technology of fermented Foods. Blackwell Publishing, Ames, Iowa, PP: 107–114.
10. Ahrens, K.E.; Ade, P.; Marten, B.; Weber, P.; Timm, W.; Ail, Y.; Glüer, C.C. and Schrezenmeir, J. (2007). Prebiotics, probiotics, and symbiotic affect mineral absorption, bone mineral content, and bone structure. J. Nutr., 137: 838-846.
11. Apostolidis, E.Y.; Kwon, I.; Ghaedian, R. and Shetty, K. (2007). Fermentation of milk and soymilk by Lactobacillus bulgaricus and Lactobacillus acidophilus enhances functionality for potential dietary management of hyperglycemia and hypertension. Food Biotechnol., 21:217–236.
12. Kligler, B.; Hanaway, P. and Cohrssen, A. (2008). Probiotics in children. Pedia. Clin. North Am., 54(6):949-967.
13. Rodriguez, C.; Medici, M.; Rodriguez, A.V.; Mozzi, F. and Font de Valdez, G. (2008).

Prevention of chronic gastritis by fermented milk made with exopolysaccharide producing *streptococcus thermophilus* strains. J. Dairy Sci., 92:2423-2434

14. Gibson, G.R.; Otaway, P.W. and Robert, A.R. (2000). Prebiotics; New development in functional foods. Chandos publishing (Oxford) Limited, London, UK
15. Lankaputhra, W.E.V. and Shah, N.P. (1995). Survival of *Lactobacillus acidophilus* and Bifidobacterium spp. In the presence of acid and bile salts. Cult. Dairy Prod. J., 30:2-7.
16. Berrada, N.; Lemeland, J.F.; Laroch, G. ; Thouvenot, O. and Piaia, M. (1991). Bifidobacterium from fermented milks, survival during gastric transit. J. Dairy Sci., 74:409-413.
17. Guarner, F.; Perdigon, G.; Corthier, G.; Salminen, S.; Koletzko, B. and Morelli,L. (2005). Should yogurt cultures be considered probiotics. Br. J. Nutr., 93:783-786.
18. Sanders, M.E. and Klaenhammer, T. R. (2001). Invited review: The scientific basis of L.acidophilus NCFM functionality as a probiotic. J. Dairy Sci., 84: 319-331.
19. Watkins, B.A. and Miller, B.F. (1983). Competitive gut exclusion of avian pathogens by Lactobacillus acidophilus in gnotobiotics chicks. Poult Sci.; 16: 1772.
20. Roberfroid, M.B. (2000). Prebiotics and Probiotics : Are they Functional foods ? AM. J. Nutr.. 71: 1682-1687.
21. Reid, G. (2000). In vitro analysis of a dairy strain of Lactobacillus acidophilus NCFM as a possible probiotic for the urogenital tract. Int. Dairy J., 10:415–419.
22. Pereira, D.I.A. and Gibson, G.R. (2002). Cholestrol Assimilation by Lactic acid bacteria and Bifidobacteria Isolated from the human gut. Appl. And Environ. Microbiol., 68:4689-4693.
23. Lin, M.Y. and Young, C.M. (2000). Folate levels in cultures of lactic acid bacteria. Int. Dairy J., 10: 409-413.
24. Oberg, C.J.; Weimer, B.C.; Moyers, L.V.; Brown, R.J. and Richardson, G.H. (1991). Proteolytic characterization of L. bulgaricus strains by the o-phyhaldialdehyde
25. Dunne, C.; Mahony, L.O.; Murphy, L.; Thorton, G.; Morrissey, D.; Halloran, S.O.; Feeney M.; Flynn, S.; Fitzgerald, G.; Daly, C.; Kiely, B.; O'Sullivan, G.C.; Shanahan, F. and Collins, J.K. (2001). In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. Am. J. Clin. Nutr., 73:386S–392S.
26. Liong, M.T. and Shah, N.P. (2005). Acid and bile tolerance and cholesterol removal ability of Lactobacilli Strains. J. Diary Sci., 88: 55-66
27. Loghavi, L.; Sastry, S.K. and Yousef, A.E. (2008). Effect of Moderate Electric Field frequency on growth kinetics and metabolic activity of *Lactobacillus acidophilus*. Biotechnol. Prog., 24:148-153.
28. Shah, N. and Jelen, P. (1990). Survival of lactic acid bacteria and their lactases under acidic conditions. J. Food Sci., 55:506-509.
29. Begley, M.; Sleator, R.D.; Gahan, C. G.M. and Hill, C. (2006). The contribution of three bile-associated loci (bSH, pva, and bt1B) to gastrointestinal persistence and bile tolerance of *Listeria monocytogenes*. Infect. Immun., 73:894-904.
30. Min, S.; Min, S.K. and Zhang, Q.H. (2003). Inactivation kinetics of tomato lipoxygenase by pulsed electric fields. J. Food Sci., 68:1995-2001.
31. Pilar, M. and Antoinio, V. (2006). Effect of PEF on enzymes and food constituents, in Pulsed Electric Fields Technology for food industry. Fundamentals and Applications by Javier Raso and Volker Heinz. U.S.A.

تأثير المجال الكهربائي النابض المعتدل على تحمل كل من الحموضة والصفراء والنمو وفعالية إنزيم البروتييز لبكتريا *Lactobacillus acidophilus* LA-K

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الخلاصة

استهدفت الدراسة تعيين مدى تأثير المجال الكهربائي النابض المعتدل على قابلية تحمل بكتريا *Lactobacillus acidophilus* LA-K للحموضة واملاح الصفراء وكذلك على نموها وفعالية انزيم البروتييز لها . تضمنت المعالجة تسليط مجال كهربائي نابض معتدل سعة 3 مايكروثانية من نوع احادي القطب الموجب ولمدة نبض 0.5 ثانية وبجهد كهربائي 1 كيلو فولط /سم وكانت سرعة جريان مرق البيتون الملقح بالبكتريا لكل من نماذج السيطرة والاخرى المعالجة بالمجال الكهربائي هي 60 مل /دقيقة عند درجة حرارة 40.5 مْ . مررت نماذج السيطرة داخل منظومة المجال الكهربائي النابض وبدون توجيه اي جهد كهربائي عليها . حدد مدى تحمل هذه البكتريا للوسط الحامضي(pH 2) بزرعها كل 30 دقيقة ، ولمدة 120 دقيقة من حضنها في مرق MRS الحامضي . عُيّن نمو هذه البكتريا بعد كل ساعة ولمدة 32 ساعة من حضنها في مرق MRS عند درجة حرارة 37 مْ . حدد مدى تحمل هذه البكتريا لاملاح الصفراء بزرعها كل ساعة ، ولمدة 16 ساعة من حضنها في مرق (MRS- Thio broth) الذي زود بكل من املاح الصفراء بنسبة 0.3 % ، وثايوكلايكوليت الصوديوم بنسبة 0.2 % . زرعت كافة النماذج اعلاه باطباق مزدوجة لكل تخفيف وباستعمال MRS agar *Lactobacilli* المحوره وحضنت في جو لاهوائي عند درجة 37 مْ ولمدة 48 ساعة .عينت فعالية انزيم البروتييز لهذه البكتريا بطريقة phthaldialdehyde - 0 للتحليل الطيفي بعد مرور 0 , 12 ,24 , 36 و 48 ساعة من حضنها في الحليب الفرز عند درجة حرارة 40 مْ . كررت كافة المعاملات اعلاه لثلاث مرات لكل تجربة و حللت النتائج احصائيا باستخدام Proc mixed model of statistical analysis system (SAS). اظهرت بكتريا *Lactobacillus acidophilus* LA-K في نماذج السيطرة كلها والاخرى التي خضعت للمجال الكهربائي النابض المعتدل قابليتها لتحمل الحموضة الى نهاية مدة الحضانة (120دقيقة) ولكن هناك انخفاض معنوي في اعداد البكتريا الحية بعد مرور كل 30 دقيقة من مدة الحضانة . عمل المجال الكهربائي النابض المعتدل على تحسين قابلية البكتريا لتحمل الحموضة . واظهرت هذه البكتريا قابليتها لتحمل أملاح الصفراء وأعطت نمو في وجود أملاح الصفراء مماثل لنمط نموها في عدم وجود املاح الصفراء ولكن وصلت هذه البكتريا الى الطور الثابت للنمو بعد مرور 12 ساعة من الحضانة في وجود أملاح الصفراء مقارنة بوصولها للطور الثابت بعد مرور 16 ساعة من الحضانة في حالة عدم وجود املاح الصفراء. نستنتج ان المجال الكهربائي النابض المعتدل لم يؤثر معنوياً في قابلية هذه البكتريا لتحمل املاح الصفراء وعزز المجال الكهربائي النابض المعتدل وبصورة معنوية ($P<0.0001$) في زيادة فعالية إنزيم البروتييز للبكتريا مقارنة بنماذج السيطرة.

الكلمات المفتاحية: النابض، المجال الكهربائي، بكتريا *Lactobacillus acidophilus*، إنزيم البروتييز, الصفراء، تحمل.