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Electroporation Enhances the Metabolic Activity of *Lactobacillus plantarum* 564

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Summary

The exposure of bacterial cells to pulsed electric fields (PEF) leads to the reversible formation of pores in the cell membrane if an applied energy is below the critical level. Therefore, the effect of electric field pulses with amplitudes below 14 kV/cm and the applied energy up to 12.2 J/cm^3 on the growth of *Lactobacillus plantarum* 564 cells was investigated. After PEF treatments, the growth of lactobacilli in De Man-Rogosa-Sharpe broth at 37 °C was monitored by isothermal calorimetry, absorbance and plate counts. All the applied treatments resulted in a higher growth rate of PEF-treated cells during early and mid-log phase, especially bacterial samples treated with lower field intensities (1.3–5.5 J/cm^3). The transport of ions and molecules through the cell membrane (which facilitates the growth of electroporated lactobacilli) was particularly evident in the mid-exponential growth phase, where the doubling time was reduced more than 3 times after the exposure to electric pulses of 5.5 J/cm^3. The heat production rate during the growth of electroporated cells was also higher, indicating the enhanced metabolic activity of PEF-treated cells. Moreover, the electroporated cells had a better acidification ability than the untreated ones. It can be summarized that the applied PEF treatments with an energy input of below 12 J/cm^3 potentially induce reversible electroporation of the cell membrane, which has a positive impact on the growth and metabolic activity of the cells of lactobacilli.

Key words: *Lactobacillus plantarum*, pulsed electric fields, isothermal calorimetry

Introduction

Many stress techniques have been used in order to change the structure of bacterial cell membranes, which affects the cell growth and lactic acid production. The attenuation of whole cells by physical or chemical agents creates a weak and permeable peptidoglycan structure, which could lead to the increase of enzymes released into the medium. Techniques that have been used to alter bacterial cell structure are: heat treatments, freeze-thawing, lysozyme treatments, electroporation, high pressure treatments, *etc.* (*1–5*). Thermal processing methods are commonly used in the food industry to maintain food safety by inactivation of pathogenic microorganisms and to in-

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crease the shelf-life of the final product. Although these methods provide safer foods, they can also unfavourably affect their sensory characteristics and nutritional quality (*6*). Pulsed electric field (PEF) technology is considered as a nonthermal alternative to the traditional pasteurization of liquid foods. The inactivation mechanism is based on electroporation of microbial cell membranes due to the repetitive application of short pulses (several microseconds to milliseconds) of high intensity electric fields (15–40 kV/cm) (*7*). In a previous study, Seratlić *et al*. (*8*) revealed that there is heterogeneity in the response of bacteria to electrical stress within the same population of lactobacilli and that the surviving population of bacteria subjected to PEF treatment (with amplitudes in the range of 22.9–31.6 kV/cm) could grow again, showing higher resistance to further PEF treatments. Also, the surviving bacterial subpopulation showed higher growth rates as the intensity of PEF treatment increased. Therefore, the evidence of bacterial persistence indicates that the PEF technology, as a nonthermal alternative to traditional pasteurization, could not completely replace thermal treatment, but can be applied as a supplementary treatment (*8*). In fact, the effect of electric field on the living cell membranes could be reversible or irreversible, depending on various physicochemical, biological and PEF treatment factors (*9–12*). Studies have shown that the site of the electric field interaction is the lipid portion of the cell membrane and the knowledge is based on the measurements of electrical currents through planar bilayer lipid membranes under the influence of high electric fields and on molecular transport into or out of the cells exposed to electric field pulses (*13*).

Electroporation is a fast process that takes place in the micro- and submicrosecond time range (*14*). If the total electrical potential across the cell membrane reaches some critical value, it becomes permeable to otherwise impermeable ions and molecules. If the electric field signals are low and short enough, causing a short stress duration, then the damage is reparable. At higher electric field strengths, where the voltage across the cell membrane is correspondingly higher, the permeability of the membrane increases to such level that the cell either needs seconds to hours to recover (reversible breakdown), or cell death may occur (irreversible breakdown) (*15*). The inability of a cell membrane to function properly and regulate electron transport that controls the entrance and the exit of small molecules could result in microbial inactivation (*16*). However, only a small group of authors investigated reversible effects of PEF on cell membranes of lactobacilli, so relatively little information is available.

In this work, the indigenous strain *Lactobacillus plantarum* 564, isolated from artisanal Serbian white cheese in brine, was selected for the research. The cells were treated in the mid-exponential growth phase by applying electric field pulses with different amplitudes, varying between 4.5 and 13.6 kV/cm. The behaviour of the PEF-treated population was monitored using isothermal calorimetry, which is used as a method for measuring the rate of heat production released during bacterial growth. The calorimetric measuring represents a criterion of the metabolic rate and provides a direct indication of the integrated metabolic response of the cells (*17*).

The main objective is to investigate the effects of different PEF treatments on the growth, acidification ability and metabolic activity of the treated lactobacilli. It is postulated that the applied PEF treatments with an energy input below 12 J/cm^3 could potentially lead to reversible electroporation of the cell membrane, which may have a positive impact on the cell growth. The study was undertaken to stimulate the growth of lactobacilli by the use of pulsed electric fields below the critical energy threshold. Since relatively little information concerning reversible PEF effect on the cells of lactobacilli is available and there has been relatively little progress in understanding cell membrane recovery, this study could set a basis for further investigation of structural changes of elecroporated cell membrane and molecular transport across the membrane due to electroporation.

Materials and Methods

Bacterial culture and sample preparation

Lactobacillus plantarum 564 strain was obtained from the culture collection of the Division of Industrial Microbiology, Faculty of Agriculture, University of Belgrade, Belgrade, Serbia. The strain was isolated from Sjenica cheese, a Serbian artisanal white cheese in brine (*18*). The stock culture was stored at –80 °C in sterile De Man-Rogosa-Sharpe (MRS) broth (Oxoid Ltd, Basingstoke, UK) with 20 % (by volume) glycerol, from which the stocks were subsequently prepared by inoculating the starter culture into the MRS broth with 15 % (by volume) glycerol to a final count of 10^7 CFU/mL and stored at –20 °C. The inocula were prepared 3 days before the experiment by overnight propagation into sterile MRS broth to a final count of 10^7 CFU/mL. After the propagation, the cell suspension was incubated for 24 h at 37 °C. On the fourth day, activated cells were PEF-treated in mid-exponential phase, which is 4 h after the inoculation.

Pulsed electric field treatments

Before the treatment, *Lactobacillus* cells were diluted with sterilized distilled water at room temperature in the ratio of 1:10 to attain a conductivity of approx. 1.3 mS/cm. The cell suspension was placed into sterile electroporation cuvettes (1 mm gap, EP-104, Cell Projects, Harrietsham, Kent, UK) and electric pulses were applied by using a CEPT pulse generator (Arc Aroma Pure, Lund, Sweden). The pulse width was set to 5 μs and the distance between pulses was 10 ms. Bacterial samples were treated with 10 monopolar square wave pulses at various nominal electric field strengths as follows: 4.5, 9.1 and 13.6 kV/cm. The voltage was monitored with a digital oscilloscope (Fluke 123, Fluke Corporation, Everett, WA, USA) that was connected to the system. The applied PEF treatments corresponded to an energy input of 1.33, 5.5 and 12.2 J/cm^3, calculated by the following equation (*19*):

$$\omega = E^2 \cdot \lambda \cdot t \qquad /1/$$

where E (kV/cm) is the electric field strength, λ (mS/cm) is the conductivity and t (s) is the treatment time. Each treatment was done in duplicates. The control bacterial

samples were placed into sterile cuvettes connected to the pulse generator but were kept untreated. After the PEF treatment, bacterial samples were inoculated into the MRS broth to a final count of 10^7 CFU/mL and incubated at 37 °C for analysis within 24 h.

Measurement of bacterial growth

The viable cell count was determined by the standard plate count method. After the PEF treatment, replicate bacterial samples were incubated for defined lengths of time at 37 °C. After each time point, samples were serially diluted in sterile 0.1 % (by mass per volume) peptone physiological salt solution (Oxoid Ltd). A volume of 1 mL of the appropriate dilutions was spread out on plastic Petri dishes and 10 mL of MRS agar were poured out on the plates. After 48 h of incubation under anaerobic conditions in a gas-pack system (Oxoid Ltd) at 37 °C, the colonies were counted.

The bacterial growth was also monitored using a turbidometry method. The absorbance was measured at a wavelength of 600 nm using a Hitachi Spetrophotometer U-1500 (Hitachi Ltd, Tokyo, Japan). The measurements were performed on bacterial samples at defined time intervals within 24 h of incubation at 37 °C.

Isothermal calorimetry analysis

The heat produced in the untreated and PEF-treated bacterial samples was continuously followed in a TAM Air isothermal heat conduction calorimeter (TA Instruments/Thermometric AB, Järfälla, Sweden), as described by Rocculi *et al*. (*20*). The sensitivity (precision) of calorimeter was ±10 μW (*21*). The instrument contains eight twin calorimeters of the heat flow type that consist of the sample and the reference side. The heat transfer takes place through the heat flow sensors that are positioned between the ampoule holders and the surrounding heat sink. The untreated and PEF-treated samples were transferred from the PEF cuvettes to 20-mL glass ampoules containing 10 mL of MRS broth to bring to a final count of 10^7 CFU/mL and sealed with a Teflon-coated septum and an aluminium crimp cap. The ampules were thermostated at 37 °C for 30 min prior to being placed into the calorimeter at 37 °C, where the heat production rate was continuously measured and the two replicates were recorded. Each calorimeter had its own reference sample containing 10 mL of water, which does not produce any heat. Isothermal measurements were conducted during 24 h and baselines (BL) were recorded before each measurement. The primary output from the heat flow sensors in the calorimeters (voltage) was recorded by a computer. The heat production rates were calculated as thermal power by the following equation:

$$P=\varepsilon\cdot(V_S-V_{BL})/m \qquad /2/$$

where P (mW/g) is the specific thermal power of the sample, ε (mW/mV) is the calibration coefficient of the calorimeter, V_S (mV) is the voltage signal from the calorimeter, V_{BL} (mV) is the corresponding voltage recorded for the baseline, and m (g) is the mass of the sample. Calibration coefficients were calculated from electrical calibrations made at 37 °C with heaters placed in the same type of ampoule as used in the experiments.

pH analysis

The acidifying properties of PEF-treated cells were evaluated by direct pH measurment of inoculated MRS broth during 6 h of incubation at 37 °C using a standard pH meter (model PHM 210, MeterLab, Radiometer Analytical SAS, Villeurbanne Cedex, France).

Statistical analysis

Statistical significance was tested by means of ANOVA analysis and the difference between individual mean values was tested using the Fisher's least significant difference (LSD) test. Significant differences were considered for $p<0.05$. Calculations were made with STATISTICA v. 6.0 PL software for Windows (StatSoft Inc, Tulsa, OK, USA).

Results and Discussion

Growth of Lactobacillus plantarum 564

The growth of *L. plantarum* 564 cells incubated in MRS broth at 37 °C for 24 h is reported in Fig. 1, starting from the second hour of incubation. According to previous experiments, the first two hours are considered as the lag phase, because bacteria need some time to adjust to a new environment, and the number of cells that entered the phase of division in that period was not significantly different from the cell number at the time of inoculation (data not shown). After 2 h of incubation, bacteria entered a constant cell division and the exponential growth phase took place in the next 7 h. Logarithmic phase is a period when the majority of the cells double their mass and divide at the same rate. However, the growth rate of the control (untreated) sample was not constant during the whole logarithmic phase. Based on the growth curve shown in Fig. 1, it can be seen that after approx. 5.5 h there was a delay in the growth lasting approx. 90 min. A similar phenomenon has been observed by Cohen *et al*. (*22*), who detected the slow growth of *L. plantarum* WCFS1 strain during late log and early stationary phases. It was also found that such strain is characterized by two consecutive log phases in MRS broth, where the sec-

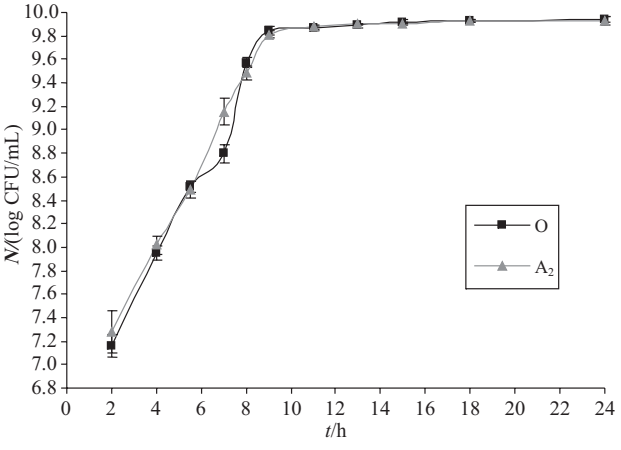

Fig. 1. The growth of *L. plantarum* 564 cells before and after the PEF treatment, incubated in MRS broth at 37 °C during 24 h. O=control (untreated) sample, A_2=PEF-treated sample with the applied energy of 5.5 J/cm^3

ond phase is characterized by a shorter doubling time between $A_{600\ nm}$=3.0 and the early stationary phase (*23*). The results of the present study are consistent with the published data, indicating a shorter doubling time in the late log phase compared to the early log growth phase (21 min compared to 47 min).

After 8 h the total cell number reached 10^9 CFU/mL and remained at the same level, indicating the beginning of the stationary phase. Since *L. plantarum* 564 was cultured in MRS medium, in a closed system bacteria reach a point where environmental factors slow down the growth rate due to overcrowding, nutrient depletion, and pH changes as a result of acid formation. Lactic acid and other organic acids that are produced during fermentation have an inhibitory effect on the bacterial growth (*24*). Although *L. plantarum* cells have higher resistance to the increased concentration of lactic acid, their growth becomes inhibited when the concentration of this organic acid reaches a critical level during fermentation (*25, 26*). The inhibitory effect of organic acids on bacterial cells is mainly caused by the diffusion of undissociated acid molecules through the cell membrane, which then dissociate within the cell (*27*). Hence, organic acids were found to be more inhibitory than strong acids, such as hydrochloric acid, which can act only on the exterior of the cell, because the cell membrane is not permeable for dissociated HCl ions (*26,28*). Lactic acid penetrates inside the cell until the equilibrium between the intra- and extracellular concentration is reached. The higher the difference between the external and intracellular pH, the greater the inhibitory effect. This explains why some microorganisms that have the ability to decrease intracellular pH are resistant to organic acids (*29,30*), and this feature has also been observed in *L. plantarum* strains (*31*).

Therefore, the cells entered the stationary phase after 9 h, when many of them are dividing, but just as many are dying, so the total number remains constant. After 5 days, a significant decline in the cell number began, when the total cell number reached the same level as at the beginning, which is characterized as the death phase (data not shown).

Pulses below 12 J/cm^3 had a positive effect on the growth of lactobacilli

The PEF treatment was applied at the exponential stage of bacterial growth, since the cell membrane is more susceptible to electroporation by the electric field treatment due to the active cell division (*32,33*). The initial cell count in MRS suspension prior to PEF treatment was approx. 10^8 CFU/mL. After the treatment, the cell suspension was immediately inoculated in MRS broth to a final count of approx. 10^7 CFU/mL and the plate counts were determined starting from the second hour of incubation at 37 °C.

The effect of different PEF treatments on the suspension of lactobacilli is reported in Table 1. It can be seen that only the treatments with applied energies of 1.33 and 5.5 J/cm^3 had a significant ($p<0.05$) influence on the increase of the total bacterial count, while the total number of cells exposed to pulses of 12.2 J/cm^3 was not statistically different from the untreated (control) samples. However, all the applied treatments caused the increase

Table 1. The effect of PEF treatments on the viability of *L. plantarum* 564 cells

PEF treatment	N(log CFU/mL)*	CV
O	$(7.16\pm0.08)^a$	1.05
A_1	$(7.28\pm0.03)^{b,c}$	0.45
A_2	$(7.38\pm0.05)^b$	0.63
A_3	$(7.22\pm0.04)^{a,c}$	0.58

O=control (untreated) sample; A_1, A_2 and A_3=PEF-treated samples with an applied energy of 1.3, 5.5 and 12.2 J/cm^3, respectively; CV=coefficient of variation; *average value±standard deviation among 4 samples; statistical significance ($p<0.05$) is shown with lower case letters in superscript

of total cell number. Increased growth is also evident in Fig. 2, where the absorbance of the cells exposed to pulse amplitudes of 4.5 and 9.1 kV/cm was significantly ($p<0.05$) higher after 6 h of incubation compared to the untreated cells and those treated with the pulse strength of 13.6 kV/cm. Given that the exposure to pulsed electric fields leads to the formation of pores in the cell membrane, this damage may be reversible if applied energy is below the critical level, which was proven by Neumann *et al*. (*34*), who investigated the influence of different PEF treatments on the cell electroporation. Ulmer *et al*. (*35*) studied the mechanisms of inactivation of *L. plantarum* in model beer by PEF in a continuous flow system and found that the viability of cells remained unaffected by the PEF treatment with amplitudes below 13.8 kV/cm and energy input below 17 kJ/kg. The authors revealed that damage to the cell membrane was reversible and that cells could recover and continue to grow normally after the treatment if incubated under optimal conditions. In fact, during the pulse application, the difference in transmembrane potential leads to the transformation of the phospholipid bilayer of the cell membrane and the induced breakdown causes pore formation in the membrane (*36*).

The mechanism of microbial inactivation by PEF treatment has not been fully elucidated (*13,37*). It is believed to be a physical response of the cell to the exposure to a high electric field (*38*). If a phospholipid bilayer of the

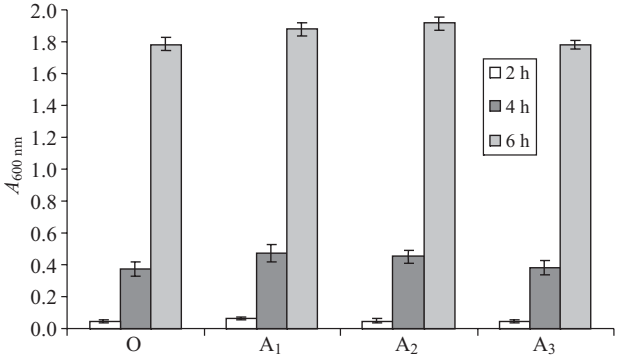

Fig. 2. The effect of PEF treatment on the absorbance of *L. plantarum* 564 cells incubated in MRS broth at 37 °C during 24 h after the treatment. $A_{600\ nm}$=the absorbance at the wavelength of 600 nm; O=control sample (0 J/cm^3); A_1, A_2 and A_3=PEF-treated samples with the applied energy of 1.33, 5.5 and 12.2 J/cm^3, respectively

cell membrane exposed to high intensity electric field pulses is temporarily destabilized in specific regions of the cell, then this phenomenon is called electroporation. During the destabilization period, the cell membrane is highly permeable to exogenous molecules present in the surrounding media (*39*). One major consequence of electroporation is a dramatic increase in the permeability called electropermeabilization, which can in some cases lead to mechanical rupture of the cell membrane (*13*). Depending on the degree of membrane changes, electropermeabilization can be reversible or irreversible (*37*). The size of electrically induced pores in the cell membrane depends on the applied electric field strength and the number of pulses. Small pores reseal after the removal of the electric field, but when the size or the number of pores is considerably large compared to the entire membrane surface, it leads to membrane rupture and destruction, which causes irreversible structural changes (*36*). Sale and Hamilton (*10*) found that the irreversible loss of membrane function and the lysis of bacterial cells occurs when the potential difference was about 1 V across the membrane (values ranged from 0.7 to 1.15 V depending on the microbial species). It can also vary depending on the pulse width, composition of the membrane, *etc*. (*39*). Ulmer *et al*. (*35*) found that the electric pulses with the field strength of 13.8 kV/cm and the energy input of 60 kJ/kg increased the critical transmembrane potential difference ($\Delta\varphi_{mem}$) in *L. plantarum* TMW 1460 cell membrane, leading to cell inactivation. In this study, the applied amplitudes varying from 4.5 to 13.6 kV/cm that correspond to energy inputs from 1.33 to 12.2 kJ/kg did not induce critical $\Delta\varphi_{mem}$ increase, so it can be assumed that reversible pore formation occurred, leading to an increased cell growth.

It has been shown that the exposure of the cell suspension to electric field pulses leads to the formation of transient aqueous pores (*15*). Although the transfer of some charged molecules and ions into and/or out of the cell during the electric pulse exists, the majority of the transport occurs after the treatment (*40,41*). When electroporated, the cells may remain viable because of the capability of pores to reseal (*40*). In this work, the transport of molecules and ions through the electropermeabilized cell membrane, which facilitates the growth of electroporated *Lactobacillus* cells, is particularly evident in the exponential growth phase. The untreated *L. plantarum* 564 cells showed a growth delay during the incubation period between 5.5 and 7 h, while PEF-treated cells grew continuously and the doubling time in this period was reduced from 141 to 41 min when an electric pulse of 5.5 J/cm^3 was applied (Fig. 1).

The reason why untreated cells showed a slower growth rate in the mid-log phase was not investigated in this work, but it is assumed that the transport of some components necessary for cell growth was slowed down in the given period, which is avoided by electropermeabilization. In fact, the transport of molecules through the permeabilized membrane can occur through three general mechanisms: diffusion, electrophoresis and electroosmosis. These mechanisms may contribute to the increased transmembrane transport of molecules during the pulse application. After the end of the pulse, until the membrane reseals completely, the only transport across the membrane can only proceed by diffusion (*42*). However, compared to the speed of pore formation, membrane recovery is a slow process, and generally depends on the temperature (*43*). Some pores remain open for a prolonged period of time and then the ions and molecules continue to diffuse through the cell (*44*). Therefore, the degree of biochemical imbalance can determine whether the cell will recover and survive or become irreversibly stressed and inactivated (*43*).

Reversible pore formation leads to the assimilation of certain nutrients from the medium into the cell, which probably affects the growth and cell development. Cells exposed to pulsed electric fields below 12 J/cm^3 had a better acidification ability than the untreated cells. The pH decrease during 6 h of incubation at 37 °C was significantly ($p<0.05$) higher in all PEF-treated samples compared to the control samples (Fig. 3), indicating better acidification activity of the electroporated cells.

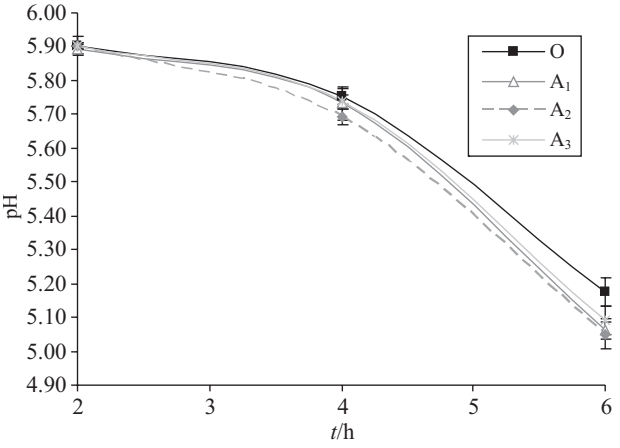

Fig. 3. Acidification activity of *L. plantarum* 564 cells before and after the PEF treatment, incubated in MRS broth at 37 °C during 24 h after the treatment. O=control (untreated) sample; A_1, A_2 and A_3=PEF-treated samples with the applied energy of 1.33, 5.5 and 12.2 J/cm^3, respectively

PEF treatment of 5.5 J/cm^3 enhances the metabolic activity of lactobacilli

Since the greater effect on the cell growth was achieved by the pulse application with energy input of 5.5 J/cm^3, the metabolic activity of the cells exposed to these PEF conditions was monitored continuously by isothermal calorimetry during 24 h at 37 °C. Calorimetric measurements of the metabolic heat produced by the growing population of untreated and PEF-treated *L. plantarum* 564 cells are shown in Fig. 4. The thermal power values generated from the second hour onwards are reported. In untreated *Lactobacillus* suspension incubated at 37 °C the thermal power increased, reaching the peak of 0.28 mW/g during the first 6 h. In the following 3 h a gradual decrease occurred, reaching 0.17 mW/g before a small peak of approx. 0.18 mW/g appeared, indicating lower heat production than in the early log phase. Thereafter, gradual decrease of the thermal power was recorded for the rest of the time. Interestingly, *Lactobacillus* cells exposed to pulses of 5.5 J/cm^3 showed higher thermal

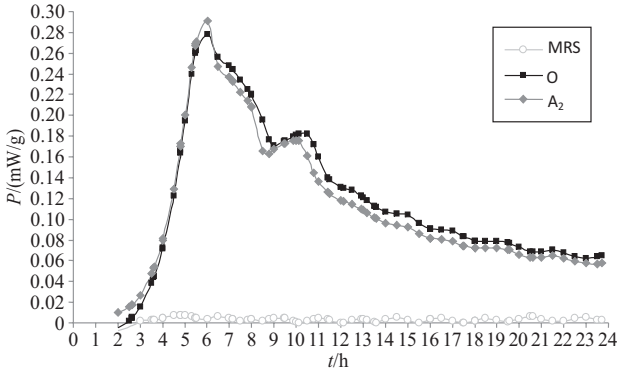

Fig. 4. Effect of PEF treatment on thermal power production of *L. plantarum* 564 cells. O=control (untreated) sample; A_2=PEF--treated sample with the applied energy of 5.5 J/cm^3. Average curves of duplicates are reported

power during the first hour of log phase, which was continued during the mid-exponential growth, reaching a slightly higher peak of 0.29 mW/g at the sixth hour of incubation. Since in the logarithmic phase the majority of proteins were involved in metabolic pathways (especially carbohydrate and energy metabolism) responsible for generating sufficient energy for cell growth (*22*), higher heat production in the early and mid log phase of PEF-treated samples indicates an enhanced growth of electroporated cells. Similar results were obtained by Lye *et al*. (*45*), who also detected an increased growth of electroporated lactobacilli during incubation. The authors found that the growth of *Lactobacillus* cells treated at 7.5 kV/cm for 4 ms was increased by 0.89–1.96 log CFU/mL after 20 h of incubation at 37 °C. In addition, the same authors found that the treated cells had a greater ability to assimilate cholesterol, which points to the fact that during electroporation the incorporation of cholesterol in PEF-treated cells was increased.

In our previous studies, where higher energies between 34.6 and 658.1 J/cm^3 were applied, the surviving *Lactobacillus* population showed a higher resistance to further PEF treatment. Besides, in the late log phase the surviving subpopulation had shown higher growth rates as the intensity of the applied energy increased (*8*). It has been reported that during the late log and early stationary phase there is an increase in the biosynthesis of the enzymes involved in fatty acid synthesis for the formation of phospholipids, strengthening the cell membrane, which prepares the cells for survival in the stationary phase (*22*). It may be that the high intensity of PEF provokes stronger cell membranes in the persistent subpopulation, while pulses with lower energy inputs (≤5.5 J/cm^3) might provoke the induction of proteins involved in the cell division, which could have a positive impact on the cell growth. This could be an interesting issue for further investigation of the application of different PEF conditions to bacterial cells at metabolic and proteomic level.

Conclusions

This research has focused on studying the behaviour of *L. plantarum* 564 cells after exposure to PEF treatments with the energy between 1.3 and 12.2 J/cm^3, and the following findings can be deduced: PEF treatment at less than critical field strengths (≤13.6 kV/cm) did not reduce the viability of *Lactobacillus* cells, and the application of pulses with lower field energies, particularly ≤5.5 J/cm^3, induced a higher growth rate when compared to the untreated lactobacilli upon incubation in MRS medium at 37 °C. This indicates that membrane damage can be repairable and pores can be resealed if the cells are incubated under optimal conditions after the PEF treatment. Reversible electropermeabilization of *Lactobacillus* cells exposed to electric field pulses with the energy input bellow critical values was also characterized by an enhanced metabolic activity of PEF-treated cells, measured by the use of isothermal calorimetry. In general, the above results provide grounds for further research that could be based on an examination of the mechanisms of membrane changes during cell growth, with particular emphasis on the logarithmic growth phase.

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