



# Bioaccumulation of zinc ions in *Lactobacillus rhamnosus* B 442 cells under treatment of the culture with pulsed electric field

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## Abstract

Pulsed electric field (PEF) technology was used to enrich *Lactobacillus rhamnosus* B 442 cells in zinc ions to obtain source of this element with high bioavailability. The highest bioaccumulation of zinc was achieved when electroporation was performed at optimal parameters: field strength of 3.0 kV/cm, pulse width of 20  $\mu$ s, electroporation time of 15 min after 20 h of culturing and at zinc concentration of 500  $\mu$ g/mL medium. The maximum bioaccumulation of ions in cells (2.85 mg Zn/g d.m.) was 164% higher than in the control sample which was supplemented with zinc but not treated with PEF. The action of PEF did not reduce the total number of microorganisms in the medium or the biomass of bacteria.

**Keywords** PEF · Zinc · *Lactobacillus rhamnosus* · Bioaccumulation

## Introduction

Zinc is one of the most important micronutrients in people's diet, as it is a component of over 200 metalloproteins and takes part in a number of biochemical reactions involving enzymes, structural proteins, and hormones [1]. The appropriate zinc concentration in cells is essential for the proper functioning of the system which protects the body from oxidative damage [2, 3]. This element participates in the metabolic processes of the cell and in gene expression and it is considered to be safe for health (has no oxidizing capacity) [4, 5].

The daily requirement for this micronutrient is age dependent—for infants, it ranges from 3 to 5 mg/day, for children—about 10 mg and for adults—from 12 mg (women) to 15 mg (men) [6]. The assimilability of zinc from diet is on

average between 15 and 35% [7] and depends on the type of food (animal or vegetable). Zinc from meat products is more bioavailable than that found in plants containing phytates, fiber, and oxalates [8]. The deficiency of this element can be partially reduced by the use of dietary supplements. However, zinc available in the form of pharmacological preparations often has low bioavailability. Moreover, it can be easily overdosed [9].

Consumption of zinc in the form of metalloproteins can be a solution of the problem with its deficiency. It has been shown that the supply of metal ions in the form of easily digestible protein complexes results in high bioavailability of the elements accumulated in the microbial cells [10–13]. Lactic acid bacteria (LAB) enriched with zinc ions and used for food production can be a valuable source of this element for people. In zinc-supplemented microorganisms it is bound in protein complexes that are absorbed in the small intestine analogously to proteins and peptides [14].

Application of electric pulses causes transient permeabilization of cell membranes. This process is called electroporation or electroporation and allows hydrophilic molecules, e.g., ions, to enter into cells. Single-cell imaging experiments revealed that the uptake of molecules depends on their chemical and physical properties such as size and charge, and takes place in well-defined membrane regions [15]. Small molecules can freely penetrate through the electroporated membrane, whereas transfer of heavier molecules involves much more complex mechanism [16].

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Pulsed electric field (PEF) technology consists in induction of short electrical impulses with a field strength of  $100 \text{ V}\cdot\text{cm}^{-1}$ – $80 \text{ kV}\cdot\text{cm}^{-1}$  at a certain time [17, 18]. Under the influence of pulsed electric field, cell membrane is permeabilized, interrupted, or damaged. The resulting changes are of permanent or temporary nature depending on the process parameters used (electric field strength, number and shape of impulses, their width and frequency) [19, 20]. The effect of PEF also depends on the shape, structure, properties and size of cells, and on their amount in suspension [21]. As a result of PEF action, permeability of the membrane surrounding the cell and the vacuole is increased [22]. PEF with very short duration and low field strength increases permeabilization of the cell membrane (reversible electroporation). With this process, e.g., ions can penetrate the cell membranes, and dyes, radiolabels, and DNA and RNA can be introduced into the cell [23, 24]. Microsecond pulsed electric fields ( $\mu\text{s}$  PEF) of about  $100 \text{ kV/m}$  are commonly used to induce permeabilisation of plasma membrane to different types of molecules small ions, drugs, or DNA [25–28].

The aim of the study was to determine the ability of *Lactobacillus rhamnosus* B 442 bacteria to accumulate zinc ions under the treatment of pulsed electric field. The zinc-supplemented microorganisms potentially could serve as the source of this element with high bioavailability in a diet.

## Materials and methods

### Materials

A bacteria strain of *L. rhamnosus* B 442 from the Agricultural Research Service Culture Collection NRRL (WDCM97) maintained in Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences and Biotechnology in Lublin, Poland, was used in the experiment. For the preparation of inoculum and culture medium, the following components were used: sterile MRS broth (BTL, Łódź, Poland)  $58.937 \text{ g/L}$ , Tween 80 (Biochemica, ICI, USA)  $1 \text{ mL/L}$ , agar (DIFCO, Detroit, MI, USA)  $15 \text{ g/L}$ , NaCl  $80 \text{ g/L}$ , glycerol (TechlandLab, Tarnobrzeg, Poland),  $\text{HNO}_3$  65% (Merck, Darmstadt, Germany) and  $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$  (Standard, Lublin, Poland) in the fixed concentrations.

### Biomass cultivation

Bacteria were passaged three times in MRS broth and incubated for 19 h at  $37^\circ\text{C}$ . Then, inoculum was prepared by transferring 3 mL of bacteria to 57 mL of sterile medium in 500-mL Erlenmeyer flasks. Flasks were incubated at  $37^\circ\text{C}$  for 48 h. The obtained inoculum (5 mL) was transferred into 85 mL of sterile medium placed in Erlenmeyer flasks. An

10-mL aliquots of zinc sulfate at fixed concentration was added to each flask (except of the control sample K1). Then, the culture was incubated at  $37^\circ\text{C}$  for 24 h.

### Optimization of zinc concentration in cells

Optimization of zinc concentration in the medium was performed by culturing of *L. rhamnosus* B 442 at different concentrations of zinc ( $\mu\text{g/mL}$  medium): 10, 100, 200, 400, 500, 750, and 1000. After 16 h of incubation, cultures were treated with PEF for 15 min at pulse width  $20 \mu\text{s}$ , electric field strength  $2.0 \text{ kV/cm}$ , using a laboratory electroporator (BTX Harward Apparatus, model ECM 830). Then, the cultures were incubated for 19 h. Simultaneously, the control cultures were conducted, respectively, K1—without zinc added to the medium and without PEF treatment and K2—with zinc added to the medium in the concentrations mentioned above and without PEF. The PEF treatment chamber consisted of four parallel plexiglas plates which had stainless steel electrodes of an area equal to  $4 \text{ cm}^2$ , facing each other with a gap of 5 mm. The culture was agitated in a chamber during PEF treatment with a magnetic stirrer. The electrical conductivity measured for the treated samples was between 2.4 and  $2.6 \text{ mS/cm}$ . Each test was performed in triplicate.

### Optimization of PEF parameters

Optimization of PEF parameters was carried out in a few stages. In the first stage, the optimal electric field strength was set by subjecting of the cultures after 16 h of incubation to electroporation the pulse amplitude in steps of 50, 100, 150, 200, 250, 500, 1000, 1500, 2000, 2500, and 3000 V, respectively, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, and  $6.0 \text{ kV/cm}$  of electric field strength, exposition time of 15 min, pulse width of  $20 \mu\text{s}$ , and frequency of 1 Hz. Microorganisms were treated with PEF at the optimal concentration of zinc ( $500 \mu\text{g/mL}$  medium) which was set earlier (“[Optimization of zinc concentration in cells](#)”). In the next stage, the time of electroporation was optimized in the range 5–25 min, at optimal electric field strength ( $3.0 \text{ kV/cm}$ ) fixed earlier. In the following step, pulse width was optimized in the range 10–75  $\mu\text{s}$  at optimal PEF parameters: electric field strength  $3.0 \text{ kV/cm}$ , electroporation time 15 min, and frequency 1 Hz. In the last stage optimization of the time of incubation after which bacteria cells were treated with PEF was performed. Cells were electroporated after 8, 12, 16, 20, and 24 h of culturing. One of the samples was subjected to a multiple treatment with PEF after 8, 12, 16, 20, and 24 h of culturing and another one was supplemented with zinc in multiple doses (0.2 of total dose every 4 h, cells treated with PEF after 24 h of culturing). In both cases, the optimal PEF parameters were applied: electric field strength  $3.0 \text{ kV/cm}$

cm, electroporation time 15 min, pulse width 20  $\mu$ s, and frequency 1 Hz. Each test was performed in triplicate.

### Determination of zinc concentration

To determine concentration of magnesium in cells, biomass was centrifuged (15 min, 3000 rpm, 1467g), supernatant was discarded, and cells were rinsed three times with deionized water. Then biomass was lyophilized in a LABCONCO freeze dryer (model 64132, Kansas City, MO, USA), and mineralized in a MARS microwave oven (CEM Corporation, USA). Samples were prepared as follows: about 0.1 g of lyophilizate was transferred to a tube and 3 ml of HNO<sub>3</sub> was added. Then, the samples were mineralized at 200 °C for 20 min. The obtained solutions were cooled down, transferred to 25 mL measuring flasks, and topped up with deionised water. Concentration of zinc ions in *L. rhamnosus* B 442 cells was determined using an electrothermal atomic absorption spectrophotometer (ET-AAS, VARIAN AA 280 FS) according to Jorhem and Engman [29] in an accredited laboratory (validation parameters: precision 5.1%, extended uncertainty 14%). The determination was performed in triplicate.

### Determination of total number of microorganisms

Total number of microorganisms was determined by plate dilution method according to American Public Health Association [30]. Colonies of microorganisms were diluted 8 and 9 times with 0.8% sterile NaCl. Then, 1 mL of each was taken and placed in Petri dishes in two replicates. The diluted bacteria were flooded with sterile liquid agar. Cultures were incubated for 48 h at 37 °C. The Petri dishes with two consecutive dilutions on which grew from 25 to 250 colonies were selected for reading. The total number of microorganisms (*L*) in 1 cm<sup>3</sup> of the sample was calculated according to the formula (1):

$$L = \frac{C}{(N_1 + 0.1N_2)} \cdot d, \quad (1)$$

where *C* is the sum of colonies on all dishes selected for counting, *N*<sub>1</sub> is the number of dishes from the first calculated dilution, *N*<sub>2</sub> is the number of dishes from second calculated dilution, and *d* is the dilution ratio corresponding to the first (lowest) dilution.

The determination was performed in triplicate.

### Determination of biomass

Biomass was determined spectrophotometrically (Spekol 11, Carl Zeiss, Jena, Germany). A sample of the culturing medium (2 mL) was centrifuged, supernatant was discarded, and cells were rinsed three times with deionized water and

brought to the original volume of 2 mL. Turbidimetric measurements were run against pure water at  $\lambda = 600$  nm, in 2 mm measurement cell. Amount of biomass was calculated using equation for the standard curve  $A_p = 1.1511c - 0.1053$ , where *A*<sub>p</sub> and *c* were apparent absorbance and concentration (mg/mL) [31]. The determination was performed in triplicate.

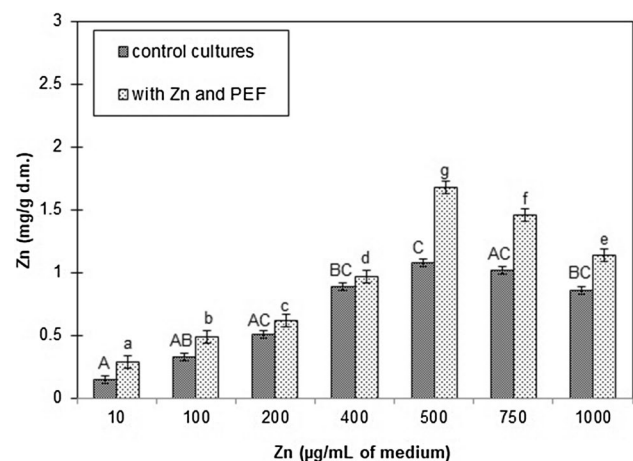
### Statistical analysis

Significant differences between particular groups were found using the Student *t* test applied to compare independent samples in pairs was used. Detailed analysis was based on Tukey's confidence intervals. The Pearson's correlation coefficients were used to evaluate linear relationships between variables. All statistical tests were carried out at significance level of  $\alpha = 0.05$ . Statistical processing of results was performed using R program version 3.1.2.

## Results and discussion

### Optimization of zinc concentration in culture medium and PEF parameters

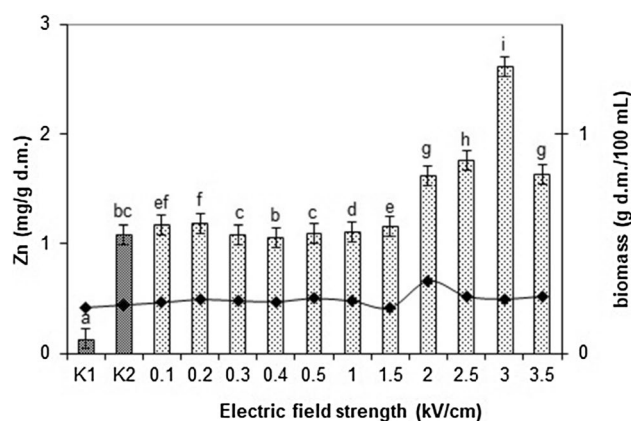
In the first stage of studies, concentration of zinc ions in the culture medium was optimized. The highest accumulation of this element (1.68 mg Zn/g d.m.) was found at 500  $\mu$ g Zn/mL medium (Fig. 1) and it was 55% higher than that noted for the sample supplemented with zinc with the same concentration but not subjected to electroporation.



**Fig. 1** Zinc concentration in the medium upon Zn<sup>2+</sup> accumulation in *L. rhamnosus* B 442 (electric field strength 2.0 kV/cm, pulse width 20  $\mu$ s, the field frequency of 1 Hz, and the time of exposure to PEF 50 min after 20 h culturing). Means with the same small or large letters are not highly significantly different ( $P < 0.05$ ;  $n = 14$ ). Control cultures absence of PEF and supplemented with ions

the whole range of Zn concentrations (10–1000  $\mu\text{g Zn/mL}$  medium) used in the experiment, higher zinc accumulation was observed in the PEF-treated cultures in comparison with the untreated ones. In the concentration range of 10–500  $\mu\text{g Zn/mL}$  medium, the strong positive correlation between accumulation of zinc ions and their concentration was observed for the electroporated cultures. However, when cultures were treated with PEF at 750  $\mu\text{g Zn/mL}$  medium and 1000  $\mu\text{g Zn/mL}$  medium, a statistically significant reduction in  $\text{Zn}^{2+}$  bioaccumulation was noted when compared to the sample electroporated at 500  $\mu\text{g Zn/mL}$  medium. Results presented in Table 1 demonstrate that the total number of microorganisms ranged from  $4.55 \cdot 10^8$  cfu/mL to  $1.13 \times 10^{11}$  cfu/mL and the lowest result was recorded for the culture supplemented with 400  $\mu\text{g Zn/mL}$  medium and subjected to electroporation. In the case of the samples not treated with PEF, the highest total number of microorganisms was found for the cultures supplemented with 1000  $\mu\text{g Zn/mL}$  medium. The biomass contents determined for both types of cultures, untreated and PEF-treated, were similar and ranged, respectively, from 0.268 to 0.304 g/g d.m. and from 0.264 to 0.323 g/g d.m.

Zinc concentration of 500  $\mu\text{g/mL}$  medium was found to be optimal for the highest accumulation of ions in *L. rhamnosus* B 442 cells and was used for subsequent experiments. In the next step, electric field strength was optimized. The studies showed that application of PEF at low field strength resulted in accumulation ranged from 1.06 to 1.17 mg Zn/g d.m. (Fig. 2). A significant increase in zinc bioaccumulation was noted when applied electric field strength ranged from 2.0 to 3.0 kV/cm. The highest amount of zinc (2.61 mg/g d.m.) was accumulated at 3.0 kV/cm and it was over 1.5 times higher than in the case of the control sample K2 (supplemented with zinc but not electroporated). The highest growth of biomass was determined for samples treated at 2.0 kV/cm (Fig. 2). At the other field strength values, the biomass ranged from



**Fig. 2** Accumulation of zinc in *L. rhamnosus* B 442 depends on the electric field strength (electric field strength between 0.1 and 3.5 kV/cm, pulse width 20  $\mu\text{s}$ , the field frequency of 1 Hz, the time of exposure to PEF 15 min after 20 h culturing, and zinc concentration in the culture medium 500  $\mu\text{g/mL}$ ). Means with the same letters are not highly significantly different ( $P < 0.05$ ;  $n = 13$ ). Control cultures K1: without zinc and absence of PEF; K2: with zinc (500  $\mu\text{g/mL}$ ) and absence of PEF

0.21 to 0.26 g/g d.m. The total number of microorganisms at 3.0 kV/cm did not show a statistically significant difference compared to the samples electroporated at: 0.1, 0.3, 0.4, 0.5, 1.0, 1.5, and 3.5 kV/cm, and the control samples K1 and K2 (Table 2). Therefore, the viability of the bacteria did not affect the accumulation of zinc in the cells. That is, the decrease in accumulation observed at the 3.5 kV/cm field intensity is the result either of a decrease in cell membrane permeability or its increase leading to the diffusion of ions from the cell into the medium.

Figure 3 illustrates an impact of electroporation time on zinc accumulation in the cells. The highest zinc bioaccumulation (2.64 mg Zn/g d.m.) was obtained after 15-min exposure (900 pulses) of culture to PEF (Fig. 3). Initially, the elongation of electroporation from 5 (300 pulses) to 15 min resulted in a gradual increase in zinc accumulation but further increasing of the exposure time to 20 (1200 pulses) and 25 min did not enhance this process or even caused its statistically significant decrease (compared to the 15 min exposure). Electroporation time did not affect significantly bacterial biomass. Its value ranged from 0.26 to 0.28 g/g d.m. and was higher than in the controls K1 or K2. The total number of microorganisms determined for the sample electroporated for 15 min was not significantly different from the samples treated for 5, 10, and 25 min and the controls K1 and K2 (Table 2). In our research, the effect of the number of pulses on permeability and thus on the increase of accumulation has the bell-shaped character, initially with the increase in the number of pulses the accumulation increases, and then, at 1200 pulses, we observe its decrease. Similar observations were reported by Mitsutake et al. [32, 33].

**Table 1** Effect of PEF on  $\text{Zn}^{2+}$  concentration in medium on total number of microorganisms and biomass of *Lactobacillus rhamnosus* B 442

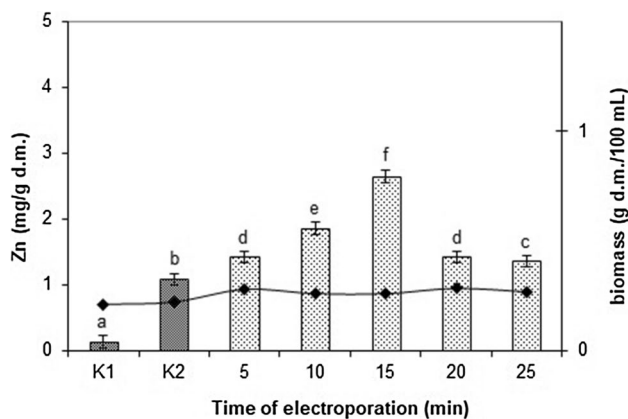
Concentration of zinc ( $\mu\text{g/mL}$ )	Total number of microorganisms (cfu/mL)		Biomass (g/g d.m.)	
	Without PEF	With PEF	Without PEF	With PEF
10	$3.90 \times 10^9$	$1.13 \times 10^{11}$	0.233	0.272
100	$2.09 \times 10^9$	$1.97 \times 10^9$	0.251	0.284
200	$1.00 \times 10^9$	$4.58 \times 10^9$	0.223	0.264
400	$6.64 \times 10^9$	$4.55 \times 10^8$	0.229	0.291
500	$1.51 \times 10^9$	$3.44 \times 10^9$	0.268	0.287
750	$1.62 \times 10^9$	$3.09 \times 10^{10}$	0.304	0.322
1000	$3.52 \times 10^{10}$	$2.25 \times 10^9$	0.284	0.323

**Table 2** Effect of electric field strength, time of exposure to PEF, pulse width and time after which cultures were treated with PEF on total number of microorganisms of *Lactobacillus rhamnosus* B 442

		Total number of microorganisms (cfu/mL)							
		At electric field strength		At various time of electroporation		At various pulse width		At various time of culturing when PEF was applied	
K1	$8.02 \times 10^9$ ab	K1	$9.55 \times 10^8$ a	K1	$2.55 \times 10^9$ ab	K1	$2.02 \times 10^9$ b		
K2	$3.91 \times 10^9$ a	K2	$9.09 \times 10^8$ a	K2	$1.50 \times 10^9$ a	K2	$5.11 \times 10^9$ d		
0.1	$3.97 \times 10^9$ a	5	$1.63 \times 10^9$ a	10	$2.71 \times 10^9$ ab	8	$9.09 \times 10^8$ a		
0.2	$4.15 \times 10^{10}$ c	10	$1.23 \times 10^9$ a	<b>20</b>	<b><math>8.73 \times 10^8</math> a</b>	12	$9.64 \times 10^8$ a		
0.3	$1.41 \times 10^{10}$ ab	<b>15</b>	<b><math>6.09 \times 10^8</math> a</b>	30	$1.59 \times 10^9$ a	16	$7.13 \times 10^9$ g		
0.4	$1.15 \times 10^9$ a	20	$1.40 \times 10^{11}$ b	40	$2.34 \times 10^9$ ab	<b>20</b>	<b><math>1.11 \times 10^{10}</math> h</b>		
0.5	$1.79 \times 10^{10}$ ac	25	$1.05 \times 10^9$ a	50	$6.36 \times 10^8$ ab	24	$6.79 \times 10^9$ f		
1.0	$7.64 \times 10^9$ ab			60	$4.86 \times 10^9$ b				
1.5	$2.64 \times 10^9$ a			75	$1.05 \times 10^9$ a				
2.0	$6.48 \times 10^{10}$ bc								
2.5	$2.80 \times 10^{11}$ d								
<b>3.0</b>	<b><math>6.53 \times 10^9</math> a</b>								
3.5	$3.64 \times 10^8$ a								

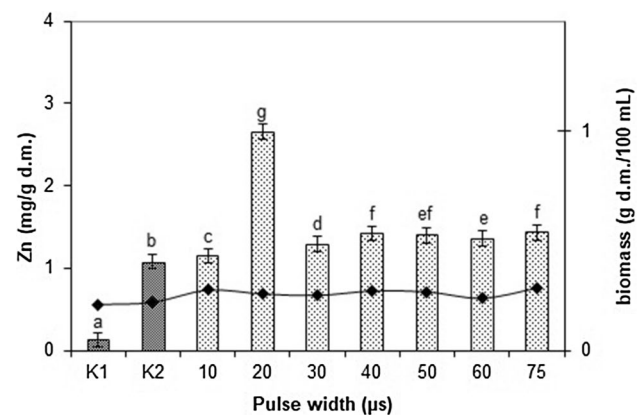
Control cultures: K1—without zinc and without PEF treatment; K2—with zinc (500 µg/mL) and without PEF treatment. Means with the same letters within a column do not differ significantly ( $P < 0.05$ )

Total number of microorganisms at optimal values of PEF parameters are in bold



**Fig. 3** Accumulation of zinc in *L. rhamnosus* B 442 cell biomass depends on the treatment time of exposure to PEF (the time of exposure to PEF between 5 and 25 min after 20 h culturing, pulse width 20 µs, electric field strength 3.0 kV/cm, at the field frequency of 1 Hz, and zinc concentration in the culture medium 500 µg/mL). Means with the same letters are not highly significantly different ( $P < 0.05$ ;  $n = 7$ ). Control cultures K1: without zinc and absence of PEF; K2: with zinc (500 µg/mL) and absence of PEF

Pulse width was another PEF parameter which was optimized. The highest zinc bioaccumulation (2.66 mg Zn/g d.m.) was obtained when cultures were treated with PEF at pulse width of 20 µs (Fig. 4). Application of pulse widths above 20 µs resulted in more than 1.5-fold decrease in zinc accumulation. Pulse width from 40 to 75 µs did not cause statistically significant changes in ion accumulation. No influence of this parameter on biomass and total number of microorganisms was observed. Results of biomass



**Fig. 4** Accumulation of zinc in *L. rhamnosus* B 442 cell biomass depends on the pulse width (pulse width between 10 and 75 µs, electric field strength 3.0 kV/cm, at the field frequency of 1 Hz, the time of exposure to PEF 15 min after 20 h culturing, and zinc concentration in the culture medium 500 µg/mL). Means with the same letters are not highly significantly different ( $P < 0.05$ ;  $n = 9$ ). Control cultures K1: without zinc and absence of PEF; K2: with zinc (500 µg/mL) and absence of PEF

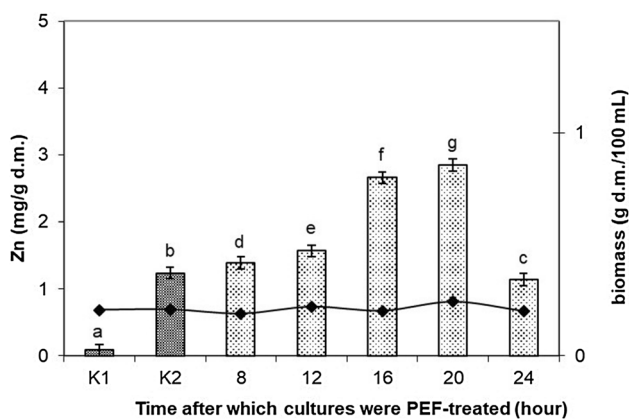
determination ranged from 0.24 to 0.28 g/g d.m. and they were higher than those obtained for the control samples K1 and K2.

In our study, the optimal pulse width for maximum accumulation of zinc ions was 20 µs. At pulse width, less than 10 µs smaller pores are formed and it is more difficult to penetrate molecules into the cell, while at pulse widths above 20 µs, it most likely that bigger pores arise and

facilitate the transfer of ions, but also diffusion of these ions from the cell to the medium may be much easier [34].

The time of culturing after which cells were treated with PEF was optimized in the final stage of the studies. It was found that this parameter significantly affected zinc accumulation in the cells of *L. rhamnosus* B 442. The highest accumulation of this element (2.85 mg Zn/g d.m.) was determined for the 20 h culture treated with PEF at field strength of 3.0 kV/cm and pulse width of 20  $\mu$ s for 15 min. (Fig. 5). The lowest amount of zinc ions was accumulated in the cells cultured for 24 h and then subjected to electroporation. Multiple exposition to PEF after 8, 12, 16, 20, and 24 h of culturing did not increase accumulation but even led to its decline by over 2.5 times in comparison with the culture treated once with PEF under optimal conditions. It was also examined whether supplementation of the culture with 0.2 of total Zn dose every 4 h will change bioaccumulation. The results showed that multiple treatment of culture with PEF and supplementation of zinc in doses did not enhance its bioaccumulation in the cells. Biomass of bacteria in the cultures treated with PEF at different times of their growth ranged from 0.19 to 0.24 g/g d.m. Time of culturing after which cells were electroporated affected significantly the total number of microorganisms. Its highest value was recorded for the culture incubated for 20 h. The addition of zinc ions did not cause a decrease in the total number of microorganisms in the samples electroporated at optimized PEF parameters after 16, 20, and 24 h of culturing (Table 2).

It is well known that zinc is an essential element for the growth and development of all living organisms. This metal is increasingly used to supplement various types of products.



**Fig. 5** Accumulation of zinc in *L. rhamnosus* B 442 cell biomass depends on time after which cultures were treated PEF (pulse width 20  $\mu$ s, electric field strength 3.0 kV/cm, at the field frequency of 1 Hz, the time of exposure to PEF 15 min after 8, 12, 16, 20, or 24 h culturing, and zinc concentration in the culture medium 500  $\mu$ g/mL). Means with the same letters are not highly significantly different ( $P < 0.05$ ;  $n = 7$ ). Control cultures K1: without zinc and absence of PEF; K2: with zinc (500  $\mu$ g/mL) and absence of PEF

Its addition to food products containing lactic acid bacteria does not inhibit the growth of microorganisms [35]. Gheisari et al. [36] observed that the total number of *Lactobacillus casei* in ice-cream enriched with zinc sulfate after a day of storage was 8.33 log cfu/g. Aquilanti et al. [35], who compared cheeses enriched with different zinc salts, reported that the total number of microorganisms was 7.0 log cfu/g in Squacquerone cheese with zinc sulfate, 7.2 log cfu/g in that with zinc gluconate and 7.8 log cfu/g in that with zinc aspartate. While in the similar samples of Caciotta cheese, the results were, respectively, 4.0 log cfu/g (zinc sulfate), 3.0 log cfu/g (zinc gluconate), and 3.2 log cfu/g (zinc aspartate).

Biosorption of zinc ions by different LAB species depends on properties of the bacteria (structure, functional groups, and surface area) and is a function of, among others, initial metal ion concentration. Mrvčić et al. [14] studied the accumulation of zinc ions by different species of LAB and they noted the lowest concentration of this element in *Lactobacillus plantarum* bacteria. The authors have shown (as in this publication) that ion accumulation was dependent on their concentration in the medium. Low concentration of zinc ions in medium (10 mg/L) resulted in their higher accumulation in cells. As the concentration of  $Zn^{2+}$  increased (up to 90 mg/L), its bioaccumulation decreased from 70 to 25% for *L. mesenteroides*, from 60 to 14% for *L. brevis*, and from 50 to 13% *L. plantarum*. The results of this study indicated that zinc supplementation of the PEF-treated cultures at a concentration range 10–500  $\mu$ g Zn/mL medium resulted in a gradual increase of bioaccumulation until its maximum value of 1.68 mg Zn/g d.m. was reached. However, when Zn concentration was above this range (750 and 1000  $\mu$ g/mL medium) its accumulation was clearly lower.

Exposure of biological membranes to a sufficiently high electric field causes a rapid and large increase of their permeability. This phenomenon, called membrane electroporation, can be either reversible or irreversible and is used to introduce various ions, molecules, or even macromolecules into cells [37]. Every cell type requires slightly different optimal process conditions that must be determined experimentally. Optimization of electric field strength, time of electroporation, and pulse width in the subsequent stages of this study showed that the highest zinc ion accumulation (2.66 mg Zn/g d.m.) was achieved when the following PEF parameters were applied: electric field strength 3.0 kV/cm, electroporation time 15 min, and pulse width 20  $\mu$ s. It was also shown that the time of culturing after which cells were treated with PEF was significant for bioaccumulation. The highest concentration of zinc was obtained by electroporation of bacteria after 20 h of culturing. Application of PEF with optimized parameters caused an increase in zinc ion accumulation (up to 2.85 mg Zn/g d.m.) in *L. rhamnosus* B 442 cells by 164% in comparison with the control sample K2 (supplemented with zinc but not treated with PEF).

Góral and Pankiewicz [38] who studied accumulation of magnesium in cells of the same strain of LAB reported that the highest amount of  $Mg^{2+}$  (2.63 mg/g d.m.) was bioaccumulated at field strength of 2.0 kV/cm. The remaining parameters of the process were the same as in this study, but level of accumulation depended on LAB species and it amounted to 4.28 mg  $Mg^{2+}$ /g d.m. for *L. rhamnosus* B 442, 1.97 mg  $Mg^{2+}$ /g d.m. for *L. rhamnosus* 1937, and 1.86 mg  $Mg^{2+}$ /g d.m. for *L. lactis* JBB 500. Pankiewicz and Jamroz [30] and Pankiewicz et al. [39] applied PEF for enhancing bioaccumulation of zinc in *Saccharomyces cerevisiae*. They also reported that optimal time of electroporation and pulse width for the highest accumulation was, respectively, 15 min and 20  $\mu$ s. This study showed that PEF treatment did not affect negatively the total number of microorganisms which oscillated between  $2.9 \cdot 10^9$  and  $1.29 \cdot 10^{11}$  cfu/mL. Seratlic et al. [40] also observed that electroporation of *L. plantarum* 564 cells at a field strength less than 13.6 kV/cm does not reduce the total number of microorganisms.

## Conclusions

To summarize, the study showed that PEF technology can be serve as an innovative method of bacteria enrichment with zinc ions. The use of PEF did not cause a decrease in the total number of microorganisms in the medium but led to higher bioaccumulation of zinc. Viability of bacteria at optimal PEF parameters was high which allows them to be used for production of food enriched with zinc of potentially high bioavailability.

## Compliance with ethical standards

**Conflict of interest** All authors declare that they have no conflicts of interest.

**Compliance with ethics requirements** This article does not contain any studies with human participants or animals performed by any of the authors.

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