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Effect of pulse electric fields (PEF) on accumulation of magnesium and zinc ions in *Saccharomyces cerevisiae* cells

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ABSTRACT

Cultures of *Saccharomyces cerevisiae* were treated with PEF to improve simultaneous accumulation of magnesium and zinc ions in the biomass.

The results showed that the ions concentration in the medium and their mutual interactions affect accumulation in cells. Increasing the concentration of one ion in the medium reduced the accumulation of the second one, in the control as well as in the cells treated with PEF. Under optimized conditions, that is, on 15 min exposure of the 20 h grown culture to PEF of 5.0 kV/cm and 20 μs pulse width, accumulation of magnesium and zinc in yeast biomass reached maximum levels of 2.85 and 11.41 mg/g d.m., respectively, To summarize, optimization of ion pair concentration and PEF parameters caused a 1.5 or 2-fold increase of magnesium and zinc accumulation, respectively, in *S. cerevisiae*.

1. Introduction

In recent years, magnesium and zinc insufficiency have been one of the most frequently diagnosed electrolytic disorders in humans. This is mainly due to the common consumption of food products deprived of a number of valuable bioelements. A common method preventing magnesium and zinc deficiency is pharmacological supplementation (Bernardo da Costa, Cornish, & Keasling, 2007; Liu, Martin, Gardner, & Ryan, 2002; MacDiarmid, Gaither, & Eide, 2000; Stehlik-Tomas, Zetic, Stanzer, Grba, & Vahcic, 2004). Preparations available on the market contain non-organic and organic salts of magnesium or zinc, e.g. chlorides, carbonates, oxides and lactates. Still, they are characterized by low bioavailability for humans. Many authors have demonstrated better availability of elements administered in the form of protein complexes (De Nicola & Walker, 2009). Such protein–mineral complexes (metalloproteins or bioplexes) are absorbed in the small intestine in a manner typical of amino acids, peptides and proteins, but not cations, which avoids the competition between trace elements for adsorption. One of the possibilities to obtain bioplexes is the supplementation of yeast biomass with magnesium and zinc, which is further used to produce protein–mineral preparations (De Nicola et al., 2007; Iwanyshyn, Han, & Carman, 2004). In bioplexes, organic links of metals may exhibit different characteristics, namely a complex of cations and amino acids, a complex of metal ion chelates and amino acids, metal albuminate and a complex of metal with polysaccharides. An additional benefit of yeasts as a potential source of bioplexes for human and animals is a short time of generation providing a high biomass yield as well as safety of the application of selected species belonging to *Saccharomyces cerevisiae* (Avery & Tobin, 1993; Cha & Cho, 2009; Vinopal, Ruml, & Kotrba, 2007). Yeast are known for their ability to accumulate metal ions from aqueous solutions by different physico-chemical interactions, e.g. by adsorption and absorption or by a metabolism-dependent mechanism. Absorption processes, during yeast growth, can be greatly affected by temperature, pH and by the presence of other metal ions (Blackwell, Singelton, & Tobin,1995; Blackwell, Tobin, & Avery,1998; Brady & Duncan, 1994b; Stehlik-Tomas et al., 2004). Accumulation of metal ions probably depends on intracellular transportation systems and on their chelating strength, by medium compounds and cellular substances. The majority of extracellular cobalt, manganese, zinc and magnesium is stored in vacuoles, where they can be bound by polyphosphates of a low molecular weigth (Błażejak, Duszkiewicz-Reinhard, Gniewosz, & Mazurkiewicz, 2004; Gadd, 1990; Williams & Frausto da Silva, 2000).

The goal of this work was to determine the ability of *S. cerevisiae* to accumulate magnesium ions in the presence of zinc (ion pair) under the conditions of pulsed electric fields (PEF). Electroporation is one of the methods used for introducing macromolecules or ions

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into cells. PEF-induced transmembrane tension facilitates the formation of pores in the membrane which leads to an increase in the cells' permeability (Aronsson, Rönner, & Borch, 2005; Marselles-Fontanet & Martin-Belloso, 2007; Sampedro, Rivas, Rodrigo, Martinez, & Rodrigo, 2007; Torregrosa, Esteve, Frigola, & Cortes, 2006; Weaver & Chizmadzhev, 1996; Zimmermann,1986). PEF is a suitable technique for element enrichment of *S. cerevisiae* (Pankiewicz & Jamroz, 2007; Pankiewicz & Jamroz, 2010; Pankiewicz & Jamroz, 2011).

2. Materials and methods

2.1. Culture maintenence and inoculum preparation

S. cerevisiae 11 B_1 (industrial strain) from the Yeast Plant (Lublin, Poland) was used. Medium for agar slants and inoculum growth contained (g/l), sucrose (20), NH_4Cl (3.2), KH_2PO_4 (2.5), Na_2SO_4 (2.0), $MgCl_2{\cdot}6H_2O$ (1.5) (POCH, Gliwice, Poland), yeast extract (YE) (5.0), agar (15) (DIFCO, Detroit, MI, USA), and unhoped wort (40.0 ml) (Lublin Breweries S.A., Lublin, Poland) at pH 5. Experimental medium for *S. cerevisiae* was composed according to Blackwell, Tobin, and Avery (1997) and contained (g/l): peptone (10) (Sigma–Aldrich CO, St. Louis, MI, USA), YE (5) and glucose (10) (POCH, Gliwice, Poland).

2.2. Biomass cultivation

Biomass cultivation was carried out according to the procedure described by Pankiewicz and Jamroz (2011). The source of metal ions was aqueous solutions of pure analytical grade salts of $MgCl_2$ and $ZnSO_4$.

2.3. Optimization of magnesium and zinc concentrations in the medium to maximize their accumulation in S. cerevisiae

Zinc and magnesium concentrations in the culture medium were set during culturing at a constant Mg^{2+} concentration (100 μg/ml) and increasing Zn^{2+} concentration (10–50 μg/ml) and, in the second configuration, at constant Zn^{2+} concentration (100 μg/ml) and increasing Mg^{2+} concentration (10–500 μg/ml). In the following experiment, cultures were treated with PEF in order to obtain higher ion accumulation in the cells. The cultures with constant Mg^{2+} concentration in the medium were treated with PEF (ECM 830 electroporator, BTX Harvard Apparatus, USA) at optimum parameters (4 kV/cm, 15 min, 20 μs, after 20 h of culturing) for the maximum accumulation of magnesium itself (Pankiewicz & Jamroz, 2010).

The PEF treatment chamber consisted of four parallel plexiglas plates which had stainless steel electrodes of an area equal to 4 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 3.9 and 7.36 mS/cm. After optimization of both ion concentrations in the medium, optimal PEF parameters for the maximum simultaneous accumulation of both ions were set. Simultaneously, voltage was optimized on the field exposition 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 kV/cm. During PEF parameter optimizations, control cultures were prepared as K1: without zinc and magnesium added to medium and without PEF treatment and K2: with zinc (150 μg/ml) and magnesium (100 μg/ml) added to medium and without PEF.

At a previously fixed optimal electric field strength, the pulse width was optimized. PEF pulse width at 10 and 20 μs was selected at 5 kV/cm, and 10, 20, 30 and 40 μs was selected at 4 kV/cm and the 15 min exposition, after 20 h of culturing, at the field frequency of 1 Hz.

Cells biomass from a 42-h culture with Mg^{2+} and Zn^{2+} concentrations of 100 μg/ml and 150 μg/ml, respectively, was treated with PEF after 20 h of culturing at an optimum electric field strength of 5 kV/cm, optimum pulse width of 20 μs and field frequency of 1 Hz. These conditions were then used to optimize the field exposure time for 5, 10, 15, 20 and 25 min.

Optimization of the time interval after which yeast cells were treated with PEF was performed after 8, 12, 16, 20 and 24 h of cultivation, at an optimum 5 kV/cm and 20 μs pulse width and a fixed optimal concentration of 100 μg Mg^{2+}/ml and 150 μg Zn^{2+}/ml.

At optimum PEF parameters and optimum magnesium and zinc concentrations, the medium was enriched with subsequent 1/4 doses of magnesium and zinc after 8, 12, 16 and 20 h of culturing. Subsequent culturing was performed under optimized conditions and these cultures were treated with PEF four times after 8, 12, 16 and 20 h. The results revealed the role of enrichment with magnesium and zinc and the number of expositions to PEF upon the magnesium and zinc accumulation in the yeast cells.

2.4. Determination of the magnesium and zinc concentration

Mineralization of yeast for the determination of the magnesium and zinc concentrations using the technique of flame atomic absorption spectrophotometry (F-AAS, VARIAN AA 280 FS) was carried out according to the procedure described by Pankiewicz and Jamroz (2011).

2.5. Determination of the yeast cell viability

Cell viability was determined in a Thoma chamber, dyeing necrotic yeast cells with a 0.01% methylene blue solution. The percentage of necrotic cells was the mean of 16 fields calculated according to the formula: % necrotic cells = (number of necrotic cells/sum of necrotic and living cells) × 100 (Pankiewicz & Jamroz, 2010).

2.6. Determination of the crop of yeast biomass

Biomass was estimated from optical density at 400 nm. Dry mass was calculated by referring to a standard curve of cell mass vs. absorbance. The fermented medium from culturing (2 ml) was centrifuged (3000 rpm), the supernatant discarded, the cells rinsed with deionized water and brought to the original volume of 2 ml. Nephelometric measurements were run against pure water in a 2 mm measurement cell. The amount of dry residue was calculated using the equation for the standard curve: $Ap = 0.4476c$, where Ap and c were apparent absorbance and concentration (mg/ml), respectively (Pankiewicz & Jamroz, 2010).

2.7. Preparation procedure for electron microscopy examinations in a transmission electron microscope (TEM–EDS)

For examinations in a transmission electron microscope, *S. cerevisiae* yeast was used that originated from a control culture: K1 – without zinc and magnesium added to medium and without PEF treatment and K2 – with zinc and magnesium added to medium and without PEF; as well as yeast from a culture treated with PEF at the optimized conditions: electric field strength 5.0 kV/cm, pulse width 20 μs, at the field frequency of 1 Hz, the time of exposure to PEF 15 min, after 20 h culturing (O).

Samples for the examinations were prepared as follows:

The yeast was fixated with a 4% solution of glutaraldehyde and washed with phosphate buffer. The preparations were subjected to contrast enhancement in a 1.5% solution of OsO_4 in 0.1 M