Annals of Microbiology, **58 (2)** 239-243 (2008)

Accumulation of selenium and catalase activity changes in the cells of *Saccharomyces cerevisiae* on pulsed electric field (PEF) treatment

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Received 11 December 2007 / Accepted 3 April 2008

Abstract - The aim of this work is the evaluation of selenium accumulation and catalase activity in pulsed electric field (PEF) treated *Saccharomyces cerevisiae* cells. Cultures of *S. cerevisiae* in the medium containing selenium as sodium selenite were treated with PEF. Applied range of field parameters (field frequency of 1 Hz, intensity of 1.5 kV/cm and pulse duration of 1 ms) does not influence the changes of catalase activity which depend only on the concentration of selenium accumulated in the yeast cells. It was noted for the non-PEF-treated cultures that the accumulation of selenium in the yeast cells increased to 60 µg/g dry mass (DM) together with the increase of selenium concentration in medium from 1 to 3 µg/mL. Consequently, the activity of extra- and intra-cellular catalase also increased. At higher selenium concentrations in medium (4-6 ug/mL) its accumulation in the cells reached 127 µg/g DM which resulted in the decrease of catalase activity. In the PEF-treated cultures, twofold higher accumulation of selenium in the cells was obtained in the whole range of concentrations applied, which caused the significant decrease of catalase activity.

Key words: selenium, *Saccharomyces cerevisiae*, pulse electric field, catalase.

INTRODUCTION

Selenium deficit in soil results in a decrease of its content in plants. Areas poor in selenium can be found in Australia, north-eastern and central China, in the northern regions of North Korea, in Nepal, Tibet and in Central Africa (Oldfield, 1999; Combs, 2001). In Poland the problem of selenium fertilisation of soil, especially acid soils, is addressed more and more frequently. The World Health Organisation recommends diurnal selenium doses from 30 µg to 40 µg /individual (EC Scientific Committee on Food, 2003; Thomson, 2004). However, even higher doses have a beneficial effect on the immune system of the organism, displaying an antineoplastic effect and alleviating the symptoms of HIV. Toxic effects of selenium appear at doses from 350 to 700 µg/day (Burbano *et al*., 2002; Rayman, 2002; Tapiero *et al*., 2003).

Selenium deficit affects glutathione metabolism by increasing its synthesis and release in the liver with simultaneous increase of GSH level in the plasma. Increase in the plasma GSH may lead to reduced cysteine levels and disturbed protein synthesis. Selenium deficit is also accompanied by reduced activity of glutathione peroxidase, with simultaneous increase in GST S-glutathione transferase activity (Hill *et al*., 1987). It is now known that selenium deficit causes a reduction in the concentration of cysteine, cystationine and homocysteine in the plasma, which may lead to disturbed synthesis of methionine and increased aggregation of blood pellets and weakening of the immune system. The condition of selenium deficit is manifested by increased toxicity of medicines and/or their weakened effectiveness. Selenium deficit is also related with reduced activity of isozymes and cytochrome P-450, and at increased activity of UDP-glucuronyltransferase (Beck *et al*., 1995; Davis and Uthus, 2002).

As opposed to other microelements, selenium is characterised by a narrow spread between the therapeutic and the toxic doses (Graczyk *et al*., 1994; Ponce de Leon *et al*., 2002). One of the methods of reducing the risk of selenium intoxication at diet supplementation with selenium preparations is to build the element into cells of *S. cerevisiae*. Replacement of sulphur with selenium in yeast protein is about 50% more efficient compared to sele-

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nium from plant sources (Gilon *et al*., 1996; Hammel *et al*., 1997; Whanger, 2004).

Cells of *S. cerevisiae* were enriched in selenium through the use of pulse electric field (PEF) (Pankiewicz and Jamroz, 2007). Electroporation is an easy and non-toxic method of macromolecule insertion in cells. In a cell subjected to the effect of PEF induced trans-membrane tension facilitates the formation of pores in the membrane and leads to an increase in its permeability (Hülsheger *et al*., 1983; Zhang *et al.*, 1994; Evrendilek *et al*., 1999; Wouters *et al*., 1999; Aronsson *et al*., 2005). Electroporation consists in temporary elimination of the integrity of the plasmatic membrane, i.e. in creating in it pores that are small enough to be able to close again after a certain period of time (Tsong, 1990; Ho and Mittal, 1996; Weaver and Chizmadzhev, 1996; Barbosa-Canovas *et al*., 1999; Aronsson and Rönner, 2001; Aronsson *et al*., 2005).

Yeast from the genus *Saccharomyces, Schizosaccharomyces* and *Candida* are an attractive source of catalase. They produce both intra- and extracellular catalase whose basic function is to decompose hydrogen peroxide (Schellhorn, 1995; Sekhar *et al*., 1999; Bukowska *et al*., 2000). Catalase is a regulator of the level of H_20_2 in organelles and functions as a specific peroxidase. In industrial processes it is used for the degradation of H_20_2, e.g. after cold pasteurisation of milk, or co-acts with glucose oxidase in removing glucose from the protein mass of eggs and in the production of gluconic acid. Isolation of the enzyme from microbial biomass or from animal organisms and its purification is very costly. The use of isolated catalase is impractical due to its rapid inactivation by H_20_2. Therefore, whole cells are used as a source of the enzymes. The main problem in their employment for the purpose is the penetration of H_20_2 through the barrier of cell wall/membrane (Hussain *et al*., 1995).

In view of the above, it appeared worthwhile to undertake a study aimed at demonstrating the effect of PEF on selenium accumulation and catalase activity in cells of *S. cerevisiae.*

MATERIALS AND METHODS

The yeast used in the study was *Saccharomyces cerevisiae* 11 B_1 originating from the yeast factory at Kunickiego Street in Lublin.

Yeast cultures were established on optimised liquid medium with the following composition (g/L): glucose (70.0), NH_4Cl (7.5), KH_2PO_4 (2.5), $MgCl_2{\cdot}6H_2O$ (2.0), Na_2SO_4 (2.0), yeast extract (YE) (5.0), and 40 mL of unhopped wort. The yeast originated from a 36-hour culture on a medium with pH 5. Shaken cultures of yeast were grown at selenium concentration in the medium (µg/mL) of 1, 2, 3, 4, 6, 8, in the form of sodium selenite. Selenium was added in five portions in the course of the culturing. In another research task, a yeast cell culture was treated with PEF at selenium concentrations of 1, 2, 3, 4, 6 µg/mL of medium after 20 h of cultivation. Electroporation of cell membranes was conducted for 15 min of field frequency of 1 Hz, intensity of 1.5 kV/cm and pulse duration of 1 ms.

When the culturing was over the mycelium was centrifuged, rinsed several times with distilled water, and then dried in a Labconco freeze-dryer (Model 64132, Kansas City, MO, USA).

Determination of selenium concentration. Selenium was determined with the method of atomic absorption spectrophotometry, using the flameless technique employing a graphite cuvette in the Varian Spectra AA-880 apparatus (Pankiewicz and Jamroz, 2007).

Determination of yeast cell viability. Cell viability was determined in the Thoma chamber, dyeing dead yeast cells with 0.01% solution of methylene blue. The percentage of dead cells was the mean of 16 fields calculated according to the formula: % of dead cells = (number of dead cells/sum of dead and living cells) x 100. Living cells was also determinated by counting of colonies grown on the complet medium (malt agar).

Scanning electron microscope (SEM) examination. To confirm the effect of PEF on *S. cerevisiae* images of electroporated and non-electroporated cells were taken by means of the TESLA BS-3000 scanning electron microscope. Specimens were fixed with 40% glutaraldehyde, and then dehydrated in alcohol solutions. Prepared specimens were sprayed with gold using the CS-100 spufron sprayer.

Determination of intra- and extra-cellular activity of catalase. Catalase activity was determined following the procedure of Fiedurek and Gromada (1997). Intra-cellular catalase was extracted several timed from homogenized mass of disintegrated cells using 0.1 M Mc Ilvane buffer with pH 7.0. Enzymatic activity was determined in the filtrates under standard conditions. The unit of catalase activity was determined as the number of µmoles of hydrogen peroxide decomposed by 1 mL of post-culturing fluid during 30 min.

RESULTS AND DISCUSSION

The study showed that selenium concentration in the culturing medium had a significant effect on its accumulation in cells of *S. cerevisiae* (Fig. 1).

At the optimum concentration of selenium in the medium, of 4 µg/mL, its maximum accumulation was recorded, at about 127 µg/g DM. Further increase in selenium concentration did not have any significant effect on its accumulation, and even caused a drop by approx. 9% (Fig. 1).

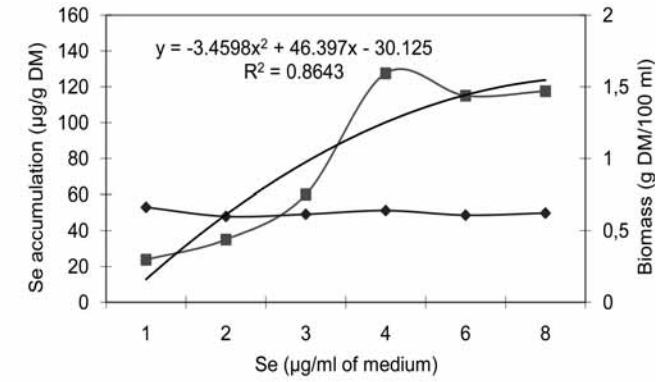

FIG. 1 - Effect of selenium (Se) concentration in nutrient medium on selenium accumulation in cells of yeast. ■: selenium accumulation, ✦: biomass.

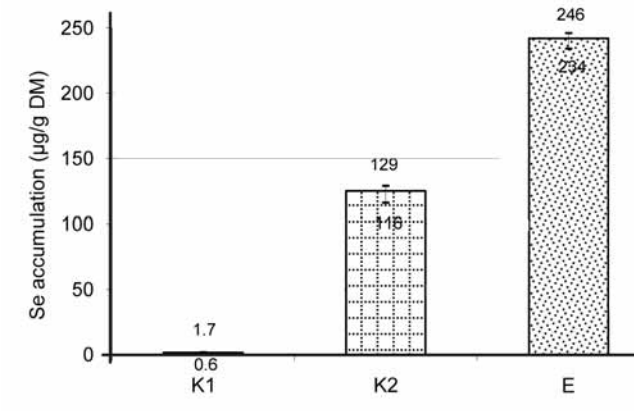

FIG. 2 - Effect of pulsed electric field (PEF) on selenium (Se) accumulation in cells of *Saccharomyces cerevisiae*. Control cultures: K1 (no Se and no PEF) and K2 (with Se and no PEF); E: with Se and 15-minute PEF exposure after 20 h of culturing.

Subsequent tests were performed to determine the viability of yeast cells. At selenium concentration from 2 to 4 µg/µL, the number of dead cells was observed to double. Further increase in selenium concentration did not have any significant effect on yeast cells viability. The density of cells treated with PEF was 2.3 x 10^8 CFU/mL.

In subsequent experiments, 20-hour cultures were treated with 15-minute pulse electric field to increase selenium accumulation in yeast cells. The highest accumulation (approx. 240 µg/g DM) was observed in cells from the culture with selenium concentration of 4 µg/mL of medium. Within the full range of applied selenium concentrations the application of PEF resulted in a doubling of selenium accumulation with relation to the culture without PEF (Fig. 2).

In order to confirm the effect of PEF on *S. cerevisiae*, images of electroporated cells were taken by SEM. In Fig. 3B one can observe numerous pores in the yeast cells, a phenomenon that is absent in the

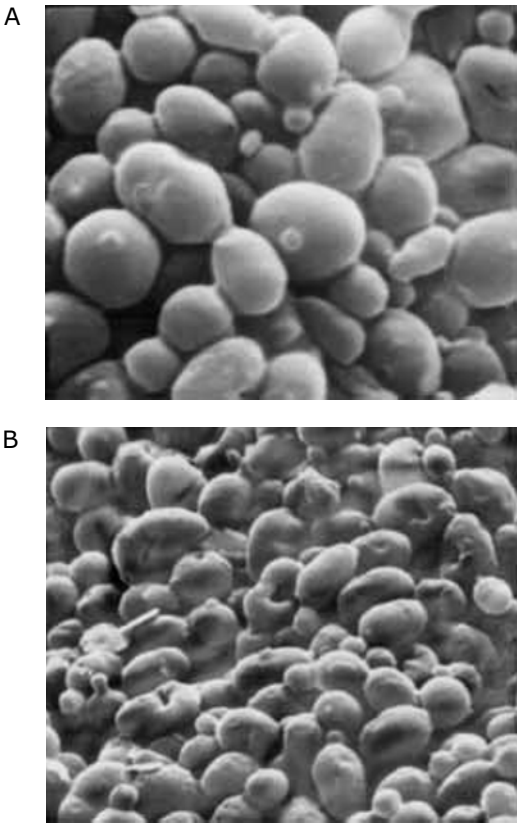

FIG. 3 - SEM photographs of cells of *Saccharomyces cerevisiae* from a 36-h culture.
A: control without PEF treatment, rate of magnifications applied, x 9300; B: treated with PEF, rate of magnifications applied, x 5500.

image of the control culture (Fig. 3A). The pores were formed due to the cells treatment with PEF, as that was the only parameter that differentiated the two cultures.

To determine the effect of selenium concentration in cells of *S. cerevisiae* on catalase activity, the post-culturing fluid was sampled or catalase was extracted from yeast cells. Determination of catalase activity, both intra- and extra-cellular, was conducted concurrently with estimation of selenium accumulation under the effect of PEF. Table 1 presents changes in catalase activity at various selenium concentrations in the culturing medium.
In the control samples without selenium, catalase activity in yeast originating from PEF-treated and non-PEF-treated cultures does not differ statistically, which means that the applied range of field parameters had no effect on the level of catalase activity. The sole parameter determining the activity of the enzyme is then selenium concentration.

Electric field may have an effect on the activity of certain enzymes. A high-intensity field usually causes partial or complete inactivation of enzymes. The activity of plasmin, an enzyme from cow milk, suspended in simulated ultrafiltrate of milk, was reduced by 90% after the application of 50 pulses of

45 kV cm^{-1} (Vega-Mercado *et al*., 1995). The activity of pepsin in a water solution decreased by 51.7 to 83.8% after PEF treatment for 126 μs at field intensities of 37.0 and 41.8 kV cm^{-1}, while the activity of lysozyme varied only slightly within the range of 0 to 38 kV cm^{-1}. On the other hand, a weak electric field may have a stimulating effect on enzymatic activity. A field of 20 V cm^{-1}, 300-1000 Hz, causes stimulation of Na^+, K^+-ATP-ase isolated from human erythrocytes (Tsong, 1992).

In non-PEF-treated culture, together with an increase of selenium concentration in medium from 1 to 3 μg/mL, it was noted that accumulation of selenium in the yeast cells increased to 60 μg/g DM. This affected the increase of extra- and intra-cellular catalase activity. However, at selenium concentrations higher than 3 μg/mL and its accumulation in the cells on the level of 127 μg/g DM, the decrease of catalase activity was observed. In the whole range of selenium concentrations applied, the higher activity of extracellular catalase was recorded in comparison with intra-cellular catalase (Table 1).

Within the full range of applied selenium concentrations, the decrease of extra- and intra-cellular catalase activity took place in the PEF-treated medium with twofold higher accumulation of selenium in the cells (Fig. 2). The activities of both extra- and intra-cellular catalase were in the range of 1.6-2.2 U/mL and 0.5-0.95 U/mL, respectively (Table 1).

Studies on the influence of selenium on enzymes activity show that it participates in protein structure as well as activates or inhibits their action by affecting the enzyme biosynthesis.

Well-defined relation between the increase of selenium accumulation in the cells and the decrease of catalase activity was noticed. On the other hand, at the lower selenium accumulation the higher activity of this enzyme was observed.

The activity of certain enzymes of the glycolytic path and the respiratory duct decreases in the presence of selenium, which is probably due to blocking of SH groups. A decrease on activity was demonstrated with relation to succinate dehydrogenase, cytochrome oxidase and arginase – enzymes containing active SH groups (Rotruck *et al*., 1973).

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TABLE 1 - Effect of selenium concentration in nutrient medium on the extra- and intra-cellular catalase activity in *Saccharomyces cerevisiae*

Selenium concentration (μg/mL)	Selenium ccumulation in yeast cells (μg/g DM)		Catalase activity (U/mL)			
			Extra-cellular		Intra-cellular	
	With PEF	No PEF	With PEF	No PEF	With PEF	No PEF
0	1	1	3.09 ± 0.15	3.15 ± 0.13	0.769 ± 0.09	0.77 ± 0.13
1	48	25	2.21 ± 0.18	2.71 ± 0.18	0.95 ± 0.12	0.66 ± 0.06
2	73	35	1.96 ± 0.14	2.67 ± 0.11	0.86 ± 0.09	0.76 ± 0.09
3	115	60	2.10 ± 0.15	3.15 ± 0.16	0.72 ± 0.08	1.094 ± 0.11
4	246	127	1.91 ± 0.13	2.77 ± 0.14	0.70 ± 0.11	0.87 ± 0.06
6	208	116	1.64 ± 0.11	2.21 ± 0.11	0.51 ± 0.11	0.78 ± 0.12

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