Effect of selenium on growth and antioxidative system of yeast cells

Marek Kieliszek¹ · Stanisław Błażejczak¹ · Anna Bzducha-Wróbel¹ · Anna M. Kot¹

Received: 18 November 2018 / Accepted: 19 January 2019 / Published online: 7 February 2019
© Springer Nature B.V. 2019

Abstract

Selenium exhibits health-promoting properties in humans and animals. Therefore, the development of selenium-enriched dietary supplements has been growing worldwide. However, it may also exhibit toxicity at higher concentrations, causing increased oxidative stress. Different species of yeasts may exhibit different tolerances toward selenium. Therefore, in this study, we aimed to determine the effect of selenium on growth and on the antioxidative system in *Candida utilis* ATCC 9950 and *Saccharomyces cerevisiae* ATCC MYA-2200 yeast cells. The results of this study have demonstrated that high doses of selenium causes oxidative stress in yeasts, thereby increasing the process of lipid peroxidation. In addition, we obtained an increased level of GSSG from aqueous solutions of yeast biomass grown with selenium supplementation (40–60 mg/L). Increased levels of selenium in aqueous solutions resulted in an increase in the activity of antioxidant enzymes, including glutathione peroxidase and glutathione reductase. These results should encourage future research on the possibility of a thorough understanding of antioxidant system functioning in yeast cells.

Keywords Selenium · Yeast, Enzymes, Antioxidative system · Glutathione

Introduction

Selenium is a biologically important element which is responsible for the proper functioning of many physiological processes. It is considered an essential trace element that performs many functions in the body of humans and animals [1, 2]. At cellular level, selenium facilitates the regeneration of cells, inhibits the proliferation of cancer cells, and has a positive effect on the functioning of the circulatory system [3]. It is a well-known component of many enzymatic proteins (selenoproteins), including glutathione peroxidase (GPx). GPx is one of the important enzymes that neutralize the effect of reactive oxygen species (ROS) and phospholipid hydroperoxides, and it maintains oxidation–reduction (redox) balance in the cells [4]. Selenium at appropriate concentrations exhibits antioxidant and regulatory properties [5] and is considered to be a potential therapeutic agent [6]. In addition, in humans, 25 genes encoding selenoproteins

that are enzymes or structural proteins have been discovered, whereas in yeasts, 12 such genes have been identified, which indicates a new role of selenium in the metabolism of eukaryotic cells [7].

At trace concentrations, selenium is considered an essential micronutrient, but in excessive levels, it can lead to selenosis [8]. Selenium is naturally found in soil and in water and in this way, it enters the food chain. The primary recipients of selenium from the soil are plants, which form the primary source of selenium in the diet of humans and animals. Differences in the content and availability of selenium in various regions of the world causes differences in the occurrence of its deficit in the diet of humans and animals. In humans, selenium deficiency inhibits the biosynthesis of selenoproteins, which in turn, affects the decreased activity of antioxidant enzymes. Selenium has a narrow range between the necessary and toxic dose for different organisms. The acceptable daily intake of selenium for an adult human body range from 50 to 70 µg/day [9, 10].

A low selenium content in soil makes it necessary to supplement it in food products. An increase in the knowledge regarding the antioxidant properties of selenium compounds has resulted in an increase in the development of selenium-enriched dietary supplements. These supplements are aimed to reduce the shortage of selenium intake in humans and

✉ Marek Kieliszek
marek-kieliszek@wp.pl; marek_kieliszek@sggw.pl

¹ Faculty of Food Sciences, Department of Biotechnology, Microbiology and Food Evaluation, Warsaw University of Life Sciences—SGGW, Nowoursynowska 159 C, 02-776 Warsaw, Poland

animals. The use of yeast biomass opens up the possibility of obtaining selenium-enriched supplements predominantly in the organic form.

Yeasts are characterized by high yield of biomass and relatively high capacity of accumulation of selenium, even when waste products from various sectors of the agri-food industry are used as the components of the culture medium (e.g., potato wastewater and glycerol) [11]. Many selenium compounds have a significant effect on cell viability, protein synthesis, and DNA integrity. Determination of optimal conditions for the production of yeast biomass enriched with organic selenium is a challenging problem faced by the food and pharmaceutical industries [12]. This is the reason why adaptation of yeast cells to stress conditions is an important topic of research. It is hypothesized that the binding process of selenium not only occurs on the surface of yeast cells but also occurs inside the cells.

Excessive intracellular accumulation of selenium is toxic to most microorganisms. At higher concentrations, it is transformed into a prooxidant, which is characterized by high cytotoxic activity [6]. Separation of selenium ions protects yeast cells against their toxic effects. However, the biotransformation of selenium into selenodiglutathione (GS–Se–SG) inside the vacuole does not contribute toward the reduction of its toxicity. Large quantities of volatile selenium are formed as a result of participation of glutathione in the detoxification process of yeast cells. This volatile selenium penetrates the vacuolar membrane and returns to the cytosol, posing a threat to the entire yeast cell [13]. This process may initiate the inappropriate incorporation of selenocysteine (SeCys) and selenomethionine (SeMet) into proteins, as well as impair their function, which may in turn lead to the development of oxidative stress, i.e., a state in which the oxidizing potential increases to the level that threatens the stability of cellular structures [14]. The consequence of this phenomenon is an imbalance in the metabolism. The processes that affect the disorganization of the cell membrane as well as the degradation of intracellular organelles are intensified, which consequently increases the oxidative stress [15]. Oxidative stress is defined as an imbalance between the prooxidative and antioxidative state, in which the processes associated with oxidation reactions are superior. In the absence of efficient repair mechanisms, cell membranes in yeast cells are oxidized, the structure and function of proteins are modified, and DNA is damaged [16]. An excessive production of oxygen radicals in the cell may also cause morphological disturbances.

Yeasts have a set of enzymatic and nonenzymatic defense systems to protect intracellular components from oxidative stress [17]. Enzymatic mechanisms include low molecular weight antioxidants and specialized enzymatic proteins that directly participate in the removal of ROS [18]. The antioxidant defense system takes place in several stages. In the

first stage, the formation of free radicals is hindered by the maintenance of appropriate redox potential in the cells, in which antioxidant enzymes play an important role. The next stage is neutralizing the effects of ROS and reconstruction of the proper structure of individual organelles. Despite a large number of studies, the mechanism of toxicity of selenium has not been fully understood, and the current level of knowledge indicates that the toxicity of selenium may be the result of various mechanisms.

Therefore, in this study, we aimed to determine the effect of selenium on the growth of yeast cells in media consisting of potato wastewater and glycerol. In parallel, the effect of selenium in aqueous solutions on the antioxidant system of *Candida utilis* ATCC 9950 and *Saccharomyces cerevisiae* ATCC MYA-2200 yeast cells was verified. Due to the products of glutathione and antioxidant enzymes transformation during the maintenance of lively active yeast biomass in aqueous solutions supplemented with selenium, their activity was determined. Changes in the enzymatic activity might help to understand and recognize the mechanisms of adaptation of microorganisms toward adverse conditions (presence of excess selenium). Studies regarding the tolerance of various yeast species toward selenium are important in case of industrial production of microorganisms as dietary supplements enriched with selenium.

Materials and methods

Biological material

In this study, we used *Saccharomyces cerevisiae* ATCC MYA-2200 and *C. utilis* ATCC 9950 yeast strains obtained from the Museum of Pure Cultures of the Department of Biotechnology and Food Microbiology of SGGW. The microorganisms were stored at 4 °C on solid YPD medium and transferred every 4 weeks to fresh medium.

Culture media

The yeast strains were stored on solid YPD medium (BTL, Poland) containing 2% glucose, 2% peptone, and 1% yeast extract. Inoculation and yeast propagation were performed in a medium containing potato wastewater enriched with glycerol (Avantor Performance Materials, Poland) in an amount of 5% (w/v). The pH of all the media was set to 5. We obtained potato wastewater from the potato processing plant PEPEES SA in Łomża (Poland) after the thermal-acid coagulation process. It was heated at 117 °C for 10 min (HMC Autoclave HG80, Germany) to precipitate the proteins contained therein. The resulting protein precipitates were separated from the medium by filtration and centrifugation methods (4000 × g, 20 min, Centrifuge 5804R

Eppendorf, Germany). The so-obtained potato wastewater was sterilized (121 °C, 20 min, HMC Autoclave HG80, Germany) and stored at a room temperature until use.

Dry substance in the deproteinated wastewater was found to be around 3.21 g/100 mL. It contained about 0.28 g of directly reducing substances and 0.9 g of total protein that could be an assimilable source of carbon and nitrogen for the yeasts [2, 19]. Glycerol was added as an additional source of carbon for the yeast cells.

Preparation of aqueous solutions of selenium (IV)

The working solution of selenium was prepared by dissolving 0.219 g Na_2SeO_3 (Sigma-Aldrich, Poland) in 100 mL deionized water (concentration 1000 mg Se (IV)/L). Aqueous solutions of selenium were prepared so that the final amount of selenium in 100 mL was in the range of 10–60 mg/L. The pH of aqueous solutions of selenium was set at 5.0. All reagents were sterilized at 121 °C for 20 min (HMC Autoclave HG80, Germany).

Preparation of yeast inoculum

The yeast inoculum was prepared in a liquid medium containing potato wastewater and 5% glycerol in a total volume of 100 mL. The medium was inoculated with a 24 h culture of *S. cerevisiae* ATCC MYA-2200 or *C. utilis* ATCC 9950 yeasts taken from the slant. The cultures were grown at 28 °C on a reciprocating shaker (SM-30 Control E. Büchler, Germany) at a vibration amplitude of 200 cycles/min. Inoculation cultures were maintained until the end of the logarithmic growth phase (24 h), i.e., obtaining the largest number of yeast cells. The resulting inoculum was used in the inoculation of control and experimental media with an addition of selenium (10–60 mg Se^{4+} /L) or aqueous solutions of selenium salts at the same concentrations.

Culturing yeasts in aqueous solutions supplemented with selenium

Culture media were inoculated with a 10% (v/v) suspension of yeast cells propagated in an inoculum culture ($5.0\text{--}8.0 \times 10^8$ cfu/mL). The cultures were grown under the same conditions as inoculation cultures (24 h, 28 °C).

The yeast cell biomass was obtained by centrifugation of culture medium ($3000 \times g$, 10 min, +4 °C, Centrifuge 5804R Eppendorf, Germany) containing potato wastewater enriched with glycerol. The pellet containing the yeast biomass was rinsed twice with sterile distilled water and then used for inoculation (in the amount of 10 g wet biomass/L ($2.5 \text{ g/g}_{\text{d.w.}}$)) of control aqueous and experimental solutions enriched with selenium. The cultures were grown under the same conditions as in case of the inoculum medium (Fig. 1).

Fig. 1 Scheme of the study

Determination of optical density (OD)

OD was recorded in order to determine the effect of selenium on the growth of yeast cells in media consisting of potato wastewater and glycerol. Briefly, 270 μL model media (potato wastewater with glycerol) and experimental media (with the addition of Se^{4+} at a concentration of 10–60 mg/L) and 30 μL of yeast from an inoculum culture were introduced to all wells of the Bioscreen C apparatus plate (Oy Growth Curves Ab Ltd., Finland). Control samples (without the addition of biological material) were prepared simultaneously. The microcultures were run for 35 h at 28 °C with continuous shaking. Measurement and registration of change in OD was performed automatically using a broadband filter at a wavelength of 420–580 nm.

Preparation of cellular extract and determination of antioxidant enzyme activity

After 24 h incubation of yeast in selenium-enriched aqueous solutions, the cell suspension was centrifuged ($3000 \times g$, 10 min, 4 °C) using a 5804R centrifuge (Eppendorf, Germany). The cell biomass was rinsed twice in 10 mL of 0.1 M phosphate buffer pH 7.4. Yeast cells were disintegrated in a laboratory mill using 0.3–0.5 mm glass balls at 4 °C. In addition, test buffers attached to commercial kits (BioVision, Mountain View, CA, USA) were used to disintegrate yeast cells in a mill, depending on the subsequent analysis of various antioxidative enzymes. After disintegration, the so-obtained cell extract (homogenate) was centrifuged ($11000 \times g$, 5 min, 4 °C), and the liquid was transferred to new test tubes and were frozen and stored at -80 °C for biochemical analyses.

Determination of protein content

Protein content in the supernatants was determined according to the Lowry method. Bovine albumin (Sigma-Aldrich, Poland) was used to prepare a standard curve [7].

Biochemical determination of antioxidant enzymes

Glutathione peroxidase (GPx, EC 1.11.1.9) activity was determined using BioVision tests (BioVision, Mountain View, CA, USA). In this method, GPx catalyzes the oxidation of glutathione (GSH) with the participation of cumene hydroperoxide (2-phenylpropane). The resulting oxidized glutathione (GSSG) becomes a substrate for the reaction catalyzed by glutathione reductase (GR). The effect of this reaction is the formation of GSH with the simultaneous oxidation of NADPH to NADP⁺. The absorbance decreases due to the oxidation of NADPH to NADP⁺, which is directly proportional to the activity of GPx. The amount of enzyme that catalyzes the oxidation of 1 μ mol of glutathione by cumene hydroperoxide in 1 min per mg of protein (mU/mg) at 25 °C is considered as one unit of GPx activity.

The activity of thioredoxin reductase (TrxR, EC1.8.1.9) was estimated using commercial kits (BioVision, Mountain View, CA, USA). It catalyzes the reduction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to 5-thio-2-nitrobenzoic acid (TNB²⁻) in the presence of NADPH as an electron donor. This reaction results in the development of a strong yellow color. The absorbance of the color developed was read at a wavelength of 412 nm after incubation at 25 °C for 20 min. One unit of TrxR activity was defined as the amount of enzyme that produces 1.0 mol (TNB) within 1 min at 25 °C.

Glutathione reductase (GR, EC 1.6.4.2) activity was determined in the extracts obtained after the process of yeast disintegration and was measured with the help of BioVision enzymatic kits (Mountain View, CA, USA). The reaction is based on the reduction of GSSG to GSH with GR in the presence of NADPH leading to the formation of a colored product, i.e., 5-thionitrobenzoate. Decrease in the concentration of DTNB due to the decrease in the production of GSH was monitored by an increase in absorbance at 405 nm for 5 min. One unit of GR activity corresponds to the amount of enzyme that catalyzes the transformation of 1 nmol of oxidized glutathione (GSSG) into its reduced form (GSH) during 1 min at 37 °C per mg of protein (mU/mg).

The level of lipid peroxidation (LPO) was determined using BioVision enzymatic tests (Mountain View, CA, USA). The method involves the formation of a colored complex of LPO products with thiobarbituric acid (TBA) in an acidic environment. The primary product of LPO is malondialdehyde (MDA), which is used to assess the effect of ROS activity. The exact content of free thiol groups and other secondary products of LPO process was calculated based on the predetermined extinction coefficient using the MDA standard.

The activity of glutathione S-transferase (GST, EC 2.5.1.18) was determined in the extracts obtained after yeast disintegration process by using monochlorobimane (MCB)

as the substrate (BioVision, Mountain View, CA, USA), which reacts with glutathione to form a fluorescent colored adduct. The reaction is catalyzed by GST. The absorbance was read at an excitation and emission wavelength of 380 and 460 nm, respectively. One unit of GST activity was equal to the amount of enzyme that catalyzed the transformation of 1 nmol of substrate into the product within 1 min per mg of protein (mU/mg).

Biochemical determination of glutathione forms

The concentration of total glutathione, its reduced (GSH) and oxidized form (GSSG), was determined in the extracts obtained after the disintegration process of yeast cells according to the manufacturer's instructions (BioVision, Mountain View, CA, USA). The reaction between the sulfhydryl groups of glutathione and *o*-phthalaldehyde (OPT), resulting in a yellow color, was used in the determination of glutathione. The intensity of the color is proportional to the amount of glutathione in the examined sample. Finally, the absorbance was measured using a universal microplate reader at a wavelength of 405 nm.

Absorbance in all biochemical analyses was read using a Multiskan SKY spectrophotometer (Thermo Scientific, Poland).

Statistical analysis

The results obtained were subjected to the analysis of variance using the Statgraphics XVII program. Significance of differences between mean values in particular groups was verified by Tukey's test for significance level $\alpha = 0.05$.

Results

Selenium effect on yeasts growth

There is a growing interest among researchers to establish alternative forms of food supplementation. Fodder yeasts are not only a valuable source of many vitamins, enzymes, or proteins, but are gaining importance as carriers of essential bioelements, e.g., selenium-enriched dietary supplements based on yeast [11, 20]. Knowledge regarding the optimal growing conditions of yeasts and the biological influence of selenium on the growth of yeast cells will improve the production of the desired biomass enriched with selenium.

After introduction of the yeast inoculum into the control medium without the addition of selenium but with the addition of potato wastewater and 5% glycerol, the OD was found to be approximately 0.3, whereas after 24 h incubation, the OD reached a value of 1.2–1.4 (Fig. 2a, b). *C. utilis* ATCC 9950 yeast culture in medium without selenium

showed higher OD values at all time variants than that of *S. cerevisiae* ATCC MYA-2200 yeast cultures.

Our results showed that selenium at concentrations in the range of 10–60 mg/L culture media significantly reduced an increase in the OD in case of *C. utilis* ATCC 9950 yeast cells compared to the control culture (Fig. 2a). The increase in OD in yeast cells in all experimental media supplemented with selenium was found to be the highest in the first 22–30 h of incubation. In all cases, the duration of both the adaptation and logarithmic phases were found to be similar which was around 2–6 h. A small increase in OD (1.18) was observed after 24 h incubation in experimental media supplemented with 10 mg Se⁴⁺/L culture media. Furthermore, after 24 h incubation in the media supplemented with 20 and 40 mg Se⁴⁺/L culture media, the growth of yeast was found to be lower by an average of 16% and 25% than that of the control samples. In case of both 10 and 20 mg Se⁴⁺/L culture medium, a similar increase in the OD (1.16) was observed after 24 h incubation. At the same time, these were the highest values obtained from all experimental culture variants. The data obtained after 30 h incubation of yeast in media supplemented with 40 and 60 mg Se⁴⁺/L culture media showed a slight increase in the OD as compared to the culture obtained after 24 h incubation. This might be due to the shift of the end of logarithmic growth phase to the time interval included between the first and second day of incubation. The lowest OD (0.927) was obtained in the medium with the addition of selenium at the highest concentration of 60 mg/L after 35 h incubation.

The OD value obtained for *S. cerevisiae* ATCC MYA-2200 yeasts at particular time intervals from experimental media at all tested selenium concentrations (10–60 mg/L) was found to be lower than that of control culture (Fig. 2b). In media supplemented with 10 and 20 mg Se⁴⁺/L culture media, the duration of the adaptation phase of yeast cells

was the same as in the control medium (3 h). OD of yeasts after 24 h incubation in the experimental medium enriched with selenium at a concentration of 40 mg/L was found to be 0.95, whereas in the medium supplemented with selenium at a concentration of 60 mg/L, it was 0.91. However, the so-close values were found to be lower in relation to the OD of the yeasts obtained from the control medium. It was noted that during the incubation with experimental media (40–60 mg/L), yeast cells took a long time to adapt to the new environment, and the duration of the adaptation phase was much longer than that in the control medium or in those supplemented with lower doses of selenium. The slowdown in yeast growth may have resulted from the occurrence of oxidative stress caused by the presence of selenium in the culture medium.

The results of this study showed that *C. utilis* yeasts were characterized by greater OD than that of *S. cerevisiae* at the inoculum culture stage. Subsequent control and experimental cultures confirmed this regularity. We hypothesize that *C. utilis* yeasts in the experimental medium were characterized by lower sensitivity to oxidative stress associated with an increase in the content of selenium in the culture medium than that of *S. cerevisiae* yeasts.

Similar results were obtained in the study by Yang et al. [21]. They showed that an increase in selenium content (> 80 µg/mL) in MRS medium resulted in a decrease in the number of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* bacteria. In addition, the presence of red coloration of the obtained biomass was observed as evidenced by the progressive detoxification process of selenium leading to the formation of elementary selenium (Se⁰).

These observations confirm the hypothesis that selenium may act as a physiological factor modifying the growth of yeasts through its inhibition. Thanks to the latest

Fig. 2 Effect of selenium on *C. utilis* (a) and *S. cerevisiae* (b) yeasts growth

biotechnological methods and knowledge of the metabolism of given microbial groups, biomass with strictly defined specificity can be obtained. Such a microbiological product rich, inter alia, in selenium in the organic form, proteins, and vitamins can be an alternative to human and animal food supplementation. As reported by Lynch et al. [5] an increased interest in the use of selenium-enriched yeast (Se-Y) preparations in animal feed products has been observed in the last few years due to the improved bioavailability by the organisms of organic forms of Se in relation to its inorganic form. In addition, it is likely that the innate differences between individual yeast strains are the primary factors that affect the diversified synthesis and deposition of various selenium compounds within the yeast fraction. Differences in the methods of selenium yeasts production may also play a role in this aspect.

Wastes of industrial origin, such as glycerol and potato wastewater, can be a good source of carbon and nitrogen, which is necessary for the development of microorganisms [22]. Thus, the direction of using these microorganisms to obtain biomass rich in functional components seems to be justified. The use of the aforementioned agri-food industry waste as ingredients of the media for the production of Se-Y may contribute to reducing the costs of this process and to solve the problem of utilization of these contaminations products.

Effect of selenium on the activity of antioxidant enzymes

Currently, the use of yeasts is widespread and plays an important role not only in the food and pharmaceutical industries but also in the biomedical and chemical industries. Recently, a number of studies have focused to obtainment various yeast metabolites that may be used in industries.

During the production of biomass, yeasts are subjected to many unfavorable environmental conditions, e.g., high temperature, inhibitory effect of ethanol, and osmotic and acid stress. Industrial yeasts must quickly identify and respond to the changing conditions that cause stress thereby adapting to the adverse environmental factors; they do this by changing their metabolic activities. This gives them a better chance of survival.

In this study, we have demonstrated that the presence of selenium has an effect on the change in the activity of antioxidant enzymes in the yeast cells. The presence of selenium may cause oxidative stress, which might lead to another phenomenon called LPO. This may lead to the damage of the yeasts cells [23]. Moreover, increased oxidative reactions in the cells contribute to an increased LPO process [24]. Low amount of selenium in aqueous solutions (≤ 10 mg Se⁴⁺/L) in relation to the control did not significantly affect the level of LPO in *C. utilis* yeasts (Fig. 3a). However, as the concentration of selenium was increased to > 20 mg Se⁴⁺/L, LPO process was found to be increased. Its highest values (60.6 mU/mg protein) were demonstrated in case of *C. utilis* yeasts obtained from experimental solutions with an addition of 60 mg Se⁴⁺/L. In case of *S. cerevisiae* yeast cells obtained from selenium-enriched aqueous solutions, it was found that the level of LPO was not significant for 40 and 60 mg Se⁴⁺/L (Fig. 3b).

A rapid increase in the activity of GPx was observed for both strains of yeasts. Its highest activity (5.39 mU/mg protein) was recorded in the biomass of *C. utilis* yeasts obtained from an aqueous solution supplemented with 60 mg Se⁴⁺/L (Fig. 4a). In case of *S. cerevisiae* yeasts, its value was found to be 3.84 U/mg protein and was 81% higher than that of the control sample (Fig. 4b). These results agree with the results presented by Fujs et al. [25]. They found that the activity of GPx from *Candida intermedia* yeasts cultured in

Fig. 3 Lipids peroxidation in the cells of *C. utilis* (a) and *S. cerevisiae* (b) yeasts

medium supplemented with 50 mg Se⁴⁺/L was on a level of 3.02 mU/mg protein.

The activity of TrxR determined in the cellular extract after disintegration from biomass of *C. utilis* and *S. cerevisiae* yeasts increased with an increasing concentration of selenium in aqueous solutions (Fig. 5a, b). At the same time, its highest value (0.38 mU/mg protein) was found to be at 60 mg Se⁴⁺/L for the *S. cerevisiae* strain. There were no significant differences in the activity of GR in the presence of lowest concentrations selenium (10 and 20 mg Se⁴⁺/L) for *C. utilis* yeast strain (Fig. 6a). In case of *S. cerevisiae*, the activity of TrxR and GR enzymes (Fig. 6b) increased with an increase in the concentration of selenium in relation to biomass obtained from aqueous control solutions. However, we did not observe significant differences in case of 40 and 60 mg Se⁴⁺/L. A

slight increase in GR activity at higher concentrations of selenium influenced the reduction of the possibility of oxidized glutathione (GSSG) transforming into its reduced form (GSH).

No significant differences were observed in this study in case of GST activity in *S. cerevisiae* yeasts obtained in the presence of 10 and 20 mg Se⁴⁺/L. In case of *C. utilis*, the activity of GST did not increase as rapidly as in case of GR after the addition of the lowest concentration of selenium to the aqueous solutions (Fig. 7a, b). GST is an enzyme involved in the conjugation reactions of GSH with a variety of electrophilic compounds to form S-conjugates. Such substances in the activated form can attack cellular macromolecules. Combinations of these compounds with GSH are then subjected to cellular processes of detoxification [25, 26].

Fig. 4 Activity of glutathione peroxidase in the cells of *C. utilis* (a) and *S. cerevisiae* (b) yeasts

Fig. 5 Activity of thioredoxin reductase in the cells of *C. utilis* (a) and *S. cerevisiae* (b) yeasts

Fig. 6 Activity of glutathione reductase in the cells of *C. utilis* (a) and *S. cerevisiae* (b) yeasts

Selenium effect on the content of various glutathione forms

Glutathione, which is the most important nonprotein low molecular weight thiol, has been shown to be involved in the detoxification of reactive metabolites [27], which are synthesized in the yeast cells after an exposure to selenium concentration. The total glutathione content in the biomass of *C. utilis* and *S. cerevisiae* yeasts increased with an increasing selenium content in aqueous solutions (Tables 1, 2). Its largest value (respectively: 8.74 and 6.95 $\mu\text{g}/\text{mg}$ protein) was obtained in the medium with the addition of 60 mg Se^{4+}/L . A slight increase in the concentration of reduced glutathione compared to the control sample was noted in the biomass of yeast obtained from aqueous solutions enriched with selenium at a concentration of 10 mg/L. Further increase in selenium content

resulted in an increase in GSH content in yeast biomass. In case of 60 mg Se^{4+}/L , there was no longer a sharp increase in GSH in the yeast cells. The results obtained confirm that the intracellular environment of yeasts cultured at a high concentration of selenium was more oxidized than in the biomass obtained from the control culture due to the inability of GSH to maintain adequate redox status in yeast cells [28]. At lower content of selenium, the ratio of GSH to GSSG did not increase significantly, probably due to the adaptive increase in GSH, which was an attempt to maintain the normal level of GSH to GSSG in the yeast cellular cytosol. According to Grant [18] an exposure to various environmental factors (selenium) causing oxidative stress leads to an increase in the concentration of glutathione in the cells. As the selenium concentration increased, both yeast strains showed an increased GSSG content.

Fig. 7 Activity of GST in the cells of *C. utilis* (a) and *S. cerevisiae* (b) yeasts

Table 1 The content of various forms of glutathione in the cells of *C. utilis* yeasts

Selenium concentration (mg Se ⁴⁺ /L)	Total glutathione (μg/mg protein)	GSH (μg/mg protein)	GSSG (μg/mg protein)	GSH/GSSG
0	2.32 ± 0.19 ^a	2.12 ± 0.23 ^a	0.20 ± 0.01 ^a	10.6
10	3.38 ± 0.22 ^b	2.89 ± 0.40 ^{ab}	0.49 ± 0.00 ^a	5.89
20	5.90 ± 0.18 ^c	3.75 ± 0.28 ^{bc}	2.15 ± 0.25 ^b	1.74
40	7.46 ± 0.24 ^d	4.74 ± 0.46 ^d	2.72 ± 0.38 ^b	1.74
60	8.74 ± 0.26 ^e	4.12 ± 0.24 ^{cd}	4.62 ± 0.41 ^c	0.89

^{a–e} means with the same letter did not differ significantly

Table 2 The content of various forms of glutathione in the cells of *S. cerevisiae* yeast

Selenium concentration (mg Se ⁴⁺ /L)	Total glutathione (μg/mg protein)	GSH (μg/mg protein)	GSSG (μg/mg protein)	GSH/GSSG
0	2.71 ± 0.15 ^a	2.53 ± 0.22 ^a	0.18 ± 0.11 ^a	14.0
10	3.89 ± 0.19 ^b	2.01 ± 0.27 ^a	1.88 ± 0.22 ^b	1.06
20	5.43 ± 0.30 ^c	2.65 ± 0.06 ^a	2.78 ± 0.30 ^c	0.95
40	6.90 ± 0.49 ^d	3.44 ± 0.38 ^b	3.46 ± 0.34 ^c	0.99
60	6.95 ± 0.37 ^d	3.46 ± 0.18 ^b	3.49 ± 0.30 ^c	0.99

^{a–d} means with the same letter did not differ significantly

Discussion

Selenium is an element that at appropriate doses can play very important functions with respect to antioxidant systems. Moreover, it is necessary for the proper functioning of the human and animal body. Recently, an interest in this element in many biotechnology fields has increased. This study investigated the tolerance and effect of this element on the yeast cells antioxidative system.

The obtained yeast growth curves (Fig. 2a, b) confirmed the assumption that selenium has the property that inhibits the growth of microorganisms. Despite the presence of this element in the media, all examined yeast strains showed the ability to grow. The highest OD in experimental media supplemented with selenium was achieved by *C. utilis* ATCC 9950 strain. The study presented by Assunção et al. [12] showed that above certain concentrations, selenium can be toxic to yeasts, causing oxidative stress. The authors observed significant differences in the survival of the examined yeasts. *Torulaspora delbrueckii* EVN 1141 strain cultured in medium supplemented with 100 μg/mL (Na₂SeO₃) demonstrated the best growth and survival. According to the data provided by Bronzetti et al. [29] sodium selenite at high doses showed a mutagenic effect on *S. cerevisiae* D7 yeast cells demonstrating a 70% decrease in cell survival compared to the control. Summing up, it should be emphasized that individual yeast species may show different tolerance to selenium. As

reported by Zhang et al. [30] the inhibitory effect of selenium on the growth of microorganisms could be based on nonspecific incorporation of seleno-amino acids (SeMet, SeCys) instead of methionine and cysteine in proteins. It is believed that the toxicity of this element results from its nonspecific substitution in place of sulfur. According to Wilson et al. [31] inhibition of yeast growth in the presence of selenium results from the formation of single or double breaks in the DNA strand. In addition, selenium can affect the inhibition of cell growth through interactions with reduced GSH [29].

The appropriate dose of selenium induces the appearance of oxidative stress in yeast cells. A high concentration of selenium in the culture medium may lead to the occurrence of so-called genotoxic effects [32]. The result of such interactions are mutations and inactivation of various metabolic pathways. Moreover, selenium changes the morphological characteristics of yeast, e.g., changing the structure of the wall and membrane complex [2, 33]. The reduction in membrane fluidity is associated with a higher content of saturated fatty acids, stronger hydrophobic interactions, and an increased content of phospholipids [34]. The phenomenon of oxidative stress causes the occurrence of membrane depolarization phenomenon, i.e., the decrease in the difference of electric potential between the cytosol and the culture environment. In addition, an excessive actin oxidation by ROS increases the aging process of yeast [35]. As a result, these effects reduce the integrity of yeast cell membranes,

thereby accelerating cell death. Selenium induces changes in the amino acid and lipid composition of yeast cells [2].

In this study, an increase in the concentration of selenium in aqueous solutions resulted in an increase in the activity of antioxidant enzymes in *C. utilis* and *S. cerevisiae* yeast strains. Our results of LPO suggest that at low levels (10 mg/L) selenium shows an antioxidant activity in yeast cells compared to the control sample. It also allows to believe that the presence of nonenzymatic scavengers of free radicals in yeasts was at an appropriate level. These compounds support the first line of cell defense against the harmful effects of oxidative stress. However, in excessive concentrations (> 20 mg/L) selenium exhibits pro-oxidative properties. Despite an increased activity of GPx, GR enzymes in *C. utilis* and *S. cerevisiae* yeasts kept in aqueous solutions with the addition of 40 and 60 mg Se⁴⁺/L, we found an increased level of LPO. This indicates that an increase in the activity of antioxidant enzymes was insufficient to protect the yeast cells from damage induced by the presence of an excessive amounts of selenium.

Changing environmental conditions and the presence of selenium can cause stress in organisms. Yeast cells can very effectively mitigate oxidative damage by applying various defense strategies. Protection of cells against the harmful effects of oxidative stress involves the activation of enzymatic and nonenzymatic components (glutathione). Enzymatic components can directly remove ROS or act by producing nonenzymatic antioxidants [36]. The ROS can cause dysfunctional protein synthesis, e.g., by erroneous mRNA translation. It has been shown in *S. cerevisiae* yeasts that erroneous translation of mRNA caused by Cr (VI) ions causes the accumulation of insoluble and toxic aggregates of carbonyl proteins consisting of abnormally synthesized, inactive proteins. This is one of the primary pathways of the toxic action of Cr (VI) on yeast cells [35]. However, the involvement of antioxidant systems in the yeast reaction to the presence of selenium is still unclear. The issue of the influence of this element on the yeast cells antioxidative processes is of interest to many research centers.

The presence of selenium in *C. utilis* and *S. cerevisiae* yeast cultures affected an increase of enzymatic activity of GPx. GPx activity may have influenced the formation of a smaller amount of superoxide anion radicals in yeast cells, which are primarily removed by other enzymes. We hypothesize that the occurrence of low concentrations of ROS in yeasts obtained from aqueous solutions supplemented with 10 mg Se⁴⁺/L did not induce the activity of GR and TRx. At the same time, our results clearly indicate that even a small concentration of selenium (10 mg/L) can have a measurable effect on ROS production in the cells, which indicated high enzymatic activity of GPx. The results obtained by Assunção et al. [12], showed that the content of selenium in the culture medium caused an increase in the SOD activity with

respect to the obtained lower GPx in selected yeast species (including *Hanseniaspora guilliermondii* and *S. cerevisiae*). Osmotic stress in yeasts caused by the presence of increasing doses of selenium in aqueous solutions induced an increase in TrxR activity and a slight increase in GST compared to control aqueous solutions. The contribution of these two enzymes in response to the increasing ROS concentration is very important. These enzymes are involved in the reduction of nonspecific connections in protein structures that were subjected to oxidization [18].

Zhang et al. [30] studied physiological and molecular mechanisms underlying the accumulation and biotransformation of selenium by *C. utilis* CCTCC M 209298 yeast cells under conditions of moderate acid stress. The authors showed that the selenium increased the amount of reduced glutathione (GSH) and ATP in yeast cells. It can be hypothesized that under conditions of stress, caused by the presence of selenium to protect cells, a high concentration of glutathione participates in the S-thiolation process, i.e., the attachment of the -SH group of proteins to the GSH molecule [18]. Such combinations are involved in the stabilization of certain proteins, protecting them against toxic oxidation processes caused by adverse environmental conditions (e.g., selenium and acid stress). Yeast cells under various stress conditions can produce large amounts of GSH involved in the protection of cells against the damage caused by hydrogen peroxide.

The high content of GSSG observed in the biomass of *C. utilis* and *S. cerevisiae* yeasts obtained from aqueous solutions with the addition of selenium (40–60 mg/L) could be the result of acute oxidative stress. The presence of complex enzymatic mechanism to eliminate stress in yeasts is a very important topic of research, e.g., GPx and GR together with glutathione. The results obtained indicate the activation of defense mechanisms and the adaptive response of yeast cells to changing culture conditions. The observed low concentration of GR in yeasts was indicative of the reduced ability of both examined yeast strains to conduct an efficient transformation of the emerging GSSG to its reduced form. GR is responsible to maintain an adequate level of reduced glutathione in the cellular cytosol. These results are consistent with those presented by Kaur and Bansal [37]. The authors showed that an increase in the content of selenium in the culture medium affected an increase in the concentration of oxidized glutathione (GSSG), GPx and GST in *S. cerevisiae* MTCC 1176 yeasts with a simultaneous decrease in GSH.

GSH is an important antioxidant since yeast strains devoid of selenium are sensitive to oxidative stress induced by peroxides, superoxide anions, as well as toxic products of LPO [18]. The primary function of glutathione is to maintain the thiol groups of proteins in the reduced state. The reduction in the concentration of GSH in yeasts might have resulted in the consumption of

selenium in reactions with free radicals, which are produced in excessive amounts, which was accompanied by an increase in the concentration of the oxidized form of glutathione (GSSG). An increase in GSSG is consistent with the role of GSH as a scavenger of free radicals and a cofactor for various antioxidant enzymes, including GPx, GST, and glutaredoxins [18]. In addition, GSH is involved in the binding of selenium, thereby contributing to the mitigation of oxidative stress effects. This is one of the additional detoxification processes of yeast cells. The effect of these processes is the reduction of the GSH/GSSG oxidation potential. It should be emphasized that the mechanism of selenium toxicity in yeasts might be associated with the process of its reduction to elemental form. This reaction is catalyzed by glutathione with the simultaneous formation of GSSG [13]. Furthermore, our results suggest that the concentration of GSSG is controlled by the effective action of GR, which requires electrons from NADPH primarily from the pentose phosphate pathway. In addition, it is a key source of electrons for many antioxidants [35]. The lack of a sharp increase in the concentration of GSH in the biomass of both yeast strains obtained from solutions supplemented with selenium at a concentration of 60 mg/L might have resulted from the fact that GR involved in this process lost its catalytic activity.

The tolerance of *C. utilis* and *S. cerevisiae* yeasts to high amount of selenium may result from the combined action of a complex antioxidant system. A decrease in the concentration of GSH in the cell leads to a rapid increase in the level of lipid peroxides, which negatively affects the regulation of metabolic processes in yeasts and causes the oxidation of unsaturated fatty acids. This multi-stage process may eventually lead to the disturbance in the structure of membrane of the vacuole (tonoplast) leading to the leakage of lytic enzymes into the intracellular environment of yeasts causing acidification [2, 38]. The consequence of such processes is the occurrence of changes in cell morphology and even death of the yeasts.

An increase in the activity of antioxidant enzymes in case of *S. cerevisiae* biomass obtained from aqueous solution supplemented with selenium at a concentration of > 20 mg/L was insufficient to protect the cells against harmful effects of selenium. This is consistent with the results obtained where a higher concentration of selenium (60 mg/L) resulted in an extension of the adaptive phase of yeast growth. In addition, the OD value obtained in experimental media for all time variants was found to be lower than that of the control culture. The accumulation of oxidized protein products may lead to changes in their tertiary structure, leading to protein dysfunction (impairing metabolic functions in yeast cells) [14]. Moreover, the oxidative effect of selenium on sulfhydryl groups of

proteins is associated with the formation of ROS such as hydroxyl radicals [39]. To sum up, the antioxidant system of yeast cells may reduce selenium toxicity in yeast cells.

Conclusion

The results of this study provide valuable information regarding the influence of selenium on the growth of yeast cells and antioxidant system functioning. According to the results, the antioxidant activity of the examined yeast strains showed similar relationships to the increasing selenium concentration in aqueous solutions. Higher activity of GPx and GR enzymes was found in case of *C. utilis* strain than that of *S. cerevisiae*. Further research aimed at understanding the functioning of antioxidant system should include molecular analysis of genes responsible for the biosynthesis of individual proteins under conditions of stress. In addition, appropriate selection of components and culture conditions through interference in yeast metabolic engineering will facilitate the development of an efficient and alternative method of various industrial waste utilization with the simultaneous possibility of obtaining selenium-enriched biomass. Understanding the functioning of the antioxidant system in eukaryotic cells is important for the assessment of factors that may affect the pro- and antioxidant balance. This can be helpful to improve the activity of primary antioxidant mechanisms in cells. This study was also referred to for further scientific works [40].

Acknowledgements This work was supported by the National Science Centre, Poland “Miniatura” (no. 2017/01/X/NZ9/00339).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest regarding the publication of this article.

OpenAccess This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Kieliszek M, Błażej S (2013) Selenium: significance, and outlook for supplementation. *Nutrition* 29(5):713–718
2. Kieliszek M, Błażej S, Bzducha-Wróbel A, Kot AM (2019) Effect of selenium on lipid and amino acid metabolism in yeast cells. *Biol Trace Elem Res* 187(1):316–327
3. Kieliszek M, Lipinski B (2018) Pathophysiological significance of protein hydrophobic interactions: An emerging hypothesis. *Med Hypotheses* 110:15–22

4. Santosh TR, Sreekala M, Lalitha K (1999) Oxidative stress during selenium deficiency in seedlings of *Trigonella foenum-graecum* and mitigation by mimosine part II. glutathione metabolism. *Biol Trace Elem Res* 70(3):209
5. Lynch S, Horgan K, Walls D, White B (2018) Selenised yeast sources differ in their capacity to protect porcine jejunal epithelial cells from cadmium-induced toxicity and oxidised DNA damage. *Biomaterials* 31(5):845–858. <https://doi.org/10.1007/s10534-018-0129-z>
6. Fernandes AP, Gandin V (2015) Selenium compounds as therapeutic agents in cancer. *Biochim Biophys Acta* 1850(8):1642–1660
7. Kieliszek M, Błażej S, Bzducha-Wróbel A (2015) Influence of selenium content in the culture medium on protein profile of yeast cells *Candida utilis* ATCC 9950. *Oxid Med Cell Longev* 659750. <https://doi.org/10.1155/2015/659750>
8. Duntas LH, Benavente S (2015) Selenium: an element for life. *Endocrine* 48(3):756–775
9. Kieliszek M, Błażej S (2016) Current knowledge on the importance of selenium in food for living organisms: a review. *Molecules* 21(5):609. <https://doi.org/10.3390/molecules21050609>
10. Rayman MP (2012) Selenium and human health. *Lancet* 379(9822):1256–1268
11. Kieliszek M, Kot AM, Bzducha-Wróbel A, Błażej S, Gientka I, Kurcz A (2017) Biotechnological use of *Candida* yeasts in the food industry: a review. *Fungal Biol Rev* 31(4):185–198. <https://doi.org/10.1016/j.fbr.2017.06.001>
12. Assunção M, Martins LL, Mourato MP, Baleiras-Couto MM (2015) Effect of selenium on growth and antioxidant enzyme activities of wine related yeasts. *World J Microbiol Biotechnol* 31(12):1899–1906
13. Kieliszek M, Błażej S, Gientka I, Bzducha-Wróbel A (2015) Accumulation and metabolism of selenium by yeast cells. *Appl Microbiol Biotechnol* 99(13):5373–5382
14. Lazard M, Dauplais M, Blanquet S, Plateau P (2017) Recent advances in the mechanism of selenoamino acids toxicity in eukaryotic cells. *Biomol Concepts* 8(2):93–104
15. White PJ (2018) Selenium metabolism in plants. *Biochim Biophys Acta* 1862(11):2333–2342
16. Seitomer E, Balar B, He D, Copeland PR, Kinzy TG (2008) Analysis of *Saccharomyces cerevisiae* null allele strains identifies a larger role for DNA damage versus oxidative stress pathways in growth inhibition by selenium. *Mol Nutr Food Res* 52(11):1305–1315
17. Jamieson DJ (1998) Oxidative stress responses of the yeast *Saccharomyces cerevisiae*. *Yeast* 14(16):1511–1527
18. Grant CM (2001) Role of the glutathione/glutaredoxin and thioredoxin systems in yeast growth and response to stress conditions. *Mol Microbiol* 39(3):533–541
19. Kieliszek M, Błażej S, Piwowarek K, Brzezicka K (2018) Equilibrium modeling of selenium binding from aqueous solutions by *Candida utilis* ATCC 9950 yeasts. *3 Biotech* 8(9):388. <https://doi.org/10.1007/s13205-018-1415-8>
20. Kubachka KM, Hanley T, Mantha M, Wilson RA, Falconer TM, Kassa Z, Oliveira A, Landero J, Caruso J (2017) Evaluation of selenium in dietary supplements using elemental speciation. *Food Chem* 218:313–320
21. Yang J, Li Y, Zhang L, Fan M, Wei X (2017) Response surface design for accumulation of selenium by different lactic acid bacteria. *3 Biotech* 7:52. <https://doi.org/10.1007/s13205-017-0709-6>
22. Bzducha-Wróbel A, Błażej S, Kieliszek M, Pobjega K, Falana K, Janowicz M (2018) Modification of the cell wall structure of *Saccharomyces cerevisiae* strains during cultivation on waste potato juice water and glycerol towards biosynthesis of functional polysaccharides. *J Biotechnol* 281:1–10
23. Garre E, Raginel F, Palacios A, Julien A, Matallana E (2010) Oxidative stress responses and lipid peroxidation damage are induced during dehydration in the production of dry active wine yeasts. *Int J Food Microbiol* 136(3):295–303
24. Xu P, Qiao K, Stephanopoulos G (2017) Engineering oxidative stress defense pathways to build a robust lipid production platform in *Yarrowia lipolytica*. *Biotechnol Bioeng* 114(7):1521–1530
25. Biłska A, Kryczyk A, Włodek L (2007) The different aspects of the biological role of glutathione. *Postępy Hig Med Dośw* 61:438–453
26. Vranova E, Inzé D, Van Breusegem F (2002) Signal transduction during oxidative stress. *J Exp Bot* 53(372):1227–1236
27. Wonisch W, Kohlwein SD, Schaur J, Tatzber F, Guttenberger H, Zarkovic N, Winkler R, Esterbauer H (1998) Treatment of the budding yeast *Saccharomyces cerevisiae* with the lipid peroxidation product 4-HNE provokes a temporary cell cycle arrest in G1 phase. *Free Radic Biol Med* 25(6):682–687
28. Pereira MD, Herdeiro RS, Fernandes PN, Eleuterio ECA, Panek AD (2003) Targets of oxidative stress in yeast sod mutants. *Biochim Biophys Acta* 1620(1–3):245–251
29. Bronzetti G, Cini M, Andreoli E, Caltavuturo L, Panunzio M, Della Croce C (2001) Protective effects of vitamins and selenium compounds in yeast. *Mutat Res* 496:105–115
30. Zhang GC, Wang DH, Wang DH, Wei GY (2017) The mechanism of improved intracellular organic selenium and glutathione contents in selenium-enriched *Candida utilis* by acid stress. *Appl Microbiol Biotechnol* 101(5):2131–2141
31. Wilson AC, Thompson HJ, Schedin PJ, Gibson NW, Ganther HE (1992) Effect of methylated forms of selenium on cell viability and the induction of DNA strand breakage. *Biochem Pharmacol* 43(5):1137–1141
32. Peyroche G, Saveanu C, Dauplais M, Lazard M, Beuneu F, Decourty L, Malabat Ch, Jacquier A, Blanquet S, Plateau P (2012) Sodium selenide toxicity is mediated by O₂-dependent DNA breaks. *PLoS ONE* 7:e36343. <https://doi.org/10.1371/journal.pone.0036343>
33. Kieliszek M, Błażej S, Bzducha-Wróbel A, Kurcz A (2016) Effects of selenium on morphological changes in *Candida utilis* ATCC 9950 yeast cells. *Biol Trace Elem Res* 169(2):387–393
34. Gharwalova L, Sigler K, Dolezalova J, Masak J, Rezanka T, Kolouchova I (2017) Resveratrol suppresses ethanol stress in winery and bottom brewery yeast by affecting superoxide dismutase, lipid peroxidation and fatty acid profile. *World J Microbiol Biotechnol* 33(11):205
35. Farrugia G, Balzan R (2012) Oxidative stress and programmed cell death in yeast. *Front Oncol* 2:64
36. Chen TF, Zheng WJ, Wong YS, Yang F (2008) Selenium-induced changes in activities of antioxidant enzymes and content of photosynthetic pigments in *Spirulina platensis*. *J Integr Plant Biol* 50(1):40–48
37. Kaur T, Bansal MP (2006) Selenium enrichment and anti-oxidant status in baker's yeast, *Saccharomyces cerevisiae* at different sodium selenite concentrations. *Nutr Hosp* 21(6):704–708
38. Schauer A, Knauer H, Ruckenstein C, Fussi H, Durchschlag M, Potocnik U, Fröhlich KU (2009) Vacuolar functions determine the mode of cell death. *Biochim Biophys Acta* 1793(3):540–545
39. Hoffman DJ (2002) Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat Toxicol* 57(1–2):11–26
40. Fujs S, Gazdag Z, Poljšak B, Stibilj V, Milacic R, Pesti M, Raspor P, Batic M (2015) The oxidative stress response of the yeast *Candida intermedia* to copper, zinc, and selenium exposure. *J Basic Microbiol* 45(2):125–135

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.