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Evaluation of sodium selenite effects on the potential probiotic *Saccharomyces cerevisiae* UFMG A-905: A physiological and proteomic analysis

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ABSTRACT

Organic forms of selenium (Se) are drawing more attention in the field of functional food. Se-enriched yeast is one of the best known approaches to supply these compounds in the form of selenomethionine. *Saccharomyces cerevisiae* UFMG A-905 is of particular interest as a nutritional supplement and pharmaceutical since it can both fix Se and has been found to have potential for use as a probiotic. The aim of this study was to evaluate the effects of sodium selenite on this strain. A comparative proteomic approach was employed, highlighting the differences in the expression of 13 proteins in a pure YPD medium control and a sample containing 100 mg/L sodium selenite. Both proteomic and phenotypic analyses revealed that oxidative stress was caused. The analyses also revealed the ability of *S. cerevisiae* to set up strategies to counteract this phenomenon. In addition, the up-regulation of a cystathionine gamma-lyase confirms the ability of the strain to produce organic forms of Se that are usually more bioavailable for humans.

1. Introduction

Selenium (Se) is an essential trace element. Recommended uptake for humans is 55 μg/day, and a level of about 40 μg/day is suggested as the minimum requirement to avoid deficiency. However, optimal Se intake is not easy to determine because there are so many interlinked factors (Alzate, Fernández-Fernández, Pérez-Conde, Gutierrez, & Cámara, 2008; Letavayová, Vlčková, & Brozmanová, 2006). Se occurs naturally

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Abbreviations: DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; DTT, dithiothreitol; GPx, glutathione peroxidase; IEF, isoelectrofocusing; MDA, malondialdehyde; SeCys, selenocysteine; SeMet, selenomethionine; TBARS, thiobarbituric acid reactive species; TNB, 2-nitro-5-thiobenzoic acid

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in both inorganic and organic forms. The two main organic forms are amino acids, selenomethionine (SeMet) and selenocysteine (SeCys) (Gromer, Eubel, Lee, & Jacob, 2005). One of the most beneficial effects that Se has in humans is its role in protecting against oxidative stress. Se is present in the active site of glutathione peroxidase (GPx), the main enzyme involved in antioxidant protection (Bordoni et al., 2008). Se-enriched yeast can be used as supplement to treat human Se-deficiency problems, because *Saccharomyces cerevisiae* strains from different origins proved to be able to absorb inorganic Se and convert it into SeMet. This compound is incorporated into proteins or associated into cell wall elements, acting as a reservoir for Se (Combs, 2001; Polatajko et al., 2004; Rayman, 2004).

Humans and animals are already consuming yeast as a probiotic. The majority of microorganisms recognized as probiotics are bacteria, and *Saccharomyces boulardii* is the only probiotic yeast approved by FDA for human consumption (Czerucka, Piche, & Rampal, 2007). However, other strains of natural yeast and yeast that's been produced for agriculture and industrial uses with similar or even better biotherapeutic properties certainly exist. Previously published results have proven that the *S. cerevisiae* strain UFMG A-905 can be isolated during the production of "cachaça", which is a popular Brazilian beverage. This strain has probiotic properties comparable to commercially available *S. boulardii* (Generoso et al., 2010; Martins et al., 2005, 2007, 2011; Tiago et al., 2012). Se has been reported to damage yeast in different ways when added at relatively high concentrations. In the literature, some papers reported DNA damage, protein damage, and oxidative stress responses with the up-regulation of genes for oxidoreductases and for proteasome protein components (Izquierdo, Casas, & Herrero, 2010; Letavayová, Vlasaková, Spallholz, Brozmanová, & Chovanec, 2008; Salin et al., 2008). There must be a balance between the ideal amount of Se needed for its antioxidant effect and the amount that will trigger a decrease in cell growth and metabolic damage in yeast cells in order for it to have its probiotic effect.

The aim of this study was to explore the use of *S. cerevisiae* UFMG A-905 as a nutritional and pharmaceutical supplement by adding its probiotic potential to its ability to supply bioavailable organic forms of Se. Different phenotypic and proteomic experimental approaches were employed focusing on the evaluation of the effects of sodium selenite on the physiology of this strain of yeast.

2. Materials and methods

2.1. Microorganism and culture conditions

The UFMG A-905 strain of *S. cerevisiae* yeast belongs to the collection of the Department of Microbiology (Biotherapeutic Agents Laboratory) of the Federal University of Minas Gerais, Brazil. It was isolated from "cachaça" production in Minas Gerais and selected for its probiotic potential (Martins et al., 2005). The yeast was grown overnight at 37 °C while being shaken at 200 rpm in YPD broth (yeast extract 1%, peptone 2%, glucose 2% – Difco, Sparks, MD, USA).

To evaluate the effects of Se on yeast viability, an overnight pre-culture of *S. cerevisiae* UFMG A-905 was used to inoculate a 10 mL culture at an initial OD_{600} (Optical Density at 600 nm) of 0.1. Sodium selenite was added to YPD broth at various concentrations: 0, 4, 10, 20, 35, 50, and 100 mg/L Na_2SeO_3 (Sigma Aldrich, St. Louis, MO, USA). The Na_2SeO_3 was obtained from a 2.19 g/L sodium selenite stock solution corresponding to 1 g/L Se. It was sterilized by filtration with a 0.20 μm single-use syringe filter from Sartorius Stedim Biotech (Goettingen, Germany). The cultures were grown at 37 °C while being shaken at 200 rpm for 9 h. After that, 0.1 mL aliquots from each culture were used to prepare the progressive dilutions that were plated in YPD agar. After 72 h of incubation, the colonies were counted to determine the number of colony forming units (CFU)/mL.

For proteomic and physiological experiments, *S. cerevisiae* UFMG A-905 was grown in 500 mL YPD broth at 37 °C while being shaken for 8 h. The same medium was fortified with 100 mg/L Na_2SeO_3. An overnight pre-culture was used to inoculate the cultures at an initial OD of 0.1. Yeast growth was monitored by both 600 nm optical density measurement (OD_{600}) and dry-weight determination.

Three biological replicates were performed for the cultures of all the experiments.

2.2. Transmission electron microscopy analysis

Yeast cells were grown in YPD broth and in the same medium fortified with 100 mg/L Na_2SeO_3. One mL aliquots were collected by centrifuge (4000 *g*, 20 min, 4 °C) after 8 and 24 h of growth and resuspended in 1 mL of sterile water. Five μL aliquots were directly applied to copper grids covered with a thin collodion layer and dehydrated at room temperature. Observations were made with CM10 transmission electron microscopy (TEM).

2.3. Proteomic analyses

2.3.1. Preparation of in toto protein extracts

The cells were harvested by centrifuge (4000 *g*, 20 min, 4 °C) in the middle of the exponential growth phase (8 h of growth). The biomass was washed twice in 50 mL of 0.85% NaCl. Pellets were obtained and were resuspended in 3 mL of pH 7.3, EDTA 1 mM 50 mM Tris-HCl. Glass beads (710–1.180 μm, Sigma Aldrich) were added, and the cells were disrupted in a vortex with 15 cycles of 1 min each. The supernatants were collected by centrifuge (4000 *g*, 4 °C, 20 min) and then ultracentrifuged (100,000 *g*, 1 h, 4 °C) in a Beckman L8-60 M Ultracentrifuge (Type 60 rotor) to fully remove cell debris. The supernatants were incubated with 10 μL/mL of nuclease mix (GE Healthcare, Pittsburgh, PA, USA) and then dialysed in 10 kDa cut-off Amicons (Sigma Aldrich) against four volumes of bidistilled water. The protein extracts were precipitated and resuspended as previously described (Lamberti et al., 2011).

2.3.2. Two-dimensional electrophoresis

Isoelectrofocusing (IEF) was performed using 13 cm IPG strips (GE Healthcare) with a *pI* linear gradient ranging from 3 to 10. Two hundred and fifty μg of protein were placed on each strip by in-gel rehydration. IEF was performed using IPGphor (GE Healthcare) at 20 °C with 50,000 Vh after 10 min of rehydration.

After IEF, the strips were prepared for the second dimension as already described (Lamberti et al., 2011).

2.3.3. *Image analysis and statistical analysis*

Two-dimensional electrophoresis gels were digitized with an SI Personal Densitometer (Amersham Biosciences/GE Healthcare). Images were analysed with Progenesis PG 220 software (Non Linear Dynamics). Spot detection was automated using the 2005 detection software algorithm and manually verified. After the establishment of some user seeds, matching was automatically performed and manually checked. Two analytical replicates for each of the three biological replicates were performed. A spot was considered significant when it was present in both the technical replicates of at least two out of three biological replicates.

2.3.4. *Protein identification by mass spectrometry*

The protein spots were excised from the dried gel and rehydrated with MilliQ water. The gel had been digested with sequencing-grade, modified porcine trypsin (Promega, Madison, WI, USA) and added to a MALDI target plate as described by Hewitson et al. (2008). The entire protocol for mass spectrometry analyses has been described previously (Lamberti et al., 2011).

2.4. *Lipid peroxidation index using thiobarbituric acid reactive species (TBARS)*

Formation of thiobarbituric acid/malondialdehyde complex (TBA-MDA) was determined using the method described by Aust (1994). Briefly, the cellular extracts obtained were incubated with 10% v/v trichloroacetic acid for 30 min on ice and centrifuged at 4000 *g* at a temperature of 4 °C for 5 min. The supernatants obtained were mixed with an equal volume of TBA and boiled for 60 min. TBARS concentrations were determined by measuring absorbance at 532 nm. A value of 156,000 M^{-1} cm^{-1} was used as molar extinction coefficient (Rice-Evans, Diplock, & Symons, 1991). The results were expressed as pmol TBARS/mg protein.

2.5. *Determination of glutathione (GSH) level*

GSH level was determined by evaluating thiol content. This was done using the Ellman method (Ellman, 1959) based on the employment of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) as a substrate. DTNB is a symmetric aryl disulphide that reacts with free thiols to form disulphide and 2-nitro-5-thiobenzoic acid (TNB). Aliquots of 0.5 mg of protein extract were diluted in pH 8.0 sodium phosphate to a final concentration of 0.01 M. Ellman reagent was added to this mix to a final concentration of 0.01 M. The reduction of chromogen was observed using a spectrophotometer. Absorbance at 412 nm was measured after 5 min of incubation. GSH level was determined from free-SH and expressed as nM/mg protein.

2.6. *Statistical analysis*

Data were compared using Student's t-test, with a value of $p < 0.05$ used to indicate a statistical significance.

3. Results and discussion

3.1. *Effect of Se on the growth of* S. cerevisiae *UFMG A-905*

S. cerevisiae UFMG A-905 was grown in agar containing different amounts of sodium selenite ranging from 0 to 100 mg/L in order to determine the minimal concentration that lead to a significant variation in yeast viability.

As shown in Fig. 1, concentration of 4 mg/L sodium selenite immediately induced a strong reduction in cell viability: 59% survival as compared to the control. Concentrations of sodium selenite up to 35 mg/L did not significantly alter yeast viability. On the contrary, 50 mg/L sodium selenite had an order of magnitude less CFU/mL than the control, resulting in 13% survival. The maximum sodium selenite concentration tested was 100 mg/L. It resulted in 7% survival with a final result of 3.33E+6 CFU/mL.

There are several reports about the influence of different sodium selenite concentrations on various *S. cerevisiae* strains in the literature. In 2006, Kaur and Bansal reported the effect of sodium selenite on the growth of *S. cerevisiae* MTCC 1766; they observed a reduction in OD_{600} of 23% at a sodium selenite concentration of 3.44 mg/L, 34% OD_{600} reduction at a sodium selenite concentration of 6.75 mg/L, and 48% OD_{600} reduction at a sodium selenite concentration of 9.86 mg/L (Kaur & Bansal, 2006). These data are in agreement with the 49% survival rate observed in this study at a sodium selenite concentration of 10 mg/L. Meanwhile, at a low sodium selenite concentration of about 4 mg/L, the growth of *S. cerevisiae* MTCC 1766 is less inhibited than *S. cerevisiae* UFMG A-905, as confirmed by the 23% OD_{600} reduction and 41% viability decrease. Ponce de Leon et al. described the effects of sodium selenite on *S. cerevisiae* 15–6252. The results were in full agreement with those in this study for *S. cerevisiae* UFMG A-905 (Ponce de Leon, Bayòn, Paquin, & Caruso, 2002). Rajashree and

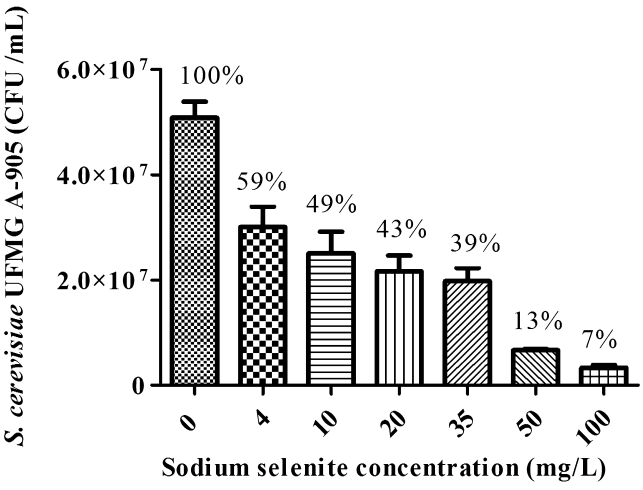

Fig. 1 – *S. cerevisiae* UFMG A-905 CFU/mL at different selenium concentrations. Results are reported as mean values ± SEM of 3 biological replicates. The survival percentage compared to the control is reported over the bars.

Muthukumar found that *S. cerevisiae* NCYC 1026 is resistant to high sodium selenite concentrations: at a sodium selenite concentration of about 110 mg/L, the authors observed a reduction of 80% in the final dry weight of the yeast (Rajashree & Muthukumar, 2013). In the same paper, the authors reported that *S. cerevisiae* NCYC 1026 was also able to grow when the concentration of sodium selenite was greater than 300 mg/L sodium selenite. 4% of the yeast survived (Rajashree & Muthukumar, 2013).

On the basis of the results obtained, 100 mg/L sodium selenite was defined as "stimulated" for all further experiments, including the comparative proteomic analyses, so that the concentration would be able to induce metabolic changes in *S. cerevisiae* UFMG A-905 without totally inhibiting yeast growth.

To evaluate the effects 100 mg/L sodium selenite would have on growth curves, liquid cultures were prepared. Growth was monitored by measuring OD_{600}. As shown in Fig. 2, the Se concentration tested strongly inhibited the growth of the strain: final biomass produced when Se is present in the solution is about 2.5-fold lower than in the control. Final OD_{600} in the stimulated sample was 4.54, while in the control it was 11.12.

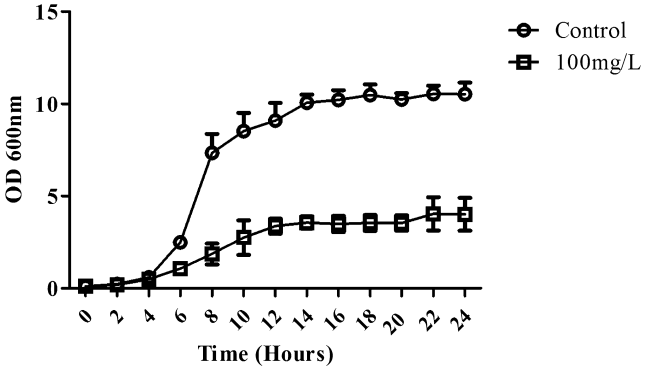

Fig. 2 – Growth curve of *S. cerevisiae* UFMG A-905 in YPD control condition and in YPD fortified with 100 mg/L sodium selenite. Results are reported as mean values ± SEM of 3 biological replicates.

Several kinds of microorganisms are known to be resistant to high concentrations of sodium selenite. It is probably related to a system of sodium selenite detoxification described in yeasts by Lewinska and Bartosz (Lewinska & Bartosz, 2008). Lamberti et al. used TEM to prove that Se deposits form at the level of the membrane or cell wall in the bacterium *Lactobacillus reuteri* Lb2 BM DSM16143 grown in 4 mg/L sodium selenite. This gave the biomass a red coloration (Lamberti et al., 2011). Since pellets of *S. cerevisiae* UFMG A-905 grown in sodium selenite solutions were red, it is reasonable to conclude that this strain also uses this detoxification mechanism to counteract the toxicity of sodium selenite. To test this hypothesis, pellets of cells were grown in YPD medium and YPD medium containing 100 mg/L sodium selenite were analysed by TEM. The experiment was performed after 8 h of growth in the exponential growth phase and after 24 h of growth in the stationary growth phase. The results are shown in Fig. 3.

Fig. 3A shows cells grown without sodium selenite. There are no visible elemental Se deposits. Fig. 3B shows cells grown in sodium selenite and harvested in the middle exponential growth phase. Cells are still intact and there are a few deposits of elemental Se that are not truly attached to the cell wall. Fig. 3C shows *S. cerevisiae* UFMG A-905 cells grown in sodium selenite harvested in the stationary growth phase. The cells are damaged and there are several elemental Se at the cell wall level. This experimental evidence confirms that the detoxification mechanism described also operates in *S. cerevisiae* UFMG A-905. Furthermore, the fact that there are more abundant elemental Se deposits in the cells in the stationary phase suggests that the mechanism is completely activated at the end of yeast growth.

3.2. *Proteomic analyses*

In toto proteome in the 4–7 *pI* range was analysed for an *S. cerevisiae* UFMG A-905 control and when stimulated with sodium selenite (Fig. 4). The images showed that Se enrichment induced the up-regulation of 8 spots, while 5 were down-regulated (Fig. 4). All the differentially expressed spots were

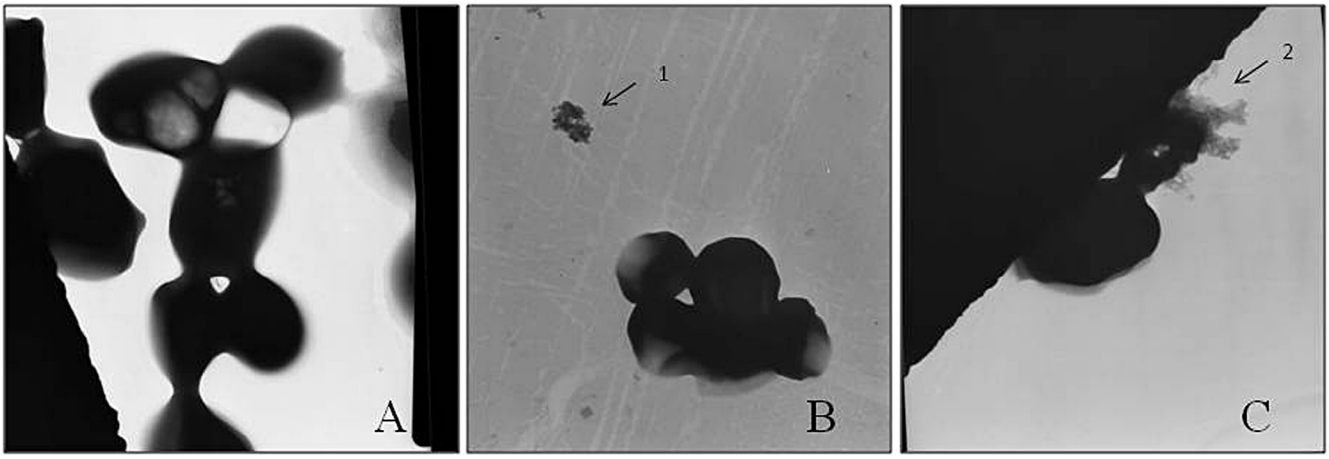

Fig. 3 – TEM images of *S. cerevisiae* UFMG A-905 grown in YPD (A), in YPD after 8 hours of growth with 100 mg/L of sodium selenite (B) and in YPD after 24 hours of growth 100 mg/L of sodium selenite (C). The arrows indicate deposits of sodium selenite.

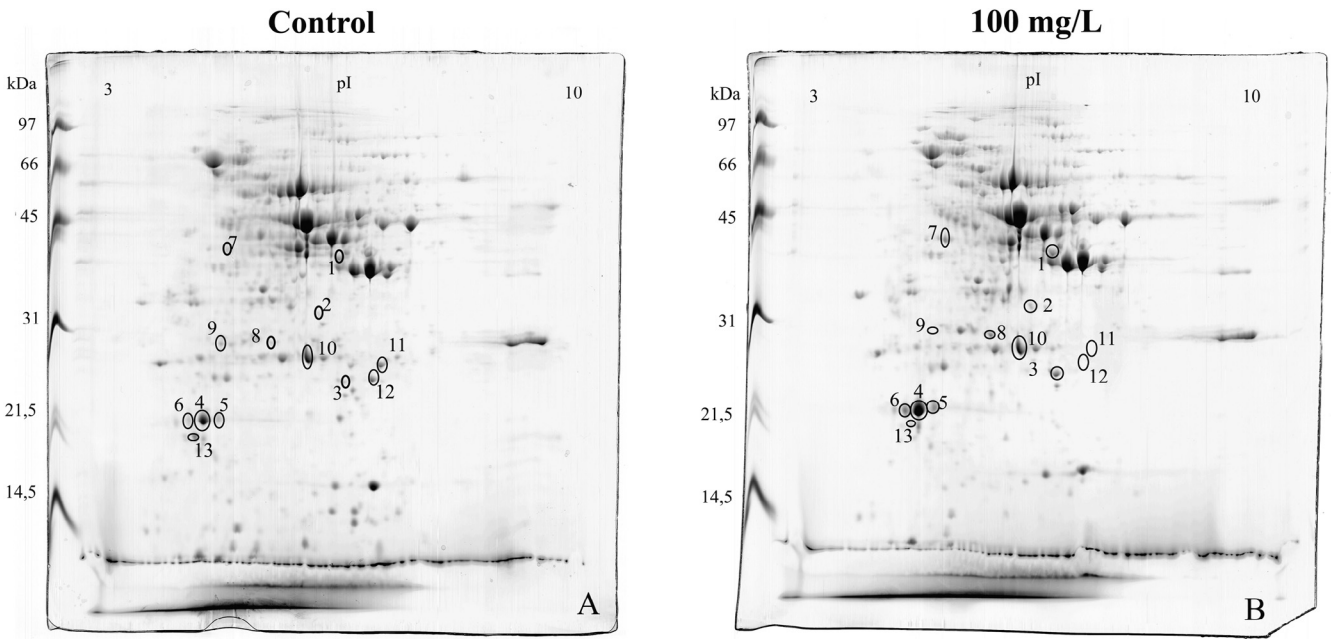

Fig. 4 – 4–7 *pI* 2-DE maps of *in toto* proteins of *S. cerevisiae* UFMG A-905 grown in YPD in control condition and in YPD + 100 mg/L sodium selenite. Highlighted spots are differentially expressed between the two tested conditions.

identified by MALDI TOF-TOF mass spectrometry and the resulting proteins were divided into four functional groups (Fig. 5).

In bacteria and mammals, Se can be incorporated both as SeCys and SeMet. In yeast, only SeMet insertion seems possible. There was a recent paper by Bierla et al. (2013) that gave the first evidence acquired using a mass spectrometer of the substitution of cysteine sulphur by Se during the production of Se-enriched yeast. Nevertheless, this phenomenon is quite irrelevant, since the percentage of SeCys is between 10 and 20%, while more than 60% of SeMet inserted. For this reason, the up-regulation of a cystathionine gamma-lyase CYS3 (spot 1) isn't surprising. This enzyme is involved in the metabolism of compounds of Se. Bockhorn et al. (2008) demonstrated that it is responsible for the conversion of SeMet to methylselenol. This reaction is catalysed by Met gamma lyase in mammals (Palace et al., 2004). Methylselenol and hydrogen selenide can

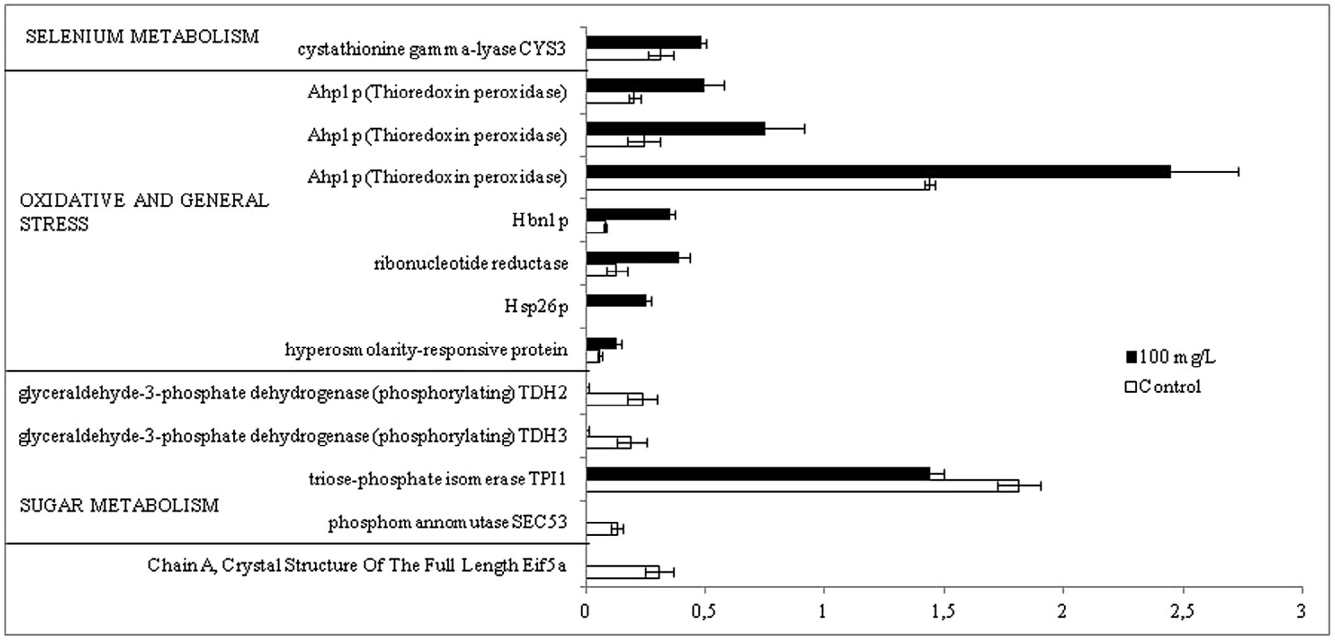

Fig. 5 – Functional classification of the proteins whose expression is significantly affected by 100 mg/L sodium selenite. For each identified protein average volumes and their variations (SEM) in the two experimental conditions (3 biological replicates and 2 technical replicates) are shown.

produce reactive oxygen species (ROS) through a glutathione-dependent reaction, causing oxidative stress within the cell. This is one of the proposed mechanisms for Se toxicity, though it is still unclear (Tarze et al., 2007). Methylselenol is an important compound in this process, and *CYS3* gene deletion leads to a high level of resistance to SeMet (Bockhorn et al., 2008).

The second functional group includes proteins involved in oxidative and general stress protection. Several metals exert toxicity through Fenton reactions that generate ROS, which can cause lipid peroxidation (Nguyên-nhu & Knoops, 2002). Though Se is classified as a non-metal, it has been reported that spontaneous reaction between selenite and free thiols generates ROS. Specifically, selenite is reduced to hydrogen selenide that in the presence of O_2 produces elemental Se and ROS (Peyroche et al., 2012). Alkyl hydroperoxide reductase 1 (Ahp1p, spots 4, 5 and 6) is a thioredoxin peroxidase of the peroxiredoxin family, mainly present at the cytosolic level (Farcasanu, Hirata, Tsuchiya, Mizuta, & Miyakawa, 1999). It is able to remove both hydrogen peroxide and alkyl hydroperoxides (Park, Cha, Jeong, & Kim, 2000) because it has a strong antioxidant capacity against organic peroxides. *In vitro*, its antioxidant function requires both thioredoxin and thioredoxin reductase (Lee, Spector, Godon, Labarre, & Toledano, 1999). The depletion of this enzyme causes an increase in lipid peroxidation, confirming its fundamental role in protecting *S. cerevisiae* from oxidative damage (Nguyên-nhu & Knoops, 2002). Another up-regulated protein is Hbn1p (spot 3), a putative nitroreductase-like protein. The nitroreductase family catalyses the reduction of nitro-substituted compounds using FMN or FAD as prosthetic groups and NADH or NADPH as reducing agents (Bryant & De Luca, 1991). Oliveira et al. demonstrated that Hbn1p is involved in oxidative stress response. It is important during oxidant exposure, such as the production of ROS induced by selenite, or when the cell activates the mechanisms required to maintain the intracellular redox balance (de Oliveira, Zanotto-Filho, Moreira, Bonatto, & Henriques, 2010). Several papers have stated that selenite directly damages DNA (Mániková et al., 2012). In *S. cerevisiae*, for example, double-strand breaks have been reported (Letavayová et al., 2008). The DNA damage linked to this kind of stress could explain the up-regulation of ribonucleotide reductase (spot 7), the essential enzyme responsible for the synthesis of 2′-deoxyribonucleotides and involved in DNA replication and repair (Lu & Holmgren, 2014), which receives electrons directly from thioredoxin (Arnér & Holmgren, 2000). Therefore, it seems that several proteins involved in repairing damage from oxidative stress are produced in the presence of 100 mg/L sodium selenite, confirming that this compound causes a significant degree of stress and, in parallel, the ability of *S. cerevisiae* UFMG A-905 to counteract this problem. Another chaperone protein, Hsp26p (spot 8), and a hyperosmolarity-responsive protein (spot 2) were up-regulated. Hsp26p is involved in protecting the cell from changes in temperature (Rossi & Lindquist, 1989), while the hyperosmolarity-responsive protein is involved in protecting the cell from hyperosmotic stress (Hyrayama, Ohto, Mizoguchi, & Shinozaki, 1995), respectively. However, as often happens with proteins involved in stress processes, they can also be employed during stress events other than the one they are mainly involved in. This phenomenon has also been demonstrated in *L. reuteri* Lb2 BM DSM16143, in which two chaperones, GroEL and GrpE, were over-expressed in the presence of Se (Lamberti et al., 2011).

Two glycolytic enzymes are among down-regulated proteins: glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) TDH2 and TDH3 (spots 11 and 12) and triose-phosphate isomerase TPI1 (spot 10). In *S. cerevisiae*, there are three isoforms of GAPDH: TDH1, TDH2, and TDH3; TDH2 and TDH3 form catalytically active homotetramers. TDH2 contributes 25–30% of the total activity of the enzyme, while TDH3 contributes 50–60% (McAlister & Holland, 1985). Triose-phosphate isomerase catalyses the reversible conversion between glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Generally, this enzyme directs DHAP towards GAP, thus improving the flow through the glycolytic pathway and producing more ATP. El-Bayoumy et al. (2012) have reported that the expression of this enzyme is repressed by Se; although the mechanism involved in this decrease is still unclear, the authors suggested that Se might disrupt glycolysis. It is reasonable to conclude that TPI1 down-regulation is in some way linked to the use of DHAP for glycerol biosynthesis instead of shifting it towards glycolysis. Actually, lipid peroxidation can cause damage to cellular membranes, leading to the need for lipid synthesis. This synthesis can be achieved by the concerted action of several enzymes. One of these enzymes is esterase, which uses glycerol as a substrate for lipid synthesis. In this model, the flux is directed to DHAP and glycerol and GAPDH is inhibited, which can make more GAP available for conversion into DHAP. Overkamp et al. demonstrated that in *S. cerevisiae tpi1Δ* mutants, DHAP accumulation is actually prevented because it is converted into glycerol (Overkamp et al., 2002). Considering that yeast cells have several energy-generating pathways, reducing glycolysis and preserving lipid integrity seems to be a feasible explanation.

Phosphomannomutase SEC53 (spot 9) is a well-conserved enzyme that converts mannose-6-phosphate to mannose-1-phosphate, a reaction that must take place in the first steps of protein glycosylation in yeast cells (Staneva, Uccelletti, Farina, Venkov, & Palleschi, 2004), for both N-linked and O-linked sugars. Protein glycosylation is essential for proteins to fold correctly. Furthermore, secretion requires proper glycosylation of proteins to be recognized as a substrate by the endoplasmic reticulum (ER) (Matynia, Salus, & Sazer, 2002). Indeed, a mutation in this enzyme causes an error in glycosylation and accumulation of proteins in the lumen of ER (Ferro-Novick, Novick, Field, & Schekman, 1984). Sodium selenite also causes the down-regulation of eIF5A (spot 13), a small eukaryotic translation initiation factor (16–18 kDa) that is ubiquitous and highly conserved among eukaryotes and archaea (Kim, Hung, Yokota, Kim, & Kim, 1998). Its real function is still unclear; at present eIF5A is known to directly stimulate protein synthesis. Its depletion causes a rapid decrease in ribosomal loading with an immediate inhibition of protein synthesis (Henderson & Hershey, 2011). This protein was also found to be repressed in a study focusing on the effect of Se on a type of baker's Se-yeast, confirming the results found here (El-Bayoumy et al., 2012).

In order to fully understand the effects caused by Se, the degree of stress it causes must be taken into consideration. Glycolysis is the main energy-generating route. It is repressed at the same time as protein synthesis is reduced. This is

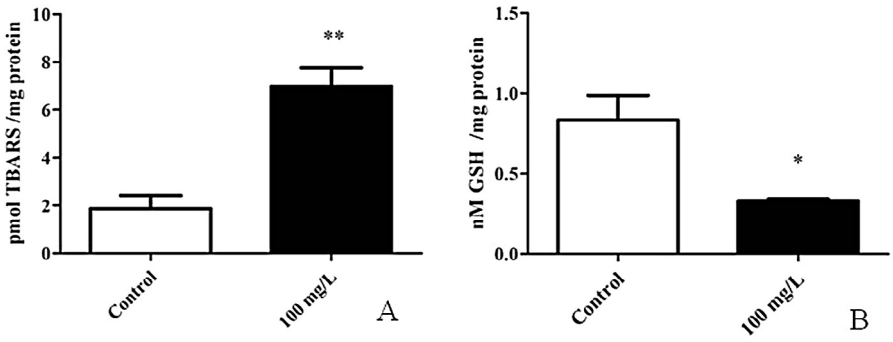

Fig. 6 – TBARS (A) production and GSH (B) levels in *S. cerevisiae* UFMG A-905 grown in YPD and in YPD + 100 mg/L sodium selenite. The values are expressed in concentration/mg protein: (*) indicates significantly compared to the control $p < 0.05$ and (**) indicates significantly compared to the control $p < 0.005$. Results are reported as mean values ± SEM of 3 biological replicates.

determined by the down-regulation of an important translation factor (eIF5A); thus, it seems that a general reduction of the whole metabolism of *S. cerevisiae* UFMG A-905 takes place, confirmed by slower, reduced growth. In parallel, this strain puts a series of actions in place that are aimed at minimizing the effects of Se toxicity: it up-regulates several proteins involved in protection against general and oxidative stress induced by Se. Through this process, internal membranes are protected from lipid peroxidation and triacylglycerol synthesis is stimulated and the nucleic acid ribonucleotide reductase is regenerated.

Another interesting observation is that Ahp1p and HSP26p, two proteins that were found to be up-regulated in this study, have already been described by several other authors as containing Se, specifically in the form of SeMet (Bierla et al., 2013; McSheehy, Kelly, Tessier, & Mester, 2005). In the case of Ahp1p, the selenized peptide MPQTVEWSK was detected in this study (Table S1). This peptide was identified by Bierla et al. using a LA ICP-MSI approach followed by capHPLC-electrospray MS identification. This finding strongly suggests that Se is also incorporated as SeMet in the *S. cerevisiae* strain analysed in this study.

3.3. *Physiological evidence of oxidative stress induction by sodium selenite*

The effect of 100 mg/L sodium selenite on the formation of both TBARS and GSH by *S. cerevisiae* UFMG A-905 was determined. Both of these molecular compounds are measured as an indicator of oxidative stress. TBARS production is directly correlated with lipid peroxidation (Lushchak & Gospodaryov, 2005). This system, based on reduction/oxidation of GSH, is one of the most common strategies to counteract radical oxygen species. GSH levels are higher when there is oxidative stress. As shown in Fig. 6, sodium selenite significantly increases TBARS production (about 7.5 pmol TBARS/mg protein compared to about 4 pmol TBARS/mg protein) and significantly reduces GSH concentration (about 2 nM/mg protein compared to about 6 nM/mg protein in the control). This physiology-based evidence confirms that when there is sodium selenite in the culture medium, it leads to oxidative stress in *S. cerevisiae* UFMG A-905 cells. These results are agreed with several studies in the literature reporting that Se induces oxidative stress (Izquierdo et al., 2010; Kaur & Bansal, 2006; Lewinska & Bartosz, 2008). Furthermore, there was a decrease in the GSH:GSSG ratio induced by Se oxidation of GSH. This phenomenon results in an inhibition of G1, G2, and S-phases of the cell cycle with a consequent reduction of growth (Combs & Gray, 1998; Kaur & Bansal, 2006). These data are also in agreement with both the growth reduction and up-regulation of oxidative stress proteins induced by sodium selenite.

4. Conclusions

Sodium selenite was found to exert significant oxidative stress on the yeast *S. cerevisiae* UFMG A-905 and several mechanisms were shown to be activated to counteract this stress. The use of physiological tests along with comparative proteomic analyses provide a powerful approach to define a clear picture of the effects of excess Se on the metabolism and physiology of *S. cerevisiae* UFMG A-905.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.jff.2015.06.048.

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