Investigation of protein expression of *Saccharomyces cerevisiae* cells in quiescent and proliferating state before and after toxic stress

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**ABSTRACT**

The focus of the present study is to determine proteins responsible for the oxidative and toxic stress response in proliferating and stationary phase (G\(_0\)) cultures. Therefore, the yeast *Saccharomyces cerevisiae* was treated with oxidative and drug compounds (H\(_2\)O\(_2\), menadione, zeocin, and ibuprofen) in both phases. These substances were chosen to determine the redox status of the yeast. *S. cerevisiae* appeared to employ different strategies to ensure their antioxidant defence metabolism. Analysis, including sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) coupled with mass spectrometry, was used in the search. The proteins were identified by SDS-PAGE, matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) mass spectrometry analysis, and Mascot database-fingerprint. The final step was determination of protein profiles of yeast *S. cerevisiae* in proliferating (M) and stationary phase (G0). Seven bands were determined and the corresponding proteins were proposed: cytochrome c peroxidase, glutathione S-transferase omega-like, NAPDH-dependent diflavin reductase, DNA replication fork-blocking protein, putative aryl alcohol dehydrogenase, AP-1-like transcription factor YAP5, GTP-binding protein. All putative proteins coincide with the literature database. A typical example of such an adaptation mechanism in the defence against oxidative damage is the synthesis of several glutathione and thioredoxin peroxidases in the yeast cell. A deeper investigation of the conserved mechanisms responsible for entry into, survival, and exit from quiescence in higher eukaryotes will help the development of new anticancer therapies, the study in the process of ageing and neurodegenerative diseases.

**Introduction**

In view of the fact that all living organisms are subject to changing environmental conditions, knowledge of stress and stress response is critical to understand how single and multicellular organisms adapt to endogenous and exogenous stress factors.

Yeast *Saccharomyces cerevisiae*, established as the most broadly researched microorganism concerning stress responses, during starvation, cease growth and enter a non-proliferating state denoted as a stationary phase or quiescence [1,2]. Moreover, cells in stationary phase develop specific differentiation programmes which allow preservation of viability for prolonged time without the presence of nutrients. And quiescent cells possess the capacity to restart growth immediately in the presence of nutrients [3]. The mechanisms by which eukaryotic microorganisms survive prolonged periods of nutrient limitation and resume proliferation remain unclear. Stationary phase cells are also unbudded and contain unreplicated DNA, characteristic of the regulatory step in G\(_1\) phase of the mitotic cell cycle [4].

Both the common mechanisms of stress response among eukaryotes and the genetic and molecular advantages of *S. cerevisiae* allow us to use them as a model system for better understanding the stationary phase in yeast cells as well as to provide novel insights into non-proliferating states in other eukaryotic cells [4]. Of considerable interest is the comprehension of different mechanisms implicated in the stress response of yeast *S. cerevisiae*, caused by different endogenous and exogenous toxic compounds.
The cells of the yeast in stationary phase could mirror cells of multicellular organisms regarding different important metabolic characteristics. It is stated that most of the energy in the G0 phase is derived from mitochondrial respiration, a process leading to considerable cellular damage over time [5]. Taking into account that cells from multicellular eukaryotic organisms spend most of their lifetime in the G0 phase, it is reasonable to use S. cerevisiae quiescent cells not only to study transcriptional regulation, but also to get valuable information for other fields like cancer, development, and ageing research [6].

This study investigated the stress response of yeast S. cerevisiae during exposure to different exogenous agents in proliferating and stationary phase. The specific protein profiles were determined in both states triggered by four different toxic compounds: H2O2, menadione, ibuprofen, and zeocin, using proteomic analysis and mass spectrometry.

**Materials and methods**

**Yeast strain and growth conditions/harvesting. Isolation of G0 cells**

The yeast strain used in this study was S. cerevisiae BY4741 with a genotype MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0, which is an auxotroph and is described by Harsch et al. as haploid [7]. It was obtained from the collection centre EUROSCARF (European S. cerevisiae Archive for Functional Analysis, Institute of Molecular Biosciences, Johann Wolfgang Goethe-University) Frankfurt, Germany. The yeast cells (0.3 g) were batch-cultivated in YPD medium (1% yeast extract, 2% bacto/peptone, 2% glucose) with a rotational Schuettel Apparatus (205 rpm) at 30°C for 20 h for logarithmically growing yeast and for 168 h at 30°C for cultures reaching stationary phase of growth, respectively. The lab flasks were inoculated with a culture of S. cerevisiae BY4741 grown on YPD agar slant for 24 h. To harvest the biomass, the broth was centrifuged at 5000 rpm for 10 min with centrifuge Sigma 3-30KS (Sigma Laborzentrifugen GmbH, Germany). The pellet was washed twice with distilled water and centrifuged following the same parameters.

The isolation of cells that have entered G0 phase was carried out through fractioning in percoll density gradient according to the method used by Allen et al. [8]. Briefly, 6 mL of the percoll solution (percoll: 1.5 mol/L NaCl) in ratio 9:1 (v/v) were pipetted in four 8-mL conical tubes. To form a gradient, the tubes were centrifuged at 13,800 rpm (19,240 g) for 15 min at 20°C. The lower and upper cell fractions were separated based on differences in their density. G0 cells formed the denser fraction (respectively the lower one) due to the thickened cell walls and accumulation of storage carbohydrates. The upper fraction containing non-quiescent cells was not a subject of the present study. Each fraction was washed twice in 40 mL 0.1 mol/L Tris-HCl buffer solution with pH 7.5 and centrifuged for 10 min at 4000 rpm at 20°C (Sigma 3-30KS, Sigma Laborzentrifugen GmbH, Germany). The cells in G0 state were presented as the high-density lower fraction [8].

**Cell survival after inhibition with H2O2, menadione, ibuprofen, and zeocin**

Survival rates of both, proliferating (Log) and G0 cultures of S. cerevisiae BY4741, were tested after treatment with oxidative and drug agents with a wide range of concentrations. IC50 was determined using a spot analysis. The yeast strain was grown on YPD agar medium which was added, after cooling, H2O2 (5, 10, and 20 mmol/L), menadione (30, 40, 50, 100, 200, 300 μmol/L), ibuprofen (0.5, 0.75, 1.1, 1.5 mg/mL), and zeocin (50, 75, 100 μg/mL) were added. Yeast cultures were grown at 30°C for 48 h together with the control without the addition of oxidative or drug agents.

**Oxidative and toxic studies of cells exposed to IC50 doses**

Oxidative and toxic studies were performed by incubating the proliferating and G0 state yeast suspensions (0.3 g biomass resuspended in 1 mL of 0.1 mol/L Tris buffer, pH 7.5) for 1 h at room temperature with IC50 doses of the following agents: hydrogen peroxide (5 mmol/L), menadione (100 μmol/L), ibuprofen (1.1 mg/mL), and zeocin (50 μg/mL, respectively. After incubation, the cells were washed twice with distilled water and subjected to mechanical disintegration. Treated and untreated yeast cells (0.3 g) were resuspended in 0.05 mol/L potassium phosphate buffer pH 7.8 and mixed with polyester beads (0.1 ± 0.5 mm) (Sigma-Aldrich, Germany) at a ratio of 1:2:1. The resuspended biomass was disintegrated by a Bullet Blender® Storm homogenizer at 8000 rpm, three times for 5 min. Cell debris was removed by centrifugation at 5000 rpm for 15 min, the resulting supernatant was clarified after centrifugation at 13,000 rpm for 20 min at 4°C and the homogenate was stored at -20°C.

**Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE (10%) was carried out using a standard procedure to examine the change in the expression of
proteins in cells treated with oxidative and drug agents in comparison to non-treated ones [9–11]. The reagents of analytical grade for SDS-PAGE N,N’,N’-tetramethylethylene-ethylenediamine (TEMED) and ammonium persulphate (APS) were supplied from Sigma®, Steinheim, Germany. Rapid Gel™ 40% liquid acrylamide was used as acrylamide-bisacrylamide reagent diluted 19:1 (USB Corporation, Cleveland, OH, USA) for casting the mixture. All bands were visualized by staining with Coomassie Brilliant Blue G-250 Sigma®, Steinheim, Germany. Unstained SDS-PAGE protein marker mixture from SERVA Electrophoresis GmbH, Heidelberg, Germany was applied as a standard identifier, including the following proteins with respective molecular masses: myosin (200 kDa), β-galactosidase (116.3 kDa), albumin bovine (67 kDa), ovalbumin (45 kDa), carboxy anhydrase (29 kDa), trypsin inhibitor (21 kDa), lysozyme (14 kDa), aprotinin (6.5 kDa). The samples were dissolved in Laemmli sample buffer (SERVA Electrophoresis GmbH) in a ratio of 1:1 and an amount of 25 µL was loaded into the wells of the PAG (polyacrylamide gel), whereas only 10 µL of standard mixture was applied. The 10% SDS-PAGE electrophoresis of intracellular crude extracts from S. cerevisiae BY4741 biomass isolated from proliferating and G0 phases was performed spot analyses revealed the degree of sensitivity of the yeast cells to the applied toxic concentrations of the selected stress agents. The results of the performed spot analyses revealed the degree of sensitivity of the yeast cells to the applied toxic concentrations, and IC50 for each of the tested compounds was defined: menadione (100 µmol/L), H2O2 (5 mmol/L), ibuprofen (1.1 mg/mL), and zeocin (50 µg/mL) (Figure 1). At IC50, the test compounds inhibited cell growth between 40% and 60% compared to the control cells and were taken as utmost concentrations at which yeast cells still retained the ability to grow. The toxicity of menadione and H2O2 is related mainly to their ability to produce intracellularly large amounts of reactive oxygen species (ROS). At high

**Database searches**

Mass spectral data were processed through FlexAnalysis 3.4 (Brucker Daltonics) and subsequently subjected to a search against different protein databases using MASCOT server (Matrixscience, London, UK). Additionally, database NCBI port and SwissProt were used to confirm the Mascot searches. For successful analysis and validation of the proteins, the following criteria were applied: (1) Type of search: Peptide Mass Fingerprint; (2) Enzyme: Trypsin; (3) Taxonomy: Saccharomyces cerevisiae (baker’s yeast); (4) Mass values: Monoisotopic, Peptide Charge State: 1; (5) Max Missed Cleavages: 1; (6) Peptide Mass Tolerance: ±2.5 Da; (7) Report top hits: 5 (WC 1229).

**Results and discussion**

**Cell viability under stress conditions**

In order to investigate the toxic effect of menadione, hydrogen peroxide, ibuprofen, and zeocin on the viability of proliferating and quiescent S. cerevisiae cells, both cell types were exposed to a range of concentrations of the selected stress agents. The results of the performed spot analyses revealed the degree of sensitivity of the yeast cells to the applied toxic concentrations, and IC50 for each of the tested compounds was defined: menadione (100 µmol/L), H2O2 (5 mmol/L), ibuprofen (1.1 mg/mL), and zeocin (50 µg/mL) (Figure 1). At IC50, the test compounds inhibited cell growth between 40% and 60% compared to the control cells and were taken as utmost concentrations at which yeast cells still retained the ability to grow.
concentration, these oxidizing compounds induce strong oxidative stress, which results in disturbance of the cellular redox homeostasis, decrease of cell viability and finally cell death.

The results from the performed spot analyses revealed that both cell types showed relative resistance to exogenous menadione up to 50 μmol/L, with colony growth detected from all dilutions. However, menadione exerted its toxic effect on both proliferating and G0 cells when added to the medium at a concentration of 100 μmol/L. The stronger inhibitory effect on growth (60%) was observed for cells in G0 state, in contrast to proliferating ones (40% growth inhibition). A concentration of 200 μmol/L menadione completely inhibited growth of the two cell types. According to Chuang et al. [14], low concentrations of superoxide-generating menadione (2 μmol/L) induce the formation of ROS which function as signal molecules in the cells. However, at high concentration, menadione triggers strong oxidative stress due to the intense formation of superoxide, radicals, and mitochondrial dysfunction and fragmentation. Having in mind that in G0 state the major source of energy is the mitochondrial respiration [5], it is not surprising that this agent has stronger effect in quiescent yeast cells.

Contrary to the results observed for menadione, the exposure of S. cerevisiae BY4741 to 5 mmol/L exogenous H2O2 led to insignificant inhibition of growth of G0 cells. However, for the exponentially grown yeasts, 40% inhibition was detected at this test concentration. The observed damage rate in proliferating cells was probably due to their higher metabolic activity and the corresponding elevated levels of oxidative damage. The results from the performed analysis with the non-steroidal anti-inflammatory drug ibuprofen revealed that at IC50 (1.1 mg/mL) of this chemical induce stronger toxic effect on proliferating yeast cells, as evidenced by weaker growth (60% growth inhibition). The quiescent S. cerevisiae cells showed visibly greater resistance with 40% inhibition of cell growth. A concentration of 1.5 mg/mL was lethal to both cell types. In the zeocin assay, both types of yeast cells showed relatively equal resistance to its toxic effect at all three tested concentrations. Knowing that zeocin causes cell death by intercalating into and cleaving DNA, it was evident that its toxicity should be comparable among both types of cells.

**Comparative analyses of the changes in the expression of proteins in proliferating (LOG) and stationary (G0) phase by SDS-PAGE**

The main objective of the present work was to determine the protein profile of the yeast strain undergoing oxidative stress. For that purpose, treated and non-treated homogenates from S. cerevisiae BY4741 were analysed by SDS-PAGE and mass spectrometry. The results from the 10% SDS-PAGE together with the
identified MALDI-TOF/MS spectra and the putative proteins and enzymes corresponding to the bands and their function are also presented. The 10% SDS-PAGE in Figure 2 gives a very intriguing visualization of the oxidative and drug stress, and the response to it, respectively. Eight fractions could be identified on lane 9 which was the control in proliferating state. The observed proteins (control in Log state) were in the size range between 25 kDa and 65 kDa. Four strongly stained protein bands at approximately 26, 32, 42, and 65 kDa were detected. Of our interest, Band 1 at 60–65 kDa and Band 6 at 32 kDa, were analysed mass spectrometrically and discussed further below. In contrast to the control in proliferating state (lane 10, control in G0), such intensive bands were not observed, except a protein band at 42 kDa. Obviously, the production of proteins was suppressed in the aforementioned lane, corresponding to the already known fact that at stationary state the cellular metabolism is lowered down [15].

Changes in the expression of proteins were recognized after treatment of the cell suspensions in proliferating and quiescent state with 5 mmol/L hydrogen peroxide observed in lane 1 and lane 2. In lane 2, a considerable increase in the protein synthesis was observed at 40–42 kDa (Band 3 and Band 4) and 26–28 kDa (Band 7). A fraction corresponding to 40–42 kDa was almost absent in lane 1. Identical expression of proteins at 62–65 kDa was recognized in both lanes 1 and 2, Band 1 and Band 2, respectively, although a few additional bands appeared around Band 2 (lane 2). Moreover, a comparison shows that most of the protein bands which were abundantly expressed in the control sample (lane 9) were less intensive in lane 1. On the contrary, in lane 2, three extensively stained bands appeared, compared to lane 10.

In lanes 3 and 4, that there was expression of proteins primarily at ~62 kDa and ~50 kDa after treatment of the suspensions of proliferating cells and cells in G0 state with 100 μmol/L menadione. Comparison between both lanes demonstrates that bands were more intensive for cells in quiescent state. Although the preliminary test for cell viability showed a low degree of inhibition both in proliferating and G0 state, it could be assumed that the experimentally determined sublethal concentration of menadione (100 μmol/L) was too high and therefore repetition of the experiments could be done by treating the homogenate with a lower concentration. Moreover, Cymne et al. [16] conducted such an experiment for viability of the same S. cervisiae strain in stationary phase, performed with 40 μmol/L of menadione. A hypothesis for the behaviour of the cell

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**Figure 2.** Denaturating 10% SDS-PAGE of proteins isolated from S. cerevisiae in Log and G0 phase: lane 1 Log phase treated with H2O2; lane 2 G0 phase treated with H2O2; lane 3 Log phase treated with 100 μmol/L menadione; lane 4 G0 phase treated with 100 μmol/L menadione; lane 5 Log phase treated with 1.1 mg/mL ibuprofen; lane 6 G0 phase treated with 1.1 mg/mL ibuprofen; lane 7 Log phase treated with 50 μg zeocin; lane 8 G0 phase treated with 50 μg zeocin; lane 9 control in Log phase; lane 10 control in G0 phase; St: Standard from Serva.
Considerable changes were noticed by the expression of proteins in lanes 5 (cells at Log phase, treated with ibuprofen) and 6 (cells at G0 phase treated with ibuprofen). One can recognize that the expression of proteins after treatment of quiescent cells with ibuprofen, was elevated. In lane 6, bands at 60–65 kDa (Band 1), 40–42 kDa (Band 4), 30–32 kDa (Band 5), and 26–28 kDa (Band 7) were more abundantly expressed than those bands in lane 5. He et al. [17] in their work mentioned that ibuprofen boosts longevity in yeast, especially in S. cerevisiae. They stated that the pro-longevity function of ibuprofen owes its activity to the noncyclooxygenase activity. Ibuprofen was considered as a drug with decreased risk of causing age-related pathologies that could be taken into account as a stepping stone to finding efficacious and safe therapeutics for humans [17]. What was also observed is that only 3 bands are dominant in lane 2. It was obvious that the fraction at 40–42 kDa was wider than the one found at the same position in lane 6. Therefore, one can assume that two proteins were responsible for responding to hydrogen peroxide stress.

Lanes 7 (metabolically active yeast cells treated with zeocin) and 8 (cells in G0 state treated with zeocin) indicated some changes in the expression of proteins. One can directly spot that proteins expressed in lane 7 were hardly discerned in comparison to bands in lanes 9 and 2. The proteins in lane 8, on the contrary to lane 7, were more visible, where the protein at 60–65 kDa was more intensively expressed. The protein band was observed in Log phase cells treated with zeocin (lane 8) not in the control suspension in G0 state (lane 7). An overall speculation for the zeocin activity proposed by Dialynaki et al. [18] was the discard of the chelated Cu²⁺ when the drug penetrates the cells. That phenomenon further results in changes in vital cell biological processes. It was also found that zeocin acts especially upon TORC1 conserved signaling pathway. Dialynaki et al. [18] suggested that treatment of yeast cells with zeocin is ensued by a switch of metabolism towards catabolism.

The selected bands marked with black dotted circles on the SDS-PAGE were excised. Consequently, tryptic digestions and mass spectrometric analyses were performed. The aim of the tests was to propose and find expressed enzymes responsible for the adaptation to oxidative stress for the following bands: Band 1 (60–65 kDa), Band 2 (60–63 kDa), Band 3 (40–42 kDa), Band 4 (40–42 kDa), Band 5 (30–32 kDa), Band 6 (30–32 kDa), and Band 7 (26–28 kDa).

### Identification of protein profile by mass spectral analysis

The putative proteins and enzymes were experimentally determined through a tryptic digest of the bands after running SDS-PAGE, and subsequently MALDI-TOF-MS data and Mascot analysis. A comprehensive view (Table 1) gives more information about the proteins and enzymes. Based on the spectra and most intensive peaks, the peptides give a clue as to what enzymes could have a role in the expression of proteins of S. cerevisiae undergoing oxidative and drug stress.

In the paragraphs below, the experimentally determined proteins and enzymes in proliferating and stationary phase cells undergoing oxidative stress are discussed.

| Band No. | Systematic name (SGD) | Coding gene | Protein name | Determined mass (Da) | Theoretical mass (Da) | Localization |
|----------|-----------------------|-------------|--------------|----------------------|-----------------------|--------------|
| 1        | YDR110W               | FOB1        | DNA replication fork-blocking protein | 65,000 | 65,309.2 | Nucleolus, rDNA heterochromatin |
| 2        | YPR048W               | TAH18       | NADPH-dependent diflavin oxidoreductase | 66,000 | 72,336.7 | Mitochondrion |
| 3        | YKR066C               | CCP1        | Cytochrome c peroxidase | 40,000 | 40,348.3 | Mitochondrion |
| 4        | YMR251W               | GTO3        | Glutathione S-transferase omega-like | 42,000 | 42,403.3 | Cytosol |
| 5        | YFL057C               | AAD 16      | Putative aryl alcohol dehydrogenase | 32,000 | 39,657 |  |
| 6        | YIR018W               | YAP 5       | AP-1-like transcription factor YAP 5 | 31,000 | 28,393.8 | Part of nucleus, part of nuclear chromatin |
| 7        | YNL093W               | YPTS5       | GTP-binding protein | 26,000 | 24,640.0 | Part of late endosome |

Table 1. Enzymes responding to oxidative stress with significant changes in expression on SDS-PAGE in Log and G0 state after treatment with H₂O₂, menadione, ibuprofen, and zeocin.
segregation of rDNA repeats in tune with Fob1 [20]. Fob1 has distant, but significant analogy to the retroviral integrase [21]. Another crucial aspect of the protein is its involvement in the regulation of cell ageing, where a null mutant exhibits lengthened replicative lifespan [22]. As far as stress in the cell is concerned, it was observed an increased resistance to methyl methanesulphonate (MMS). An increased general stress and a progressive lengthening of the cell cycle for the last few cell divisions is the essence for ageing of the wild-type cells. Moreover, the aforementioned features are much less apparent in the long-lived FOB1 deletion mutant. The individual cells revealed that there are various forms of cell death being associated with diverse terminal cell morphologies and varying levels of stress and lifespan [22].

Band 2
The result from Mascot determined that Band 2 corresponds to the enzyme NAPDH-dependent diflavin reductase with a coding gene TAH18 and an approximate mass of 65 kDa. Fe-S clusters play a role as an inorganic cofactor in proteins taking part in a number of vital cell processes. The essential flavoprotein Tah18 is one of the components of the early step in the cytosolic and nuclear iron–sulphur protein assembly (CIA) machinery. The CIA machinery appears to be functionally conserved from yeast to humans through the human Ndor1–Ciapin1 proteins. The work of Netz et al. [23] pointed out that the complex of Tah18 and Dre2 constitutes an electron transfer chain delivering electrons from NADPH via the FAD (flavin adenine dinucleotide)- and FMN (flavin mononucleotide)-containing Tah18 to the Fe-S cluster of Dre2p. Other studies have shown that the mitochondrion in tah18 mutants owes its protection against H₂O₂-induced damage to cytochrome c [24]. Tah18 is involved in the positive regulation of hydrogen peroxide-mediated programmed cell death having an apoptotic (pro-death) role under oxidative stress, with a key process to eliminate dead cells. The protein complex, Dre2-Tah18 controls yeast cell death in response to high levels of hydrogen peroxide. In the absence of exogenous oxidative stress, Tah18 and Dre2 physically interact outside the mitochondria. Respectively, in the presence of oxidative stress, Tah18 is targeted to the mitochondria and controls mitochondrial integrity and cell death [24]. Tah18p-dependent nitric oxide synthesis provides high-temperature stress tolerance and could be possibly an applicant for development of antifungal drugs [25].

Figure 3 shows the mass spectrum obtained from Band 2 (lane 2 on the SDS-PAGE) with extensive peptide masses, expressed as [M + H]⁺ ions.

Band 3
In lanes 2 and 6, two proteins were determined at circa 42 kDa. The first putative protein/enzyme determined from Band 3 is the glutathione S-transferase omega-like GTO3 with a measured mass of ~42 kDa. The S. cerevisiae genome encodes three proteins that have analogy to human GSTos (omega-class glutathione S-transferase) hGSTO1-1 and hGSTO2-2. GSTos possess different features from most of the GSTs and bear a resemblance to Grx (glutaredoxin) proteins which, from the point of detoxifying the cell, are not included in the enzymatic defence of the cell against oxidative stress [26]. The three yeast proteins have been labelled as Gto1, Gto2, and Gto3, and their purified recombinant forms operate as thiol transferase (glutaredoxins) against model disulphide HED (β-hydroxyethyl disulphide), as dehydroascorbate reductases, and as dimethylarsinic acid reductases, while no activity against the standard GST substrate CDNB (1-chloro-2, 4-dinitrobenzene) is found [26]. Their glutaredoxin activity is also detectable in yeast cell extracts. The enzyme activity characteristics of the Gto proteins contrast to those of another yeast GST, Gtt1. The latter is active against CDNB and displays glutathione peroxidase activity against organic hydroperoxides such as cumene hydroperoxide, but is not active as a thiol transferase [27–30]. Gtt1 is linked to the endoplasmic reticulum and is involved in thermotolerance. Moreover, according to the work of Choi et al. [31], it is shown that Gtt1 arises after a diauxic shift and its high level of expression lasts during the stationary phase. The GSTos belong to a family of multipurpose isoenzymes that take part in the cellular defence against several exogenous and endogenous compounds having distinct structural and functional characteristics, which differ from those of other GSTs. Lately, genetic and molecular researches demonstrated evidence that regulating the MAPK signalling pathway GstO1 includes a protective function against H₂O₂ using the Drosophila system. On the other hand, GstO2 is required to trigger mitochondrial ATP synthase in the Drosophila neurodegenerative disease model [32].

Band 4
The second putative protein is mitochondrial cytochrome c peroxidase with measured mass ~40 kDa (theoretical mass 40348.3 kDa, Table 1), a haeme oxidoreductase which functions as degrading ROS in mitochondria, thus being involved in the response to oxidative stress [33,34]. This enzyme, determined from Band 4, is localized in the mitochondrial intermembrane space and its main biological function is
thought to be reduction of H\textsubscript{2}O\textsubscript{2} generated during aerobic respiration [35]. The determination of that enzyme, having a key function of detoxifying ROS, especially H\textsubscript{2}O\textsubscript{2}, is proposed in other papers. van der Klei et al. [36] in their work suggest that the scavenging role of cytochrome c peroxidase is an alternative mechanism of catalase enzyme [36,37]. As mitochondria are one of the most vulnerable organelles to oxidative stress, and cytochrome c peroxidase has no recognizable paralog in the genome, it is assumed that another alternative mechanism should exist which would be responsible for the removal of the hydrogen peroxide generated in this cellular compartment [38]. Our result confirms the idea of Martins et al. [34] that the peroxidase activity from cytochrome c is autonomous and the enzyme also plays a role in sensing hydrogen peroxide and regulation of the catalase activity. Kathiresan et al. [33] assume in their recent studies that CCP1 behaviour reminds more of a mitochondrial H\textsubscript{2}O\textsubscript{2} sensor rather than of a H\textsubscript{2}O\textsubscript{2} detoxifying catalytic protein.

**Band 5**
Putative aryl alcohol dehydrogenase AAD16 corresponds to Band 5 from the spectral Mascot analysis where the molecular mass is around 32 kDa as determined from the SDS-PAGE. Seven genes encoding proteins are found in *S. cerevisiae* with a high degree of more than 85% (>85%) of amino acid sequence identity to the aryl alcohol dehydrogenase of the lignin-degrading filamentous fungus, *Phanerochaete chrysosporium*. It is shown that among the seven-gene set only one is not telomere associated. That means that only one is not related to the other six at the nucleotide sequence grade, but the protein level of that one bears a resemblance to the fungal aryl alcohol dehydrogenase. Moreover, a search in the sequences of AAD genes shows a close match to the DNA-binding site of the Yap1p transcriptional activator upstream of chemicals, such as diamide and diethyl maleic acid ester (DEME) that provoke an oxidative shock by inactivating the glutathione (GSH) reservoir of the cells generated by expression of the AAD genes. On the contrary, the oxidizing agent hydrogen peroxide has no effect on exhibiting these genes [39]. It is evident that the present experimental work shows the evocation of AAD 16 during ibuprofen induced oxidative stress (lane 6) and no expression when *S. cerevisiae* in G\textsubscript{0} is treated with hydrogen peroxide (lane 2).

Experiments of single and multiple aad deletants indicate that only AAD4 (YDL243c) and AAD6 (YFL056/57c) respond to the oxidative stress. Previously, results showed that only AAD4 and AAD6 responded to oxidative stress [39].

**Band 6**
Band 6 (visible in the untreated proliferating cells) represents an AP-1-like transcription factor with a molecular mass of \textasciitilde32 kDa. Specific transcriptional factors emerge when there is a change in the sufficient
growth conditions. Iron is an essential element, but on the other hand, could be potentially toxic. Therefore, the iron homeostasis is sustained through transcriptional control. The aforementioned factors include the group of basic leucine zipper (bZIP) iron sensing transcription factors, and the AP-1-like transcription factor YAP5 as well. According to Zampar et al. [40], Yap5p is also involved in the diauxic shift. The diauxic shift in S. cerevisiae happens at the end of the logarithmic and the beginning of the stationary phase of growth as the cell metabolism readjusts from glycolytic to gluconeogenic operation. The diauxic shift is fulfilled by three major events that are organized with growth time. First, the glycolytic flux is diminished and the building of depot compounds is mediated before glucose limitation by down-regulation of phosphofructokinase and pyruvate kinase reactions. Second, when glucose is exhausted, the reversion of carbon flow through glycolysis and beginning of the glyoxylate cycle operation is elicited by an up-regulation of the enzymes. They act as catalysts of malate synthase and cytosolic citrate synthase reactions. The last stage of the adaptation is connected to the quitting of the pentose phosphate pathway with a switch in NADPH regeneration [40–42].

**Band 7**

In the present study concerning Band 7 (visible in G0 state), a GTP-binding protein (Ypt53) with molecular mass ≈ 26 kDa is determined in terms of oxidative stress in stationary phase. Representatives of the Rab family of small GTPases are responsible for the regulation of the macromolecular traffic of proteins and lipids in the secretory and endocytic pathway via recruitment of various effectors [43–46]. The functional homology between mammalian and yeast cells is well known. There is high level of identity between rab5 and Vps21p, Ypt52p, and Ypt53p. A considerable increase in Ypt53 over time occurred when cells were grown to post-log phase in glucose-containing medium but was almost undetectable during the log phase. Similar behaviour of the adaptation is connected to the quitting of the pentose phosphate pathway with a switch in NADPH regeneration [40–42].

**Conclusions**

The present work is focused on comparative analysis of the protein expression levels of treated and untreated cells of S. cerevisiae BY4741 in actively proliferating and quiescent state. IC50 doses of four toxic substances (menadione, hydrogen peroxide, ibuprofen, and zeocin) were determined and applied. Through SDS-PAGE and subsequent MALDI-TOF/TOF tests, protein profiles of the cells upon enduring oxidative stress were generated. That allowed the analyses of the key enzymes responsible for overwhelming toxic disbalance in the proliferating and quiescent cells, respectively. That could serve as a basis for further investigations on specific enzyme mechanisms of action and revealing the relationships between them using distinguishing proteomic analyses, 2D SDS-PAGE, and as a result, a specific proteomic map could be defined.

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**Disclosure statement**

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

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