

UNIVERSITY OF PÉCS

Biological and Sportbiological Doctoral School

An investigation of the external stress induced by mycotoxin citrinin and the internal stress induced by the deletion of the *ERG5* gene and their consequences in yeasts

PhD Thesis

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1. Introduction

Homeostasis is a dynamic equilibrium in cells in which, in parallel with oxygen uptake, the cells are affected by reactive oxygen species (ROS), but this does not disturb the cells in their growth or vital processes. In fact, the presence of ROS is indispensable for various physiological processes. Stress can be defined as the opposite of homeostasis, i.e. unbalanced homeostasis. Two forms of stress can be distinguished: redox homeostasis as an equilibrium can be changed by (i) external factors (e.g. oxidative stressors, xenobiotics, mycotoxins, transition metals, etc.), and (ii) internal genetic modifications (mutations, alterations in the number of chromosomes, etc.) may also induce an altered oxidoreduction state. The importance of these changes is reflected by the findings of the most recent researches: among others Alzheimer's disease and Parkinson's disease are associated with an unbalanced redox state of the cells. By means of an evolutionarily evolved antioxidative system, cells can compensate oxidative stress (depending on its extent) in the processes of adaptation. If the level of stress exceeds the adaptation ability of the cells, apoptotic/necrotic processes will occur, resulting in cell death.

In the research work relating to my dissertation, model systems were created for the investigation of external and internal stress. For these purposes, the petite-negative, haploid fission yeast *Schizosaccharomyces pombe* (*S. pombe*) and the petite-positive, haploid budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) were applied.

In the case of external stress, unbalanced oxidoreduction homeostasis can be triggered, e.g. (i) the cadmium cation induces glutathione (GSH) depletion, i.e. the lack of a central antioxidant molecule, and this is manifested in the accumulation of ROS, and especially the superoxide anion radical ($O_2^{\bullet-}$), the hydroxyl radical ($\bullet OH$), peroxides, etc.. Another example: the chromate anion also causes GSH depletion and a $\bullet OH$ concentration increment. Besides these, many oxidative stressors are known, and we investigated the mode of action of one of these, the mycotoxin citrinin (CTN), and the processes leading to altered oxidative stress conditions in *S. pombe* cells.

A random observation led to the investigation of external stress. In our previous experiments, ergosterol and phospholipid biosynthesis-damaged *S. cerevisiae* deletion mutants were found to possess an altered susceptibility to oxidative stressors in comparison with the parental strain. This was a novel observation, because little information is available about mutation-induced oxidative stress. We investigated what processes occur after lipid peroxide treatment; we characterized the oxidative stress state and examined how a mutation, in this instance deletion of the *ERG5* gene, can induce an altered redox state in the strain.

The recognition of these processes and an understanding of the regulation and the consequences of oxidative stress may lead to knowledge which promotes the protection of animals' health (e.g. determination of the permissible concentrations of mycotoxins in foods and feeds) and in the case of mutation-induced oxidative stress, an understanding and the possibility of curing oxidative stress-related diseases (e.g. Alzheimer's disease, Parkinson's disease, diabetes, etc.).

2. Aims

At the Department of General and Environmental Microbiology, Faculty of Sciences, University of Pécs, projects relating to oxidative stress processes and membrane dynamics have been ongoing since 1993. I joined in this work as an undergraduate in 2007 and continued as a Ph.D. student from 2012.

1. The first part of my study was an investigation of the mode of action of CTN. Blaskó et al. (2013) in our Department earlier determined the fluidizing effects of CTN on the plasma membrane through direct interactions with the free sulfhydryl group of the integral membrane proteins. Disorganization of the plasma membrane resulted in the efflux of essential potassium ions. On this basis, we set out to investigate the acute cytotoxic effects and oxidative stress-inducing effects of CTN in the haploid, eukaryotic, fission yeast *S. pombe*.

The aims of our examinations were:

- 1.1. To check on the pH-dependent cytotoxicity of CTN, described by Haraguchi et al. (1987).
- 1.2. At the pH optimum, to investigate the growth inhibition properties of CTN and in further experiments to determine subinhibitory concentrations, where at least 70% of the cells are viable.
- 1.3. To acquire information concerning the uptake of CTN and its effective concentration, we characterized the kinetics of its uptake.

In acute tests:

- 1.4. To investigate the CTN-induced oxidative stress processes by quantitative determination of the intracellular ROS content and each ROS species separately. To compare the levels of oxidative stress induced by H₂O₂, a well-characterized positive control was applied.
- 1.5. To identify transcription factor(s) responsible for regulation at the cell and molecular levels.
- 1.6. To investigate the regulation of CTN-induced oxidative stress processes, the GSH content and the specific activities of antioxidant enzymes were measured.

1.7. To investigate the oxidative stress-induced fragmentation of the nuclei and cell cycle arrest.

2. The second part of my Ph.D. studies comprised an investigation of the consequences of *ERG5* mutation (as an internal stress factor) and of *t*-BuOOH (as an external stressor) in the BY4741 parental and *erg5* Δ mutant strains.

The aims of our examinations were:

2.1. To determine the susceptibility of the BY4741 parental strain and its ergosterol biosynthesis-deficient mutants to oxidative stressors and amphotericin B. To choose the strain most sensitive to the lipid peroxidation induced by *t*-BuOOH.

2.2. To compare the sterol and fatty acid contents of the BY4741 parental and *erg5* Δ mutant strains. To measure the membrane fluidity changes, resulting from the altered plasma membrane composition. The altered composition and biophysical parameters of the plasma membrane suggest modification of the biological functions. To check on this, the glycerol assimilation of the cells was investigated.

2.3. To investigate the *ERG5* mutation-induced unbalanced oxidoreduction state through measurement of the intracellular ROS content and the activity of the antioxidative defense system.

2.4. In long-term acute tests to study the plasma membrane composition and fluidity changes induced by *t*-BuOOH treatment.

2.5. To characterize the redox processes induced by *t*-BuOOH treatment through quantitative measurements of ROS and the activities of the antioxidant system.

3. Materials and methods

The heterothallic, *ura4*-D18 h-, uracil auxotrophic *S. pombe* strain was applied to investigate the mode of action of CTN. For investigation of signal transduction (MAPK) pathways, the parental strain (*leu* 1-32 *ura4*-D18 *his* 7-366 *ade6*-M210, h⁺) and its deletion mutants Δ *atf1* (*atf1*::*ura4* *leu* 1-32 *ura4*-D18, h⁺) and Δ *pap1* (*pap1*::*ura4* *leu* 1-32 *ura* 4-D18, h⁺) were used.

The *S. cerevisiae* parental strain BY4741 (MATa, *his3* Δ 1, *leu2* Δ 0, *met15* Δ 0, *ura3* Δ 0) and its deletion mutant in ergosterol biosynthesis *erg5* Δ (*erg5* Δ ; isogenic to BY4741 with YMR015c::kanMX4) were applied to characterize the *ERG5* deletion-induced unbalanced oxidoreduction state.

In all experiments, mid-log-phase cultures were used to ensure the same physiological condition of the cells. The cells were washed by centrifugation at 1017 g (3000 rpm) for 5 min. A stock solution of CTN (250 mM) was prepared in acetonitrile, and *t*-BuOOH was

dissolved in distilled water. The concentration of the active substance in the solvent was 0.8% (v/v).

Determination of the pH-dependent cytotoxic effects, the inhibition of growth, the survival rate, the adaptation and CTN uptake

The inhibition of the growth of *S. pombe* cells by 0, 125, 250 and 500 μM CTN, and of *S. cerevisiae* by 0, 0.4 and 0.6 mM *t*-BuOOH was followed with a starting concentration of 10^6 cells ml^{-1} . The cell multiplication was determined spectrophotometrically at 595 nm. The MICs of CTN, the sensitivities of the MAPK deletion mutant strains and the pH-dependent cytotoxic effects of CTN were determined by the standard microdilution method of NCCLS M27-A.

The susceptibility of the *erg5* Δ mutant to antifungal drugs was determined on the surface of WO medium. The survival rates after CTN and *t*-BuOOH treatments and the adaptation after CTN pretreatment were determined by streaking on 10^7 cells ml^{-1} .

10^7 cells ml^{-1} vegetative cells and protoplasts of *S. pombe* were treated with 1000 μM CTN in 0.6 M KCl, and samples were taken at several time points (0, 1, 3, 5, 10, 20, 40, 60 and 90 min). The cells were centrifuged and the supernatants were measured at an excitation wavelength of 340 nm and an emission wavelength of 509 nm. The concentration of the toxin taken up by the cells or protoplasts was determined by means of a calibration curve.

Measurement of ROS

To estimate the intracellular peroxides, $\text{O}_2^{\cdot-}$ and total ROS, the dye dihydrorhodamine 123 (DHR 123), dihydroethidium (DHE) and 2',7'-dichlorofluorescein diacetate (DCFDA) were used to label 10^7 cells ml^{-1} . The total ROS content was measured with a Hitachi F-7000 fluorescence spectrophotometer; peroxides and $\text{O}_2^{\cdot-}$ were measured with a BD FACSCalibur flow cytometer. The excitation wavelength for DCFDA and DHE was 488 nm and that for DHR 123 was 505 nm, while the emission wavelength for DCFDA and DHR 123 was 525 nm and that for DHE was 610 nm.

Determination of specific enzyme activities and glutathione concentration

The specific activities of CuZn superoxide dismutase (SOD_{CuZn}), Mn superoxide dismutase (SOD_{Mn}), glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD) and catalase (CAT) and the intracellular concentrations of GSH, oxidized glutathione (GSSG), thiols and protein content were determined by well-established colorimetric assays as indicated below.

Determination of cell cycle arrest and fragmentation of nuclei

In cell cycle arrest experiments, 10^7 cells ml^{-1} were exposed to 1000 μM CTN for 60 min and samples were then prepared by a standard protocol (ForsburgLab Protocols). The percentages of cells in each stage of the cell cycle were determined with a Becton Dickinson flow cytometer.

To detect and quantify the fragmentation of nuclei, a suspension of mid-log-phase cells (10^7 cells ml^{-1}) was treated with 1000 μM CTN for 60 min, and 4-5- μl suspensions pipetted onto slides were stained with 10 $\mu\text{g ml}^{-1}$ 4',6-diamidino-2-phenylindole (DAPI). The nucleus morphology was examined in 300 cells in each sample with a Nikon Eclipse 80i fluorescent microscope equipped with an UV filter.

Determination of fluorescence anisotropy, lipid and sterol contents and glycerol assimilation

10^7 cells ml^{-1} *S. cerevisiae* were treated with 1 mM *t*-BuOOH for 60 min and then labeled with 2 μM TMA-DPH for 5 min. The excitation wavelength was 340 nm and fluorescence emission was detected at 430 nm with a Perkin-Elmer spectrofluorometer.

An early stationary culture of *S. cerevisiae* (10^7 cells ml^{-1}) was treated with 1 mM *t*-BuOOH for 5 h, and the total sterol and fatty acid contents were extracted and evaluated by GC-MS analysis.

The glycerol assimilation of the BY4741 parental and *erg5* Δ mutant strains was determined spectrophotometrically at 595 nm.

4. Results and discussion

Investigation of pH-dependent cytotoxic effects of CTN, and determination of subinhibitory concentrations and uptake of CTN

In 1987, Haraguchi et al. reported the pH-dependent cytotoxic effects of CTN, stating that the toxin possesses a stronger cytotoxic effect at acidic pH, but this was not taken into consideration in other publications. To check on this, we investigated the pH-dependent cytotoxicity of CTN by the microdilution method. Our results revealed non-linear dose and pH dependences. The MIC values of CTN for *S. pombe* cells at pH 4.5 and pH 6.0 were 175 and 1000 μM , respectively.

CTN treatment induced dose-dependent growth inhibition. In comparison with the control cultures, exposure to 125 μM CTN caused a slight delay in the entry of *S. pombe* cells into the logarithmic phase, but did not significantly influence the growth yield. The generation time changed from 3.1 h to 4.1 h. Treatment of cells with 250 or 500 μM CTN resulted in 90.4% and 96.1% decreases, respectively, in the growth yield at the end of a 44-h cultivation.

In further experiments, we determined the subinhibitory concentration of CTN to be 1000 μM . Treatment of cells with this concentration for 60 min caused a 21% decrease in the colony-forming ability (cells exposed to 500 and 2000 μM CTN proved to exhibit a 2% and 98% decrements, respectively).

The MICs of CTN at pH 4.5 and pH 6.0 were relatively high and we presumed that this was a consequence of the high bioadsorption capacity of the yeast cell wall. Unexpectedly, only 30% of the CTN was taken up from 1000 μM (subinhibitory) CTN solution by the vegetative cells within 20 min, suggesting the non-occurrence of bioadsorption by the cell wall. However, after enzymatic digestion of the cell wall, the CTN uptake was doubled (58%), indicating that the cell wall is a barrier limiting CTN uptake. The curve of uptake revealing saturation may point to the lack of active transport and metabolism of CTN in *S. pombe* cells.

CTN-induced oxidative stress processes and their consequences

Growth inhibition experiments suggested the possibility of adaptation. In adaptation experiments, therefore, cells were pretreated with the subinhibitory concentration for 60 min and then treated with 1000 or 2000 μM CTN for a further 60 min. As a result, the survival rate increased from 79% to 98% at 1000 μM , and from 2% to 50% at 2000 μM .

In *S. pombe*, both the external and the internal oxidative stress responses are regulated via MAPKs. The growth-inhibitory concentration of CTN was determined by the microdilution method in the range 0-1000 μM for the *S. pombe* (5×10^3 cells ml^{-1}) parental strain and its transcription factor deletion mutants, $\Delta pap1$ and $\Delta atf1$. In comparison with the parental strain, exposure of the $\Delta atf1$ and $\Delta pap1$ strains to 250 μM CTN resulted in a 27.1% and 52.4% decrease, respectively in the growth yield. This suggests that the altered redox state is regulated determiningly by the transcription factor Pap1, but activation of the factor Atf1 is also necessary for the cells to cure the CTN-induced oxidative stress.

A 2-fold increment in total ROS content was measured after treatment for 60 min with 1000 μM CTN. This was a consequence of the 1.5-fold higher peroxide content, since no alteration was detected in the intracellular $\text{O}_2^{\bullet-}$ content.

Enzyme activity changes result from the CTN-induced accumulation of peroxides and hence the regulation of antioxidant genes via the oxidative stress stimulus-induced transcription factor Pap1 and the MAPK-activated transcription factor Atf1. The down-regulation of CAT (a H_2O_2 -splitting enzyme) and the up-regulation of GPx (which catalyzes the oxidation of GSH to GSSG with the concomitant consumption of H_2O_2) were detected. It appears reasonable that the opposite regulation of these enzymes is a consequence of the CTN-induced overproduction of peroxides and GSH, and the down-regulation of GR.

To test the effects of CTN on the cell cycle, the DNA content was measured. Flow cytometric analysis of the DNA content of the cells revealed a significant decrease in G1/G2/M (G2/M arrest) from the control 85.01 ± 2.00 to 77.82 ± 3.30 %, and an increase in the S phase from 11.18 ± 1.91 to 16.62 ± 2.60 %, as a result of the subinhibitory CTN treatment.

These results suggested the possibility of CTN-induced alterations in the nucleus morphology, and these were investigated with DAPI-stained cells. 1000 μ M CTN treatment for 60 min resulted in a 3.2-fold increase in the fragmentation of the nuclei in comparison with the untreated cells.

The effect of ERG5 mutation on plasma membrane-related processes

In comparison with their parental strain BY4741, the gene deletion mutants (*erg2 Δ* , *4 Δ* , *5 Δ* and *6 Δ*) of *S. cerevisiae* exhibited significantly increased MICs to amphotericin B, which was attributed to the absence of ergosterol in the cell membrane. Among the yeast sterols, ergosterol forms the strongest plasma membrane-disorganizing complex with amphotericin B.

To evaluate the consequences of mutation-induced alterations in the plasma membrane of the control *erg5 Δ* cells in comparison with BY4741, the plasma membrane fluidity was measured by means of steady-state fluorescence anisotropy. The fact that the *erg5 Δ* cells exhibited a significantly higher *r* value than that of the parental strain BY4741 showed that the alterations in its membrane composition decreased the mobility of the fluorophore, manifested in a more rigid plasma membrane structure.

The increased plasma membrane rigidity was a consequence of the increased total sterol content (by 49.8%) and the increased amount of unsaturated fatty acids of polar phospholipids.

The altered plasma membrane composition resulted in a lower glycerol assimilation of the cells.

Investigation of the redox homeostasis of the *erg5 Δ* mutant

The responses of *S. cerevisiae* ergosterol-less mutants were tested to oxidative stressors such as *t*-BuOOH, H₂O₂, menadione, Cd²⁺ and Cr(VI). The cells of *erg5 Δ* were 2.5-fold more sensitive to *t*-BuOOH than the parental strain and also exhibited an unbalanced redox state. In the untreated mutant cells, significantly lower O₂^{•-} and H₂O₂ concentrations were detected, which may be a consequence of the significantly elevated specific activities of SOD_{Mn} and CAT in the cells. The GSH concentration and the GR specific activity were not

changed in *erg5Δ*, but the GSSG concentration was significantly higher, resulting in an elevated specific activity of GST.

t-BuOOH treatment modified the plasma membrane composition, and affected the redox state of the cells

In order to study the plasma membrane-modifying ability of 1 mM *t*-BuOOH, treatment for at least one generation, during 5 h, was applied. The data suggested that *t*-BuOOH elicited adaptation processes, which compensated the *t*-BuOOH-induced lipid peroxidation in the unsaturated fatty acids of the plasma membrane via modification of the distributions of the sterol and fatty acid components. After *t*-BuOOH treatment, a 15-86% decrement was observed in the ratio unsaturated/saturated fatty acids.

Besides the increased unsaturated fatty acid content, significantly decreased total sterol contents were detected in both strains exposed to *t*-BuOOH. This and the decrement in the fluorescence anisotropy *r* value indicated that the *t*-BuOOH treatment exerted fluidizing effects on the plasma membrane of the strains. However, it must be mentioned that the results derived from the sterol and fatty acid analyses cannot be correlated directly with the fluorescence anisotropy measurements because of the different exposure times.

In the *t*-BuOOH-treated cells of BY4741 and *erg5Δ*, the intracellular O₂^{•-} concentrations were increased 6.7-fold and 2.3-fold, and the H₂O₂ concentrations were increased 6.8-fold and 3.2-fold, respectively, in comparison with the untreated cells. For the reduction of O₂^{•-} to H₂O₂, significantly elevated activities of mitochondrial SOD_{Mn} in BY4741 and of cytoplasmic SOD_{Cu/Zn} in *erg5Δ* were detected. In response to the increased peroxide contents, different modes of regulation were observed in the two strains. For the detoxification of H₂O₂, the specific activity of CAT was increased in the cells of BY4741 as a consequence of the very high concentration of peroxides, but not in those of *erg5Δ*, where the *t*-BuOOH-induced stress processes may be regulated by the GSH-GPx-GR pathway. There exists a selenium-independent type of GST which possesses GPx activity and reacts with organic peroxides (lipid peroxides). In the *t*-BuOOH-exposed BY4741 strain, a significantly higher GST activity was detected.

5. Summary

The haploid eukaryotic fission yeast *S. pombe* was used to investigate the dose-, time- and pH-dependent cytotoxic effects and oxidative stress-generating effects of the mycotoxin CTN, and the regulation of the stress processes evolved at cellular and molecular levels in acute toxicity tests.

1.1. The pH-dependent cytotoxic effect of CTN was confirmed and taken into consideration in the further experiments. The MIC₉₀ of CTN was 1000 μM at pH 6.0 and 175 μM at pH 4.5; the latter is optimal for the growth of the applied model organism.

1.2. Dose- and time-dependent cytotoxic effects of CTN were observed and the subinhibitory concentration of the toxin (1000 μM) was determined at which at least 70% of the cells were viable.

1.3. The kinetics of the uptake was characterized. Due to the barrier function of the cell wall, only 30% of the CTN was taken up by the vegetative cells from 1000 μM CTN solution. Moreover, the saturation curve of CTN uptake may point to the lack of active transport and metabolism during the interval involved in our investigations.

1.4. As a consequence of CTN-induced oxidative stress, a 2-fold increase in total ROS level was measured, solely as a consequence of the 1.5-fold increase in peroxide concentration. To compare the levels of induced oxidative stress, H₂O₂, a well-characterized positive control, was applied, which testified to the reliability of our results.

1.5. This was the first documentation of the adaptation capability of CTN-treated cells. Pretreatment of the cells with a subinhibitory concentration of CTN for 60 min caused significantly increased survival rates when these cells were treated with 1000 or 2000 μM CTN for a further 60 min: the colony-forming ability increased from 79% to 98% and from 2% to 50% in the cases of 1000 and 2000 μM CTN-treated cells. The adaptation process was proved to activate primarily the redox-sensitive transcription factor Pap1, since the impact of CTN-induced oxidative stress was not sufficient to activate the Atf1 pathway. This was manifested in a decreased growth yield of *pap1Δ*, though the factor Atf1 is also necessary for the regulation on the basis of the observed 35% growth yield.

1.6. Via the oxidative stress stimulus-induced transcription factor Pap1 and the MAPK-mediated Atf1, in response to the increment in peroxide concentrations, oxidative stressor-specific regulation of the antioxidant system was detected. We demonstrated that with at least 70% cell viability the elevated GSH concentration is unambiguous; in contrast with others, the results we obtained on the cultures revealed low cell viability. The increased GSH concentration up- or down-regulated some of the antioxidant enzymes responsible for GSH homeostasis (GPx, GR, G6PD and GST). The results of H₂O₂ treatments were in good agreement with literature findings.

1.7. The accumulation of ROS-induced oxidative DNA damages was manifested in an increased number of fragmented nuclei. The cells strived to correct the oxidative damage and the ROS-mediated microtubule disorders by arrest of the cell cycle in the G2/M phase.

In the second part of our work, the haploid budding yeast *S. cerevisiae*, the *erg5Δ* deletion mutant and its parental strain BY4741 were applied to investigate the consequences of the absence of ergosterol (deletion in the *ERG5* gene) on the lipid composition and on the biophysical and biological functions of the plasma membrane. We also determined the oxidoreduction states of the strains and investigated the consequences of *t*-BuOOH treatment on the above-mentioned parameters.

2.1. In comparison with the parental strain BY4741, the *erg5Δ* mutant exhibited a significantly higher tolerance to amphotericin B, which forms a complex with ergosterol. Although, the end-product of ergosterol biosynthesis was not produced, the total sterol content of the cells was 49% higher. As a consequence of sterol overproduction, the rigidity (*r* value) of the plasma membrane of *erg5Δ* was elevated in comparison with the parental strain.

2.2. This is the first report that *erg5Δ* cells are 2.5-fold more sensitive to *t*-BuOOH than the parental strain. The explanation of this phenomenon is that the amount of unsaturated fatty acids (primary targets of lipid peroxidation) is higher. Our data also point to the altered sensitivity of the mutants of ergosterol biosynthesis to oxidative stressors.

2.3. The *erg5Δ* mutant possesses an unbalanced redox state, as confirmed by the determination of intracellular $O_2^{\bullet-}$ and peroxide contents. Both ROS were significantly lower in untreated *erg5Δ*, which may be a consequence of the significantly elevated specific activities of SOD_{Mn} and CAT. The higher concentration of GSSG induced an elevated specific activity of GST in *erg5Δ*, indicating the unbalanced redox state of the strain. *erg5Δ* cells are therefore under continuous, low-level, but tolerable stress, resulting in the continuous up-regulation of antioxidant enzymes.

2.4. In agreement with the literature, *t*-BuOOH-induced fluidization of plasma membrane was observed in the BY4741 strain and the *erg5Δ* mutant. This can be explained by the decreased total sterol content of *t*-BuOOH-treated cells.

2.5. Besides the realignment of the plasma membrane, this is the first description of a more than 6-fold increment in the concentrations of $O_2^{\bullet-}$ and peroxides in the parental strain, and a 2.4-fold increase in the former and a 3.2-fold increase in the latter in the case of *erg5Δ*, after *t*-BuOOH treatment, resulting in different antioxidant responses. In BY4741, elevated GSH concentration and enzyme activities responsible for GSH homeostasis were detected, and the specific activities of CAT and SOD_{Mn} were also increased. In contrast with this, the *erg5Δ* deletion mutant exhibited a 50% higher GSH increment than that of the parental strain, whereas the specific activity of CAT did not change and, instead of SOD_{Mn} , the specific activity of SOD_{CuZn} was increased.

6. Publications

Publications related to the thesis

Gazdag, Z., **Máté, G.**, Čertík, M., Türmer, K., Virág, E., Pócsi, I., Pesti, M., 2014. *Tert*-butyl hydroperoxide-induced differing plasma membrane and oxidative stress processes in yeast strains BY4741 and *erg5Δ*. J. Basic Microbiol. 54, 50-62. (2013 **IF 1.822**)

Máté, G., Gazdag, Z., Mike, N., Papp, G., Pócsi, I., Pesti, M., 2014. Regulation of oxidative stress-induced cytotoxic processes of citrinin in the fission yeast *Schizosaccharomyces pombe*. Toxicon 90, 155-166. (2013 **IF 2.581**)

Publications not related to the thesis, submitted publications or publications in manuscript

Blaskó, Á., Gazdag, Z., Gróf, P., **Máté, G.**, Sárosi, Sz., Krisch, J., Vágvölgyi, Cs., Pesti, M. Effects of clary sage oil and its main components, linalool and linalyl acetate, on the plasma membrane of *Candida albicans*: An in vivo EPR study. (submitted)

Máté, G., Kovács, D., Gazdag, Z., Pesti, M. Regulation of oxidative stress-induced cytotoxic processes of the antioxidant linalool in the human pathogen *Candida albicans*. (in manuscript)

Papp, G., **Máté, G.**, Mike, N., Gazdag, Z., Pesti, M. Regulation of antioxidant system in cells of the fission yeast *Schizosaccharomyces pombe* after combined treatment with patulin and citrinin. (in manuscript)

Lectures and posters related to the thesis

Gazdag, Z., Kőszegi, B., Čertík, M., **Máté, G.**, Thürmer, K., Belágyi, J., and Pesti, M.: Examination of oxidative stress sensitivity and oxido-reduction state of *Saccharomyces cerevisiae* Δ *erg5* ergosterol mutant. 39th Annual Conference on Yeasts, Smolenice, Slovakia, 2011, p. 73, poster abstract.

Gazdag, Z., **Máté, G.**, Čertík, M., Kőszegi, B., Türmer, K., Belágyi, J., Pesti, M.: *Saccharomyces cerevisiae* Δ ERG5 ergosterin mutáns törzs oxidatív stressz érzékenységének és oxido-redukciós állapotának vizsgálata. V. Hungarian Mycological Conference, Budapest, Hungary, 2012, pp. 38-39, abstract of a lecture.

Máté, G., Blaskó, Á., Mike, N., Gazdag, Z., Papp, G., Nagy, L., Vágvölgyi, Cs., Pesti, M.: Cytotoxic effects of citrinin on the fission yeast *Schizosaccharomyces pombe*. ISM-Mycored International Conference Europe 2013., Martina Franca, Italy, 2013, p. 273, poster abstract.

Máté, G., Gazdag, Z., Papp, G., Mike, N., Pócsi, I., Pesti, M.: Citrinin-induced unbalanced oxido-reduction state of *Schizosaccharomyces pombe* cells. 4th Central European Forum for Microbiology, Keszthely, Hungary, 2013, pp. 184-185, abstract of a lecture.

Máté, G., Gazdag, Z., Mike, N., Papp, G., Pócsi, I., Pesti, M.: Regulation of citrinin-induced oxidative stress processes in the yeast *Schizosaccharomyces pombe*. II. Interdisciplinary Doctorandus Conference, Pécs, Hungary, 2013, pp. 207-209, abstract of a lecture.

Other lectures and posters

Máté, G.: *Saccharomyces cerevisiae* Δ ERG6 mutáns oxidatív stressz érzékenységének vizsgálata. Szentágotthai János Szakkollégium Scientific Conference, Pécs, Hungary, 2011, lecture.

Máté, G.: *Saccharomyces cerevisiae* ΔERG6 mutáns oxidatív stressz érzékenységének vizsgálata. TÁMOP 4.3.2. „Nyitott Egyetem – a PTE tudásbázisának disszeminációja“, Pécs, Hungary, 2011, lecture.

Blaskó, Á., Gazdag, Z., Gróf, P., **Máté, G.**, Sárosi Sz., Czuni, L., Krisch, J., Vágvölgyi, Cs. and Pesti, M.: Effects of clary sage oil and its main components linalool and linalyl acetate on *Candida albicans*. 4th Central European Forum for Microbiology, Keszthely, Hungary, 2013, pp. 119-120, abstract of a lecture.

Mike, N., Papp, G., Gazdag, Z., **Máté, G.**, Czibulya, Zs., Kunsági-Máté, S., Čertik, M., Pesti, M.: Non-estrogenic, oxidative stress inducing effects of the mycotoxin zearalenone in the fission yeast. II. Interdisciplinary Doctorandus Conference, Pécs, Hungary, 2013, p. 205-206, abstract of a lecture.

Mike, N., Papp, G., Gazdag, Z., **Máté, G.**, Czibulya, Zs., Kunsági-Máté, S., Čertik, M., Pesti, M.: The oxidative stress inducing ability of zearalenone – a non-estrogen specific effect in the fission yeast. 4th Central European Forum for Microbiology, Keszthely, Hungary, 2013, p. 188, abstract of a lecture.

Mike, N., Papp, G., Gazdag, Z., **Máté, G.**, Türmer, K., Czibulya, Zs., Kunsági-Máté, S., Ember, I., Vágvölgyi, Cs., Čertik, M., Pesti, M.: Cytotoxic effects of zearalenone mycotoxin on the cells of fission yeast *Schizosaccharomyces pombe*. 30th International Specialised Symposium on Yeast. Stará Lesná, Slovakia, 2013, p. 89, poster abstract.

Mike, N., Papp, G., Gazdag, Z., **Máté, G.**, Türmer, K., Czibulya, Zs., Kunsági-Máté, S., Ember, I., Vágvölgyi, Cs., Čertik, M., Pesti, M.: Regulation of zearalenone-induced oxidative stress process in the fission yeast *Schizosaccharomyces pombe*. 7th International Fission Yeast Meeting. London, England, 2013, p. 186, poster abstract.

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