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The kinetic reduction of Cr(VI) by yeast *Saccharomyces cerevisiae, Phaffia rhodozyma* and their protoplasts*

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Chromium in the sixth oxidation state may easily penetrate cellular membranes *via* non-specific sulfate transporters due to its tetrahedral symmetry (high similarity to SO_4^{2-} and HPO_4^{2-}). This feature makes chromium a toxic and hazardous pollutant responsible for the deterioration of midland water quality. The aim of the study was to evaluate the capacity of two yeast species — *Saccharomyces cerevisiae* and *Phaffia rhodozyma* — and their protoplasts to reduce Cr(VI) to lower oxidation states. The study also deals with the behavior of the yeasts upon the presence of elevated sulfate ions as a competitive inhibitor of chromate transport by the sulfate transporters. The chromate-reducing activities were monitored by determination of Cr(V) free radical form with the use of L-band (1.2 GHz) EPR (electron paramagnetic resonance) spectroscopy. It was observed that both of the studied yeast strains exhibited the ability to reduce Cr(VI) applied at 4 mM. The cells of *P. rhodozyma* showed about 3.5 times higher reduction than *S. cerevisiae*. The reduction efficiency was significantly improved when the protoplasts of both strains were used and reached 100% in the first 10 minutes of the reduction process which suggests that the cellular wall may have a notable influence on the uptake and/or inhibition of chromium reduction process. The reduction effect of *P. rhodozyma* cells and protoplasts may be associated with the more sufficient production of metabolites (such as glutathione and cysteine), which may also be responsible for the increased tolerance of the strain towards high concentrations of toxic chromium.

Key words: chromium, *Phaffia rhodozyma*, pollution, *Saccharomyces cerevisiae*

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INTRODUCTION

Contamination with heavy metals is a serious environmental problem. Pollution with chromium originating from several industrial processes such as leather tanning, electroplating, textile industries, nuclear power plant, water cooling, pulp production, ore and petroleum refining processes are the reason for deterioration of midland water quality. In trace amounts, being beneficial to living organisms, chromium serves as an essential element for glucose and fat metabolism as well as for the stabilization of the tertiary structure of proteins and nucleic acids (Pas *et al.*, 2004). However, at higher concentrations it is extremely toxic, causing allergies, egzema, and respiratory track disorders. It is also a strongly mutagenic and cancerogenic agent, especially in its oxidized form, Cr(VI) (Barceloux, 1999). Once inside the cell Cr(VI) is being reduced to the Cr(V) and can react with H_2O_2, which leads to the production of hydroxyl radicals ($^{\bullet}OH$) *via* the Fenton-like reaction. Cr(V) causes DNA breaks and various mutations in chromosomes.

Due to high toxicity, chromium is regarded as a priority pollutant by the US EPA. The adverse health effects and diverse cellular and molecular reactions make the studies on chromium toxicology and metabolism very crucial in terms of environmental protection and clinical medicine. Such studies are performed using eukaryotic organisms, mainly yeast, plants, mammalian cells and transgenic mice (Hedlam & Lay, 2001; Cervantes *et al.*, 2001; Abbas *et al.*, 2011). Among these, yeast has proved to be a very suitable model for the research of eukaryotic cell response to chromium stress and Cr bioremediation pathways (Ksheminska *et al.*, 2005; 2010). Chromium, in the sixth oxidation state, can easily enter into the living cells because Cr(VI) ion has tetrahedral symmetry (same as SO_4^{2-} and HPO_4^{2-}, unlike Cr(III) which has octahedral symmetry) and can get into the cell membrane through non-specific sulfate transporters by facilitated diffusion (Pereira *et al.*, 2008). The competition between chromate and sulfate for the transporter has been reported by several authors, both in Procariota and Eucariota (Yoshimoto *et al.*, 2002; Wysocki & Tamas, 2010). In *Saccharomyces cerevisiae* sulfate is transported into yeast cells via the system involving two permeases, one with a high and the other with a low sulfate affinity (Cherest, 1997).

Then water-soluble Cr(VI) compounds are reduced to various unstable reactive intermediates, like Cr(V) and/or Cr(IV) and reach the stable and less toxic Cr (III), which can be easily removed by precipitation (Cieslak-Golonka, 1996). The gradient of Cr(VI) between the two sides of the cell membrane is maintained metabolically and it is continuously reduced by both enzymatic and non-enzymatic pathways (Jamnik & Raspor, 2003). It is unclear whether enzymatic/non-enzymatic and both intra — and extracellular reductions of Cr(VI) play a decisive role of Cr(VI) detoxification in eukaryotic microorganisms. Ascorbic acid, glutathione, cysteine, methionine are efficient non-enzymatic reducers of Cr(VI) to Cr(III) at physiological conditions of living cells (Smutok *et al.*, 2011). The enzymatic mechanism of the Cr(VI) reduction is more complicated as the chromate causes a strong decrease in sulfur assimilation and induction of genes and enzymes of the sulfur amino acid pathways (Pereira, 2008) as well as stress induced proteins

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Abbreviations: Cr, Chromium; *P. rhodozyma, Phaffia rhodozyma*; *S. cerevisiae, Saccharomyces cerevisiae*

Table 1. Viability of *S. cerevisiae* cells during the incubation with different Cr(VI) concentrations.

Cr (VI) concentration (mM)	Viability after 120 min incubation (%)
0.5	100
1.0	99
2	97
3	89
4	50
5	0

Table 2. Viability of P. *rhodozyma* cells during the incubation with different Cr(VI) concentrations.

Cr (VI) concentration (mM)	Viability after 120 min incubation (%)
0.5	100
1.0	100
2	100
3	95
5	89
7	78
9	63

(Jamnnik & Raspor, 2003; Wysocki & Tamas, 2010). The increase of intra-cellular glutathione reductase and glucose-6-phosphate dehydrogenase activity of *Schizosaccharomyces pombe* was observed in the 2 mM presence of Cr(VI) (Pesti *et al*., 2002) and it indicates significant involvement of the enzyme in the detoxification process. Recently in the experiments with *Hansenula polymorpha* it was suggested that Cr(VI) can be reduced intra-cellularly by flavocytochrome b_2, a non-selective protein in respect of electron acceptors (Smutok *et al*., 2011).

The mechanisms of Cr(VI) reduction with yeast strains, *Saccharomyces cerevisiae* and *Phaffia rhodozyma* and their protoplasts are the subject of the studies.

The study also evaluates the behavior of the yeasts in the presence of elevated sulfate ions as a competitive inhibitor of chromate transport by the sulfate transporters.

MATERIALS AND METHODS

The yeast strains, *Saccharomyces cerevisiae* and *Phaffia rhodozyma* were a kind gift from prof A. Sibirny Laboratory, Lviv, Ukraine.

Two species of yeast *Saccharomyces cerevisiae* and *Phaffia rhodozyma* were used to evaluate their behavior to reduce Cr(VI) to lower oxidation state, Cr(V). Yeast were cultured in Petri dish containing 10 ml of growth medium YPD (Yeast extract Peptone Dextrose) at 30°C for *S. cerevisiae* and 25°C for *P. rhodozyma*. After 14 days yeast were passaged to 250 ml flasks containing 50 ml fluid growth medium and incubated on a rotary shaker at appropriate temperatures for 24h (*S. cerevisiae)* and 48h (*P. rhodozyma)*. The composition of YPD medium was changed — the glucose was replaced by saccharose in terms of avoiding the glucose non-specific reduction of Cr(VI). The composition was as follows (g/l of medium): peptone, 20; yeast extract, 10; saccharose, 20; agarose, 20. The composition of liquid medium was (g/l of medium): peptone, 2: yeast extract 2 and 50 ml of mineral salts containing (g/l): $(NH_4)_2SO_4$, 60; $MgSO_4$, 6; KH_2PO_4, 10; $CaCl_2$, 2,4 and the saccharose was added as a source of energy. Cells were harvested at the logarithmic phase of growth, washed with distilled water and after that suspended in the phosphate buffer, pH 7.5. The same concentrations of yeast biomass were used in all the experiments.

Viability of yeast cells after incubation with Cr(VI). The viability study of *S. cerevisiae* and *P. rhodozyma* yeast cells was carried out applying plate and methylene blue staining methods. The plate method involves seeding cells at various decimal dilutions on YPD solid medium. After every 24 h the colonies were counted and converted to the number of cells in 1 ml of suspension. Methylene blue staining method is based on a combination of the dye with the yeast cells. The dye penetrates inside the dead cells and stains them blue (Sami M *et al*., 1994).

The yeast samples were treated with $K_2Cr_2O_7$ in different concentrations given in Table 1.

On the basis of the viability data, the concentration of chromate equal to 4 mM was chosen for both the experiments with *S. cerevisiae* and *P. rhodozyma*. 4 mM of Cr(VI) do not affect viability of *P. rhodozyma* as strongly as *S. cerevisiae*, but for the studies on the influence of sub-lethal dosage of chromium on physiological responses of both strains, 4 mM concentration is appropriate.

The preparation of yeast strain free culture medium. The yeast samples (not incubated with chromium) of *P. rhodozyma* and *S. cerevisiae* were collected in the lag phase of growth, centrifuged and then the supernatant, free of yeast cells was taken to the reduction process measurements.

Determination of the minimum content of sufate ions necessary for the growth of the yeast. Free of sulfate medium (modified composition of Bushnell-Haas medium) was tested in the yeast viability experiments. In order to find minimal concentration of sulfate necessary for cell growth the Bushnell-Haas mediums with varying sulfate concentrations were tested. It was found out that the minimal sulfate concentrations in which yeast cells avoid sulfur starvation was approximately 100 μM, which corresponds to the literature (Pereira *et al*., 2008).

Protoplast isolation procedure. The protoplasts were isolated using the method developed and optimized by the authors with the use of the Zymolyase enzyme produced by a culture of *Arthrobacter luteus*.

Yeast cells were incubated until the log phase of growth was reached (24 h for *S. cerevisiae* at 30°C and 48 h, 25°C for *P. rhodozyma*, respectively). Next the cells were washed 3 times with distilled water and the sediment was suspended in the 4 ml of buffer pH 7.5 with addition of 0.4 mg Zymolyase 20T and 50 mM DTT (dithiothreitol). Cells were incubated for 1h in 30°C temperature on a rotary shaker. After the incubation, 6 ml of buffer (pH 7.5) was added to the mixture of protoplasts and fragments of the cell wall. Then the mixture was gently centrifuged for 5 min, 400 G. Protoplasts were irrigated twice in 10ml of buffer (pH 7.5) by gentle centrifugation (400G, 5 min).

EPR — L band chromium reductionmeasurments. Reduction process was carried out in 120 minutes with the final concentration of Cr(VI) equal to

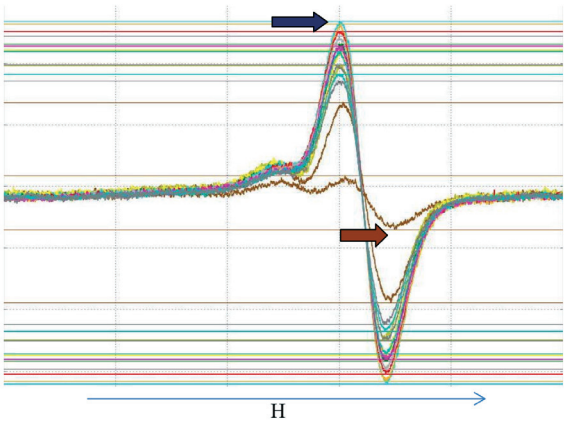

Figure 1. EPR-L band spectra of Cr(V).
The upper arrow indicates maximum reduction of Cr(VI) in the middle of experiment. The lower arrow indicates minimum reduction of Cr(VI) at the end of the process.

4 mM in each probe. The scan of the spectra was recorded 5 times for each measurment.

The kinetics of chromate reduction were monitored by means of Cr(V) free radical form determination using the L-band (1.2 GHz) EPR (electron paramagnetic resonance) spectrometer. The spectrometer was equipped with a microwave bridge with the operating frequency of 1.2 GHz and an extended surface coil-type high frequency resonator having an internal diameter of 16 mm. The following settings of the spectrometer were typically used: 40 mW maximum microwave power, 27 kHz field modulation frequency, 40 G magnetic field scan. The 5 scans of EPR signal were averaged. The typical EPR-L band spectra of Cr (V) is shown in Fig 1. Every EPR signal was standardized to free-radical probe (TEMPO) at constant concentration. The spectrum of the probe has been taken at the beginning of each measurement. The procedure allows to compare amplitudes of all samples signals.

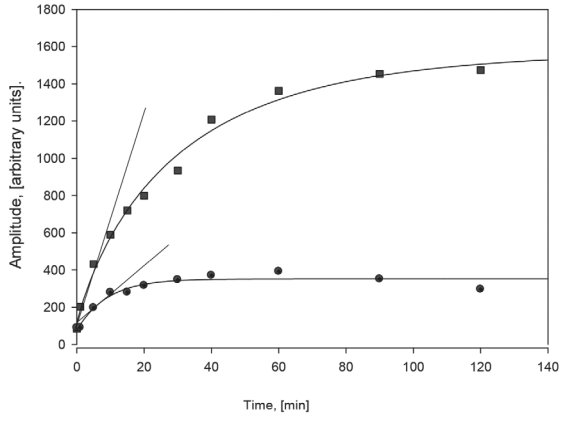

Figure 2. EPR signal of Cr(V) during the reduction process of 4 mM Cr(VI) by *Phaffia rhodozyma* (■) and *Saccharomyces cerevisiae* (●).

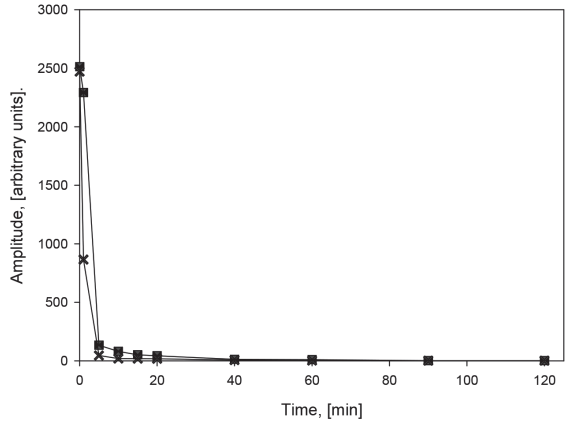

Figure 3. Comparison of 4mM Cr(VI) reduction by protoplasts of (x) *Phaffia rhodozyma* and (■) *Saccharomyces cerevisiae*.

RESULTS

The reduction of Cr(VI) by the cellular reducing system of living organisms generates paramagnetic long-lived reactive intermediate, Cr(V). The data given in Fig. 2 show the formation of Cr(V) during the reduction process conducted by *S. cerevisiae* and *P. rhodozyma* which is much faster in the case of *Phaffia rhodozyma*. The initial slopes of the curves illustrating Cr(V) formation reaction in the time-depending mode suggest that the reduction process is 2.5 times faster in the case of *Phaffia rhodozyma* compared to the *Saccharomyces cerevisiae* cells.

This result was expected because *Phaffia rhodozyma* is far more resistant to Cr(VI) than *Saccharomyces cerevisiae.* The conclusion also can be drawn out from the viability experiments — data shown in Table 1 and other literature (Horvath *et al.*, 2011; Smutok *et al.*, 2008). It was interesting to verify whether the faster reduction of Cr(VI) by *Phaffia rhodozyma* is connected with chemical groups of cell wall or/and low molecular mass reductants secreted to the extra-cellular space by the yeast cells. The other explanation is the effective penetration of chromate into the cells and subsequently enzymatic and non-enzymatic reduction. In order to check occurrence of above mentioned phenomena the reduction of Cr(VI) was done with protoplasts (cells without the cell wall) of both strains. The data is shown in Fig. 3.

As can be seen in Fig. 3 the rate of chromium reduction is very fast and there is almost no difference between the protoplast of both strains. It means that the lack of significant differences in fast reduction of the metal by the *P. rhodozyma* and *S. cerevisiae* cells is not related with intra-cellular both enzymatic and non-enzymatic processes. The differences is explained by the data given in Fig. 4, where the reduction Cr(VI) by the free-cell medium is shown.

In the case of *P. rhodozyma* free-cell medium treated with 4 mM of Cr(VI) the reaction of Cr(V) formation reaches the maximum after 30 min while in the medium of *S. cerevisiae,* the reduction process is running during whole time of the experiment. The gradual decrease in the amplitude signals was observed after 180 min in the case of *S. cerevisiae* (data not shown). These results suggest that the *P. rhodozyma* medium culture, after rejection of the intact cells, was more active in terms of Cr(VI) reduction. The difference in such activity is about 70 times bigger in the case of free-medium of *P. rhodozyma* than *S. cervisiae,* which can be judged from the initial slope of the curves presented in Fig. 4.

Such difference can be caused by metabolic mechanisms responsible for secretion of the reductants to the extra-cellular space in *P. rhodozyma* cells. Some metabolic pathways of the reductant synthesis can be triggered by

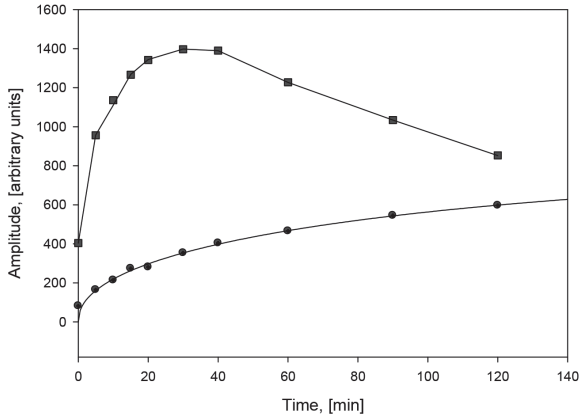

Figure 4. Comparison of the reduction process of 4 mM Cr(VI) by the cell-free culture media of *Phaffia rhodozyma* (■) and *Saccharomyces cerevisiae* (●) used in the cells growth.

chromium (Jamnik *et al*., 2003). If so, it can be verified with the use of sulfate (added before chromium) which blocks the Cr(VI) transporters. The 30 mM and 100 mM sulfate concentrations were chosen based on the experiments of (Pepi, Baldi 1992). The data of the Cr reduction in the presence of sulfate in the media were shown in Fig. 5 and 6.

The presented data was normalized to the signal of paramagnetic spin probe, and it is possible to compare sample values of EPR signals coming from different set of measurements. As can be seen in Fig. 5 the formation of free-radical Cr(V) by the *S. cerevisiae* cells in the presence of 30 mM sulfate ions is enhanced about 2 times, compared to the sample in the absence of sulfate (Fig. 2). Analyzing the time-dependent formation of Cr(V) with addition of sulfate and without sulfate (Fig. 2) one can observe that after 30 min the reduction of Cr(VI) by *S. cerevisiae* cells achieve a plateau. While in the presence of 30 mM sulfate after 35 min the formation of Cr(V) reaches the maximum and after that the signal decreases (Fig. 5). It is worth noticing that in the presence of 30 mM of sulfate the EPR amplitude is significantly higher than in the case of sulfate absence and it can suggest that sulfate induces the production of chromium reductants. The 100 mM concentration of the sulfate inhibits the reduction process about 2 times in the case of *Saccharomyces cerevisiae* (Fig. 2, 5).

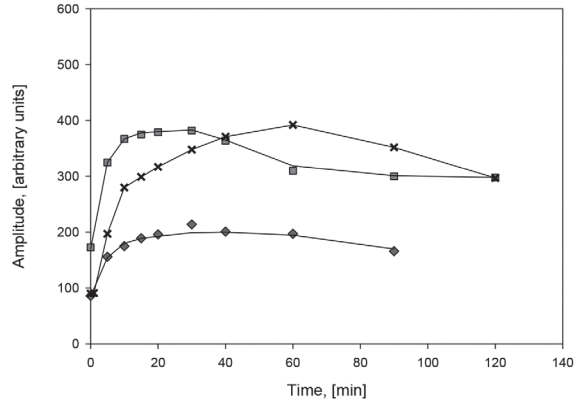

Figure 5. Reduction of 4 mM Cr(VI) by *Saccharomyces cerevisiae* in the present of sufate ions: 30 mM (■), 100 mM (◆), control (x).

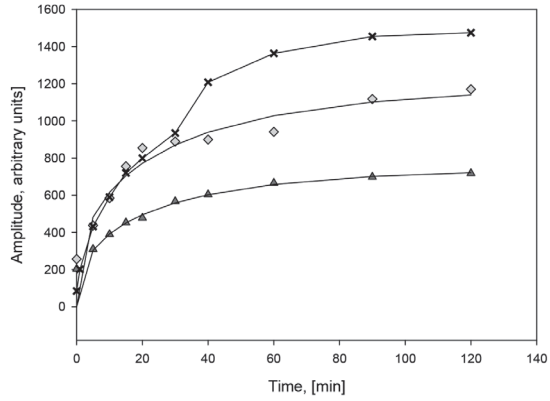

Figure 6. The reduction of 4 mM Cr(VI) by *Phaffia rhodozyma* in the presence of sulfate: 30 mM (▲), 100 mM (◆), control (x).

An opposite situation is observed in the case of *P. rhodozyma* as illustrated in Fig. 6. The signal of Cr(V) is reduced in the presence of sulfate ions compared to the measurements without sulfate (Fig. 2 and Fig. 6). What is surprising, the decrease of the signal is significant in the presence of 30 mM of sulfate during 60 min, whereas the 100 mM of sulfate concentration reduces the signal in a less degree.

DISCUSSION

The reduction of chromate by living organisms has been demonstrated by various methods in animal system (Appenroth *et al*., 1996), bacteria (Shi *et al*., 1994) and plant (Kaszycki *et al*., 2005). The EPR-L band method was used successfully to monitor the Cr (VI) reduction processes. The usage of the L-band techniques has several advantages, mainly the formation of paramagnetic species can be measured continuously *in vivo* with minimal disturbance of the living systems. Samples placed into surface coil resonator are not disturbed and there is no need to cut or disintegrate them as required in other method, e.g X-band spectroscopy. With the EPR L-band spectroscopy direct kinetic studies can be carried out in whole cells under physiological condition. Using this method the chromate reduction by *S. cerevisiae* and *P. rhodozyma* was undertaken.

In the study those yeast strains were chosen because one of them, *P. rhodozyma* is highly resistant to the toxic chromate, much more resistant than moderate *S. cerevisiae*. Their response to sub-lethal concentration of the metal might reveal some differences in the reduction mechanism.

The data presented in Fig. 2, Tables 1 and 2 confirm that the *P. rhodozyma* is much more resistant to chromium than *S. cervisiae* (Nechay *et al*., 2009). Based on the results shown in Figs. 3 and 4 it can be suggested that the higher resistance of *P. rhodozyma* to toxic chromate is related to the extra-cellular reductants. The dominant role of the extra-cellular reductants of Cr(VI) was suggested by the Gonchar group (Ksheminska *et al*., 2006) and the suggestion was based on "disappearance" of Cr(VI) which resulted in the detection of Cr(III) complexes in extra-cellular space of some yeast species. The data shown in Fig. 4 for the first time illustrates direct role of extra-cellular compounds secreted by the yeast strains in terms of free-radical scavenging. The reduction capacity of

P. rhodozyma overwhelms reduction abilities of the *S. cerevisiae*. One can suppose that the characteristic feature of *P. rhodozyma* to synthesize the astaxanthin can be responsible for the reduction level. However, recently published data exclude such a possibility as a "carotenoidless" *P. rhodozyma* mutant exhibited higher resistance to chromium than astaxantin-producer strain (Horvath *et al*., 2011). The explanation of such a big difference can be associated with effective secretion to the medium of S-aminoacids, glutathione and other antioxidants. The literature data (Ksheminska *et al*., 2010) report the presence of at least two Cr(V) — intermediates with EPR X-band in the extra-cellular media of *Pichia quillermondii* which can prove an existence of different Cr(V) complexes with extra-cellular compounds.

It is widely known that sulfate and chromate compete for the sulfur transport channels into cells. Inside the cell chromate has an influence on genes and enzymes of sulfur metabolism (Pereira *et al*., 2008) and the metabolite pools of sulfur pathway are decreased. On the other hand, in the case of *Candida intermedia* the strain exposed to 100 μM Cr(VI) showed elevated content of glutathione (Jamnik *et al*., 2003) and such an influence can be a result of a general response of the cell to stress. In the case of *P. rhodozyma* the data presented in Fig. 2 and Fig. 6 indicates that the Cr(VI) reduction is diminished in the presence of sulfate. However, the higher reduction of the chromate by *P. rhodozyma* can be observed in the presence of 100 mM sulfate concentration compared to 30 mM sulfate (Fig. 6) judging on EPR amplitude signals. It can be suggested that 100 mM of sulfate can induce biosynthesis of reductants more effectively in the yeast strain. The results shown in Fig. 2 and Fig. 5 concerning *S. cerevisiae* confirmed the data obtained by Babyak *et al*., (2005), where the authors observed the inhibition of Cr(VI) accumulation in yeast cells in the presence of 100 mM sulfate. This effect is understandable in terms of competition between ions for sulfur transporter. In the case of *P. rhodozyma* such high inhibition does not occur. However, the data in Fig.4 and Fig. 5 indicate that the reduction of chromate by *Saccharomyces cerevisiae* still occurs in the presence of 100 mM sulfate. The effect can be explained by the residual extra-cellular activity in the yeast cells (Fig. 4) or by the assumption that there is another and distinct transport of chromate into cell. Such assumption was suggested by Perirea (2008).

In the case of *S. cerevisiae* it was observed that amplitudes of Cr(V) do not change drastically in the 30 mM sulfate concentration compared to control (Fig. 5). Only the rate of reduction was higher up to 30 min.

However, in the case of *P. rhodozyma* the reduction decrease is surprisingly influenced. Reduction of Cr(VI) is bigger in the presence of 100 mM of sulfate than 30 mM. The reduction behaviour of *S. cerevisiae* in sulfate presence is understandable, because the 30 mM sulfate induce synthesis of several sulfur metabolites, particularly S-Ado-homocysteine, methionine and glutathione, which can reduce chromate effectively (Pereira *et al*., 2008). The simple explanation of elevated reduction activity of *P. rhodozyma* in the presence of 100 mM sulfate can be a fact that this concentration induce synthesis of radical scavengers in higher level and can suggest that cellular response for high sulfur concentration is biochemically and physiologically different than in *S. cerevisiae*. The investigation of this effect requires further studies.

CONCLUSION

Protoplasts of both strains showed couple times faster reduction of chromium compared to intact cells which suggests the high influence of the cell wall on uptake into the cell and the reduction of Cr(VI). What is more *P. rhodozyma* has demonstrated greater Cr(VI) reduction than *S. cerevisiae* both in intact cells and its isolated protoplasts what could be connected with more sufficient production of metabolites like glutathione and cysteine which also can be a factor responsible for its resistance for high concentrations of toxic chromium.

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REFERENCES

Abbas Ch, Sibirny (2011) A genetic control of biosynthesis and transport of riboflavin and flavin nucleotides and construction of robust biotechnological producers. *Microbiol Mol Biol Rev* **75**: 321–360.

Appenroth D, Rost M, Friedrich M, Schulz O (1996) Contribution to the mechanism of chromate nephrotoxicity in developing rats: EPR investigations. *Arch Toxicol* **71**: 25–30.

Babyak L, Kseminskaya G, Gonchar M, Yanovich D, Fedorovich D. (2005) Selection and properties of mutant yeast *Pichia guilliermondii* strais resistant to chromium (VI) *Appl Biochem Microbiol* **41**: 177–181.

Barceloux DG (1999) Chromium. *J Toxicol Clin Toxicol* **37**: 173–194.

Cervantes C, Campos-Garcia J, Devars S, Gutierez-Corona F, Loza-Tavera H, Torres-Guzman JC, Moreno-Sanchez R (2001) Interactions of chromium with microorganisms and plants. *FEMS Microbiol* **25**: 335–347.

Cherest H, Davidian JC, Thomas D, Benes V, Ansorge W, Surdin-Kerjan Y (1997) Molecular characterization of two high affinity sulfate transporters in *Saccharomyces cerevisiae*. *Genetics* **145**: 627–635.

Cieślak-Golonka (1996) Toxic and mutagenic effects of chromium (VI). A Review. *Polyhedron* **21**: 3667–3689.

Fedorovych D, Ksheminska H, Babyak D, Kaszycki P, Koloczek H. (2001) Hexavalent chromium stimulation of riboflavin synthesis in flavinogenic yeast. *BioMetals* **14**: 23–31.

Hedlam H, Lay P (2001) EPR spectroscopic studies of the reduction of chromium (VI) by methanol in presence of peptides. Formation of long-lived chromium (V) peptide complexes. *Inorg Chem* **40**: 78–86.

Horvath E, Papp G, Gazdag Z, Belagyi J, Blasko A, Deli J, Vagvolgyi Cs, Pesti M (2011) Characterization of stress processes of *Phaffia rhodozyma* stress-resistant mutant. *Acta Biol Hung* **62**: 204–210.

Jamnik P, Raspor P (2003) Stress response of yeast *Candida intermedia* to Cr(VI). *Biochem Mol Toxicol* **17**: 316–323.

Kaszycki P, Gabryś H, Appenroth K.J, Jaglarz A, Sedziwy S, Walczak T, Koloczek H (2005) Exogenously applied sulphate as a tool to investigate transport and reduction ofchromate in the duckweed *Spirodela polyrhiza*. *Plant Cell Environ* **28**: 260–268.

Ksheminska H, Fedorovych D, Babyak L, Yanovych D, Kaszycki P, Koloczek H (2005) Chromium(III) and (VI) tolerance and bioaccumulation in yeast: a survey of cellular chromium content in selected strains of representative genera. *Process Biochem* **40**: 1565–1572.

Ksheminska HP, Honchar T, Gayda G, Gonchar M (2006) Extracellular chromate-reducing activity of the yeast cultures. *CEJB* **1**: 137–149.

Ksheminska H, Honchar T, Usatenko Y, Gayda G, Gonchar M (2010) The chromate resistance phenotype of some yeast mutants correlates with a lower level of Cr(V)-species generated in the extra-cellular medium. *Biometals* **23**: 633–642.

Nechay HI, Ksheminska P, Kolisnyk HV, Grządka M, Gonchar MV (2009) Reduction of chromate and carotene-synthesizing activity of selenite-resistant mutants of the yeast *Xanthophyllomycesdendrorhous (Phaffia rhodozyma)*. *Biopolymers and Cell* **25**: 633–642.

Pas M, Milacic R, Draslar K, Pollak N, Raspor P (2004) Uptake of chromium (III) and chromium (VI) compunds in the yeast cell structure. *BioMetals* **17**: 25–33.

Pepi M, Baldi F (1992) Modulation of chromium(VI) toxicity by organic and inorganic sulfur species in yeasts from industrial wastes. *Biometals* **5**: 179–185.

Pereira Y, Lagniel G, Godat E, Baudouin-Cornu P, Junot Ch, Labarre J (2008) Chromate causes sulfur starvation in yeast. *Toxicol Sci* **106**: 400–412.

Pesti M, Gazdag Z, Emri T, Farkas N, Koosz Z, Belagyi, Pocsi I (2002) Chromate sensitivity in fission yeast is caused by increased

glutathione reductase activity and peroxide overproduction. *J Basic Microbiol* **42**: 408–419.
Sami M, Ikeda M, Yabuuchi S (1994) Evaluation of the alkaline methylene bluestaining method for yeast activity determination. *J Ferment Bioengin* **78**: 212–216.
Shi X, Dong Z, Dalal NS, Gannet PM (1994) Chromate-mediated fre radical generation from cysteine, penicillamine, hydrogen peroxide and lipid hydroperoxides. *Biochim Biophys Acta* **1226**: 65–72.
Smutok O, Broda D, Smutok H, Dmytruk K, Gonchar M (2011) Chromate-reducing activity of *Hansenula polymorpha* recombinant cells over-producing flavocytochrome b_2. *Chemosphere* **83**: 449–454.
Wysocki R, Tamas MJ (2010) How *Saccharomyces cerevisiae* cope with toxic metals and metalloids. *FEMS Microbiol* **34**: 925–951.
Yoshimoto N, Takahashi H, Smith FW, Yamaya T, Saito K (2002) Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in *Arabidopsis* roots. *Plant J* **29**: 465–473.