Biometals (2008) 21:613–621
DOI 10.1007/s10534-008-9147-6

Responses of *Rhodotorula* sp. Y11 to cadmium

ZhiJian Li · HongLi Yuan

Received: 14 December 2007 / Accepted: 17 May 2008 / Published online: 29 May 2008

Abstract Some aspects of the cellular responses to cadmium were extensively investigated in the yeast *Rhodotorula* sp. Y11. Scanning electron microscopy indicated that accumulation of cadmium in the Y11 did not cause any visible effects on cell morphology. More than 20% yeast cells still showed viability after 15 h of cadmium accumulation under 100 mg l^{-1} cadmium concentration, and transmission electron microscopy analysis showed that plasmolysis and thickened cell wall were not observed in all of the cells. In the presence of cadmium, the activities of superoxide dismutase (SOD) and catalase (CAT) were all greater than the control, but the increase was in a dose-independent manner. Changes in SOD and CAT activities were also dependent on the time of exposure. Therefore, it suggests that antioxidative defenses play an important role in cadmium tolerance in *Rhodotorula* sp. Y11. Nondenaturing polyacrylamide gels revealed only one SOD isoforms in Y11 even under exposure to cadmium.

Keywords Cadmium · Catalase · *Rhodotorula* sp. · Resistance · Superoxide dismutase

Z. Li · H. Yuan (✉)
College of Biological Sciences, Key Laboratory of Agro-Microbial Resource and Application, Ministry of Agriculture, China Agricultural University, Beijing 100094, People's Republic of China
e-mail: hlyuan@cau.edu.cn

Introduction

Pollution by heavy metals is now widespread and it is causing serious ecological problems in many parts of the world. Amongst these heavy metals, cadmium, which becomes a serious pervasive environmental pollutant commonly generated from mining, smelting, electroplating, and painting is attracting increased concern, since it ultimately reaches and accumulates in animal and human tissues throughout the food chain (Sanità di Toppi and Gabbrielli 1999; De Silóniz et al. 2002).

Conventional physicochemical treatments methods, which have been practiced for several decades for the removal of toxic heavy metals from wastewaters, may be ineffective or very expensive. These disadvantages become more pronounced at metal concentrations less than 100 mg l^{-1} (Kapoor and Viraraghavan 1995). The potential of using bioaccumulation or biosorption, which have been used for the removal of heavy metal ions by microorganisms, has become an attractive subject over the past decade. Many microbial biomasses have been screened for their biosorption capabilities (Kapoor and Viraraghavan 1995; Vieira and Volesky 2000). Despite the known potential of yeasts for the removal of heavy metal cations from aqueous solutions (Blackwell et al. 1995), there is little information relating to the relationship between cadmium and *Rhodotorula* sp. It is recently reported *Rhodotorula* spp. are able to accumulate cadmium and lead and some heavy metal

accumulation mechanisms were also suggested (Salinas et al. 2000; Cho and Kim 2003; Li and Yuan 2006; Li et al. 2008). However, detailed information by which *Rhodotorula* sp. can survive and grow in the presence of potentially toxic concentrations of cadmium has to be fully characterized further.

Nonviable biomass has several advantages for metal biosorption. Nevertheless, application of active and growing cells is also a better option sometimes due to its unique advantages, such as their ability of self-replenishment and continuous metabolic uptake of metals after physical adsorption (Malik 2004). Resistance mechanisms to heavy metals by microorganisms is important issue when removal of metals by growing biomass is considered. Metal resistance of microorganisms is a complex process and the mechanisms of metal ion accumulation and resistance differ according to microorganisms and metal ions. So an overall analyses of the metal tolerance in the same isolate needs to be performed (Blackwell et al. 1995; Nies 1999; Pereira et al. 2006). In this way, we could better understand how different mechanisms may interact together to enhance heavy metal resistance (Pereira et al. 2006). Furthermore, the understanding of the heavy metal tolerance mechanisms can also be of great value to design efficient techniques for heavy metal removal.

Several heavy metal-resistance mechanisms have been proposed in yeast, including sequestration of heavy metals by metallothioneins through their high cysteine content, adsorption of heavy metal cations to the cells and ion exchange (Kierans et al. 1991; Blackwell et al. 1995; Suh et al. 1998; Gomes et al. 2002; Cho and Kim 2003). Particularly the yeast vacuole is considered to be a site for cadmium storage (Volesky et al. 1993). Superoxide dismutases (SOD) have also been described for its response to heavy metals. SOD is involved in the dismutation of superoxide anion to dioxygen and hydrogen peroxide, which is subsequently detoxified by catalases (CAT) or peroxidases (Adamis et al. 2004; Schmidt et al. 2007). Since metals in the natural environment can exacerbate the levels of reactive oxygen species (ROS), which are responsible for most cellular injuries, any cell exposed to heavy metal stress has to cope with ROS and the induction of antioxidant enzymes is of great importance (Adamis et al. 2004; Schmidt et al. 2007). It is reported that expression of SOD is involved in high tolerance to copper in the deep-sea yeast, *Cryptococcus* sp. N6 (Abe et al. 2001; Miura et al. 2002). The manganese and iron SODs protect *Escherichia coli* from heavy metal toxicity (Geslin et al. 2001). In the vanadate-tolerant yeast *Hansenula polymorpha*, vanadate and copper induce overlapping oxidative stress responses in terms of the induction of SOD and CAT activity (Mannazzu et al. 2000).

In preliminary experiments, *Rhodotorula* sp. Y11 showed higher tolerance to cadmium and physiological parameters response to cadmium have been partly elucidated (Li and Yuan 2006; Li et al. 2008). However, the effects of cadmium on morphology, viability and antioxidant defence systems of Y11 have not been studied. Therefore, in this study, these aspects of the cellular responses of Y11 are determined.

Material and methods

Microorganism, medium and culture conditions

The strain *Rhodotorula* sp. Y11, isolated from mine soil could survive under 2000 mg l^{-1} cadmium (Wang et al. 2004). *Rhodotorula* sp. Y11 was maintained and activated in YPD medium comprising of glucose, 20 g l^{-1}, yeast extract, 10 g l^{-1}; peptone, 20 g l^{-1}. The pH of the medium was adjusted to 5.8.

The cells were grown in 500 ml Erlenmeyer flasks containing 100 ml of media. Flasks were incubated on an rotary shaker at 150 rpm for 20 h at 28°C unless stated otherwise. The biomass was harvested by centrifugation at 4,000*g* for 5 min. Once harvested, the biomass was washed twice with deionized distilled water and used for the experiments directly.

Cadmium uptake and cell viability

Biomass (30 mg dry weight) prepared from cadmium free medium was added to 20 ml 100 mg l^{-1} cadmium solutions with natural pH at 28°C on an rotary shaker at 150 rpm. After incubation, cells were separated at defined intervals of time by centrifugation at 12,000*g* for 20 min. The biomass in deionized distilled water without cadmium was used as control.

Extracellular and intracellular cadmium was determined according to the method of Li et al. (2008). Samples were withdrawn from the culture flasks at

defined intervals harvested by centrifugation at 4,000 rpm for 5 min. The pellet was washed three times with distill water and the pellet was divided into two portions. One portion was washed three times in 0.02 M EDTA (10 min each time). The amount of cadmium associated with the cell biomass was removed as the EDTA washable fraction present at the cell surface, thereby allowing only intracellular cadmium to be measured. Another portion was washed by distilled water under the same condition as control and the total cadmium adsorbed by cells was measured in this portion. The concentration of cadmium was determined by inductively-coupled plasma atomic emission spectrophotometry (ICP-AES, IRIS Advantage).

The viability of the cells taken at defined intervals was assessed by the classical spread-plating method to assess the cadmium toxicity (Soares et al. 2002).

Small-scale preparation of cell extracts, enzyme assay and activity staining

The cells grown in cadmium-supplemented medium (0–200 mg l^{-1}) were harvested by centrifugation at 6,000 rpm for 5 min and washed with 20 mM potassium phosphate (pH 7.8). Following resuspension in 0.4 ml of the same buffer, cells were disrupted with 0.45 mm glass beads by vibrating for 15 pulses of 1 min each in Eppendorf tube on a vortex mixer, with 1 min intervals on ice between pulses. Cell debris was removed by centrifugation at 12,000*g* for 15 min at 4°C. Cell extracts were stored at −20°C to be used for CAT and SOD analyses. Protein was estimated by the dye-binding method of Bradford (1976) with bovine serum albumin as a standard protein.

SOD activity in extracts was determined by using the modified pyrogallol autoxidation method, as described previously (Marklund and Marklund 1974). One unit of SOD enzyme activity is defined as the amount of enzyme that inhibits the autoxidation rate of pyrogallol by 50%. Gradient native polyacrylamide gel electrophoresis (PAGE) was stained for SOD activity by the photochemical nitro blue tetrazolium reaction as described previously (Beauchamp and Fridovich 1971). Fifty microgram protein were loaded per lane. CAT activity was measured spectrophotometrically as a decrease in H_2O_2 absorbance at 240 nm using an absorption coefficient of 0.039 mM^{-1} cm^{-1} from an initial H_2O_2 concentration of 3 mM (Aebi 1983). One unit CAT activity is defined as the amount of enzyme that decomposed 10 μmol H_2O_2 in 1 min at 25°C.

All solutions of $Cd(NO_3)_2$ used were diluted by distilled deionized water from the stock solutions. All experiments were repeated two or more times and the results reported in this article are the average values ± SD value. The significant differences between two samples were calculated using Student's *t*-test wherever applicable.

Electron microscopy observation

Scanning electron microscopy (SEM) assay was carried out as described by Srivastava and Thakur (2006). Biomass growing in medium without or with cadmium (100 mg l^{-1}) was harvested and placed in a phosphate-buffered glutaraldehyde fixative (2.5%) overnight. The fixative was removed with two 15 min washes with ice-cold 0.1 M phosphate buffer (pH 7.3). The phosphate buffer was removed and the sample was washed at 10 min intervals with ethanol solutions ranging from 30 to 100% (v/v). The ethanol was decanted and the sampler was washed at 20 min intervals with 75:25 ethanol:amyl acetate, 50:50 ethanol:amyl acetate, 25:75 ethanol:amyl acetate solutions, and pure amyl acetate. The samples were then coated with a thin layer of gold and assayed using a SEM (Hitachi S-570). Transmission electron microscopy (TEM) observation was conducted as previously described (Yuan et al. 2007). Biomass prepared from cadmium free medium was added to 100 mg l^{-1} cadmium solutions with natural pH and incubated on an rotary shaker at 150 rpm for 15 h at 28°C. After incubation, cells were separated by centrifugation at 12,000*g* for 20 min. The biomass in deionized distilled water without cadmium was used as control. The cells prepared fixed with 2.5% glutaraldehyde in 0.1% phosphate-buffered saline (PBS), washed in 0.1% PBS for four times and post-fixed in 1% osmium tetroxide. The samples were dehydrated in a graded acetone series and embedded in Spurr with equal volume of acetone and then in pure Spurr resin each for 24 h. Sections of 50 nm were cut with a LKB-V ultramicrotome. TEM (JEOL-100S) was used to examine the cadmium precipitation in the cells.

Results

Effect of cadmium on yeast cell morphology

Assessment of morphological changes of Y11 in response to cadmium was observed by SEM. Figure 1a shows the cells grown in medium without cadmium exposure. It was observed that the usual cells shapes range from round to oval and the length from 3 to 4.5 μm. No ring shaped bud scars could be clearly seen on the surface of mother cells. The presence of 100 mg l^{-1} cadmium in the medium did not induce significant changes of morphological properties of yeast cells (Fig. 1b).

Cadmium uptake and cell viability

The total cadmium uptake and cell viability during cadmium accumulation by living cells is shown in Fig. 2. As total cadmium fast accumulated on the cells with little intracellular cadmium during the first 45 min, cell viability showed similar decrease comparing with the control. Even when some cadmium penetrated into the cytoplasm at 2 h, it did not cause significant reduction of cell viability as compared to the control ($P > 0.05$). Whereas the viability of yeast cells exposed to cadmium decreased fast and dramatically in comparison with the control after 2 h due to the toxic effect of cadmium ($P < 0.05$). After 15 h, 23% of cadmium treated cells was still alive.

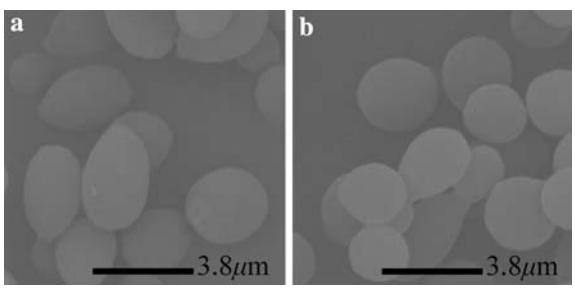

Fig. 1 Scanning electron microphotographs of *Rhodotorula* sp. Y11 grown in medium without (**a**) and with cadmium (**b**)

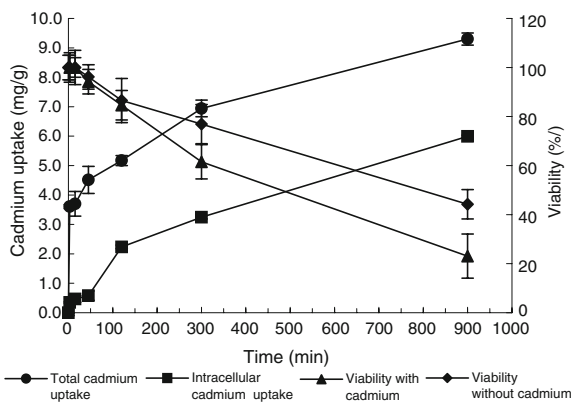

Fig. 2 Cadmium uptake and cell viability. Data from three replicate determinations are shown mean ± SD values

TEM was performed for the identification of cadmium accumulation within cells in order to investigate the cadmium accumulation mechanisms of Y11. Figure 3a shows the original Y11 cell which was not exposed to cadmium solutions. There was essentially no cadmium on the cell surface, cell membrane, and in the cytoplasm. It was observed that the cell wall was shown as a dark and the cell wall of Y11 had a tendency to wrinkle. Organelles within the cell could be distinguished. However, when samples of biomass exposed to cadmium solutions for 15 h, significant darkening of the cell inner occurs, which may be due to cadmium staining the components inside the cell. Even so normal organelles are still observed (Fig. 3b). No evident cadmium granular deposits were observed to accumulate along the outer surface of the cell wall and within the cell. Furthermore, plasmolysis of some cell was often observed by TEM after 15 h exposure to cadmium (Fig. 3c), and consequently it was impossible to distinguish organelles within the cell. It was found that the cell wall thickened and were slightly light compared with that of the normal cells. The formation of electron-dense granules was found in and around inner cell walls.

Effect of cadmium on the levels of SOD and CAT

The levels of SOD and CAT activities in the red yeast *Rhodotorula* sp. Y11 cells during growth exposure to 0–200 mg l^{-1} cadmium were determined for up to 34 h. In the present study, cadmium accumulation in *Rhodotorula* sp. Y11 resulted in a significant changes in the activity of the two enzymes (Table 1). In general, it was observed that Y11 grown in medium amended cadmium contained about 1.5 and 1.2 times higher relative abundance of SOD when compared with normal amount of SOD of cells grown in medium without cadmium at 10 and 20 h of the growth phase, respectively ($P < 0.05$). Especially, the SOD activity increased more than twofold upon

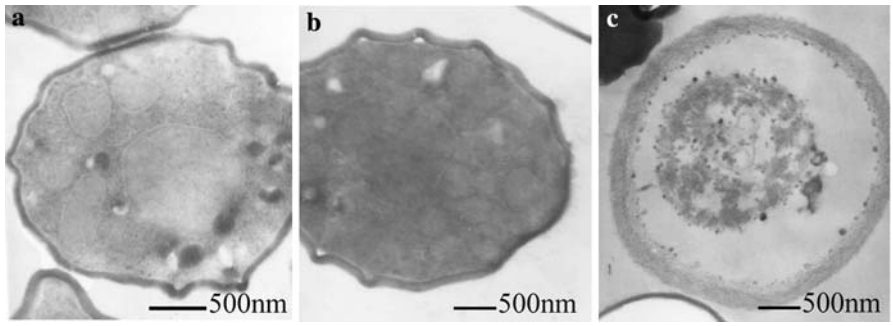

Fig. 3 Transmission electron microphotographs of *Rhodotorula* sp. Y11 without (**a**) and with cadmium (**b**, **c**)

Table 1 Effects exerted by cadmium on antioxidant enzyme levels in *Rhodotorula* sp. Y11

	Time (h)	Cadmium (mg l^{-1})			
		0	50	100	200
SOD (U mg^{-1} protein)	10	2.1 ± 0.13	3.2 ± 0.25	3.3 ± 0.28	4.39 ± 0.20
	20	3.8 ± 0.19	4.6 ± 0.25	4.7 ± 0.14	4.8 ± 0.46
	34	5.8 ± 0.87	5.9 ± 0.06	5.8 ± 0.11	5.6 ± 0.16
CAT (U mg^{-1} protein)	10	48.5 ± 3.2	142.6 ± 6.5	140.0 ± 7.1	152.6 ± 10.6
	20	200.4 ± 5.8	306.6 ± 17.9	341.3 ± 21.4	340.4 ± 16.5
	34	273.8 ± 21.4	430.8 ± 14.6	477.1 ± 25.6	467.5 ± 17.3

Data are shown as mean ± SD of three independent experiments

treatment with 200 mg l^{-1} cadmium at 10 h. Despite all that, different cadmium concentration induced comparable increments in the levels of specific SOD activity. Therefore, cadmium highly significantly stimulated the SOD production at the mid (10 h) and late (20 h) of the exponential growth phase, but SOD increase was not in a cadmium dose-dependent manner. Whereas cadmium had no significant effect on SOD activity in *Rhodotorula* sp. Y11 compared with the controls at 34 h. CAT activity in the cells exhibited a similar increase pattern as SOD except that significant increase (more than 1.5-fold) was also observed further at 34 h when compared to the control ($P < 0.05$). This means CAT still play an important role in the scavenging of the potentially harmful oxidant at 34 h. The obtained results show that in the samples treated with cadmium induced the antioxidative defence systems, which play a protective role against the toxicity of ROS.

Activity patterns under nondenaturing PAGE

The SOD was tested by activity staining to elucidate their response to cadmium. Nondenaturing PAGE analyses shows the SOD activity profile of *Rhodotorula* sp. Y11 (Fig. 4). Basically, only a single kind of SOD isoenzyme electrophoretic band pattern was seen at normal culture conditions. To check if there were cadmium-induction SOD enzymes in Y11, an induction experiment was performed by adding cadmium at final concentration of 100 mg l^{-1}. In

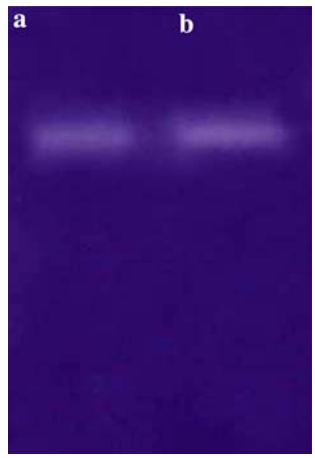

Fig. 4 Staining for SOD activity of *Rhodotorula* sp. Y11 in absence (**a**) and presence (**b**) of cadmium (100 mg l^{-1})

contrast, the electrophoretic pattern of Y11 remains unaltered with only a single band revealed at the same distance of migration (Fig. 4). Inhibition studies indicated that the SOD isoenzyme in *Rhodotorula* sp. Y11 was Mn-SOD (data not shown).

Discussion

The presence of 100 mg l^{-1} cadmium in the medium was in the range of cadmium concentration (0–150 mg l^{-1}) having no significant effect on Y11 growth (Li et al. 2008), The SEM results further confirm the resistance of Y11 to cadmium. It has been found that raising concentrations of copper affected both morphology and physiological parameters of the viable yeast (De Silóniz et al. 2002). Doshi et al. (2007) showed that the difference in the surface morphology of *Spirulina* sp. was evident after cadmium uptake. Morphological properties of yeast cells *Candida intermedia* were not changed under 1 mM chromium (III), but ten times higher concentration of chromium (III) in the medium affected the cell shapes as well as their budding pattern (Paš et al. 2004).

The delayed intracellular cadmium accumulation means the cell wall may act as a barrier against cadmium into the cytoplasm and the following intracellular cadmium uptake reveals in the cell there are intracellular mechanisms that help to cope with metal stress. During heavy metal accumulation by *Saccharomyces cerevisiae* NCYC 1190, there was about 99% loss of viability of copper-treated cells in the first 20 min, and a less pronounced lethal effect was detected after 1 h of contact with cadmium or lead (Soares et al. 2002). Cadmium showed a dramatic effect on *S. cerevisiae* cell viability. Although no loss of viability was observed at 50 μM cadmium concentration, there was approximately 90% loss of viability at 500 μM after 5 min contact with cadmium (Gadd and Mowll 1983).

TEM demonstrated that the cell wall and inner parts of these cells play a major role in cadmium accumulation. The increased cell wall thickness of the *Acremonium pinkertoniae* myceliar walls in response to copper was clearly visible, which preventing the toxic ions from reaching the interior of the cells (Zapotoczny et al. 2007). The thicker mannan layers seemed to contribute to the high cadmium uptake capacity of *S. cerevisiae* ATCC 834 in comparing with *S. cerevisiae* ATCC 24858 (Park et al. 2003). Volesky et al. (1993) reported that cadmium was mainly deposited in the vacuoles of *S. cerevisiae*, but Suh et al. (1998) showed that cadmium accumulated both on the cell surface and inside the cell of *S. cerevisiae*. Perkins and Gadd (1993) indicated that majority of lithium accumulated in *S. cerevisiae* X2180-1B was located in the vacuoles and the enlarged vacuoles observed during lithium loading may be associated with an intracellular toxic response. There was little evidence for intracellular accumulation of silver and the majority of cell-associated silver was present as electron-dense granules within and around cell walls, or sometimes associated with extracellular polysaccharide as in *Rhodotorula rubra* (Kierans et al. 1991). In *Kluyveromyces marxianus*, the outer cell wall surface concentrate uranium, the significant darker inner cellular matrix implies a role for inner cellular mass in biosorption of uranium by this form of biomass (Bustard et al. 1997).

From the results, it also suggested that not all of the cells showed the same contribute to the cadmium accumulation. A similar phenomenon was observed for uranium accumulation by *S. cerevisiae* and silver deposits in bacterial populations (Strandberg et al. 1981; Goddard and Bull 1989). Kierans et al. (1991) also observed uneven accumulation of silver, as indicated by the formation of electron-dense granules in and around cell walls. In *A. pinkertoniae*, the younger mycelia developing on the culture border were always white which was similar to those growing on control media with trace amounts of copper. Only in the oldest parts of the culture the crystalloids were eventually fully exposed (Zapotoczny et al. 2007).

The average value content of cadmium uptake by spectrophotometric data do not take the role of every cell in cadmium detoxification into account. The TEM gives us more detailed knowledge on the cell heterogeneity. It was suggested that this was either due to population heterogeneity in respect of metal uptake capacity and/or attrition during growth in the bioreactors (Kierans et al. 1991). The latter hypothesis was discounted in by fixing and embedding yeast colonies in situ (Kierans et al. 1991). Furthermore, *S. cerevisiae* NCYC 1190 cells propagated under defined laboratory conditions, stopping the growth at

the same time, were believed to be always in the same physiological state (Soares et al. 2002). So, from a evolutionary perspective, the profound signification under the uneven metal uptake ability may represents a resistance mechanism to heavy metals rather than a simple population heterogeneity. During the process of heavy metal accumulation, the ability of some cells to accumulate more metals, which reducing the concentration of bioavailability of heavy metals in the surrounding microenvironment, increases the probability of any remaining cells to survive. Although the process is deleterious to individual cell, the group is still able to persist in heavy metal environments without killing the entire population.

SOD play a primary defence mechanism in the scavenging of the potentially harmful superoxide radicals and represent a critical enzyme of the antioxidant defence system (Fridovich 1983; Fujs et al. 2005). It is clearly important to establish whether the exposure of Y11 to cadmium causes a detrimental or stimulatory effect on the enzymes involved in this detoxification process. Although redox inactive metals ion, such as cadmium, nickel, and zinc, do not directly generate ROS, microorganisms exposed to these elements often accumulate ROS and undergo oxidative stress. Some possible relationship between cadmium and oxidative stress was suggested (Vido et al. 2001). There have been previous studies that have examined the effect of cadmium on SOD and CAT activity in microorganisms. A fourfold increase in SOD activity is induced by copper in the marine yeast, *Debaryomyces hansenii* (Orozco et al. 1998). Cu, Zn-SOD activity of *S. cerevisiae* is stimulated in the presence of copper, while it is lightly inhibited in the presence of cadmium (Romandini et al. 1992). In *E. coli*, the absence of SODs was associated with an sensitivity to cadmium, nickel and cobalt ions, which suggests that intracellular generation of superoxide by cadmium, nickel and cobalt is toxic in *E. coli*. It also supports the *sod* genes in the protection of *E. coli* cells against metal stress (Geslin et al. 2001). In the vanadate-tolerant strain of *H. polymorpha*, the presence of both vanadate and copper in the growth medium caused a significant increment in the activity of the two antioxidant enzymes SOD and CAT. Interestingly, while vanadate and copper induced comparable increments in the levels of SOD activity, dramatic differences were observed in the effects of the two metal ions on CAT activity, which showed an 8.4 and 1.7-fold increment by vanadate and copper, respectively (Mannazzu et al. 2000). Interestingly, no clear increase of SOD activity in *Streptomyces subrutilus* P5 was observed on addition of lead ions to the medium, and it is concluded that the strain *S. subrutilus* P5 precipitate of lead ions by the extracellular SOD, which might reduce the intracellular generation of superoxide radicals caused by metal toxicity, hence relieving the protective task of intracellular SODs and confering a biological advantage for survival over the other microorganisms in a highly lead-polluted environment (So et al. 2001). CAT level in *S. cerevisiae* remains almost unchanged in the conditions tested (Romandini et al. 1992). The CAT activity of the *Candida albicans* cells treated with 1 mM cadmium first decreased, and then rose at 24 h to about 2.6 times that of the controls. The increase of peroxisomes and CAT activity following cadmium stress gives credence to the hypothesis that cadmium toxicity is related to its potential to induce oxidative stress in cells (Chen et al. 1995).

Since the carotenogenic yeast, like *Rhodotorula mucilaginosa*, has been reported to contain only Mn-SOD and the absence of Cu, Zn-SOD in a pigmented yeast has been accepted as a general rule (Moore et al. 1989). However, Hernández-Saavedra (2003) reports the presence of an active Cu, Zn-SOD after copper induction in pigmented yeast *Udeniomyces puniceus*. It suggests that the absence of Cu, Zn-SOD in pigmented yeast is complemented by the presence of carotenoproteins that act as an extra mitochondrial antioxidant (Moore et al. 1989). Mn-SOD is present only in the mitochondrial (Moore et al. 1989; Schroeder and Johnson 1993), therefore, it is thought to be a major scavenger of reactive oxygen metabolite(s) in the mitochondrial matrix, like Cu, Zn-SOD in the cytoplasm. Hwang et al. (2003) found that mitochondrial Mn-SOD is important for stress responses in *C. albicans*. In the present study, a possible explanation to the comparable level of SOD and CAT at different cadmium concentration could be due to the limited induction level by ROS and there must be other molecular approaches, like carotenoids, to improving ROS tolerance.

Abe et al. (2001) suggest SOD have a role in the defensive mechanisms against high concentrations of Cu in the yeast *Cryptococcus* sp. N6. The activity of

SOD in cell extract was markedly stimulated from <1 total protein to 25.8 and 110 mU μg^{-1} total protein by 1 mM $CuSO_4$ and 10 mM $CuSO_4$, respectively. Further study showed that *Cryptococcus* sp. N6. have two distinct SOD activity bands one band, with higher mobility, appeared when the cells were grown without $CuSO_4$, and the other band appeared when the cells were grown in presence of 10 mM $CuSO_4$ (Miura et al. 2002). Schmidt et al. (2007) reported that the response of *Streptomyces acidiscabies* E13 on heavy metal stress seems to rely rather on Ni-SOD induction while Fe, Zn-SOD expression is unchanged or even repressed under heavy metal stress.

Conclusion

In conclusion, *Rhodotorula* sp. Y11 could tolerate high cadmium concentration without changing cell morphology. There was still approximately 23% of cells without loss of viability after 15 h contact with 100 mg l^{-1} cadmium. TEM confirmed that not all of the cells showed the same response and contribution to the cadmium accumulation. Antioxidative defence systems was an important factor compensating cadmium stresses in Y11. Active staining revealed that only one kind of SOD enzyme exist in Y11, whether in presence or absence of cadmium. Yeast response to heavy metal stress was a complex phenomenon and superior antioxidative defenses could not fully explain the higher cadmium tolerance of Y11. It was that different mechanisms acting together confer the higher levels of tolerance observed. Cadmium tolerance of *Rhodotorula* sp. Y11 showed significant potential for the decontamination of toxic metals from contaminated wastewater.

Acknowledgements This study was financially supported by the Major State Basic Research Development Program: Study on the Environmental Pollution Mechanisms and Control Principles in Beijing and Surrounding Area (G1999045709) and Tai Hu Project (No. BK 2007741) from Jiangsu Science and Technology.

References

Abe F, Miura T, Nagahama T, Inoue A, Usami R, Horikoshi K (2001) Isolation of a highly copper-tolerant yeast, *Cryptococcus* sp, from the Japan Trench and the induction of superoxide dismutase activity by Cu^{2+}. Biotechnol Lett 23:2027–2034

Adamis PDB, Gomes DS, Pereira MD, de Mesquita JF, Pinto MLCC, Panek AD, Eleutherio ECA (2004) The effect of superoxide dismutase deficiency on cadmium stress. J Biochem Mol Toxicol 18:12–17

Aebi HE (1983) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol III, 3rd edn. Verlag Chemie, Weinheim, pp 273–286

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Blackwell KJ, Singleton I, Tobin JM (1995) Metal cation uptake by yeast: a review. Appl Microbiol Biotechnol 43:579–584

Bradford MM (1976) A rapid sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Bustard M, Donnellan N, Rollan A, McHale AP (1997) Studies on the biosorption of uranium by a thermotolerant, ethanol-producing strain of *Kluyveromyces marxianus*. Bioproc Eng 17:45–50

Chen T, Li W, Schulz PJ, Furst A, Chien PK (1995) Induction of peroxisome proliferation and increase of catalase activity in yeast, *Candida albicans*, by cadmium. Biol Trace Elem Res 50:125–133

Cho DH, Kim EY (2003) Characterization of Pb^{2+} biosorption from aqueous solution by *Rhodotorula glutinis*. Bioproc Biosyst Eng 25:271–277

De Silóniz MI, Balsalobre L, Alba C, Valderrama MJ, Peinado JM (2002) Feasibility of copper uptake by the yeast *Pichia guilliermondii* isolated from sewage sludge. Res Microbiol 153:173–180

Doshi H, Ray A, Kothari IL (2007) Biosorption of cadmium by live and dead *Spirulina*: IR spectroscopic, kinetics, and SEM studies. Curr Microbiol 54:213–218

Fridovich I (1983) Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol 23:239–257

Fujs Š, Gazdag Z, Poijšak B, Stibilj V, Milačič R, Pesti M, Raspor P, Batič M (2005) The oxidative stress response of the yeast *Candida intermedia* to copper, zinc, and selenium exposure. Basic Microbiol 45:125–135

Gadd GM, Mowll JL (1983) The relationship between cadmium uptake, potassium release and viability in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 16: 45–48

Geslin C, Llanos J, Prieur D, Jeanthon C (2001) The manganese and iron superoxide dismutases protect *Escherichia coli* from heavy metal toxicity. Res Microbiol 152: 901–905

Goddard PA, Bull AT (1989) Accumulation of silver by growing and non-growing populations of *Citrobaeter intermedius* B6. Appl Microbiol Biotechnol 31:314–319

Gomes DS, Fragoso LC, Riger CJ (2002) Regulation of cadmium uptake by *Saccharomyces cerevisiae*. Biochim Biophys Acta 1573:21–25

Hernández-Saavedra NY (2003) Cu, Zn superoxide dismutase in Rhodotorula and Udeniomyces spp isolated from sea water: cloning and sequencing the encoding region. Yeast 20:479–492

Hwang CS, Baek YU, Yim HS, Kang SO (2003) Protective roles of mitochondrial manganese containing superoxide dismutase against various stresses in *Candida albicans*. Yeast 20:929–941

Kapoor A, Viraraghavan T (1995) Fungal biosorption: an alternative treatment option for heavy metal bearing wastewaters A review. Bioresour Technol 53:195–206

Kierans M, Staines AM, Bennett H, Gadd GM (1991) Silver tolerance and accumulation in yeasts. Biol Metals 4: 100–106

Li ZJ, Yuan HL (2006) Characterization of cadmium removal by *Rhodotorula* sp Y11. Appl Microbiol Biotechnol 73:458–463

Li ZJ, Yuan HL, Hu XD (2008) Cadmium-resistance in growing *Rhodotorula* sp Y11. Bioresour Technol 99: 1339–1344

Malik A (2004) Metal bioremediation through growing cells. Environ Int 30:261–278

Mannazzu I, Guerra E, Ferretti R, Pediconi D, Fatichenti F (2000) Vanadate and copper induce overlapping oxidative stress responses in the vanadate-tolerant yeast *Hansenula polymorpha*. Biochim Biophys Acta 1475:151–156

Marklund S, Marklund G (1974) Involvement of the superoxide anionradical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Bur J Biochem 47:469–474

Miura T, Abe F, Inoue A, Usami R, Horikoshi K (2002) Superoxide dismutase is involved in high tolerance to copper in the deep-sea yeast, *Cryptococcus* sp N6. Biotechnol Lett 24:1069–1074

Moore MM, Breedveld MW, Autor AP (1989) The role of carotenoids in preventing oxidative damage in the pigmented yeast, *Rhodotorula mucilaginosa*. Arch Biochem Biophys 270:419–431

Nies DH (1999) Microbial heavy-metal resistance. Appl Microbiol Biotechnol 51:730–750

Orozco MR, Hernandez-Saavedra NY, Valle FA, Gonzalez BA, Ochoa JL (1998) Cell yield and superoxide dismutase activity of the marine yeast *Debaryomyces hansenni* under different culture conditions. J Mar Biotechnol 6:255–59

Park JK, Lee JW, Jung JY (2003) Cadmium uptake capacity of two strains of *Saccharomyces cerevisiae* cells. Enzyme Microb Tech 33:371–378

Paš M, Milačič R, Drašlar K, Pollak N, Raspor P (2004) Uptake of chromium (III) and chromium (VI) compounds in the yeast cell structure. BioMetals 17:25–33

Pereira SIA, Lima AIG, de Figueira EMAP (2006) Screening possible mechanisms mediating cadmium resistance in *Rhizobium leguminosarum* bv. *viciae* isolated from contaminated Portuguese soils. Microbial Ecol 52: 176–186

Perkins J, Gadd GM (1993) Accumulation and intracellular compartmentation of lithium ions in *Saccharomyces cerevisiae*. FEMS Microbiol Lett 107:255–260

Romandini P, Tallandini L, Beltramini M, Salvato B, Manzano M, de Bertoldi M, Rocco GP (1992) Effects of copper and cadmium on growth, superoxide dismutase and catalase activities in different yeast strains. Comp Biochem Physiol C 103:255–262

Salinas E, Elorza de Orellano M, Rezza I (2000) Removal of cadmium and lead from dilute aqueous solutions by *Rhodotorula rubra*. Bioresour Technol 72:107–112

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Schmidt A, Schmidt A, Haferburg G, Kothe E (2007) Superoxide dismutases of heavy metal resistant *streptomycetes*. J Basic Microbiol 47:56–62

Schroeder WA, Johnson EA (1993) Antioxidant role of carotenoids in *Phaffia rhodozyma*. J Gen Microbiol 139:907–912

So NW, Rho JY, Lee SY, Hancock IC, Kim JH (2001) A lead-absorbing protein with superoxide dismutase activity from *Streptomyces subrutilus*. FEMS Microbiol Lett 194:93–98

Soares EV, Duarte APRS, Boaventura RA, Soares HMVM (2002) Viability and release of complexing compounds during accumulation of heavy metals by a brewer's yeast. Appl Microbiol Biotechnol 58:836–841

Srivastava S, Thakur IS (2006) Biosorption potency of *Aspergillus niger* for removal of chromium (VI). Curr Microbiol 53:232–237

Strandberg GW, Shumate SE, Parrott JR (1981) Accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* Microbial cells as biosorbents for heavy metals. Appl Environ Microbiol 41:237–245

Suh JH, Yun JW, Kim DS (1998) Comparison of Pb^{2+} characteristics between live and dead cells of *Saccharomyces cerevisiae* and *Aureobasidium pullulans*. Biotechnol Lett 20:247–251

Vido K, Spector D, Lagniel G, Lopez S, Toledano MB, Labarre J (2001) A Proteome analysis of the cadmium response in *Saccharomyces cerevisiae*. J Biol Chem 276:8469–8474

Vieira RH, Volesky B (2000) Biosorption: a solution to pollution. Int Microbiol 3:17–24

Volesky B, May H, Holan ZR (1993) Cadmium biosorption by *Saccharomyces cerevisiae*. Biotechnol Bioeng 41: 826–829

Wang NF, Yuan HL, Ying JY (2004) Characteristics of Cd^{2+} accumulation by a yeast strain isolated from mine soil. J Agro Environ Sci 23:674–676 (In Chinese)

Yuan HL, Li ZJ, Ying JY, Wang ET (2007) Cadmium(II) removal by a hyperaccumulator fungus *Phoma* sp F2 isolated from blende soil. Curr Microbiol 55:223–227

Zapotoczny S, Jurkiewicz A, Tylko G, Anielska T, Turnau K (2007) Accumulation of copper by *Acremonium pinkertoniae*, a fungus isolated from industrial wastes. Microbiol Res 162:219–228