Biosorption of Cadmium Ions by Different Yeast Species

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Toxicity and accumulation of Cd^{2+} in yeasts were studied in eight different yeast species. The adaptation to toxic concentration of this metal was dependent on the production of extracellular yeast glycoproteins. The highest concentration of Cd^{2+} ions in the growth medium was tolerated by a *Hansenula anomala*, strain while the lowest tolerance was found by the strain of species *Saccharomyces cerevisiae.* Extracellular glycoproteins of *Hansenula anomala* absorbed nearly 90% of the total content of Cd^{2+} ions bound by yeast cells, while extracellular glycoproteins of *Saccharomyces cerevisiae* bound only 6% of the total amount of cadmium. This difference is caused by the variable composition of the saccharide moiety in the extracellular glycoproteins. The composition of extracellular glycoproteins changed during the adaptation of the yeast cells to the presence of Cd^{2+} ions.

Introduction

Environmental pollution caused by toxic heavy metals in industrial effluents is one of the most pressing problem in many densely populated cities worldwide. Microorganisms are potent bioremediators, removing metals via active or passive uptake mechanisms. All yeasts are capable of accumulation of various heavy metals, but the research is preferentially pointed out to the accumulation of potentially toxic metals in significant values (Kweon *et al.*, 2001; Brady and Duncan, 1994; Mowell and Gadd, 1984). Fungal metal uptake is essentially a biphasic process consisting of a metabolism-independent and metabolism-dependent steps. The initial biosorption step is independent on temperature, metabolic energy, the presence of a metabolizable energy source, and the presence of metabolic inhibitors (Blackwell *et al.*, 1995). The initial binding is thought to involve the microbial cell wall, although extracellular polymers may be responsible in some cases. Binding is attributed to ion-exchange, adsorption, complexation, precipitation and crystallisation within the multilaminate, microfibrillar cell wall structure (Mowell and Gadd, 1984). Li *et al.* (1997) described binding of cadmium by glutathione with the resulting cadmium-bisglutathionato complex and consecutive transport into the vacuole in *S. cerevisiae.*

The aim of the study was to investigate the ability of some yeast strains to absorb Cd^{2+} ions into the cells and to study of the role of exopolymers in resistance of these strains.

Materials and Methods

Organisms, media and growth conditions

Eight different yeast species isolated from both soil and water samples were used: *Aureobasidium pullulans* CCY 27-1-111, *Cryptococcus laurentii* CCY 17-3-16, *Cystofilobasidium capitatum* CCY 10-1-3, *Hansenula anomala* CCY 38-1-22, *Pichia fermentans* CCY 29-97-15, *Rhodotorula rubra* CCY 20-7-29, *Saccharomyces cerevisiae* CCY 21-4-100, *Sporobolomyces roseus* CCY 19-6-4 (Sláviková and Vadkertiová, 1997, 2000). All strains are maintained at the Culture Collection of Yeasts (Institute of Chemistry, Bratislava, Slovakia).

The basic medium used for cultivation contained ($g \cdot l^{-1}$): yeast extract 4; $(NH_4)_2\,SO_4$ 10; glucose 20; KH_2PO_4 1; $K_2HPO_4 \cdot 3H_2O$ 0.2; NaCl 0.1;

$CaCl_2$ 0.1; $MgSO_4 \cdot 7H_2O$ 0.5; and 1 ml microelement solution (mg/l): H_3BO_4 1.25; $CuSO_4 \cdot 5H_2O$ 0.1; KI 0.25; $MnSO_4 \cdot 5H_2O$ 1; $FeCl_3 \cdot 6\ H_2O$ 0.5; $(NH_4)_2\ Mo_4\ O_{24}$ 4 H_2O 0.5 and $ZnSO_4 \cdot 7H_2O$ 1. The maximum concentration of Cd^{2+} ions ($CdCl_2$ 2.5 H_2O) for each strain was measurement by the optical density at 660 nm during growth in three different concentrations.

Strains of species *R. rubra, Cr. laurentii, Cys. capitatum* and *Sp. roseus* were grown at 18 °C while other strains at 28 °C in 250 ml cultivation medium in 500 ml flasks on the orbital shaker (80 cycles min^{-1}).

Isolation and analysis

When the culture reached the late exponential phase, the cells were separated by centrifugation. The *exopolymer samples* were isolated by precipitation from the supernatant after addition of two volumes of 96% (*v/v*) ethanol, and subsequent centrifugation. The ethanol precipitate was dissolved in distilled water and repeatedly precipitated with ethanol, dissolved in distilled water, and freeze-dried.

Isolated cells were suspended in distilled water and subjected to ultrasound treatment (Person-Ultragen UZD 300, Nitra, Slovakia) at 20 kHz, 3×2 min, at about 21 °C and 110 W (Stratilová *et al*., 1998). The cells were separated by centrifugation and the *wall sample* was precipitated with ethanol (96%) from supernatant (1:2 *v/v*). The sediment was frozen by liquid nitrogen. After defrosting the *cytosol sample* was isolated by ethanol precipitation from supernatant and sediment was used as *cell sample.* All samples were freeze-dried and analysed. Data represent the mean percentage from three independent experiments.

Capacity of sorption was calculated as percentage of residual Cd^{2+} after desalinisation on molecular sieves PD 10 (Pharmacia Sweden).

Monosaccharides were analysed after hydrolysis (4 M HCl, 8 h, 100 °C). Descenting paper chromatography was performed on Whatman No. 1 paper using elution system ethylacetate-pyridine-water (8:2:1 v/v/v). Monosaccharide components were determined as alditol trifluoroacetates by GC-mass spectrometry.

Total phosphorus was assayed after Breierová *et al.*(1996).

Determination of Cd^{2+} ions, was performed by, inductively coupled plasma-optical emission spectrometry (ICP–OES) in axial configuration. The samples were mineralised and Cd^{2+} content was determined using calibration curve at 226.5 nm for Cd^{2+} ions.

Infrared spectra were obtained on a NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software. 128 scans at a resolution of 4 cm^{-1} were averaged. The samples were pressed into KBr pellets with a sample/KBr ratio about 2/200 mg.

Results and Discussion

The diversity of intracellular organelles and biomolecules provides a wide range of potential binding sites (Gadd, 1990). Extracellular polymers in the conditions of osmotic stress the important role in the regulation of cytosolic concentration (Breierová *et al*., 1997).

The biomass of the studied yeast strains was capable to accumulate Cd^{2+} ions from solutions of the chloride salts in different ways. The sensitivity of strains is significantly dependent on the ability or inability to absorb the heavy metal into the cells (Table I). The sorption capacity of extracellular polymers enabled to tolerate high concentration of Cd^{2+} ions in the extracellular space. These polymers produced a protective barrier against the ac-

Table I. Accumulation of Cd^{2+} ions in yeast cell compartments.

Species	Wall	Cytosol	Cell	Exopolymer	Cd^{2+} ions in cultivation medium [$g.l^{-1}$]
	% Cd^{2+}				
S. cerevisiae	36	35	23	6	0.006
Sp. roseus	19	10	29	42	0.022
A. pullulans	18	4	27	51	0.034
P. fermentans	23	8	14	55	0.034
Cy. capitatum	6	6	20	68	0.034
Cr. laurentii	11	5	20	64	0.045
R. rubra	5	1	19	75	0.045
H. anomala	5	2	2	90	0.056

cumulation of the heavy metal ions into cells. The most sensitive strain was *S. cerevisiae* that demonstrated noticeable accumulation of the Cd^{2+} ions in the intracellular compartments (cytosol and cell) and in lesser amount into the wall sample (surface of cells). However, extracellular polymers of *H. anomala* (resistant strain) absorbed the maximum amount of Cd^{2+} ions (90%), while the cytosol and cell samples contained only 4% of total amount absorbed by this strain.

The extracellular polymers excreted into the cultivation medium during growth of yeast cultures, are an activeprotective product of the yeast under stress conditions. These polymers are composed of saccharide and protein moieties. The capacity of absorption and/or binding Cd^{2+} ions related to a structural element of exopolymers and is dependent on the yeast species. The saccharide moieties of stress exopolymers contain mainly mannose and less glucose, galactose and arabinose residues in comparison with the exopolymers produced under optimum conditions (Table II). Differences in the phosphorus content in the exopolymers isolated from optimum and stress conditions were observed. Stress exopolymers contained higher amount of phosphorus in comparison with the exopolymers produced in optimum conditions.

Table II. Composition of exopolymers produced with or without Cd^{2+} ions.

Strain No.	Glc	Gal	Man	Ara	P
			%[a]		
27/Cd	68.1	6.1	25.7	–	4.3
27	72.3	9.3	18.4	–	3.2
17/Cd	30.5	12.4	28.5	28.5	6.2
17	20.6	22.4	41.6	15.4	3.6
19/Cd	32.2	35.2	21.3	11.3	5.1
19	32.7	6.2	42.4	15.7	6.1
38/Cd	20.4	4.3	56.8	18.5	6.4
38	33.7	5.8	48.2	12.3	4.7
10/Cd	34.5	19.0	28.7	17.8	7.7
10	63.2	8.2	23.2	5.4	4.9
29/Cd	75.3	5.2	19.5	–	6.6
29	66.3	6.2	27.5	–	5.8
21/Cd	25.2	10.6	64.2	–	6.2
21	33.1	10.8	56.1	–	5.0
20/Cd	32.8	17.5	38.3	11.4	9.4
20	31.2	8.6	39.2	21.0	5.4

[a] Mean percentage values of monosaccharides (w/w) Glc – glucose, Gal – galactose, Man – mannose, Ara – arabinose and P – total phosphorus in exopolymers produced in cultivation medium + Cd (No./Cd) and minus Cd (No.) *Aureobasidium pullulans* 27, 27/Cd, *Cryptococcus laurentii* 17, 17/Cd, *Sporobolomyces roseus* 19,19/Cd, *Hansenula anomala* 38,38/Cd, *Cystofilobasidium capitatum* 10,10/Cd, *Pichia fermentans* 29, 29/Cd, *Saccharomyces cerevisiae* 21, 21/Cd, *Rhodotorula rubra* 20, 20/Cd.

The presence of Cd^{2+} ions influenced the content of glutamic acid while the content of other amino acids in protein moiety of exopolymers remained stable. It is known that stress proteins contain strongly hydrated amino acids with non-polar or polar residues (Fig. 1) (Jaenicke, 1991). The similar change in the content of glutamic acid was reported under the other stress conditions (Stratilová *et al*., 1998). Exopolymers isolated from to cultivation medium contaminant with Cd^{2+} ions contained higher amount of glutamic acid in the protein moiety. This change eliminated toxic effect of Cd^{2+} ions. Most but not all cell wall proteins are O- and N-glycosylated. The O-chains, which are attached to serine or threonine are short and linear while the N-chains which are linked to aspartic acid are represented by highly branched oligosaccharides (Klis, 1994). The influence of stress is reflected in a decrease in level of serine and threonine and increase of aspartic acid. These data are in the agreement with the previous results (Breierová *et al*., 1996).

IR data of the physiological exopolymers and stress exopolymers samples are listed in Table III. In the 1200–1000 cm^{-1} region of the FT-IR data each particular polysaccharide has a specific band maximum (Mathlouti and Koenig, 1986; Kačuráková *et al*., 2000). This region is dominated by ring vibration overlapped with stretching vibration of (C-OH) side groups and the (C-O-C) glycosidic bond vibration. The anomeric region between 950–750 cm^{-1} is typical for the tertiary structure of saccharides.

In the case of isolated samples, the IR absorption maximum at 1060 cm^{-1} points gave evidence about the dominance of an α-mannan component. The characteristic "anomeric region" absorption bands for α-mannans showed frequency positions at 916, 883 and 813 cm^{-1} (Fig. 2A). In the case of exopolymers (capsular polysaccharides) produced by *Cr. laurentii* 17, 17/4 there a shift of the band at 896 cm^{-1} was found and the absorption bands

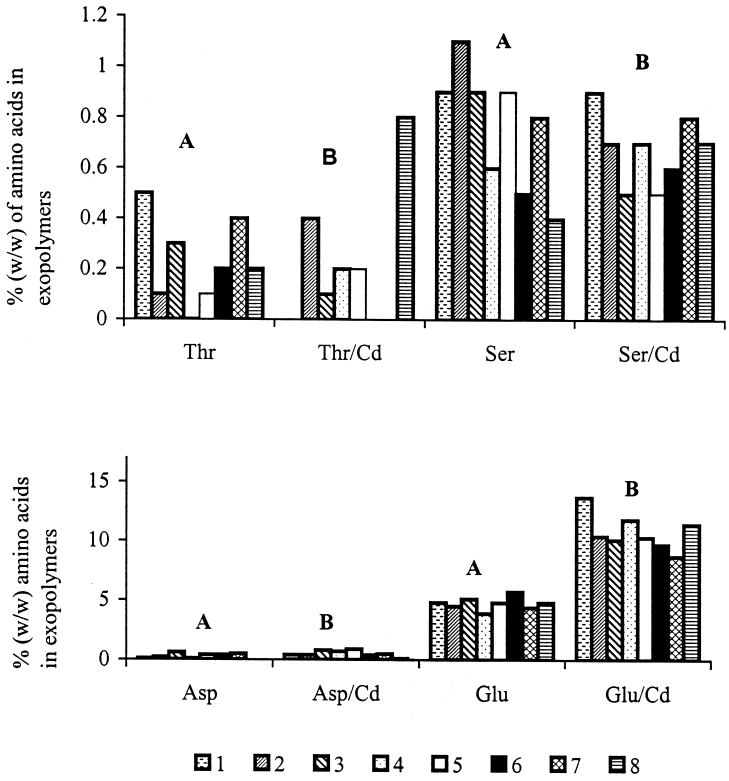

Fig. 1. Changes in content of amino acids of the exopolymers: **A** aspartic acid-Asp, glutamic acid-Glu, threonine-Thr and serine-Ser in isolated exopolymers minus Cd, and **B** Asp/Cd, Glu/Cd, Thr/Cd, Ser/Cd plus Cd in cultivation medium. **1** – *A. pullulans*, **2** – *R. rubra*, **3** – *Cr. laurentii*, **4** – *Cy. capitatum*, **5** – *S. cerevisiae*, **6** – *P. fermentans*, **7** – *H. anomala*, **8** – *Sp. roseus.*

Table III. FT-IR frequencies observed in the solid compounds measured in KBr pellets.

Frequency [cm^{-1}]				Band assignment
21, 21/Cd	38, 38/Cd	20, 20/Cd	17, 17/Cd	
		1726	1726	carboxyl of uronic acids
1654	1658	1659	1651	amid I
1541	1540	1540	1540	amid II
1455–1375	1455–1375	1453–1375	1457–1376	CH deformation
1246	1248	1245	1250	CO, CC of ring
1132	1132	1131	1129	C–O–C of glycosidic link/ring
1058, 1029, 977	1058, 1029, 977	1058, 1029, 974	1058, 1043, 980	CO, CC, ring
916, 882, 813	916,882, 813	914,881, 812	918, 896, 807	anomeric region (C1–H), ring

are overlay bands arabinan (Kačuráková *et al*, 2000). The intensity decrease at 1726 cm^{-1} was observed in the samples of strains with protective capsular polysaccharides *Cr. laurentii* 17/4 and β-carotene *R. rubra* 20/4 (Fig. 2B). The content of amino acids is indicated by the bands of amid I at 1650 cm^{-1} and of amid II at 1540 cm^{-1}. The yeasts can be used in living forms, as well as their exopolymers produced into extracellular space after growth of cultures as metal biosorbents. The metal sorption capacity of exopolymers is dependent on the composition of the saccharide moiety of these polymers and the way of sorption of these ions. Our results may indicate that the adsorption of

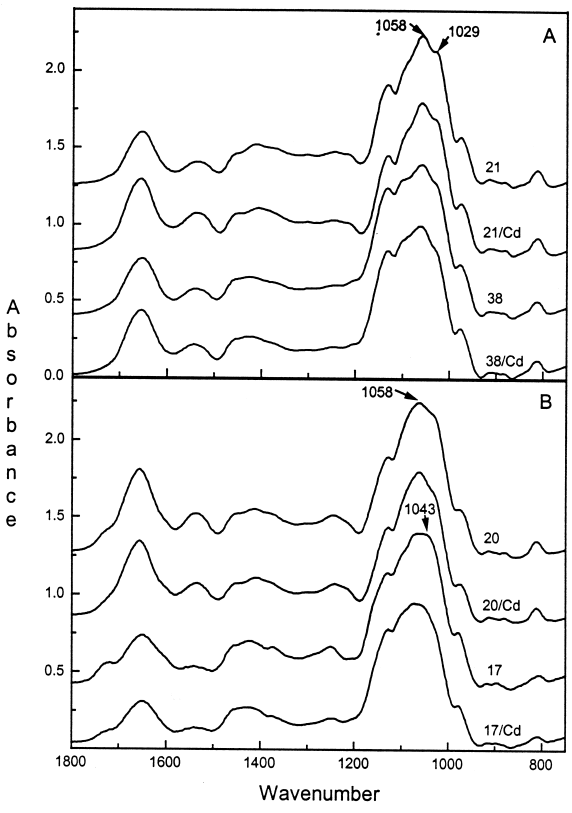

Fig. 2. FT-IR spectra of exopolymers A: 38 – *H. anomala* with maximum tolerance and 21 – *S. cerevisiae* with minimum tolerance to Cd^{2+} ions and B: 17 – *Cr. laurentii* with production capsular polysaccharides of and 20 – *R. rubra* with production β-carotene pigmets.

the metal ions on exopolymers proceeded not only through the ionic interactions but also via physical entrapments (Table IV).

Cadmium is a toxic agent to microorganisms however, there are yeasts strains resistant to this metal (Prahaland and Seenayya,1988; Trevors *et al.*, 1986) The mechanisms of resistance depend on the ability of the yeast to transform the absorbed metal into complex polymeric compounds not toxic for cells.

Acknowledgements

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Strain	Content of Cd^{2+} [$mg \cdot g^{-1}$] in exopolymers		Capacity of sorption
	Before using PD 10	After using PD 10	%*
S. cerevisiae	1103.2	62.3	5.6
Sp. roseus	2359.7	362.8	15.4
A. pullulans	1264.0	51.8	4.1
P. fermentans	1632.7	109.9	6.7
Cy. capitatum	1600.9	732.8	45.8
Cr. laurentii	5328.5	2757.8	51.8
R. rubra	4711.5	38.1	0.8
H. anomala	6534.9	2175.3	33.3

Table IV. Capacity of Cd^{2+} sorption of the isolated exopolymers before and after using the moleculr sieves PD 10.

* % = (befor using PD10 / after using PD10) × 100.

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