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Cytoplasmic inorganic polyphosphate participates in the heavy metal tolerance of *Cryptococcus humicola*

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Abstract The basidiomycetous yeast *Cryptococcus humicola* was shown to be tolerant to manganese, cobalt, nickel, zinc, lanthanum, and cadmium cations at a concentration of 2.5 mmol/L, which is toxic for many yeasts. The basidiomycetous yeast *Cryptococcus terreus* was sensitive to all these ions and did not grow at the above concentration. In the presence of heavy metal cations, C. *humicola*, as opposed to *C. terreus*, was characterized by the higher content of acid-soluble inorganic polyphosphates. In vivo 4′,6′-diamino-2-phenylindole dihydrochloride staining revealed polyphosphate accumulation in the cell wall and cytoplasmic inclusions of C. *humicola* in the presence of heavy metals. In *C. terreus*, polyphosphates in the presence of heavy metals accumulate mainly in vacuoles, which results in morphological changes in these organelles and, probably, disturbance of their function. The role of polyphosphate accumulation and cellular localization as factors of heavy metal tolerance of *Cryptococcus humicola* is discussed.

Introduction

Heavy metals are widespread environmental pollutants hazardous to human health. Exposure to these toxic metal ions may result in growth cessation, apoptosis, and cell death of many microorganisms. Several mechanisms of heavy metal toxicity in microorganisms have been proposed: they replace the essential metal in metalloproteins, allosterically inhibit some enzymes by binding to their catalytic sites, indirectly cause oxidative stress, and exhibit membrane-damaging and chaotropic effects (Macomber and Hausinger 2011; Vagabov et al. 2008; Cray et al. 2013a). Cd^{2+} caused endoplasmic reticulum stress in *Saccharomyces cerevisiae* by inducing the unfolded protein response, and Cd^{2+} toxicity is believed to be the direct consequence of accumulation of this cation in the endoplasmic reticulum (Gardarin et al. 2010). This effect may be chaotropicity mediated. It should be noted that microbial cells are able to tolerate chaotropic stressors better at low temperatures (Chin et al. 2010).

The mechanisms of heavy metal tolerance in microorganisms are extensively studied (Spain 2003; Culotta et al. 2005; Thorsen et al. 2009; Wysocki and Tamás 2010). Multiple genes and signaling pathways are responsible for yeast viability under heavy metal excess. The two sets of metal-responsive genes were revealed under the exposure of the yeast *S. cerevisiae* to copper, silver, zinc, cadmium, mercury, and chromium (Jin et al. 2008). The mechanisms of heavy metal tolerance in yeasts include environmental sensing, sulfur and glutathione biosynthesis, vacuolar and endosomal transport, and sorting (Thorsen et al. 2009). Two hundred and thirty-seven genes involved in Cd^{2+} tolerance in *Schizosaccharomyces pombe* were revealed (Kennedy et al. 2008). These genes represent a number of pathways including sulfate assimilation, phytochelatin synthesis and transport, ubiquinone biosynthesis, stress signaling, cell wall biosynthesis and cell morphology, gene expression and chromatin remodeling, vacuole function, and intracellular transport of macromolecules (Kennedy et al. 2008). The concept of Mn^{2+} homeostasis in *S. cerevisiae* is based on the involvement of a considerable number of genes in this process (Culotta et al. 2005). *Candida tropicalis* isolated from wastewater was found to be resistant to the high concentrations of Zn^{2+}, Ni^{2+}, Hg^{2+}, and Pb^{2+}; the synthesis of glutathione increased, and its involvement in metal tolerance was suggested (Rehman and Anjum 2011). It should be noted that some stress responses and adaptations in yeast do not involve gene-mediated membrane processes (Permyakov et al. 2012; Mollinedo 2012).

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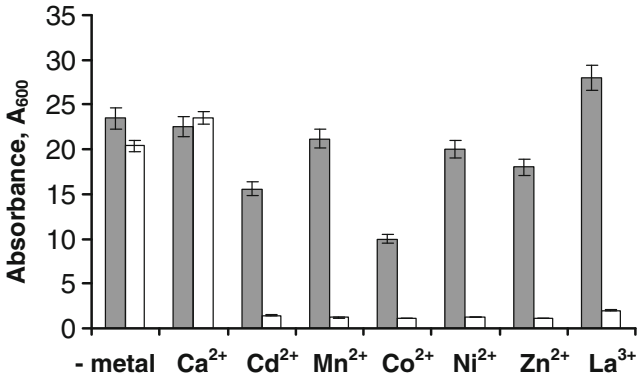

Fig. 1 The culture absorbance of *Cryptococcus humicola* (*gray*) and *Cryptococcus terreus* (*white*) at 48-h cultivation in the media containing 2.5 mmol/L metal cations and 1.6 mmol/L P_i

The heavy metal tolerance of basidiomycetous yeasts has been observed in some species (Vadkertiová and Sláviková 2006; Vreulink et al. 2010; Singh et al. 2013) but little studied as yet. The study of heavy metal tolerance is of interest for understanding the changes in yeast biodiversity under conditions of environmental pollution.

Inorganic polyphosphates (PolyP) are an important biopolymer participating in many regulatory processes in microbial cells (Kulaev et al. 2004; Rao et al. 2009; Hirota et al. 2010; Achbergerová and Nahálka 2011). The interrelationship between phosphorus metabolism, PolyP, and heavy metal resistance was observed in *Escherichia coli* (Keasling et al. 2000), in the archaea *Sulfolobus* (Remonsellez et al. 2006), in *Trichoderma harzianum* (De Lima Freitas et al. 2011), and in insects (Gomes et al. 2012). Disruption of the *pho84* gene encoding the phosphate transporter of the *S. cerevisiae* plasma membrane, PHO84, which is responsible for Mn^{2+} uptake via manganese-phosphate complexes, results in a manganese-resistant phenotype (Jensen et al. 2003). The *pho80* mutants of *S. cerevisiae* defective in phosphate uptake, storage, and metabolism exhibit a wide range of defects in metal homeostasis (Rosenfeld et al. 2010). The ability of *S. cerevisiae* to adapt to toxic Mn^{2+} concentrations after an unusually long lag phase has been demonstrated. The adaptation correlated with the triggering of PolyP metabolism: the drastic increase in the rate and chain length of acid-soluble PolyP (Andreeva et al. 2013).

We have chosen the following two basidiomycetous yeast species as objects of our research: *Cryptococcus humicola* isolated from plant surface and *Cryptococcus terreus* isolated from soil (Golubev and Shabalin 1994). The cells of these two species substantially differed in the ability to take up phosphate from the medium under growth limitation: *C. humicola*, in contrast to *C. terreus*, proved to be an effective phosphate-accumulating organism (Breus et al. 2012). The aim of this study was to compare the two basidiomycetous yeasts, *C. humicola* and *C. terreus*, with respect to heavy metal tolerance and to determine whether these two species have any peculiarities of their PolyP pools.

Materials and methods

Strains and growth conditions

The yeasts were obtained from the All-Russian Collection of Microorganisms (Russian Academy of Sciences) and maintained on malt slants. *C. terreus* VKM Y-2253 and *C. humicola* 9-6 were grown on a shaker (140 rpm) at 29 °C in 200 mL of the medium containing (g/L) glucose, 10; yeast extract, 4; and peptone, 5.

The culture growth was estimated by absorbance measured in a 1-cm cuvette at 600 nm with a SF-26 spectrophotometer (Russia) using appropriate dilutions. After 72-h cultivation (the stationary phase), the cells were placed in fresh media. The high initial absorbance (A_{600} ~1.4) was used to obtain sufficient biomass even in case of growth suppression.

The concentrations of heavy metal cations and phosphates in the media are indicated in the tables and figure legends. The salts ($MnSO_4 \cdot 4H_2O$, $CoSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, $La(NO_3)_3 \cdot 6H_2O$, $Cd(CH_3COO)_2 \cdot 2H_2O$) were of analytical grade. The glucose peptone medium contained 1.6 mmol/L phosphate (P_i) derived from yeast extract and peptone. KH_2PO_4 was added to the media with enhanced P_i concentration (11.5 mmol/L).

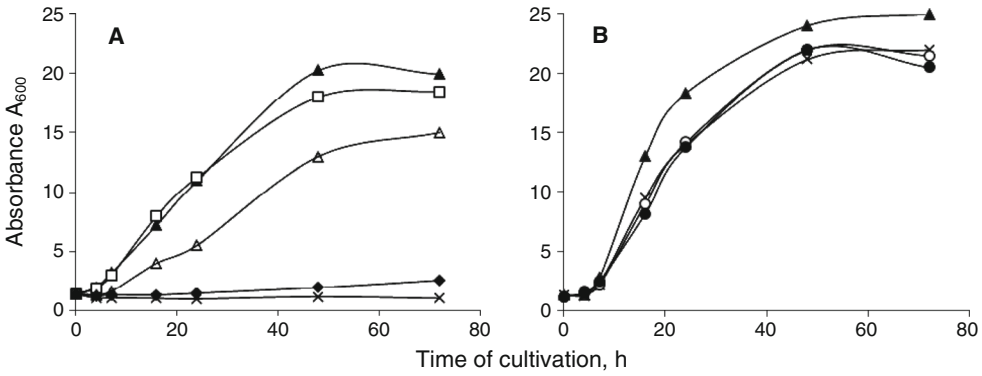

Fig. 2 The growth curves of *Cryptococcus terreus* (**a**) and *Cryptococcus humicola* (**b**) in the media with 1.6 mmol/L P_i. *Filled triangle*, without Mn^{2+}; with Mn^{2+}, mmol/L: *empty square*, 0.1; *empty triangle*, 0.5; *filled diamond*, 1.25; *times symbol*, 2.5, *empty circle*, 5, *filled circle*, 10

Table 1 The content of manganese at 48 h of cultivation in culture liquid and biomass (percent of initial quantity in the culture medium)

Yeast species	P_i in the medium, mmol/L	Manganese content,%		
		Culture medium	Extract of biomass with citrate buffer	Rest biomass
Cryptococcus humicola	1.6	72	2	26
	11.5	54	19	27
Cryptococcus terreus	1.6	95	4	1
	11.5	91	8	1

Polyphosphate and manganese assay

For PolyP extraction and assay, the cells were harvested by centrifugation at 5,000*g* for 10 min and washed twice with 0.1 mol/L citrate buffer, pH 4.5. PolyP fractions were extracted and assayed as described earlier (Vagabov et al. 2000). The PolyP1 fraction obtained by extraction with 5 % $HClO_4$ (Vagabov et al. 2000) is indicated in this work as acid-soluble PolyP. Other fractions, PolyP2, PolyP3, PolyP4, and PolyP5, were obtained and assayed as described earlier (Vagabov et al. 2000). The total content of these fractions is presented in the tables and figures as acid-insoluble PolyP. Manganese concentration in the samples was determined by atomic emission spectroscopy after the exposure at 180 °C in 32 % $HClO_4$ for 24 h (Lichko and Okorokov 1976).

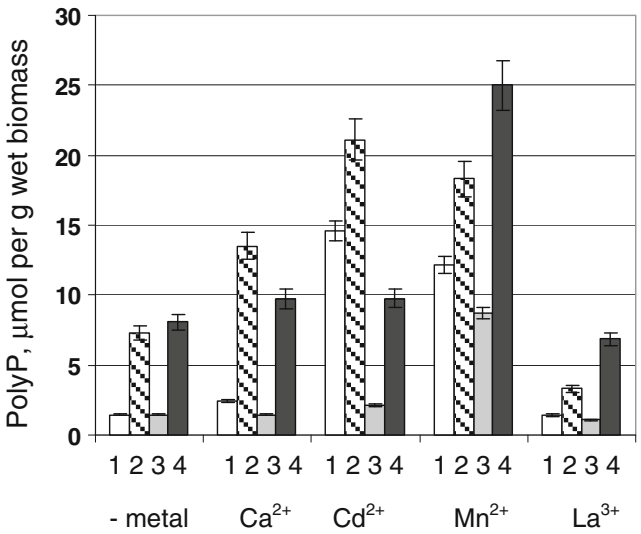

Fig. 3 The content of PolyP in yeast cells at 48-h cultivation in the media containing 2.5 mmol/L metal cations and 1.6 mmol/L P_i: *1* acid-soluble PolyP, *Cryptococcus humicola*; *2* acid-insoluble PolyP, *Cryptococcus humicola*; *3* acid-soluble PolyP, *Cryptococcus terreus*; *4* acid-insoluble PolyP, *Cryptococcus terreus*

Table 2 The effect of 2.5 mmol/L Mn^{2+} on the PolyP content (micromole P per gram wet biomass) in the cells of *Cryptococcus humicola* and *Cryptococcus terreus* under cultivation with 11.5 mmol/L of P_i for 48 h

PolyP fraction	*Cryptococcus humicola*		*Cryptococcus terreus*	
	$-Mn^{2+}$	$+Mn^{2+}$	$-Mn^{2+}$	$+Mn^{2+}$
Acid soluble	3.51±1.1	43.1±10.0	2.92±1.3	10.7±0.5
Acid insoluble	10.0±0.88	30.0±2.35	19.7±0.53	42.7±1.5

Fluorescence microscopy

Fluorescence microscopy with the fluorochrome 4′,6′-diamino-2-phenylindole dihydrochloride (DAPI) is one of the methods for determining PolyP localization in living cells (Serafim et al. 2002; Pavlov et al. 2010; Puchkov 2010; Martin and Van Mooy 2013). For DAPI staining, 10 μg/mL of DAPI (Sigma, USA) was added to cell cultures followed by incubation at room temperature for 30 min. The samples were analyzed under fluorescent and phase contrast microscopes (AXIO Imager A1, ZEISS, Germany), filter set 49 (ZEISS), with the excitation maximum at 359 nm and the emission maximum at 460 nm.

Results

The effects of heavy metals on yeast growth

C. humicola and *C. terreus* differ in heavy metal tolerance (Fig. 1). The growth of *C. terreus* was inhibited by all of the tested cations except for calcium at a concentration of 2.5 mmol/L. Ca^{2+} had no effect on the growth of both cultures. The yeast *C. humicola* proved to be tolerant to the excess of heavy metal cations. The Co^{2+} and Cd^{2+} ions inhibited the growth of this yeast but partially.

The effects of Mn^{2+} on the growth of both yeasts were investigated more thoroughly (Fig. 2). The cells of *C. humicola* grew even at 10 mmol/L of Mn^{2+}, whereas the cells of *C. terreus* stopped to grow at 1.25 mmol/L of the cation. The increase in phosphate concentration from 1.6 to 11.5 mmol/L had no effect on the growth of both cultures in the presence and absence of Mn^{2+} (data not shown). The *C. terreus* was cultivated for 7 days in the media containing manganese (2.5 mmol/L) and phosphate (1.6 or 11.5 mmol/L), but no growth was observed.

Both yeast species were jointly cultivated in the media with and without Mn^{2+}. They are well differentiated by light microscopy: *C. humicola* cells are rod-shaped and *C. terreus* cells are round. The number of cells of each type was counted under a light microscope. The media were inoculated with a suspension containing the cells of *C. humicola* (0.5 %) and

Table 3 The rates of acid-soluble PolyP in the total PolyP content (percent) of *C. humicola* and *C. terreus*. The yeasts were cultivated for 48 h in the medium with 1.6 mmol/L of phosphate

Yeast species	Culture conditions		
	Without heavy metal	2.5 mmol/L Mn^{2+}	2.5 mmol/L Cd^{2+}
C. humicola	20	40	41
C. terreus	15	23	18

C. terreus (99.5 %). After 48-h cultivation without manganese, the culture contained 40 % of *C. terreus* cells. *C. humicola* was predominant (97.4 %) in the medium with 2.5 mmol/L of Mn^{2+}. This result suggests the possibility of replacement of sensitive species by resistant species under heavy metal pollution of the environment.

Manganese in the medium and biomasses

We determined the content of manganese after 48-h cultivation in the culture liquid, in the extracts from biomass samples with 0.1 mol/L citrate buffer (pH 3.0), and in the rest of the biomass (Table 1). Under the exposure of C. *terreus* in the presence of 2.5 mmol/L Mn^{2+}, the decrease of manganese in the medium was insignificant (Table 1). It was not surprising as no culture growth was observed. However, the biomass contained 12 μmol manganese per g wet biomass. Under the cultivation of C. *humicola*, the content of manganese in the culture medium decreased depending on P_i concentration. After washing

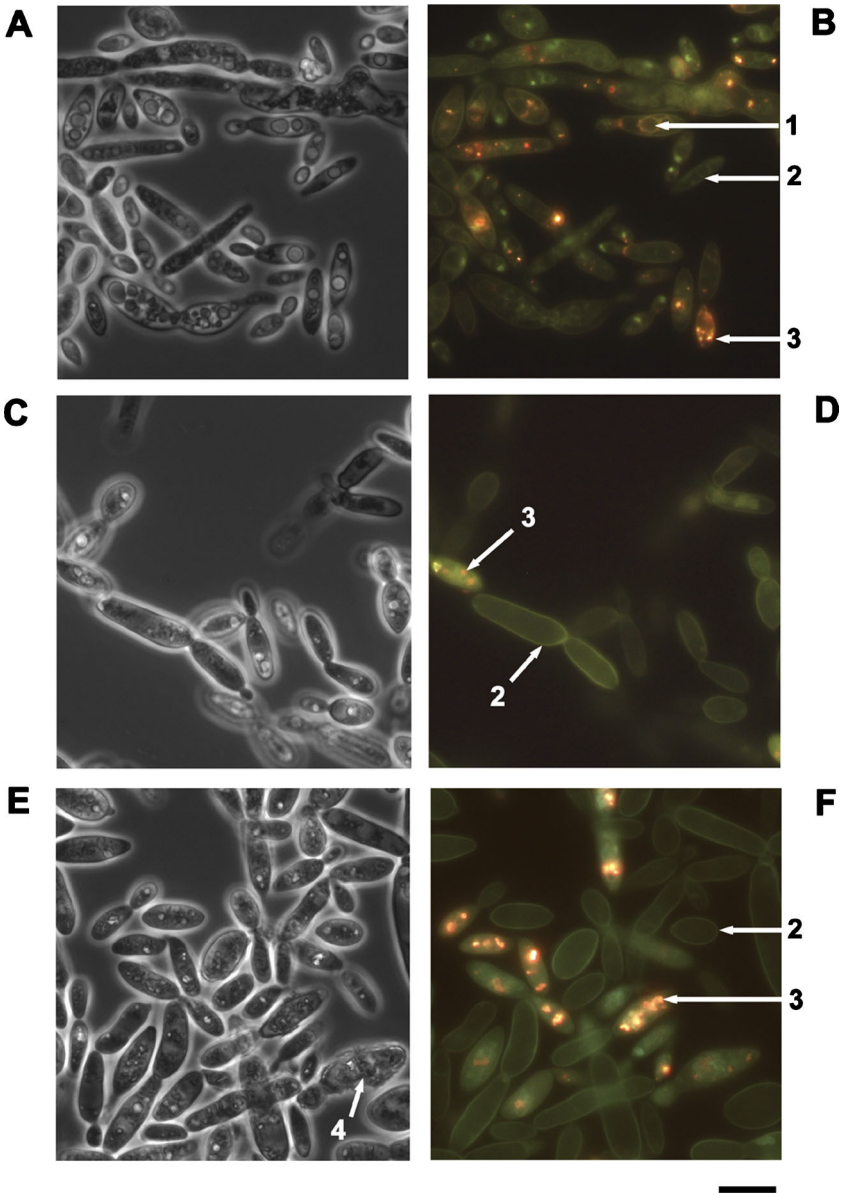

Fig. 4 The micrographs of the cells of *Cryptococcus humicola* (48 h of cultivation). **a**, **c**, **e** phase contrast microscopy; **b**, **d**, **f** fluorescence microscopy of DAPI-stained cells. *Scale bar*= 5 μm. **a**, **b** Control cultivation in the medium containing 1.6 mmol/L P_i; **c**, **d** cultivation in the presence of 2.5 mmol/L Mn^{2+} and 1.6 mmol/L P_i; **e**, **f** cultivation in the presence of 2.5 mmol/L Mn^{2+} and 11.5 mmol/L P_i. *1* Vacuole; *2* cell wall; *3* PolyP containing cytoplasmic inclusions; *4* light-absorbing component associated with the cell wall

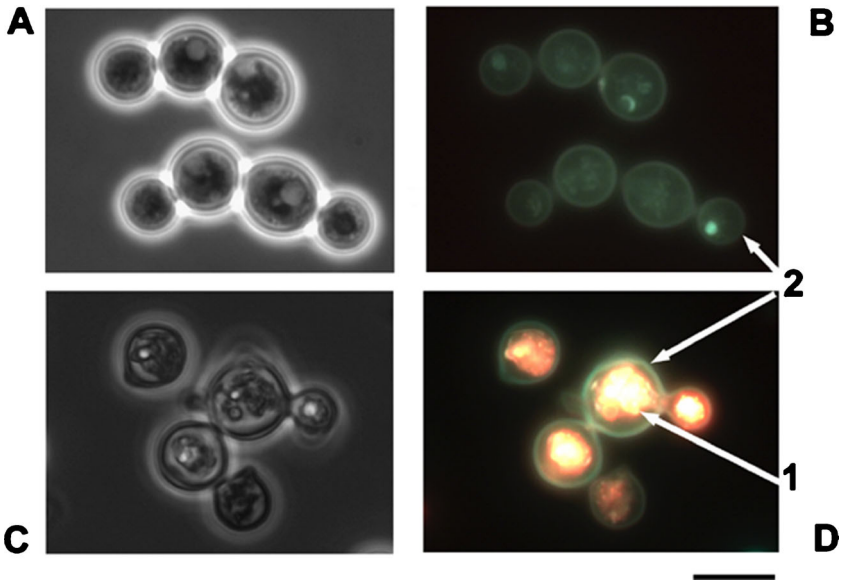

Fig. 5 The micrographs of *Cryptococcus terreus* cells (48 h of cultivation). **a**, **c** Phase contrast microscopy; **b**, **d** fluorescence microscopy of DAPI-stained cells. *Scale bar*=5 μm. **a**, **b** Control cultivation in the medium containing 1.6 mmol/L P_i; **c**, **d** cultivation in the presence of 2.5 mmol/L Mn^{2+} and 1.6 mmol/L P_i. *1* Vacuole; *2* cell wall

with citrate buffer, the content of manganese in the biomass was 33 μmol per g wet biomass independent of P_i concentration. The difference in manganese content in the culture medium at 1.6 and 11.5 mmol/L P_i can be explained by manganese absorption on the cell surface at high P_i concentration. This manganese can be easily extracted by citrate buffer (Table 1). Thus, the tolerance of C. *humicola* to excess manganese cannot be explained by the lower uptake of the cation.

The effects of heavy metals on PolyP content in *C. humicola* and *C. terreus*

Figure 3 shows the content of acid-soluble and acid-insoluble PolyP in the cells of *C. humicola* and *C. terreus* in the presence of heavy metal cations in the medium with 1.6 mmol/L phosphate. The content of PolyP without metals was similar in the cells of both species.

The effects of metal cations on PolyP content in both species were different. The presence of Ca^{2+}, Cd^{2+}, and La^{3+} had a little influence on PolyP content in the cells of *C. terreus*. The content of PolyP in *C. terreus* cells increased 3.5-fold in the presence of Mn^{2+}. The content of PolyP in *C. humicola* cells increased 1.8-, 3.9-, and 3.4-fold in the presence of Ca^{2+}, Cd^{2+}, and Mn^{2+}, respectively. The content of PolyP in *C. humicola* cells decreased 2-fold in the presence of La^{2+}.

PolyP content increased in the cells of both yeasts only in the presence of Mn^{2+}. This increase was more marked at a higher phosphate concentration (11.5 mmol/L) (Table 2). It was probably due to the stimulation of PolyP synthesis in the presence of Mn^{2+}. It is known that Mn^{2+} stimulates PolyP synthesis by the Vtc4 protein in *S. cerevisiae* (Hothorn et al. 2009).

Enhanced contribution of acid-soluble PolyP in the presence of Cd^{2+} and Mn^{2+} is a distinctive feature of *C. humicola* (Table 3). The contribution of this fraction increased to 30–40 % in *C. humicola* during the cultivation with Co^{2+}, Ni^{2+}, and Zn^{2+} (data not shown). A correlation was revealed between heavy metal tolerance and the accumulation of acid-soluble PolyP, which is considered to be localized in the cytosol (Kulaev et al. 2004).

The effects of heavy metals on cell morphology and DAPI staining

The cells of *C. humicola* grown in the medium with 1.6 mmol/L P_i and without metals had numerous small inclusions in the cytoplasm (Fig. 4a). Their DAPI fluorescence was in the orange, yellow, and green region of the spectrum (Fig. 4b), which is typical for DAPI-PolyP complexes (Serafim et al. 2002; Puchkov 2010). The periphery of vacuoles was stained with DAPI and looked like a bright-colored ring. *C. terreus* in the same medium showed green fluorescence of the cell envelope and diffuse fluorescence of the cytoplasm, but no marked PolyP inclusions were found in the cytoplasm (Fig. 5b).

The cultivation with excess Mn^{2+} ions resulted in the changes in cell morphology revealed by phase contrast and fluorescent microscopy after DAPI staining. The *C. humicola* cells grown in the presence of Mn^{2+} were larger than the control cells (Fig. 4c). In the medium with 11.5 mmol/L P_i in the presence of Mn^{2+}, a light-absorbing component was associated with the cell wall (Fig. 4e). This component did not fluoresce after DAPI staining. Probably, it may be insoluble manganese salts which are washed with citrate (Table 1). The intensity of cell wall fluorescence in the presence of Mn^{2+} was much higher than in the control, independent of P_i concentration (Fig. 4d, f). Intensively fluorescing cytosolic inclusions look larger than in the control (Fig. 4f). However, these inclusions are visible not in all cells. We suppose that this

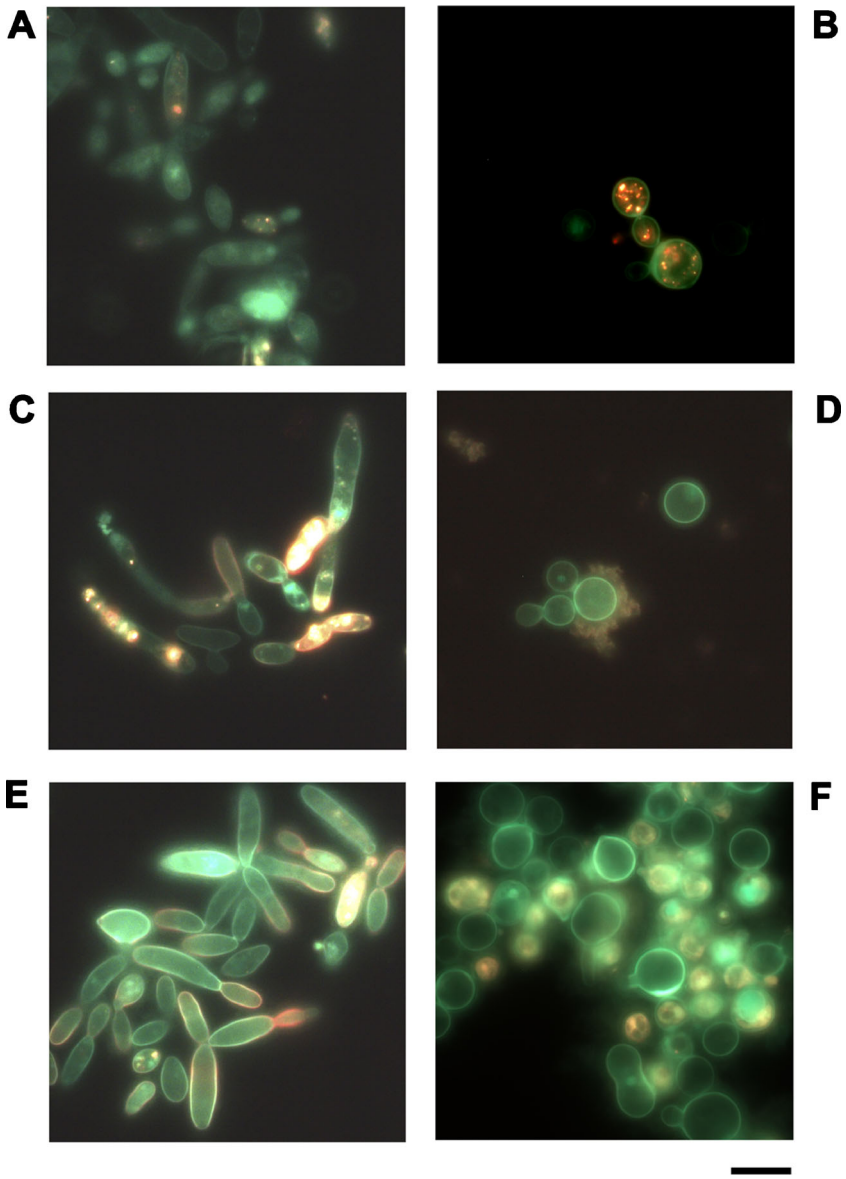

Fig. 6 The micrographs of DAPI-stained cells of *Cryptococcus humicola* (**a**, **c**, **e**) and *Cryptococcus terreus* (**b**, **d**, **f**) after 48-h cultivation in the media with 1.6 mmol/L P_i and 2.5 mmol/L Ca^{2+} (**a**, **b**), 2.5 mmol/L Cd^{2+} (**c**, **d**), and 2.5 mmol/L La^{3+} (**e**, **f**). *Scale bar*=5 μm

effect is due to culture heterogeneity in PolyP content and to disturbance of the PolyP/DAPI complex by Mn^{2+}.

In the presence of Mn^{2+}, the cells of *C. terreus* have the enlarged bright-orange fluorescent vacuole containing numerous small inclusions (Fig. 5d). It seems that the essential part of PolyP accumulating in the presence of Mn^{2+} is localized in the vacuoles of this species. It correlates with the accumulation of acid-insoluble PolyP (Fig. 3). The intensity of cell wall fluorescence in the presence of Mn^{2+} increased similar to *C. humicola* cells. The two yeast species under study are substantially different in PolyP localization under manganese excess: the tolerant *C. humicola* cells accumulate PolyP in cytosolic inclusions, while the sensitive *C. terreus* accumulates PolyP in vacuoles.

During cultivation in the presence of 2.5 mmol/L Ca^{2+}, the fluorescence of DAPI-stained *C. humicola* cells was similar to the control (Figs. 4b and 6a). During the cultivation of *C. terreus* cells in the presence of 2.5 mmol/L Ca^{2+}, numerous DAPI-fluorescent granules were observed in the cytoplasm but not in vacuoles (Fig. 6b).

In the presence of Cd^{2+} (Fig. 6c), the cell wall fluorescence of *C. humicola* cells becomes more intense, and the number of fully fluorescent cells increases. Cd^{2+} causes the lysis of some part of *C. terreus* cells. A considerable amount of extracellular material is formed in the *C. terreus* culture in the presence of Cd^{2+}; this material is associated with the cell walls and fluoresces after DAPI staining (Fig. 6d). In the presence of La^{3+} (Fig. 6e), the cell wall of *C. humicola* fluoresces most brightly compared to other cations under study. The fluorescence of the *C. terreus* cell wall also increases; however, many cells are damaged and fluoresce entirely (Fig. 6f).

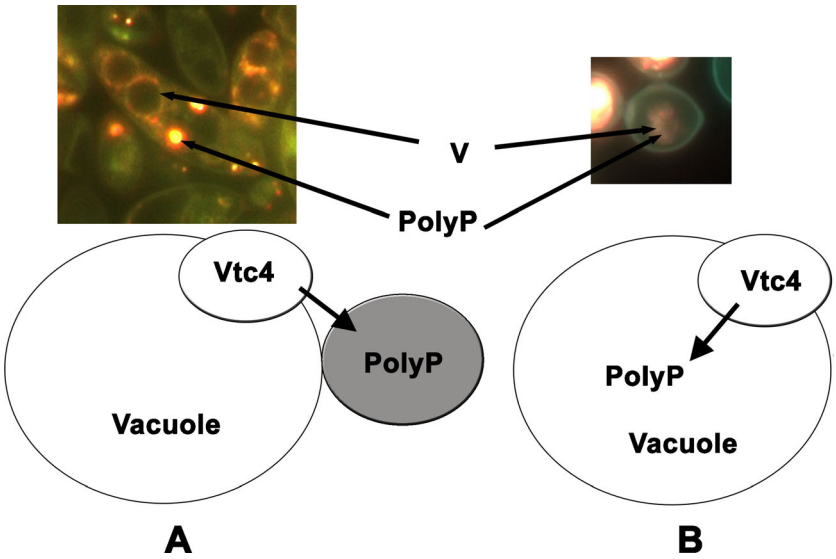

Fig. 7 The hypothetical scheme of localization of PolyP synthesized by the vacuolar PolyP synthetase Vtc4: *A*—*Cryptococcus humicola*, *B*—*Cryptococcus terreus*

Discussion

In this work, we have studied two basidiomycetous yeast species with different resistance to heavy metal cations. *C. humicola* and *C. terreus* showed tolerance and sensitivity, respectively, to all of the cations used. Since PolyP is believed to be an important factor of microbial resistance to heavy metal cations (Kulaev et al. 2004), we have attempted to reveal the differences in the state of these polymers in the cells of both yeast species under heavy metal excess. The content of acid-soluble PolyP increased in the tolerant C. *humicola* but not in the sensitive C. *terreus*. DAPI fluorescence shows that the PolyP accumulating in *C. humicola* in response to excess heavy metal cations are localized in cytoplasmic inclusions and in the cell wall, while in C. *terreus*, they are localized in vacuoles. Moreover, the morphology of vacuoles in C. *terreus* changed and their function was probably disturbed. It is supposed that acid-soluble PolyP is presumably localized in the cytoplasm (Kulaev et al. 2004). The increase in the content of this PolyP and the enlargement of DAPI-stained PolyP inclusions in the cytoplasm of *C. humicola* confirm this suggestion. The PolyP-synthesizing enzymes in yeast are little studied as yet. Two of them have been revealed in *S. cerevisiae*: dolychylpyrophosphate:polyphosphate transferase responsible for the synthesis of the minor part of yeast cell polyP localized mainly in the cell wall (Kulaev et al. 2004) and Vtc4 protein, a transport chaperone localized in the vacuolar membrane (Hothorn et al. 2009).

DAPI fluorescence data (Fig. 7) suggest that the significant portion of PolyP in the cells of C. *terreus* and C. *humicola* is associated with vacuoles. The hypothetic scheme of PolyP accumulation in the cells of both species is given in Fig. 7. In *C. humicola* cells, some part of PolyP is agglomerated close to the vacuolar membrane on the cytoplasmic side, and then, the formed PolyP granules are released into the cytoplasm. In *C. terreus* cells, DAPI staining revealed a different situation: the accumulated PolyP is localized inside the vacuole. The mechanism of Vtc4 functioning revealed in *S. cerevisiae* provides the translocation of the growing PolyP chain across the membrane (Hothorn et al. 2009). It suggests the possibility of PolyP translocation mainly into the vacuoles or cytoplasm, depending on the peculiarities of Vtc4 proteins in different yeasts. The suggested differences in Vtc4 functioning in the cells of C. *humicola* and C. *terreus* may cause the difference in PolyP localization.

The results suggest that the accumulation of PolyP in cytoplasmic inclusions may be one of the factors providing the heavy metal tolerance of C. *humicola* cells by forming cation/PolyP complexes. It seems that *C. humicola* realizes many pathways of cell protection from excess toxic cations. The cultivation of *C. humicola* in the media with 2.5 mmol/L Mn^{2+} and 11.5 mmol/L P_i for more than 72-h results in pH alkalization from the initial value 5.8 to 7.2. The poorly soluble $Mn(OH)_2$ formed in the alkaline medium is easily oxidized in the air to $MnO(OH)_2$, since the cultivation takes place under aeration. As a result, the medium and the biomass become brown, which is typical of $MnO(OH)_2$. Alkalization of the medium to 8.0 during long-term cultivation is typical of *C. humicola* and does not depend on the presence of excess manganese. This alkalization, resulting in the formation of low-soluble Mn^{2+} compounds, seems to be an additional factor of manganese resistance. As regards *C. terreus*, during its growth without manganese, the medium is alkalized from pH 5.8 to 7.0–7.5. In the presence of excess manganese, when the culture does not grow, pH of the medium remains 5.8.

Thus, *C. humicola*, like *C. tropicalis* (Rehman and Anjum 2011), is a heavy metal-resistant yeast. The study of such microorganisms from natural ecotopes is of interest for understanding the changes in the species set under technogenic pollutions of soil and water (Liu et al. 2012).

The study of the yeasts from extreme environments has revealed that membrane fluidity fluctuation is an important factor of stress tolerance: the high absolute fluidity fluctuation is associated with the lower survival. Fluidity and its variations therefore reflect the survival strategy and fitness in extreme environments and are good indicators of adaptability of microorganisms (Turka et al. 2011). The *C. humicola* strain used in this study seems to have adaptive mechanisms for overcoming cellular membrane disorder, because this yeast strain produces a membrane-damaging extracellular glycolipid (Puchkov et al. 2002). *C. terreus* is highly sensitive to this glycolipid, indicating the lower ability of its membranes to overcome stress factors. It may be one of the causes of the high sensitivity of *C. terreus* to heavy metal stress. The resistance to membrane-damaging and chaotrophic agents and long-chain polyphosphate accumulation may be the factors enhancing the competitive ability of some *Cryptococcus* species in some ecological niches (Kachalkin and Yurkov 2012; Cray et al. 2013b).

It should be noted that the fungi tolerant to heavy metals may be a protective factor for other organisms. For example, mycorrhization protects roots from Cd-induced injury by preventing the access of cadmium to plant cells (Schützendübel and Polle 2002).

Back to PolyP, this widespread biopolymer is the key stress-overcoming factor in microorganisms. It participates in induction of the synthesis of RpoS, the RNA-polymerase subunit in bacteria responsible for stress and stationary phase gene expression and in the regulation of the level of the stringent response factor, guanosine 5′-diphosphate 3′-diphosphate (ppGpp) (Rao et al. 2009). They may be a source for ATP synthesis under stresses (Castro et al. 1999) and protect from environmental stresses under phosphate limitation conditions (Jahid et al. 2006). Finally, numerous data suggest the potential involvement of polyphosphates in detoxification of heavy metal cations by their complexation and sequestration (Kulaev et al. 2004). *C. humicola* may be a promising model for studying the mechanisms of heavy metal tolerance.

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