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Arsenate and phosphate interaction in *Saccharomyces cerevisiae*

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Abstract: In the present study, arsenate(As(V)) and phosphate(P(V)) interactions were investigated in growth, uptake and RNA content in yeast(*Saccharomyces cerevisiae*). Yeast grew slowly with As(V) concentrations increasing in the medium. However, the maximal population density was almost the same among different As(V) treatments. It was in the late log phase that yeast growth was augmented by low As(V), which was maybe due to the fact that methionine metabolism was stressed by vitamin B_6 deprivation, so As(V) treatments did not affect maximal population density. However, with P (V) concentrations increasing, the maximal population density increased. Therefore, the maximal population density was determined by P (V) concentrations in the medium but not by As(V) concentrations in the medium. Ycf1p(a tonoplast transpor) transports $As(GS)_3$ into the vacuole, but arsenic(As) remaining in the thalli was 1.27% with As(V) exposure for 60 h, from which it can be speculated that the percentage of As transported into vacuole should be lower than 1.27%. However, the percentage of As pumped out of cell was 71.49% with As (V) exposure for 68 h. Although two pathways (extrusion and sequestration) were involved in As detoxification in yeast, the extrusion pathway played a major role in As detoxification. RNA content was the highest in the early-log phase and was reduced by As(V).

Keywords: yeast; maximal population density; arsenic essentiality; arsenic speciation

Introduction

Arsenate (As (V)), the dominant form of arsenic (As) in aerated conditions, is taken up by plants and microorganisms via the phosphate(P(V)) transport systems because of the chemical similarity between As (V) and P(V)(Dixon, 1997). It has been demonstrated that As(V) inhibits P(V) uptake by yeast(*Saccharomyces cerevisiae*), phytoplankton, *Arabidopsis thaliana* and the As hyperaccumulator, Chinese brake fern *Pteris vittata* (Clark *et al.*, 2003; Wang *et al.*, 2002). Similarly, P (V) suppresses As (V) uptake by phytoplankton, rice, *Lupinus albus*, the As tolerant plants *Holcus lanatus* and *Cytisus striatus*, and the As hyperaccumulator *P. vittata* (Wang *et al.*, 2002; Abedin *et al.*, 2002; Esteban *et al.*, 2003; Bleeker *et al.*, 2003; Meharg and Macnair, 1992; Tu and Ma, 2002). However, how yeast develops a population under As(V) stress and how P(V) detoxifies As(V) in the population level are little understood.

Arsenic compounds have been abundant at near toxic levels in the environment. In response, microbes have evolved mechanisms for As resistance and enzymes that oxidize As(III) to As(V) or reduce As(V) to As (III) (Mukhopadhyay *et al.*, 2002). In yeast, As (V) is taken up via several P (V) transporters (Bun-ya *et al.*, 1996). In the cytoplasm, As(V) is reduced to As (III) by Acr2p (the first identified eukaryotic arsenate reductase) prior to extrusion or sequestration (Bobrowicz *et al.*, 1997; Mukhopadhyay and Rosen, 1998). Acr3p (a plasma membrane transporter), located in plasma membrane, pumps As (III) out of cell, and Ycf1p (a tonoplast transporter), located in the tonoplast, transports As $(GS)_3$ into the vacuole and confers As(III) resistance in yeast(Ghosh *et al.*, 1999; Kala *et al.*, 2000). Although two pathways(extrusion and sequestration) are involved in As detoxification in yeast, it is not clear which one plays a major role. The aim of the present work was to assess the contributions of two pathways to As detoxification in yeast. It is also needed to investigate how yeast population develops under As(V) stress and how P(V) detoxifies As(V) in the population level.

1 Materials and methods

1.1 Growth response to As(V) exposure

Cells were grown to the log phase on YNB medium without phosphorus(P) (Qbiogene Company, USA) amended with 100 μmol/L KH_2PO_4, and then sub-cultured into the same medium (100 ml) to obtain OD_{660}=0.02. When OD_{660} reached 0.08, As(V) (as Na_2HAsO_4) was added at the concentrations ranging from 0 to 200 μmol/L. At time intervals, samples were taken for OD_{660} measurement. From Section 1.1 to 1.5, yeast was cultured in shaking-bed with 250 r/min and 30℃.

1.2 Detoxification of As(V) by P(V)

Cells were grown to the log phase on YPD(Yeast Extract/Peptone/Dextrose) medium, washed with YNB medium without P three times and then

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suspended in the same volume of YNB medium without P. It was then sub-cultured into the YNB medium amended with uniform 20 μmol/L Na_2HAsO_4 and five P(V)(as KH_2PO_4) concentrations ranging from 10 to 500 μmol/L. At time intervals, samples were taken for OD_{660} measurement.

1.3 Determination of P and As concentrations in the thalli of yeast

Cells were grown to the log phase on the YNB medium with 100 μmol/L P(V), and then sub-cultured into the YNB medium amended with 180 μmol/L P(V) and 20 μmol/L As(V). After 60 h, thalli of yeast was collected by centrifugation at 1000 g for 10 min. After being washed 3 times by the YNB medium without P, thalli of yeast was digested in 5 ml of high-purity nitric acid, first at 80℃ for 2 h and then at 120℃ for 20 h. P concentrations in the digestion solution were determined by ICP-OES (inductively coupled plasma-optical emission spectrometry, Perkin Elmer Optima 2000 DV). Arsenic concentrations in the digestion solution were determined using an AF-610A atomic fluorescence spectrometer (Beijing Ruili Analytical Instrument Co., Beijing, China). Tea-leaf samples (obtained from the Chinese Center for Standard Materials and with P and As concentrations of 2840 ±60 and 0.28 ±0.03 mg/kg, respectively) were used as a standard reference material for quality assurance during digestion and analysis by ICP-OES and atomic fluorescence spectrometry.

1.4 Selectivity of P(V) and As(V) by yeast

Cells were grown to the log phase on the YNB medium amended with 100 μmol/L P (V), and then sub-cultured into the YNB medium amended with P(V) and As(V) to obtain OD_{660}=0.02. There were three treatments of P(V) and As(V) (in μmol/L): 20+80, 50+50 and 80+20, i.e. 3 ratios of P(V) to As(V), 1:4, 1:1 and 4:1, respectively. At the end of 24, 48 and 68 h, samples were taken for OD_{660} measurement. At 68 h, the medium was centrifuged at 1000 g for 10 min. The supernatant was collected and filtered by 0.22 μmol/L filter membrane and then As speciation was detected by HPLC-HG-AFC immediately according to the methods of He *et al.*(2000) and Yuan *et al.*(2005).

1.5 RNA content at different growth phases

Cell culture was the same as in section 1.1, but there were just two As(V) treatments(0 and 2 μmol/L). At 4, 8 and 12 h, freshly harvested cells were used for RNA purification using enzymatic lysis method (RNeasy mini kit, Qiagen Companies).

2 Results

2.1 Growth response to As(V) exposure

As(V) showed high toxicity to yeast(Fig.1). Yeast grew slowly with increasing As (V) concentrations in the medium. However, maximal population density was almost the same among different As(V) treatments. pH decreased slowly with increasing As (V) concentrations in the medium (Table 1). Ten μmol/L As (V) increased yeast growth from 24 to 48 h, which was confirmed several times using As (V) concentrations lower than 10 μmol/L. Fig.2 also showed that 2 μmol/L As(V) increased yeast growth from 16 to 32 h. It was in the late log phase that yeast growth was augmented by low As(V).

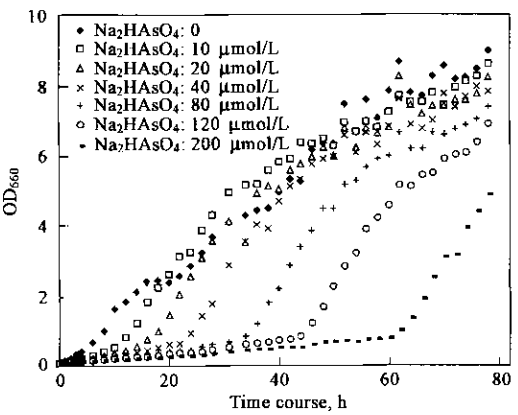

Fig.1 *S. cerevisiae* response to As (V) exposure (0 to 200 μmol/L Na_2HAsO_4)
The medium was YNB with initial P(V) of 100 μmol/L(as KH_2PO_4)

Table 1 pH in the medium for yeast (*S. cerevisiae*) after 78 h exposure to As(V) from 0 to 200 μmol/L(as Na_2HAsO_4)

As(V), μmol	0	10	20	40	80	120	200
Final pH	3.1	3.18	3.2	3.22	3.3	3.37	3.53

Notes: The medium was YNB with initial P(V) of 100 μmol/L (as KH_2PO_4) and initial pH 5.7

2.2 Detoxification of As(V) by P(V)

At fixed As (V) concentration of 20 μmol/L, increasing P (V) from 10 to 500 μmol/L increased yeast growth significantly (Fig.3), and the maximal population density was different among different P(V) treatments. Maximal population density of yeast increased with increasing P (V) concentrations in the medium.

2.3 Percentages of As and P remaining in the thalli of yeast

Initial As(V) and P(V) concentrations were 20 and 180 μmol/L in the medium, respectively. But after 60 h exposure, the percentages of As and P remaining in the thalli of yeast were 1.27% and 70%, respectively (Table 2).

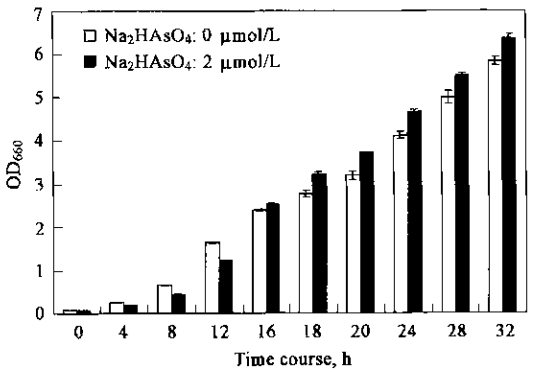

Fig.2 *S. cerevisiae* response to As (V) exposure (0 to 2 μmol/L Na_2HAsO_4). The medium was YNB with initial P (V) of 100 μmol/L (as KH_2PO_4). The error bars represent one *SE* of the mean from three replicates

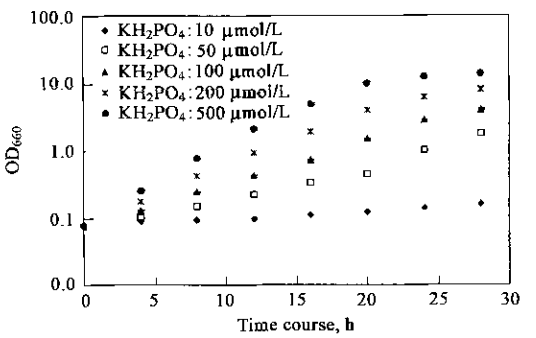

Fig.3 *S. cerevisiae* response to P nutrition from 10 to 500 μmol/L (as KH_2PO_4) under the stress of 20 μmol/L As(V) in the YNB medium. The error bars represent one *SE* of the mean of three replicates

Table 2 The percentages of As and P in the thalli of *S. cerevisiae*

	In thalli, μg	In the medium, μg	The percentages in the thalli
P	171.8±0.9	246.9	70.0±0.4
As	0.84±0.05	66	1.3±0.08

Notes: Yeast grew in the medium with initial P (V) of 180 μmol/L and As(V) of 20 μmol/L for 60 h, values are the means of three replicates ± one standard error

2.4 Selectivity of As(V) and P(V) by yeast

The ratios of P (V) to As (V) had a significant effect on yeast growth. Yeast grew the best under the ratio of 4:1 compared to the others(ratios of 1:1 and 1:4)(Fig.4). For example, at 68 h, yeast biomass under the ratio of 4:1 was 7 and 123 times more than that under the ratios of 1:1 and 1:4. Furthermore, only As(III) and As (V) were detected, and no organic As was detected in the medium at 68 h. Percentages of As (III) in the medium increased with increasing ratios of P(V) to As(V)(Fig.5). The percentage of As(III) in the medium was 71.49% under the ratio of 4:1, 0.6 and 2.9 times more than that under the ratios of 1:1 and 1:4, respectively.

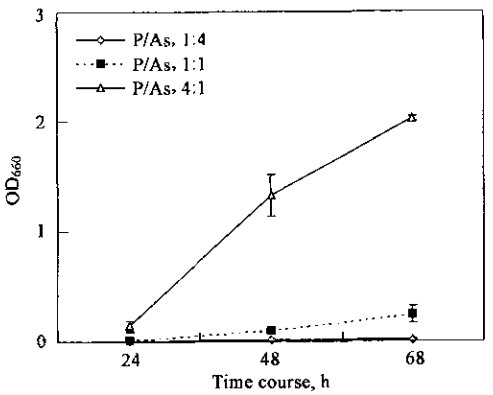

Fig.4 Growth response of *S. cerevisiae* to different P(V)/As(V) ratios. The ratio 1:4, 1:1 and 4:1 referred to the amount of P (V) and As (V) 20+80, 50+50, 80+20 μmol/L, respectively. The error bars represent one *SE* of the mean from three replicates

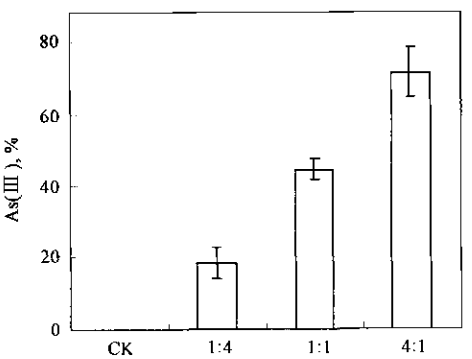

Fig.5 The percentages of As (III) in total arsenic in the YNB medium. *S. cerevisiae* grew in the YNB medium amended with different P(V)/As (V) ratios for 68 h

The ratios of 1:4, 1:1 and 4:1 referred to the amount of P (V) and As(V) 20+80, 50+50, 80+20 μmol/L, respectively. The CK treatment referred to the treatment amended with 80 μmol/L As(V) and without yeast. The error bars represent one *SE* of the mean from three replicates

2.5 RNA content at different phase

Without As(V) in the medium, RNA content was the highest at 4 h, and then decreased with time, and at 12 h, RNA content was very low (Table 3). However, with As (V) at 2 μmol/L in the medium, RNA content was the highest at 8 h. RNA content was very low at 4h and 12 h.

Table 3 RNA purified from freshly harvested *S. cerevisiae* cells exposed to As(V) at 2 μmol/L(as Na_2HAsO_4)

Sample time, h	As, μmol/L	OD_{660}	Sample volume, ml	Total RNA	
				Ratio	Content, μg
4	0	0.477	100	2.05	75
	2	0.317	200	1.7	2.4
8	0	1.25	42	2.01	45
	2	0.82	84	2.1	68
12	0	2.19	45	2.4	4
	2	1.78	45	2.4	6

Notes: The medium was YNB with initial P(V) of 100 μmol/L (as KH_2PO_4); samples were taken every 4 h

3 Discussion

Yeast growth was augmented by low As(V) in the late log phase(Figs.1 and 2). Uthus(1992) found that As is of physiological importance, especially when methionine metabolism is stressed (e.g. pregnancy, lactation, methionine deficiency, vitamin B6 deprivation). In YNB medium used in our experiments, pyridoxine hydrochloride(vitamin B6) was contained. The amount of pyridoxine hydrochloride would decrease gradually with yeast growth, so methionine metabolism could be stressed in the late log phase. This may explain why low As (V) increased yeast growth in the late log phase.

Fps1 gene is involved in As (III) uptake in yeast and expression of the Fps1 gene is down regulated upon As(III) and antimonite addition(Wysocki *et al.*, 2001). It is believed that with yeast growth amended with As(V) in the medium, As(III) (reduced from As (V)), was pumped out of the cell (Ghosh *et al.*, 1999), and As(III) pumped out of the cell should not be taken up by yeast again because of the close of the Fps1p channel and thus would not exert toxicity to yeast. This hypothesis was confirmed by the fact that As(V) treatments inhibited yeast growth in the early-log phase, but maximal population density was almost the same among different As (V) treatments(Fig1). Maximal population density was determined by P(V) concentrations in the medium and was not discounted by As(V) concentrations in the medium. This conclusion was confirmed by Fig.3, showing that at higher P(V) concentrations, there was higher maximal population density of yeast. There were two reasons why P(V) increased yeast growth. On one side, P, an essential element increased yeast growth; on the other side, P(V) decreased As(V) uptake because As(V) entered into cell via P(V) transporters (see Introduction) and thus increased yeast growth.

In the CK treatment (no inoculation of yeast), no As (III) but just As (V) was detected in the medium, which meant that sterilization at 121℃ for 25 min and culture at 30℃ for 68 h did not change As speciation in the medium. Ycf1p transports $As(GS)_3$ into the vacuole(Kala *et al.*, 2000), but As remaining in the thalli was just 1.27% (Table 2), from which it can be speculated that the percentage of As transported into vacuole should be lower than 1.27%. However, the percentage of As pumped out of cell was 71.49% under the ratio of 4:1(Fig.5), which was much higher than the percentage of As transported into vacuole. Although two pathways (extrusion and sequestration) involved in As detoxification in yeast, the extrusion pathway played a major role in As detoxification.

Many factors may have effects on RNA content in cells. For a usual population of yeast, RNA content was the highest in the early-log phase and RNA content was low in the steady phase(Table 3). Nakane *et al.* (1997) also found that aged spontaneously hypertensive rats showed a significant decrease in the RNA to DNA ratio in the CA1 subfield of the hippocampus (3.79 ± 0.61) compared to adult spontaneously hypertensive rats (5.27 ± 0.81). As (V) reduced RNA content in the early-log phase (Table 3), maybe due to P in the cell (such as ATP or RNA) replaced by As. Gao *et al.* (1999) also found that transretinoic acid and retinol palmitate reduced the DNA and RNA contents of rat Ito cells. As a model microorganism, yeast has been studied by many researchers in As uptake and detoxification, but there are still much left needed to study, such as crystal structure of protein involved in As metabolism.

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