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Heterologous Expression of OsPDR Enhances The Tolerance to Cobalt in Yeast*

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Abstract A cadmium-responsive metal ion transporter OsPDR, which was highly upregulated in the shoot of rice (*Oryza sativa* ssp. *japonica cv*. Nipponbare) at 24 h after treatment with 50 μmol/L Cd, was identified in cadmium-responsive transcriptome profiles analyzed by RNA-Seq. In this study, we isolated *OsPDR* from rice (*Oryza sativa cv*. Nipponbare) and the metal ions transport activity of OsPDR was analyzed. The results of metal tolerance experiments showed that overexpression of *OsPDR* can enhance yeast's tolerance to Co, but not to Zn, Ni or Cd, and higher accumulation of Co was observed compared to the empty vector-transformed yeast as determined by inductively coupled plasma mass spectrometry (ICP-MS). The EGFP-OsPDR fusion protein was localized to the vacuolar membrane using confocal microscopy. These data suggested that OsPDR might play an important role in Co homeostasis. Additional studies are needed to understand the function of *OsPDR* in plants.

Key words *OsPDR*, cobalt, transport, yeast
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Cobalt (Co) is an essential trace element for plant growth and is a component of vitamin B12. Low concentrations of Co can promote plant growth properly, but if the concentration of Co in the soil solution reaches 10 mg/L, it inhibits the growth and development of plants or even causes death, which would have a significant effect on human health [1–3]. With the development of modern industry, an increasing number of cultivated rice fields are polluted by the heavy metal Co. Co, once entering the soil, is stored in the cultivated layer by adsorption, precipitation and complexation [4–5]. Additionally, Co can be highly transported and is easily absorbed through the roots of plants and, in turn, accumulates in the vegetative and reproductive organs of plants, damaging not only the yield and quality of crops but also endangering human health by entering the human body through the food chain[6–7].

The ATP-binding cassette (ABC) transporter subfamily, a family of membrane proteins with a strong metal transport function, is mainly located in the plasma membrane, endoplasmic omentum, mitochondrial membrane and vacuolar membrane[8–11]. All of the members of this subfamily contain 4 or 6 highly hydrophobic transmembrane domains and ATP binding and/or nucleoside binding domains (NBDs) in the peripheral cytoplasm [8]. The ABC transporter proteins in yeast can be classified into subfamilies, from ABCB to ABCG, according to their sequence similarity to mammalian NBDs[12].

Recent research has revealed that ABC transporters can export harmful substances, extracellular toxins and targeted membrane modules, including substances that are related to resistance to biotic and abiotic stress in plants[13]. The known ABC genes can be classified into three subfamilies in plants:

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multidrug resistance (MDR), multidrug resistance-associated protein (MRP) and pleiotropic drug resistance (PDR)[14–15]. MRP genes play a role in many processes, such as detoxification and the transport of vacuolar flavonoid [16–17]. The MRP and MDR subfamilies are mainly related to Cd transportation in plants[18–20]. MRPs may be involved in the transport of the PC-Cd or GS-Cd complex on the vacuolar membrane[21–23]. PDR proteins, which are the subject of this study, have been found in plants that can respond to abiotic and biotic stress[24]. cDNA of *SpTUR2* was cloned as the first *PDR* gene in *Spirodella polyrhiza* and encodes a PDR5-like ABC transporter [25]. The expression of *PDR* genes is induced by pathogens, and PDR proteins can transport products to the cell surface[15]. PDR proteins are widely found in the plant cell membrane and can transport many cell substrates, such as metal ions [26–27]. Furthermore, studies have shown that the absence of some ABCG/PDR transporters on the plasma membrane of rice can increase its sensitivity to heavy metals[28]. Several *PDR* genes have been found in plants. *NtPDR3* was induced under iron-deficiency in *Nicotiana tabacum* [29–30]. The expression of *AtPDR8* in *Arabidopsis thaliana* increased, and the tolerance of transgenic plants with overexpression of *AtPDR8* to Cd and Zn also increased [31–32]. Further studies showed that the expression of *OsABCG43*/*PDR5* had effects on the distribution of Cd in yeast cells [33]. *OsPDR9* can be induced by Cd and Zn in rice[34]. Finally, the expression of *AtPDR9* can detoxify the herbicide 2,4-D in *Arabidopsis thaliana*[35]. However, there are few reports of *PDR* genes that are related to the transport of Co.

To analyze the function of OsPDR involved in heavy metal transport in yeast, confocal microscopy was used to observe the subcellular localization of OsPDR. Changes in the phenotype of yeast with *OsPDR* over-expressed were observed and the heavy metals content of *OsPDR* expressed in yeast was also measured to analyze the transport functions of OsPDR. The mechanism for regulating the Co content was defined, and this mechanism may underlie a theoretical foundation to support the breeding of rice with low Co accumulation in the future.

1 Materials and methods

1.1 Plant materials and growth conditions

Wild-type rice (*Oryza sativa cv*. Nipponbare) was used as the experimental material for gene cloning. The rice seeds were germinated in sterile water in glass dishes and were kept at 37℃ for 2-3 d in the dark and then were transferred to 1/2 Hoagland's solution that was renewed every 2 d[36]. The materials were cultured in a greenhouse at 25℃ under a 16 h/8 h light/dark cycle[37].

1.2 Gene cloning and plasmid construction

The *OsPDR* gene was obtained from the transcriptome profiles of cadmium-responsive metal ion transporters identified in rice [38]. Total RNA was isolated from seedlings of wild-type rice (21-day-old) using RNAsio Plus (TaKaRa, Japan). The cDNA was synthesized by the HiScript 1st Strand cDNA Synthesis Kit (Vazyme, China). The PCR products were cloned into the pEASY ® -Blunt Zero Cloning Vector (Transgen biotech, China) and were used as templates. The full-length sequences of *OsPDR*, *OsPDR-EGFP* and *EGFP* were cloned into the *pYES2* vector between *Hin*d Ⅲ and *Eco*R Ⅰ restriction sites using the ClonExpress® Ⅱ TM One-Step Cloning Kit (Vazyme), and the primers are listed in Table 1.

Table 1 List of primers used to amplify OsPDR sequences

Name	Sequence(5′→3′)	Vector
OsPDR-F	ATGCTCACTGGACCAGCAAC	*pEASY®-Blunt-OsPDR*
OsPDR-R	GAAAAGTTACCTTCCTGCAGC	
pYES2-OsPDR-F	ACTATAGGGAATATT**aagctt**ATGCTCACTGGACCAGCAAC	*pYES2-OsPDR*
pYES2-OsPDR-R	TGATGGATATCTGCA**gaattc**GAAAAGTTACCTTCCTGCAGC	
pYES2-OsPDR-F	ACTATAGGGAATATT**aagctt**ATGCTCACTGGACCAGCAAC	*pYES2-OsPDR-EGFP*
EGFP-OsPDR-R	CCCTTGCTCACTCTAGACATCCTTCCTGCAGCAGGTGCAA	
OsPDR-EGFP-F	TTGCACCTGCTGCAGGAAGGATGTCTAGAGTGAGCAAGGG	
EGFP-pYES2-R	TGATGGATATCTGCA**gaattc**TTACTTGTACAGCTCGTCCA	
pYES2-EGFP-F	ACTATAGGGAATATT**aagctt**ATGTCTAGAGTGAGCAAGGG	*pYES2-EGFP*
EGFP-pYES2-R	TGATGGATATCTGCA**gaattc**TTACTTGTACAGCTCGTCCA	

Restriction sites are in bold letters.

1.3 Yeast strains and growth conditions

The yeast strains used in this study included BY4741 (his3D1, leu2D0, met15D0, ura3D0, mating type α) and YK44 mutant strains (ura3-52, his3-200, ZRC, Cdt1, mating type α), which were received from Euroscarf (Frankfurt, Germany). The LiOAc/PEG method was used for yeast transformation, and the positive clone was selected from synthetic dropout medium without uracil (SD-Ura)[39]. The Yeast Extract Peptone Dextrose Medium (YPD) was used for the growth assay and glucose (Glu) in YPD medium was replaced by 2% (*w*/*v*) galactose (Gala) (YPG) was used for inducing the expression of genes.

1.4 Subcellular localization in yeast cells by fluorescence microscopy

pYES2-EGFP and *pYES2-OsPDR-EGFP* were transformed into BY4741 and YK44 and were used to observe the subcellular localization of OsPDR in yeast. Transformants were pre-cultured on SD-Ura medium and then on YPG for induction [40]. The transformed yeast cells were selectively stained with FM4-64, a marker of vacuolar membranes [41–42]. The yeast cells were incubated with 5 μmol/L FM4-64 for 30 min at 30℃ in the dark, washed three times with YPG medium, cultured with 3 ml YPG for 2 h and then washed three times with 0.05 mol/L PBS (NaCl 137 mmol/L, KCl 2.7 mmol/L，Na_2HPO_4 10 mmol/L, KH_2PO_4 2 mmol/L, pH 7.4) before observation with a confocal laser scanning microscope (LSM 710 NLO; Carl Zeiss, Jena, Germany)[43].

1.5 Metal tolerance experiments

For the complementation assays, *pYES2-OsPDR* and *pYES2* plasmids were transformed into yeast BY4741 and yeast YK44 on YPD medium and YPG medium containing 500 μmol/L or 1 mmol/L $CoCl_2$ using the lithium acetate method [44]. The transformed yeast cells were cultured overnight at 30℃ until reaching an A_{600} value of 0.5 and then were successively diluted to A_{600} values of 0.5, 0.05, 0.005 and 0.0005. Diluted yeast cultures (4.5 μl) were used for the drop-test experiments with metal solutions (500 μmol/L and 1 mmol/L $ZnSO_4$, 50 μmol/L and 100 μmol/L $CdSO_4$, 500 μmol/L and 1 mmol/L $NiCl_2$, and 500 μmol/L and 1 mmol/L $CoCl_2$) added to the solid medium. Three biological replicates were analyzed for all of the drop-test experiments.

1.6 ICP-MS experiments

To detect the metal content in yeast cells，*OsPDR* expressed in the yeast strains BY4741 and YK44 were pre-cultured on SD-Ura medium at 30℃ for 30 h and grown for 12 h on the induction medium to induced the expression of *OsPDR* by a dilution of culture broth (1∶1 000). The A_{600} was diluted to 0.8 and subsequently incubated on the induction medium supplemented with 150 μmol/L $CoCl_2$ and 0 μmol/L $CoCl_2$ (control) for 48 h. Ethylenediamine tetra-acetic acid (EDTA, 10 mmol/L) was used to absorb metal ions bound to the precipitate after centrifugation, rinsed with deionized water three times, then stored at 50℃ for 3 d. The concentration changes of Co in yeast were determined after microwave digestion (Milestone, Italy) by inductively coupled plasma mass spectrometry (ICP-MS).

2 Results

2.1 Isolation and phylogenetic analysis of the OsPDR

Cadmium-upregulated transcriptome profiles were identified in rice roots and shoots under Cd treatment by RNA-Seq analysis[38]. We focused on one gene that was highly responsive under Cd stress identified by the Cufflinks program [38]. The open reading frame of *OsPDR* (chr07: 20207865.. 20213557) was 900 bp and analyzed using DNASTAR Lasergene v7.1 software[45]. OsPDR can be classified as a member of the ABCG/PDR subfamily through a phylogenetic analysis (Figure 1a). The *OsPDR* gene was 77% identical to *TuPDR5* at the amino acid level using sequence alignment and encoded a deduced protein of 299 amino acids (Figure 1b)[25]. OsPDR has 3 transmembrane domains (TMs) predicted by TMHMM with an extracellular N terminus (http://www.cbs.dtu.dk/services/TMHMM/)(Figure 1c,d)[46–47]. SWISS-MODEL provides the information of the model, such as the oligomeric state, ligands and cofactors. The modelling process and reliability of the model was estimated by the global quality estimation score (GMQE:0.55) and the local composite scoring function (QMEAN.: –5.23). The combined quality estimate showed that the resulting GMQE was 0.20, which combines the QMEAN with the GMQE acquired from the alignment between target and template. The tertiary structure of OsPDR credible predicted by the SWISS MODEL was generated based on the template of the ATP-binding cassette subfamily G member 2 (ABCG2), which has 33% sequence similarity, 23.29% sequence identity with ABCG2 and the coverage was 0.98 (Figure 2).

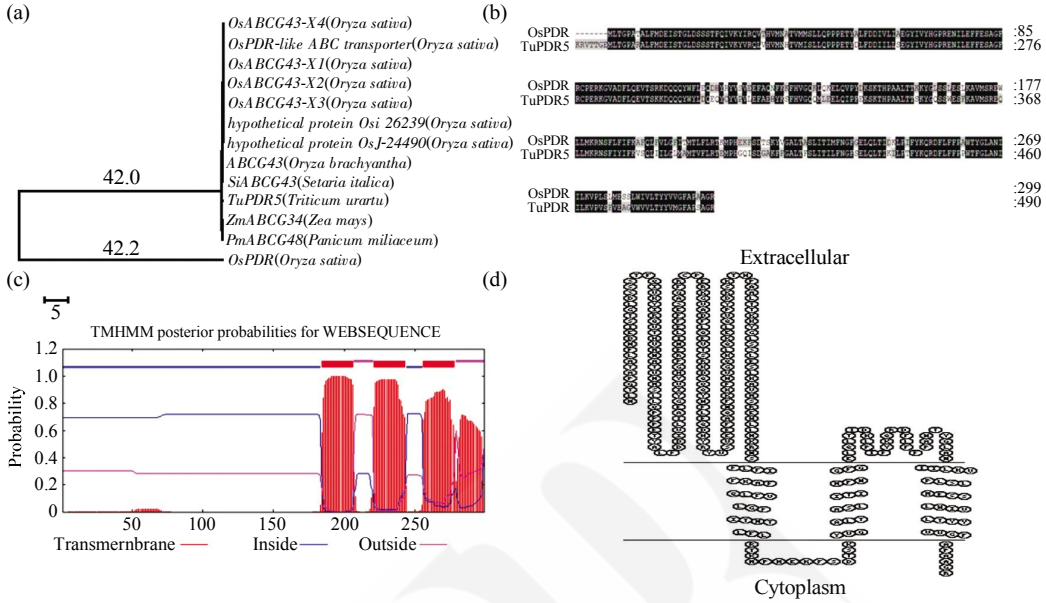
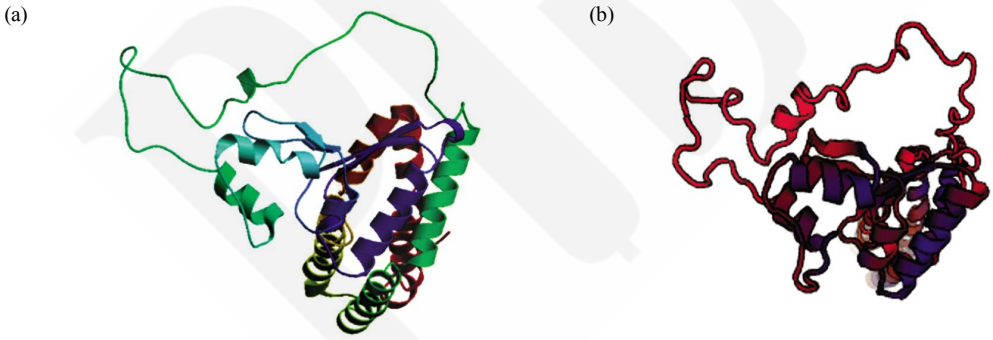


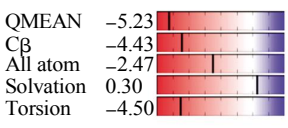
Fig. 1　Sequence features of OsPDR

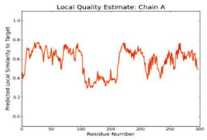
(a) A phylogenetic tree of *OsPDR* was speculated by the Maximum-Likelihood method and analyzed with MEGA6. ABC (ATP-binding cassette transporter), ABCG (ATP-binding cassette subfamily G), PDR (pleiotropic drug resistance). (b) Amino acid sequence alignment between OsPDR and TuPDR5. The alignment was conducted by ClustalW. White letters in black indicate identical amino acid residues. The predicted transmembrane domains of OsPDR are denoted by TM1-TM3. (c) Transmembrane domains predicted by TMHMM. (d) Topological analysis of OsPDR by SACS HMMTOP.

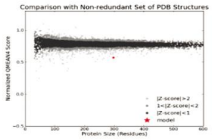


(c)

Model #01	File	Built with	Oligo-state	Ligands	GMQE	QMEAN
	PDB	ProMod3 Version 1.1.0	Monomer(matching prediction)	None	0.55	−5.23







Template	Seq Identity	Oligo-state	QSQE	Found by	Method	Resolution	Seq similarity	Range	Coverage	Description
5nj3.1.A	23.29	Homo-dimer	0.20	BLAST	EM	NA	0.33	1-297	0.98	ATP binding cassette subfamily G member 2

Fig. 2　Tertiary structure of OsPDR predicted by the SWISS MODEL (https://www.swissmodel.expasy.org/)

(a) Template of the ATP-binding cassette subfamily G member 2(ABCG2). (b) The model of OsPDR. (c) Modelling results. An analysis of the quality estimation information. The oligomeric structure (matching prediction) was a monomer without ligand and the model was built with ProMod3 Version 1.1.0. The score of GMQE was 0.55 and the score of QMEAN was −5.23. Local model quality estimates were presented as a per-reside plot by the QMEAN scoring function and the overall model quality based on global QMEAN estimates was provided as a Z-score, which the obtained values in relation to the global score caculated by a series of PDB structures with high-resolution. The combined quality estimate showed that the resulting GMQE (combines the QMEAN with the GMQE obtained from the alignment between target and template) was 0.20. The sequence identity was 23.29% and the sequence similarity was 33% between OsPDR and the template ABCG2.

2.2 OsPDR is localized to the vacuolar membrane in yeast

To confirm the subcellular localization of OsPDR, the OsPDR-EGFP fusion protein and EGFP protein were heterologously expressed in BY4741 yeast respectively. For the EGFP protein expressed in BY4741 yeast, green fluorescence was observed throughout nearly all of the cell, indicating that the protein is localized in the cytoplasmic matrix[48−49]. To further verify the subcellular localization of the fusion protein, the lipophilic dye FM4-64 was used to specifically stain the vacuolar membrane. Merging the GFP and FM4-64 images demonstrated that the EGFP-tagged OsPDR localized to the vacuolar membrane when analyzed in the stationary phase (Figure 3b).

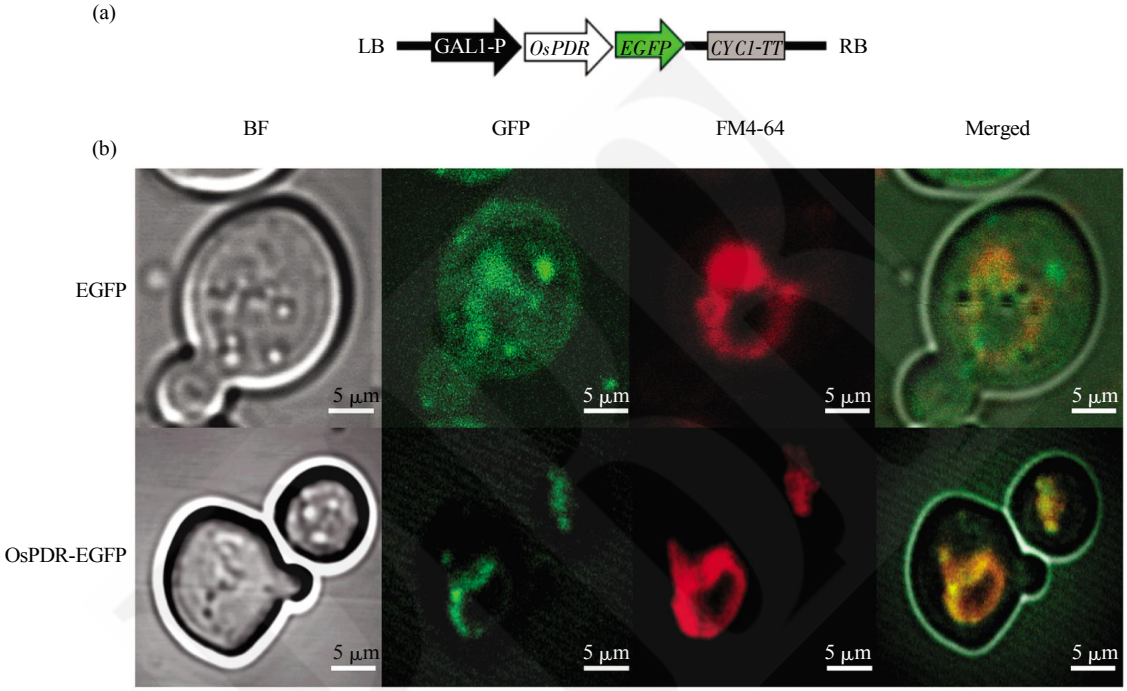


Fig. 3 Subcellular localization of OsPDR

(a) Schematic representation of *pYES2-OsPDR-EGFP*. (b) The localization of EGFP and OsPDR-EGFP fusion proteins. *pYES2-EGFP* and *pYES2-OsPDR-EGFP* expressed in the yeast strain BY4741. *pYES2-EGFP* and *pYES2-OsPDR-EGFP* expressed in yeast were cultured in SD-Ura until they reached the stationary phase. The cells were then induced in YPG medium overnight. From left to right, the figure shows the bright-field images, green fluorescence signals, FM4-64 fluorescence signals and the merged images with FM4-64 in red and GFP in green for pYSE2-EGFP or pYSE2-OsPDR-EGFP.

2.3 Overexpression of OsPDR in yeast confers Co tolerance

The *pYES2* and *pYES2-OsPDR* plasmids were transformed into the wild-type yeast strain BY4741 and the yeast mutant YK44 that is sensitive to Zn, Cd, Co and Ni. Compared with *pYES2* transformed into yeast, *pYES2-OsPDR* expressed in BY4741 and YK44 grew more efficiently in the presence of 500 μmol/L $CoCl_2$ and 1 mmol/L $CoCl_2$ plus Gala. The growth of *pYES2* and *pYES2-OsPDR* in BY4741 and YK44 on the YPD medium was uniform (Figure 4b and 4c). However, there were no apparent phenotypic differences between *pYES2* and *pYES2-OsPDR* in BY4741 and YK44 on the medium after a series of drop-test experiments were carried out with different concentrations of Zn, Cd and Ni. These results indicate that the expression of *OsPDR* in yeast BY4741 and YK44 can enhance the tolerance to heavy metal Co and may transport Co into vacuoles together with the result of vacuolar membrane localization.

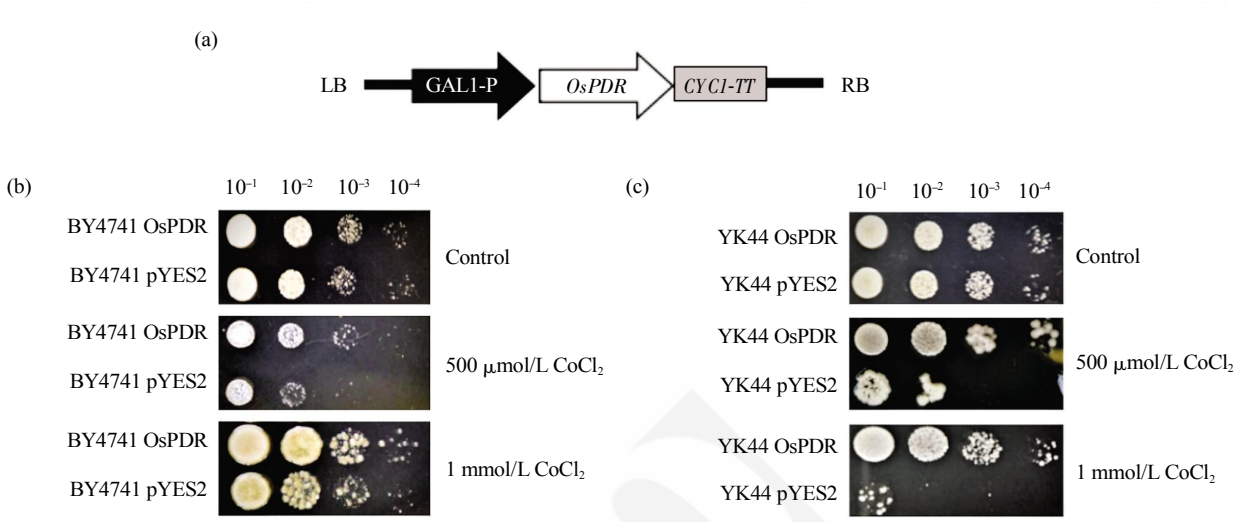


Fig. 4 Co tolerance of OsPDR over-expressed in BY4741 and YK44 (sensitive to Zn/Cd/Co/Ni)

(a) Schematic representation of *pYES2-OsPDR*. (b and c) Sensitivity of yeast mutants on solid medium containing heavy metals. BY4741 and YK44 were transformed with *pYES2* or *pYES2-OsPDR* and *pYES2-OsPDR*-transformed BY4741 and YK44 grows more efficiently than the *pYES2*-transformed BY4741 and YK44 (control) in the presence of 500 μmol/L $CoCl_2$ and 1 mmol/L $CoCl_2$. The YPG medium supplemented with 500 μmol/L $CoCl_2$ and 1 mmol/L $CoCl_2$ or no additional metal (control) was uesd for and incubated for 3–5 d at 30℃.

2.4 The overexpression of OsPDR affected the content of Co in yeast

The Co content of *pYES2* and *pYES2-OsPDR* transformed into BY4741 and YK44 strains was measured by ICP-MS. When *OsPDR* was expressed in BY4741 and YK44, the Co content was increased compared to that found in the *pYES2*-only transformants treated with 150 μmol/L $CoCl_2$(Figure 5).

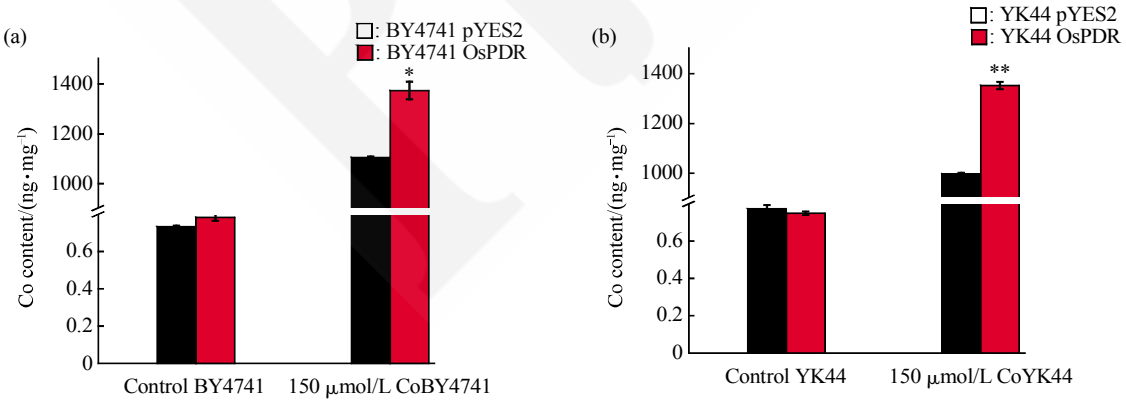


Fig. 5 ICP-MS measurement of the Co content in yeast BY4741 and YK44 with pYES2-transformed and pYES2-OsPDR-transformed

(a)The content of Co in BY4741 cells grown in the presence of 150 μmol/L Co or no additional metal added to the YPG culture medium. (b) Content of Co in YK44 cells grown in the presence of 150 μmol/L Co or no additional metal added to the YPG culture medium. The error bars represent the *SD* of three replicates (*$P < 0.05$, **$P < 0.01$).

The Co content did not change significantly between *pYES2* and *pYES2-OsPDR*-transformed yeast of BY4741 and YK44 under the condition of no additional metals. The content of Co in *pYES2-OsPDR*-transformed yeast of BY4741 was significantly increased compared to that of *pYES2*-only transformed yeast of BY4741 after 150 μmol/L $CoCl_2$ treatment (Figure 5a), and the same results were obtained in

yeast Yk44 (Figure 5b). The *pYES2-OsPDR*-transformed yeast in BY4741 and YK44 can be more tolerant to Co compared with the empty vector *pYES2* expressed in yeast BY4741 and YK44. The same results were obtained in yeast strains BY4741 and YK44. The result is consistent with the results of the metal tolerance experiments in yeast, which further proved that heavy metal Co can be stored in vacuoles where can isolate Co from causing damage to the yeast cells (Figure 4).

3 Discussion

3.1 OsPDR can enhance Co tolerance in yeast

Previous studies have shown that the expression of *OsPDR* is upregulated in shoots of rice (43-fold) with 24 h Cd exposure and exhibits no difference in the roots between 1 and 24 h. The gene for *PDR5* (the homolog of yeast *OsPDR*) cloned from *Saccharomyces cerevisiae* is linked to the excretion of cytotoxic metabolites [25]. Here, we analyzed the function of *OsPDR* in yeast. In this study, we found no Cd sensitivity in yeast expressing *OsPDR*, however, we found that heterologous expression of *OsPDR* in yeast BY4741 and YK44 can result in increasing tolerance to Co when we tested the transport ability of OsPDR (Figure 4). The functions of vacuoles are diverse and can involve the following points: maintaining osmotic pressure, participating in and regulating the accumulation and transportation of intracellular substances, maintaining the stability of cells and their internal environment and isolating wastes which have detoxifying effects. These data suggest that OsPDR is a vacuolar membrane-localized Co transporter that can pump Co into vacuoles for storage. Our results in yeast differ from a previous study that showed that *OsPDR* was highly responsive to cadmium exposure in the rice shoot. This inconsistency may be largely due to the heterologous expression of plant genes in yeast that can yield a difference from the functions of genes in rice plants. The reason that only *OsPDR* expressed in yeast BY4741 and YK44 showed tolerance to Co with no effect on other metals such as Zn, Ni or Cd is not clear. We hypothesize that the differences may be related to the different mechanisms for compartmentalizing metals and/or the toxicity of these metals toward yeast[50].

3.2 Overexpression of OsPDR increased the Co content in yeast under Co stress

Empty vector-transformed yeast and *OsPDR*-transformed yeast showed higher Co contents for both BY4741 or YK44 when treated with 150 μmol/L $CoCl_2$ compared with other metals. This difference may be related to the greater content of Co transported from the culture medium into cells. The content of Co was almost unchanged between yeast cells (BY4741 and YK44) expressing *pYES2* or *pYES2-OsPDR* without any Co in the medium with Gala. However, with 150 μmol/L $CoCl_2$ supplied in the medium, the Co content was higher in *OsPDR*-transformed yeast compared with empty vector-transformed yeast (Figure 5), which means that the expression of *OsPDR* can be more tolerance to Co. The localization of EGFP-tagged OsPDR to the vacuolar membrane also supported that supposition (Figure 3). This evidence strongly suggests that the metal tolerance of yeast strains with transformed *OsPDR* might be due to the sequestration of Co into vacuoles through the activity of OsPDR.

Taken together, our results show that OsPDR can function as a Co influx pump that can sequester extra Co into vacuoles in yeast. To further understand the function of *OsPDR*, additional studies need to be carried out in plants.

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References

[1] Clemens S M, Palmgreen G, Kramer U. A long way ahead: understanding and engineering plant metal accumulation. Trends in Plant Science, 2002, **7**(7): 309−315

[2] Ernst W H O, Verkleij J A C, Schat H. Metal tolerance in plants. Acta Botanica Neerlandica, 1992, **41**(3): 229−248

[3] Peralta-Videaa J, Martha L L, Mahesh N, *et al*. The bioehemistry of environmental heavy metal uptake by plants: Implications for the food chain. The international Journal of Biochemistry and Cell Biology, 2009, **41**(8): 1665−1677

[4] Jarup L. Hazards of heavy metal contamination. British Medical Bulletin, 2003, **68**(1): 16−182

[5] Zhou Q, Guo J J, He C T, *et al*. Comparative transcriptome analysis between low- and high-cadmium-accumulating genotypes of pakchoi (*Brassica chinensis L.*) in response to cadmium stress. Environmental Science and Technology, 2016, **50**(12): 6485−6494

[6] Sugiyama M. Role of cellular antioxidants in metal induced damage. Cell Biology and Toxicology, 1994, **10**(1): 1−22

[7] Ueno D, Yamaji N, Kono I, *et al*. Gene limiting cadmium

accumulation in rice. Proc Natl Acad Sci USA, 2010, **107** (38): 16500-16505

[8] Rea P A, Li Z S, Lu Y P, *et al*. From vacuolar GS-X pumps to multispecific ABC transporters. Annual Review of Plant Physiology and Plant Molecular Biology, 1998, **49**(49): 727-760

[9] Bovet L, Eggmann T, Meylan-Bettex M, *et al*. Transcript levels of *AtMRPs* after cadmium treatment: induction of *AtMRP*3. Plant Cell and Environment, 2003, **26**(3): 371-381

[10] Bovet L, Feller U, Martinoia E. Possible involvement of plant ABC transporters in cadmium detoxification: a cDNA sub-microarray approach. Environment International, 2005, **31**(2): 263-267

[11] Brü le S V D, Muller A, Fleming A J, *et al*. The ABC transporter SpTUR2 confers resistance to the antifungal diterpene sclareol. Plant Journal, 2002, **30**(6): 649-662

[12] Paumi C M, Chuk M, Snider J, *et al*. ABC transporters in *Saccharomyces cerevisiae* and their interactors: new technology advances the biology of the ABCC (MRP) subfamily. Microbiology and Molecular Biology Reviews, 2009, **73**(4): 577-593

[13] Martinoia E, Klein M, Geisler M, *et al*. Multifunctionality of plant ABC transporters-more than just detoxifiers. Planta, 2002, **214**(3): 345-355

[14] Jasinski M, Ducos E, Martinoia E, *et al*. The ATP-binding cassette transporters: structure, function, and gene family comparison between rice and *Arabidopsis*. Plant Physiology, 2003, **131** (3): 1169-1177

[15] Crouzet J, Trombik T, Fraysse A S, *et al*. Organization and function of the plant pleiotropic drug resistance ABC transporter family. Febs Letters, 2006, **580**(4): 1123-1130

[16] Lee E K, Kwon M, Ko J H, *et al*. Binding of sulfonylurea by AtMRP5, an *Arabidopsis* multidrug resistance-related protein that functions in salt tolerance. Plant Physiology, 2004, **134** (1): 528-538

[17] Gaedeke N, Klein M, Kolukisaoglu U, *et al*. The *Arabidopsis thaliana* ABC transporter AtMRP5 controls root development and stomata movement. Embo Journal, 2001, **20**(8): 1875-1887

[18] Klein M, Geisler M, Suh S J, *et al*. Disruption of *AtMRP4*, a guard cell plasma membrane ABCC type ABC transporter, leads to deregulation of stomatal opening and increased drought susceptibility. Plant Journal, 2004, **39**(2): 219-236

[19] Kitamura S, Shikazono N, Tanaka A. *TRANSPARENT TESTA 19* is involved in the accumulation of both anthocyanins and proanthocyanidins in *Arabidopsis*. Plant Journal, 2004, **37** (1): 104-114

[20] Hipfner D R, Gauldie S D, Deeley R G, *et al*. Detection of the M(r) 190,000 multidrug resistance protein, MRP, with monoclonal antibodies. Cancer Research, 1994, **54**(22): 5788-5792

[21] Grant C E, Valdimarsson G, Hipfner D R, *et al*. Overexpression of multidrug resistance-associated protein (MRP) increases resistance to natural product drugs. Cancer Research, 1994, **54**(2): 357-361

[22] Klein M, Burla B, Martinoia E. The multidrug resistance-associated protein (MRP/ABCC) subfamily of ATP-binding cassette transporters in plants. Febs Letters, 2006, **580**(4): 1112-1122

[23] Kolukisaoglu H U, Bovet L, Klein M, *et al*. Family business: the multidrug-resistance related protein (MRP) ABC transporter genes in *Arabidopsis thaliana*. Planta, 2002, **216**(1): 107-119

[24] do Amaral A M, Saito D, Formighieri E F. Identification of citrus expressed sequence tags (ESTs) encoding pleiotropic drug resistance (PDR)-like proteins. Genetics and Molecular Biology, 2007, **30**(3): 857-865

[25] Smart C C, Fleming A J. Hormonal and environmental regulation of a plant PDR5-like ABC transporter. Journal of Biological Chemistry, 1996, **271**(32): 19351-19357

[26] van den Brûle S, Smart C C. The plant PDR family ABC transporters. Planta, 2002, **216**(1): 95-106

[27] Verrier P J, Bird D, Burla B, *et al*. Plant ABC proteins a unified nomenclature and updated inventory. Trends in Plant Science, 2008, **13**(4): 151-159

[28] Stein M, Dittgen J, Sanchez-Rodriguez C, *et al*. *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. Plant Cell, 2006, **18**(3): 731-746

[29] Sasabe M, Toyoda K, Shiraishi T, *et al*. cDNA cloning and characterization of tobacco ABC transporter: *NtPDR1* is a novel elicitor-responsive gene. Febs Letters, 2002, **518**(1): 164-168

[30] Ducos E, Fraysse A S, Boutry M. *NtPDR3*, an irondeficiency inducible ABC transporter in *Nicotiana tabacum*. Febs Letters, 2005, **579**(30): 6791-6795

[31] Kim D Y, Bovet L, Maeshima M, *et al*. The ABC transporter AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. Plant Journal, 2007, **50**(2): 207-218

[32] Kobae Y, Sekino T, Yoshioka H, *et al*. Loss of AtPDR8, a plasma membrane ABC transporter of *Arabidopsis thaliana*, causes hypersensitive cell death upon pathogen infection. Plant Cell Physiology, 2006, **47**(3): 309-318

[33] Oda K, Otani M, Uraguchi S, *et al*. Rice *ABCG43* is Cd inducible and confers Cd tolerance on yeast. Bioscience, Biotechnology and Biochemistry, 2011, **75**(6): 1211-1213

[34] Moons A. *Ospdr9*, which encodes a PDR-type ABC transporter, is induced by heavy metals, hypoxic stress and redox perturbations in rice roots. Febs Letters, 2003, **553**(3): 370-376

[35] Ito H, Gray W M. A gain-of-function mutation in the *Arabidopsis* pleiotropic drug resistance transporter PDR9 confers resistance to auxinic herbicides. Plant Physiology, 2006, **142**(1): 63-74

[36] Hoagland D R, Arnon D I. The water-culture method for growing plants without soil. Circular California Agricultural Experiment Station, 1950, **347**(5406): 357-359

[37] Arnon D I, Hoagland D R. Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Science, 1940, **50**(1): 463-485

[38] Oono Y, Yazawa T, Kawahara Y, *et al*. Genome-wide transcriptome analysis reveals that cadmium stress signaling controls the expression of genes in drought stress signal pathways in rice. Plos One, 2014, **9**(5): e96946

[39] Gietz R D, Schiestl R H. High-efficiency yeast transformation using the LiAc/SS carrier DNA/PEG method. Nature Protocols, 2007, **2**(1): 31−34

[40] Sun T, Zhang Y X, Chai T Y. Cloning, characterization and expression of the *BjEXPA1* gene and its promoter region from *Brassica juncea L*. Plant Growth Regulation, 2011, **64**(1): 39−51

[41] Xiang S Q, Feng S S, Zhang Y X, *et al*. The N-terminal degenerated metal-binding domain is involved in the heavy metal transport activity of TaHMA2. Plant Cell Reports, 2015, **34**(9): 1615−1628

[42] Vida T A, Emr S D. A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. The Journal of Cell Biology, 1995, **128**(5): 779−792

[43] Courbot M, Willems G, Motte P, *et al*. A major quantitative trait locus for cadmium tolerance in *Arabidopsis halleri* colocalizes with *HMA4*, a gene encoding a heavy metal ATPase. Plant Physiology, 2007, **144**(2): 1052−1065

[44] Ito H, Fukuda Y, Murata K, *et al*. Transformation of intact yeast cells treated with alkali cations. Journal of Bacteriology, 1983, **153**(1): 163−168

[45] Larkin M A, GBlackshields G, Brown N P. Clustal W and ClustalX version 2.0. Bioinformatics, 2007, **23**(21): 2947−2948

[46] Xu J, Chai T Y, Zhang Y X, *et al*. The cation-efflux transporter BjCET2 mediates zinc and cadmium accumulation in *Brassica juncea L*. leaves. Plant Cell Reports, 2009, **28**(8): 1235−1242

[47] Feng S S, Tan J J, Zhang Y X, *et al*. Isolation and characterization of a novel cadmium-regulated Yellow Stripe-Like transporter (SnYSL3) in *Solanum nigrum*. Plant Cell Reports, 2017, **36** (2): 281−296

[48] Baekgaard L, Mikkelsen M D, Sorensen D M, *et al*. A combined zinc/cadmium sensor and zinc/cadmium export regulator in a heavy metal pump. Journal of Biological Chemistry, 2010, **285** (41): 31243−31252

[49] Jahn T P, Schulz A, Taipalensuu J, *et al*. Post-translational modification of plant plasma membrane H+-ATPase as a requirement for functional complementation of a yeast transport mutant. Journal of Biological Chemistry, 2002, **277**(8): 6353−6358

[50] Mills R F, Krijger G C, Baccarini P J, *et al*. Functional expression of AtHMA4, a P1B-type ATPase of the Zn/Cd/Cd/Pb subclass. Plant Journal, 2003, **35**(2): 164−176

OsPDR 在酵母中异源表达增强了酵母对钴的耐受性 *

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摘要 在通过 RNA-Seq 技术得到的镉响应转录组图谱中，用 50 μmol/L Cd 处理 24 h 后，一个镉响应金属离子转运蛋白 *OsPDR*被鉴定出其在水稻(*Oryza sativa* ssp. *japonica cv*. Nipponbare)茎中的表达量显著上调．本研究中，从水稻(*Oryza sativa cv*. Nipponbare)中分离了 *OsPDR* 基因，并对其金属离子转移活性进行了分析．金属耐受性实验结果表明，过表达 *OsPDR* 能提高酵母对 Co 的耐受性，但对 Zn、Ni 和 Cd 的耐受性不强，并且经电感耦合等离子体质谱法(ICP-MS)测定 Co 含量后，与空载体转化酵母相比，过表达 *OsPDR* 的酵母中 Co 的积累更高．利用共聚焦显微镜观察发现，EGFP-OsPDR 融合蛋白定位于液泡膜上．这些数据表明 OsPDR 可能在 Co 稳态中起着重要作用．*OsPDR* 在植物中的作用，还需要进一步的研究．

关键词 *OsPDR*，钴，转运，酵母

学科分类号 Q5，Q7

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