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# Cell Surface Display of Four Types of *Solanum nigrum* Metallothionein on *Saccharomyces cerevisiae* for Biosorption of Cadmium

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We displayed four types of *Solanum nigrum* metallothionein (SMT) for the first time on the surface of *Saccharomyces cerevisiae* using an α-agglutinin-based display system. The SMT genes were amplified by RT-PCR. The plasmid pYES2 was used to construct the expression vector. Transformed yeast strains were confirmed by PCR amplification and custom sequencing. Surface-expressed metallothioneins were indirectly indicated by the enhanced cadmium sorption capacity. Flame atomic absorption spectrophotometry was used to examine the concentration of $Cd^{2+}$ in this study. The transformed yeast strains showed much higher resistance ability to $Cd^{2+}$ compared with the control. Strikingly, their $Cd^{2+}$ accumulation was almost twice as much as that of the wild-type yeast cells. Furthermore, surface-engineered yeast strains could effectively adsorb ultra-trace cadmium and accumulate $Cd^{2+}$ under a wide range of pH levels, from 3 to 7, without disturbing the $Cu^{2+}$ and $Hg^{2+}$. Four types of surface-engineered *Saccharomyces cerevisiae* strains were constructed and they could be used to purify $Cd^{2+}$-contaminated water and adsorb ultra-trace cadmium effectively. The surface-engineered *Saccharomyces cerevisiae* strains would be useful tools for the bioremediation and biosorption of environmental cadmium contaminants.

**Keywords:** Cadmium, surface display, metallothionein, *Solanum nigrum*, *Saccharomyces cerevisiae*, flame atomic absorption spectrophotometry (FAAS)

## Introduction

Heavy metal ions are released into the natural environment by a variety of industrial processes with the fast development of modern industry. Moreover, their strong toxicity, non-degradable nature, and food-chain bioaccumulation have become a global environmental concern [8, 20]. Taking too much heavy metal ions can lead to health hazards to human beings [5, 29]. Many studies focusing on ion exchange, reverse osmosis, chemical precipitation, activated carbon adsorption, electrocoagulation, electrodialysis, ultrafiltration, and solvent extraction on the removal of heavy metal ions have been carried out [3, 14]. However, conventional physical and chemical methods for heavy metal ion removal have limitations, such as high costs, incomplete removal, secondary pollution, and the lack of metal specificity [1, 6, 7]. Many researchers have paid more attention to the microbial biomass-based biosorption methods because they are cheap, effective, and environmentally friendly, and can work well at relatively low concentrations [3, 12, 15–18, 21, 26].

$Cd^{2+}$, one type of toxic but useful heavy metal ion [22], has been widely used in many industrial processes, all of which can lead to the release of it into the environment by many different ways. Human beings often take $Cd^{2+}$ directly or indirectly from food because of its food-chain bioaccumulation property [2]. In order to reduce the effect of cadmium on human health, many researchers have carried out studies on removing $Cd^{2+}$ from the environment. K. Kuroda and Ueda [17, 18] successfully displayed yeast metallothionein (YMT), a tandemly fused YMT and hexa-His to adsorb $Cd^{2+}$. In another study, a short metal-binding peptide was displayed on the yeast surface for selective preconcentration of ultra-trace $Cd^{2+}$ by Yang *et al.* [31],

which was very useful for detection of this heavy metal ion by graphite furnace atomic absorption spectrometry (GFAAS). Many other exogenous proteins were also displayed on the cell surface of microbe to adsorb heavy metal ions [13, 27]. To purify $Cd^{2+}$-contaminated water by biological property inherent with microorganisms, especially with cell-surface engineered microbes, might be a potential method to this problem [11, 26].

*Solanum nigrum* is a plant that exhibits high $Cd^{2+}$ affinity ability due to its metallothionein (SMT), which is a cysteine-rich protein [10]. SMT genes encode *Solanum nigrum* metallothionein, which can be classified as SMT1 (subtypes a, b, c), SMT2 (subtypes a, b, c, d, e), and SMT3 (subtypes a, b, c). Among them, SMT2a, SMT2c, SMT2d, and SMT2e play an important role in heavy metal detoxification [10, 25]. However, SMTs have not been displayed on the cell surface of *Saccharomyces cerevisiae* yet. In order to enhance the adsorption and resistance properties of *Saccharomyces cerevisiae* to cadmium and remove cadmium from polluted water, we displayed SMTs on it by the cell surface engineering approach and investigated the property of the cell-surface-engineered *Saccharomyces cerevisiae* in this study.

## Materials and Methods

### Strains and Plasmids

The host used for recombinant DNA manipulation in this study was *Escherichia coli* DH5α [$F^-$, *endA1*, *hsdR17* ($r_k^-m_k^+$), *supE44*, *thi-1*, $\lambda^-$, *recA1*, *gyrA96*, *ΔlacU169*(*φ80lacZΔM15*)]. The yeast strains and plasmids used in this study were *Saccharomyces cerevisiae* CEN.PK113-5D and pYES2, pMD18-T vector. *E. coli* was grown on Luria-Bertani (LB) medium (1% (w/v) tryptone, 0.5% (w/v) yeast extract, 1% (w/v) sodium chloride) plates containing 1.5% agar and 100 μg/ml ampicillin to choose a positive colony and then amplified it in LB medium. The yeast strains bearing recombinant plasmids were selected on SC Minimal Medium and amplified in SC Minimal Medium lacking of uracil at 30°C.

### Plasmids Construction

pYES2 was used as the original plasmid in this study. First, the inducer promoter *GAL1* in pYES2 was replaced by the constitutive expression promoter triose-phosphate isomerase (*Tpi*) with SpeI and EcoRI. Second, the yeast secretion signal peptide alpha factor gene with MfeI, SpeI, and NotI was inserted after *Tpi* into the plasmids. Finally, the 3′ half sequence of the yeast agglutinin gene (*AG*) with EcoRI, MluI, and NotI was linked with the alpha factor gene in this plasmid. We named the modified plasmid as pYES2-*Tpi-α-AG*, whose structure is shown in Fig. 1A. The primers used in this study are summarized in Table 1.

*Solanum nigrum* metallothionein RNA was extracted following the standard method, and then its cDNA was obtained by reverse transcription. The SMT genes were amplified by polymerase chain reaction (PCR). The SMT genes analyzed in this study included SMT2a (EU760481.1), SMT2c (EU760483.1), SMT2d (EU760484.1), and SMT2e (EU760485.1). The primers used to amplify the SMT genes and construct the constitutive expression plasmid are shown in Table 1.

The amplified products were transformed into *E. coli* DH5α with the T-vector pMD-18 and sequenced by the dideoxynucleotide chain termination method [23]. The successful plasmids were extracted from *E. coli* DH5α, digested with EcoRI and MluI, and then inserted into the modified pYES2, which was also digested with EcoRI and MluI.

The resulting plasmid was named pYES2-*Tpi-α*-SMT-*AG*. It was sequenced to confirm that the SMT gene had inserted into the correct site of the plasmid. The successful integration plasmid for display of SMT (pYES2-*Tpi-α*-SMT-*AG*) and the integration plasmid (pYES2-*Tpi-α-AG*) with its SMT-encoding sequence deleted were

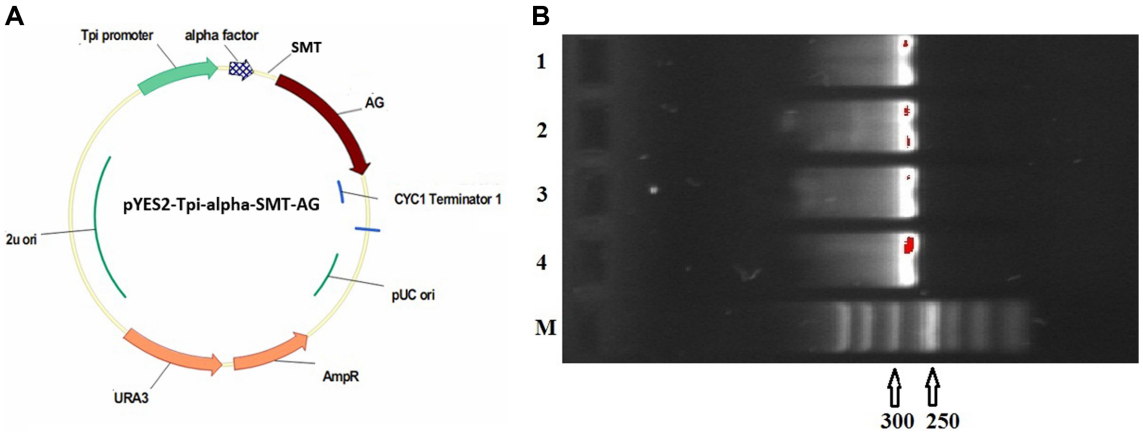


**Fig. 1.** Plasmid constructed for display of SMT on yeast cell surface (**A**) and colony PCR of transformants (**B**).
M: DNA Marker Ladder 500; 1, 2, 3, and 4: genes SMT2a, SMT2c, SMT2d, and SMT2e obtained from transformants.

**Table 1.** Primers used in this study.

| Primer ID | Sequence (5'--3') | Restriction site |
|---|---|---|
| TPI-F | GC*ACTAGT*ATATGACAGAGTCGC | SpeI |
| TPI-R | C*GAATTC*CTGTATGTGTTTTTTG | EcoRI |
| Alpha factor-F | TC*CAATTG*ATGAGATTTCCTTC | MfeI |
| Alpha factor-R | GA*ACTAGT*TCGCGGCCGCCCTA | SpeI, NotI |
| AGα1-F | G*GAATTC*CTCTAG*ACGCGT*CGCCAAAAGC | EcoRI, MluI |
| AGα1-R | GCGT*GCGGCCGC*TTTGATTATGTTCTTTCTAT | NotI |
| SMT-2a-F | GC*GAATTC*ATGTCTTGCTGTGGAGGA | EcoRI |
| SMT-2a-R | GG*ACGCGT*TTAGAGCAAGTGCAAGGG | MluI |
| SMT-2c-F | CG*GAATTC*ATGTCTTGCTGTGGAGGA | EcoRI |
| SMT-2c-R | GC*ACGCGT*TTGCAAGGGTCACATGTG | MluI |
| SMT-2d-F | CG*GAATTC*ATGTCTTGCTGTGGAGGA | EcoRI |
| SMT-2d-R | GC*ACGCGT*TTGCAAGGGTCGCACTTG | MluI |
| SMT-2e-F | CG*GAATTC*ATGTCTTGCTGTGGAGGA | EcoRI |
| SMT-2e-R | GC*ACGCGT*GTGCAAGGGTCACATTTG | MluI |

Restriction sites are underlined and italicized.

transferred into *Saccharomyces cerevisiae* CEN.PK113-5D similarly.

**Transformation of *S. cerevisiae***

The constructed plasmids were transformed to the *S. cerevisiae* cells with the method of electrotransformation. The transformants were isolated by incubation at 30°C for 48–72 h on selective SC medium plates.

**$Cd^{2+}$ Adsorption by SMT-Displaying Yeast Cells**

Prior to adsorption, the surface-engineered yeasts were precultivated and cultivated up to the stationary growth phase in SD medium containing 0.5% (w/v) casamino acids without uracil. The cells were harvested by centrifugation and then washed with 50 mM HEPES (pH 6.5). After washing, it was incubated for 2 h at 30°C and 180 rpm in 50 mM HEPES (pH 6.5), which contained 150 μM $CdCl_2$. It was centrifuged at 600 ×*g* after adsorption, and washed again with 50 mM HEPES to remove $Cd^{2+}$ on the cell surface but not bound to it. Finally, the resulting concentration of $Cd^{2+}$ in the rest supernatant was measured directly by flame atomic absorption spectrophotometry (FAAS), and the cell pellet's dry weight was measured after lyophilizing for 24 h. We used the maximum adsorption capacity of cadmium as the last result and six parallel experiments were done for each type of yeast. The data are shown in Table 2.

**Table 2.** Adsorption of $Cd^{2+}$ by different kinds of yeast.

| Yeast strains | Adsorption of $Cd^{2+}$ (mg/g dry weight) |
|---|---|
| *Saccharomyces cerevisiae*[a] | 12.49 ± 0.28 |
| *Saccharomyces cerevisiae*[b] | 24.59 ± 0.54 |
| *Saccharomyces cerevisiae*[c] | 22.86 ± 0.38 |
| *Saccharomyces cerevisiae*[d] | 24.45 ± 0.43 |
| *Saccharomyces cerevisiae*[e] | 23.56 ± 0.32 |

[a]Yeast containing pYES2-*Tpi-α-AG*

[b]Yeast containing pYES2-*Tpi-α*-SMT2a-*AG*

[c]Yeast containing pYES2-*Tpi-α*-SMT2c-*AG*

[d]Yeast containing pYES2-*Tpi-α*-SMT2d-*AG*

[e]Yeast containing pYES2-*Tpi-α*-SMT2e-*AG*

**$Cd^{2+}$ Adsorption Under Different pH Values**

The effect of pH on the adsorption ability was determined through $Cd^{2+}$-binding experiment in 50 mM HEPES at pH levels between 3 and 7.

**Effects of $Cu^{2+}$ and $Hg^{2+}$ on Bioadsorption for $Cd^{2+}$**

Influence of $Cu^{2+}$ and $Hg^{2+}$ on the adsorption of $Cd^{2+}$ was investigated through co-existence of $Cu^{2+}$ and $Cd^{2+}$, $Hg^{2+}$, and $Cd^{2+}$, respectively. The pH of the $Cd^{2+}$-containing solution was 6.5.

**Adsorption of Ultra-Trace $Cd^{2+}$ by SMT-Displaying Yeast Cells**

The method used was similar to that described in the "$Cd^{2+}$ adsorption by SMT-displaying yeast cells" section, with the difference being that the concentration of $Cd^{2+}$ was adjusted to 100 μg/l. After adsorption by yeasts, the $Cd^{2+}$ in the water was extracted by diphenylthiocarbazone-ethanol and ionic liquids. The concentration of the concentrated $Cd^{2+}$ was measured by FAAS.

**Growth in $Cd^{2+}$-Containing Medium**

Transformants were harvested from SC medium at the stationary phase and then diluted with the SC medium containing 0.5 mM $CdCl_2$. The final culture broth at 600 nm was 0.2. After that, cells

were cultivated at 30°C and 160 rpm and cell growth was measured by absorbance of the culture broth at 600 nm.

The EMBL accession numbers of the SMT genes analyzed in this study are EU760481.1 (SMT2a), EU760483.1 (SMT2c), EU760484.1 (SMT2d), and EU760485.1 (SMT2e).

## Results

### Vector Construction, Transformation, and Positive Transformants Confirmation

The multicopy plasmid pYES2-*Tpi-α*-SMT-*AG* was constructed for the display of SMT. As a control plasmid, the modified multicopy plasmid pYES2-*Tpi-α-AG* lacking SMT was also constructed. The main components of cell surface displayed plasmid are as follows: *Tpi* promoter, yeast secretion signal alpha factor, SMT-encoding gene, and 3′ half sequence of anchoring protein-encoding gene AG. The SMT gene was located between the alpha factor and AG. The construction methods were shown in the Materials and Methods section. The structure of the plasmid is shown in Fig. 1A.

These plasmids constructed above were introduced into *S. cerevisiae* CEN.PK113-5D with the method of electrotransformation. The positive transformants were selected from SC plates and cultivated in SC at 30°C and 160 rpm for 48 h. The colony PCR was conducted to confirm that the transformants were correct. The results are shown in Fig. 1B.

### Adsorption of $Cd^{2+}$ by Surface-Engineered Yeast

The adsorption of heavy metal-containing water (150 μM $CdCl_2$) by five types of yeast in this study was examined to determine the effect of SMT display. SMT-displaying cells with pYES2-*T-a*-SMT-*AG* showed higher adsorption ability at the cell surface than cells harboring the control plasmid pYES2-*T-a-AG* and the wild types (Table 2). This enhancement of adsorption ability at the cell surface indicated that the displayed SMT was functional as a chelator of heavy metal ions and contributed to the improvement of cell adsorption ability. As was shown in Table 2, the cells displaying SMT2a had the highest adsorption ability. However, there was no significant difference between the four types of displayed yeasts on the adsorption ability. All of the cell-surface-engineered yeasts could effectively adsorb $Cd^{2+}$ and be applied for the biosorption and bioremediation of $Cd^{2+}$-contaminated water.

### Tolerance to $Cd^{2+}$ by Cells Displaying SMT

The adsorption of heavy metal ions at the cell surface may serve to protect cells from the ions and contribute to tolerance to them. To investigate the protective effect of the displayed SMT against $Cd^{2+}$ toxicity, surface-engineered yeasts grown to the stationary phase were inoculated into synthetic liquid medium supplemented with 0.5 mM $CdCl_2$ (Fig. 2). SMT-displaying cells with pYES2-*T-a*-SMT-*AG* exhibited a dramatically enhanced $Cd^{2+}$ tolerance in comparison with cells only harboring pYES2-*T-a-AG*. This result suggests that all of the SMTs displayed on the cell surface have an important effect on growth in $Cd^{2+}$-

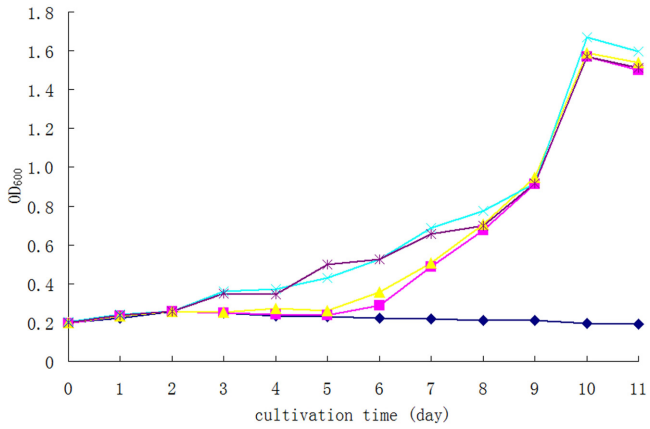


**Fig. 2.** Yeast growth in cadmium-containing medium.
Yeast: (◆) containing pYES2-Tpi-α-AG; (■) containing pYES2-Tpi-α-SMT2a-AG; (▲) containing pYES2-Tpi-α-SMT2c-AG; (×) containing pYES2-Tpi-α-SMT2d-AG; (✳) containing pYES2-Tpi-α-SMT2e-AG.

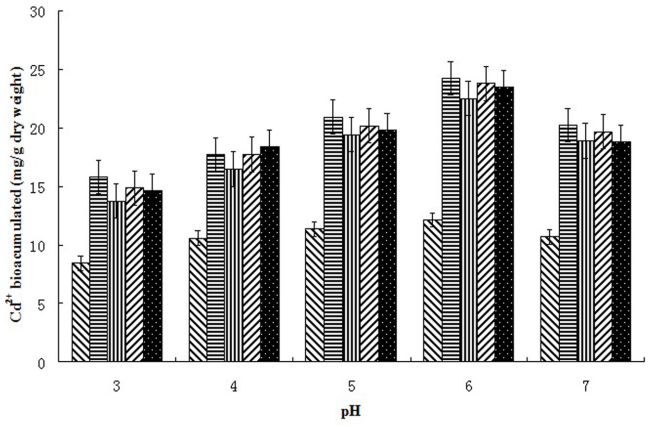


**Fig. 3.** the pH influence on the adsorption ability of different four kinds of displayed yeasts.
yeast: (▨) containing pYES2-Tpi-α-AG; (▤) containing pYES2-Tpi-α-SMT2a-AG; (▥) containing pYES2-Tpi-α-SMT2c-AG; (▨) containing pYES2-Tpi-α-SMT2d-AG; (■) containing pYES2-Tpi-α-SMT2e-AG.

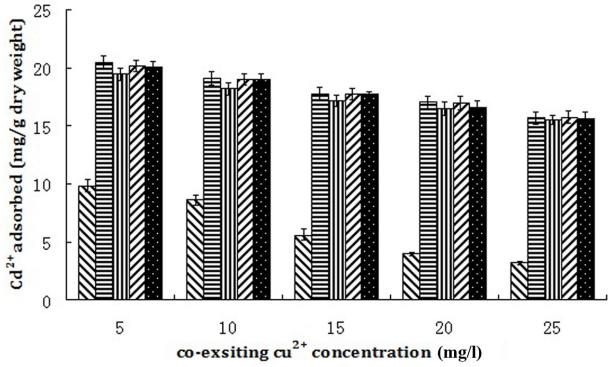


**Fig. 4.** The effect of co-existing $Cu^{2+}$ on the adsorption of $Cd^{2+}$ by different strains of *Saccharomyces cerevisiae*.

yeast: (▨) containing pYES2-Tpi-α-AG; (▤) containing pYES2-Tpi-α-SMT2a-AG; (▥) containing pYES2-Tpi-α-SMT2c-AG; (▨) containing pYES2-Tpi-α-SMT2d-AG; (■) containing pYES2-Tpi-α-SMT2e-AG.

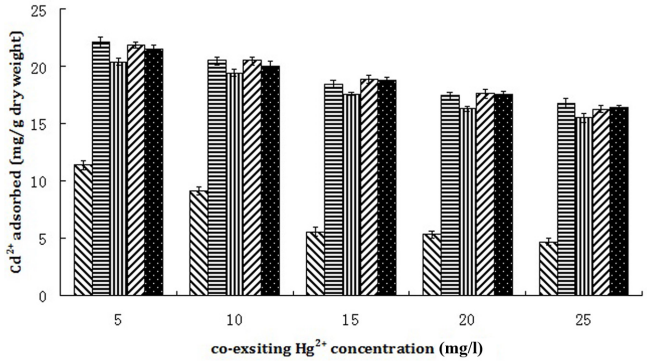


**Fig. 5.** The effect of co-existing $Hg^{2+}$ on the adsorption of $Cd^{2+}$ by different strains of *Saccharomyces cerevisiae*.

yeast: (▨) containing pYES2-Tpi-α-AG; (▤) containing pYES2-Tpi-α-SMT2a-AG; (▥) containing pYES2-Tpi-α-SMT2c-AG; (▨) containing pYES2-Tpi-α-SMT2d-AG; (■) containing pYES2-Tpi-α-SMT2e-AG.

containing medium and enhance cellular tolerance to $Cd^{2+}$.

### Adsorption of $Cd^{2+}$ under Different pH Values by Surface-Engineered Yeast

The pH influence experiment suggested that the transformants can effectively adsorb $Cd^{2+}$ at pH values from 3 to 7. The effect of pH on the adsorption ability of cell-surface-engineered yeast was lower than the control. The highest adsorption ability appeared at the pH levels about 6. The result is shown in Fig. 3. This result indicated that the transformants can be used to purify water with a wide range of pH that is polluted by $Cd^{2+}$. For water with pH level of about 6, this type of yeast can display their highest adsorption ability. Therefore, this kind of yeast might be very useful in treating $Cd^{2+}$-polluted water.

### Adsorption of $Cd^{2+}$ with the Co-Existing Heavy Metal Ions $Cu^{2+}$ and $Hg^{2+}$

Figs. 4 and 5 demonstrate that the affinity of surface-exposed SMT for cadmium exceeded the competing heavy metals ions, such as $Cu^{2+}$ and $Hg^{2+}$. In both cases, although high concentrations of $Cu^{2+}$ and $Hg^{2+}$ seriously inhibited $Cd^{2+}$ adsorption, the $Cd^{2+}$ uptake capacity of surface-engineered cells only decreased by 31.65%, 32.02%, 33.50%, 30.26%, and 36.13%, 32.21%, 35.54%, 33.66% as the $Cu^{2+}$ and $Hg^{2+}$ concentration increased to 25 mg/l, respectively. In contrast, the control decreased by 74.51% and 62.63%, respectively, when the $Cu^{2+}$ and $Hg^{2+}$ concentration increased to 25 mg/l.

We can infer that the surface-engineered yeasts can adsorb $Cd^{2+}$ effectively when the polluted water contains $Cu^{2+}$ and $Hg^{2+}$ at the same time. This is very useful to purify polluted water in the environment that is polluted by several types of heavy metal ions simultaneously.

**Table 3.** Adsorption of ultra-trace $Cd^{2+}$ by different types of yeast.

| Strains | Adsorption ratio |
|---|---|
| Yeast contained pYES2-*Tpi-α-AG* | 45.08% |
| Yeast contained pYES2-*Tpi-α*-SMT2a-*AG* | 98.06% |
| Yeast contained pYES2-*Tpi-α*-SMT2c-*AG* | 92.13% |
| Yeast contained pYES2-*Tpi-α*-SMT2d-*AG* | 95.43% |
| Yeast contained pYES2-*Tpi-α*-SMT2e-*AG* | 94.55% |

Original $Cd^{2+}$ concentration: 100 ug/l; yeast cells concentration: 10 g/l.

### Adsorption of Ultra-Trace $Cd^{2+}$ by Surface-Engineered Yeast

The adsorption ability of surface-engineered yeast was significant higher than that of the control. As can be seen from Table 3, the surface-engineered yeast could effectively adsorb ultra-trace $Cd^{2+}$. This is a valuable characteristic of the surface-engineered yeast for it can purify $Cd^{2+}$-polluted water much more thoroughly than the control.

## Discussion

Compared with intracellular adsorption, adsorption at the cell surface has many advantages, for the following reasons: first, it is easy to recover and recycle adsorbed heavy metal ions from the surface-engineered yeasts without disintegrating them [3], which is very important in considering how to treat the heavy metal ions after their

removal from wastewater. In contrast, one has to disintegrate the cells when one wants to recover adsorbed heavy metal ions from intracellular accumulated sources or plant bioremediation, which is inconvenient and costly [30]. Second, it is economically advantageous to use surface-engineered yeast cells because they can be repeatedly used as bioadsorbents, as the cells are not severely damaged during the recovery process [4]. Therefore, the system can be easily used for adsorption, recovery, and recycling of heavy metal ions.

Four types of SMT genes were cloned from *Solanum nigrum*, which has the ability of hyperaccumulation to $Cd^{2+}$ [10], in this study. Accordingly, four types of cell surface display plasmids were constructed with pYES2 and SMT genes. The constitutive expression plasmid constructed from pYES2 was very important for this study, as the high adsorption ability property of engineered cells largely relied on the successful construction of this plasmid.

Four types of engineered cell surface display yeasts were constructed based on the successful construction of the cell surface display plasmid described above. Colony PCR and adsorption test demonstrated that SMTs were successfully expressed and displayed on the cell surface of *Saccharomyces cerevisiae*. Successful display of SMTs that have the ability to adsorb $Cd^{2+}$ on the yeast cell surface resulted in enhanced adsorption and recovery of heavy metal ion $Cd^{2+}$ at the cell surface without cellular disintegration. This result demonstrated that SMTs displayed on the cell surface were active and successfully served as a chelator of heavy metal ion $Cd^{2+}$. As can be seen from Table 2, display of SMT on the yeast cell surface is effective for the adsorption of $Cd^{2+}$ and enhances the yeasts' adsorption ability and produces valuable bioadsorbents. The adsorption ability of the engineered yeasts was almost twice that of the control, which illustrated that SMTs can effectively adsorb $Cd^{2+}$ from contaminated water. As can be seen from Table 2, cells that displayed SMT2a have the highest adsorption ability. However, the adsorption ability among the engineered yeasts has no significant difference, which is consistent with the difference between their structures [22].

The resistance to $Cd^{2+}$ by displayed SMT yeast results from the enhanced adsorption of toxic heavy metal ions on their surface (Fig. 2), which is similar to the resistance to $Cd^{2+}$ by YMT and hexa-His-displaying yeast cells [17, 18] Binding heavy metal ions at the cell surface is probably a mechanism of resistance like intracellular sequestration and efflux pumping [9, 24, 28]. The cell division of non-engineered yeasts was inhibited by 80 μM $Cd^{2+}$ [16] whereas the engineered ones lived in the 0.5 mM $Cd^{2+}$-containing medium (Fig. 2). It is considered that the surface-engineered yeast resistance to $Cd^{2+}$ was by reducing the opportunity of metal permeation into the cytosol through adsorption and binding them at the cell surface. Therefore, it is feasible that the trapping of heavy metal ions on the cell surface is a functional phenomenon, serving as a mechanism for tolerating the presence of toxic heavy metal ions. As can be seen from Fig. 2, the inhibitory effect of $Cd^{2+}$ on cell division is thought to result in a longer proliferation period in the $Cd^{2+}$-containing medium. However, the clarification of the mechanism and the reason for the long lag period require further investigations.

Compared with control yeast cells, the engineered yeasts are influenced less by pH variations and can effectively adsorb $Cd^{2+}$ at wide pH levels, as shown in Fig. 3, which is very useful for their use in different contaminated waters. The SMT may be functional and their construction was probably very stable under a wide range of pH. However, the detail of this specific mechanism and the reason for this stable property still need to be investigated.

Several works on yeast surface display of metal-binding proteins/peptides have been carried out [16–18]. Although these surface-engineered cells showed high affinity to a wide range of heavy metals, they lacked selectivity to certain heavy metals. The display of cadmium-binding protein on the yeast surface obviously changes its binding property to the metal of interest. In this particular case, the improvement on binding selectivity was one of the main focus. Figs. 4 and 5 demonstrate that co-existing heavy metal ions $Cu^{2+}$ and $Hg^{2+}$ did not affect the adsorption ability for $Cd^{2+}$ of engineered yeast significantly. These cells exhibit preference for $Cd^{2+}$ adsorption rather than for $Cu^{2+}$ and $Hg^{2+}$. These data suggest that SMTs displayed on *S. cerevisiae* could selectively adsorb $Cd^{2+}$ from mixed heavy metal ions contaminated water.

When the surface-engineered yeasts to were exposed ultra-trace heavy metal ions, they exhibited higher adsorption ability than that of the control. The main reason for this difference probably is that the cadmium-binding protein displayed on the cell surface and could detect the $Cd^{2+}$ more easily. The controls do not have cell surface displayed protein, so they have much more difficulty in detecting ultra-trace $Cd^{2+}$.

Microorganism–metal interactions have been reported and classified into active and passive mechanisms [11]. Living cells have been reported to preferentially adsorb $Cd^{2+}$ by energy-dependent metabolism, whereas non-living ones adsorb heavy-metal ions by metabolism-independent surface binding method [19]. Accordingly, both the living

and non-living cells constructed in this study can be used as effective bioadsorbents of $Cd^{2+}$.

The potential ability of surface-engineered yeasts displaying proteins as bioadsorbents is largely due to the properties of the displayed protein. Therefore, in order to get engineered cells with high adsorption ability, we should focus future research on selecting proteins with high metal-binding capacity.

## Acknowledgments

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