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ORIGINAL PAPER

Cell Surface Display of MerR on *Saccharomyces cerevisiae* for Biosorption of Mercury

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Abstract The metalloregulatory protein MerR which plays important roles in *mer operon* system exhibits high affinity and selectivity toward mercury (II) (Hg^{2+}). In order to improve the adsorption ability of *Saccharomyces cerevisiae* for Hg^{2+}, MerR was displayed on the surface of *S. cerevisiae* for the first time with an α-agglutinin-based display system in this study. The *merR* gene was synthesized after being optimized and added restriction endonuclease sites *EcoR* I and *Mlu* I. The display of MerR was indirectly confirmed by the enhanced adsorption ability of *S. cerevisiae* for Hg^{2+} and colony PCR. The hydride generation atomic absorption spectrometry was applied to measure the Hg^{2+} content in water. The engineered yeast strain not only showed higher tolerance to Hg, but also their adsorption ability was much higher than that of origin and control strains. The engineered yeast could adsorb Hg^{2+} under a wide range of pH levels, and it could also adsorb Hg^{2+} effectively with Cd^{2+} and Cu^{2+} coexistence. Furthermore, the engineered yeast strain could adsorb ultra-trace Hg^{2+} effectively. The results above showed that the surface-engineered yeast strain could adsorb Hg^{2+} under complex environmental conditions and could be used for the biosorption and bioremediation of environmental Hg contaminants.

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Keywords *Mer operon* system · Mercury adsorption · α-Agglutinin-based display system · Hg-contaminated water · Bioremediation

Introduction

Mercury (Hg) is a highly toxic heavy metal to human health, and the Agency for Toxic Substances and Disease Registry had ranked it the third in the priority list of hazardous substances [1, 2]. Hg was released to environment through many industrial processes and could be accumulated by plants or animals which might be used for foods by humans [3, 4]. In recent years, most of the Hg in the environment was released by natural processes and human activities including oceanic emission, biomass burning, mining, metal manufacturing and fossil fuel burning, which resulted in environmental pollution and potential danger to human beings [5–8]. A large portion of Hg existed in the environment was Hg^{2+}, and it can influence human health seriously [8–10].

The impacts of Hg contamination on environment and human health impelled researchers to find effective ways for treating this toxic metal. The remediation techniques for Hg contamination mainly include physical treatment, chemical remediation and bioremediation [11, 12]. Conventional physico-chemistry methods used to remove Hg from contaminated environment were often inadequate and lack of selectivity to reduce Hg^{2+}; they were usually unfriendly to environment and expensive [13–15].

Bioremediation technology had been wildly applied for treating Hg contamination due to its cheap, green and environmentally friendly characters [16–18]. Microbial-based remediation technology is a critical important part of bioremediation because of the certain properties of microorganisms [12, 19]. A lot of microbes had been examined for

bioremediation of Hg [20, 21]. The *mer* (mercury resistance) *operon* system in bacteria could transfer Hg^{2+} into the cell where it was reduced to Hg^0 and volatilized from the bacterial cell to the atmosphere at last [22, 23]. Therefore, the bacteria-owned *mer operon* system had been attempted to be used to reduce Hg^{2+} contamination [24–26]. But this microbial volatilization system is often inhibited by high concentration of heavy metal ions [15]. In order to enhance the tolerance and adsorption ability for Hg of the microorganisms, researchers were trying to modify these microbes. Kiyono and Pan-hou [27] constructed an engineered *Escherichia coli* expressing Hg transport system and organomercury lyase for accumulation and transformation of Hg. Engineered bacterium expressing Hg transport system and metallothionein was constructed for biosorption of Hg^{2+} by Deng and Jia [28]. However, the Hg^{2+} adsorbed by these engineered bacteria was usually difficult to recover because it was in the cytoplasm or volatilized to the atmosphere [29, 30]. These problems might be solved by displaying the target proteins that have high affinity to Hg^{2+} on the cell surface. MerR is a metalloregulatory protein in the *mer operon* system which contained in many bacteria [18, 22, 31] and it can selectively adsorb Hg^{2+} [32]. *Escherichia coli*'s Hg^{2+} adsorption ability was enhanced by displaying MerR on its cell surface [33]. The cell surface-engineered microorganisms might be a potential effective way for treating Hg pollutions.

The *Saccharomyces cerevisiae* is one of the most suitable microorganisms for the development of cell surface display system, and many heterologous proteins had been displayed on it [34–36]. We hypothesized that *S. cerevisiae* displaying MerR might adsorb Hg^{2+} effectively and not result in secondary pollution. It might be a potential method to purify Hg^{2+}-polluted water. In this study, we displayed MerR on the cell surface of *S. cerevisiae* with an α-agglutinin-based display system to enhance its adsorption ability for Hg^{2+}. The properties of the engineered yeast showed that it could adsorb Hg^{2+} effectively and could be used to purify Hg-polluted water.

Materials and Methods

Strains, Media and Plasmids

Escherichia coli DH5α [F^-, *endA1*, *hsdR17* (r_k^- m_k^+), *supE44*, *thi-1*, λ^-, *recA1*, *gyrA96*, *ΔlacU169*($^{\varphi}$*80lacZΔM15*)] was used as the host for recombinant DNA in this study. *Saccharomyces cerevisiae* CEN.PK113-5D (named C5D) was used as the host strain for genetic engineering. The Luria–Bertani (LB) medium plate used for selecting positive *E. coli* was made of 0.5% (w/v) yeast extract, 1% (w/v) tryptone, 1% (w/v) sodium chloride, 1.5% (w/v) agar, 100 μg/mL ampicillin, 40 μg/mL 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) and 1 mM isopropyl–D-thiogalactopyranoside (IPTG). The Luria–Bertani (LB) medium used for amplifying *E. coli* was made of 0.5% (w/v) yeast extract, 1% (w/v) tryptone, 1% (w/v) sodium chloride and 100 μg/mL ampicillin. The recombinant yeast strain was chosen on the SC minimal medium containing 0.67% (w/v) yeast nitrogen base (YNB), 2% (w/v) glucose, 0.01% (w/v) (adenine, arginine, cysteine, leuine, lysine, threonine, tryptophan), 0.005% (w/v) (aspartic acid, histidine, isoleucine, methionine, phenylalanine, proline, serine, tyrosine, valine) and 2% (w/v) agar. The plasmids used in this study were pYES2 and pMD18-T.

Plasmids Construction

The original plasmid used in this study was pYES2. Firstly, we used the constitutive expression promoter triose-phosphateisomerase promoter (*Tpi*) to replace the inducible promoter *GAL1* in pYES2. Secondly, we inserted the yeast secretion signal peptide gene after *Tpi*. Finally, the alpha factor gene was connected with the 3′ half sequence of yeast agglutinin gene (*AG*) [37]. The structure of the constructed plasmids is shown in Fig. 1, and it was named as pYES2-*Tpi-α-AG*.

The *merR* gene sequence (CP019338.1:1310586-1311020) was downloaded from NCBI GeneBank database, and the sequence was optimized by JCat (http://www.jcat.de/). The *EcoR* I and *Mlu* I sites were added to the sequence after adaptation and then synthesized by Sangon Biotech company (shanghai). At last, the sequence was digested by *EcoR* I and *Mlu* I and then inserted between the alpha factor gene and the 3′ half sequence of yeast agglutinin gene (*AG*) in the modified pYES2 which was also digested by *EcoR* I and *Mlu* I. The obtained cell surface display plasmid was named as pYES2-*Tpi-α-merR-AG* (Fig. 2), and the *merR* gene was confirmed in the right site through sequencing with the Sanger dideoxynucleotide chain termination method [38]. The successful integration plasmid for displaying MerR (pYES2-*Tpi-α-merR-AG*) and the integration plasmid (pYES2-*Tpi-α-AG*) without MerR-encoding sequence were transferred into the *S. cerevisiae* similarly.

Transformation of *Saccharomyces cerevisiae*

The electrotransformation method [39] was used to transform the aforementioned integration plasmids to the *S. cerevisiae* competent cells. The competent *S. cerevisiae* cells were obtained through dealing with lithium acetate (LiAc) and dithiothreitol (DTT) [40]. The transformants were cultured on the selective SC medium plate and cultivated at 30 °C until the colony appeared. The yeast strains were named C5D-C and C5D-MerR, respectively.

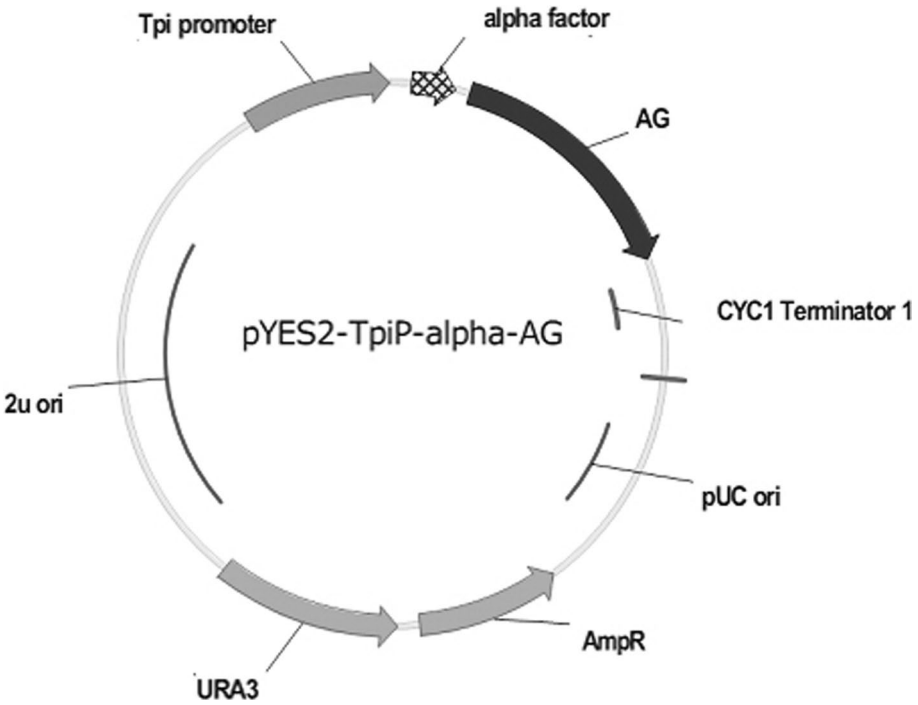

Fig. 1 Structure of the plasmid pYES2-*Tpi-α-AG*

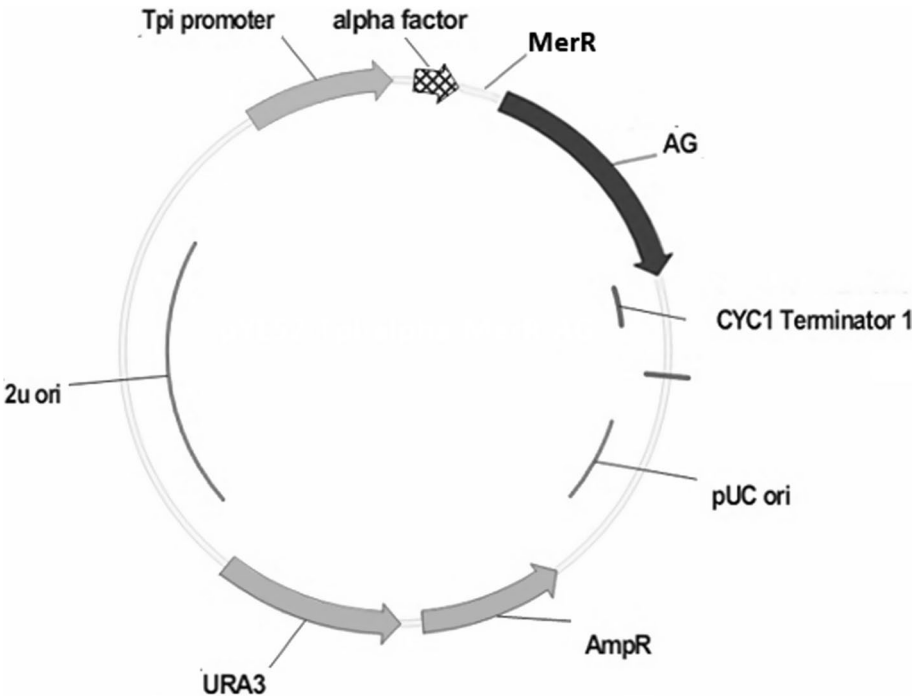

Fig. 2 Structure of the plasmid pYES2-*Tpi-α-merR-AG*

Colony PCR

The colony PCR was conducted based on the transformants according to the following procedures: 94 °C 5 min, 94 °C 30 s, 58 °C 40 s, 72 °C 45 s and 72 °C 10 min. The primers used were *merR*F (5′ GAATTCATGGAAAACAACT3′) and *merR*R (5′ACGCGTCTGTGGTGGTGGT3′).

Hg^{2+} Adsorption by MerR-Displaying Yeast Cells

Firstly, the yeasts were grown up to stationery growth phase before adsorption experiment. The yeast cells were harvested with the method of centrifugation and then washed with 50 mM HEPES (pH 7.0). The cells were put in 20 mg/L $HgCl_2$ solution after washing and incubated for 2 h. After adsorption, the yeast cells were harvested and washed again. The weight of the yeast cells was calculated after lyophilizing for 24 h. The final concentration of Hg^{2+} in the rest supernatant was measured directly with the method of hydride generation atomic absorption spectrometry. We did six parallel experiments for each type of yeast strain.

Hg^{2+} Adsorption Under Different pH

The Hg^{2+} solution with pH levels between 4 and 8 was prepared before adsorption experiment. The yeasts cells were obtained through the method described in the "Hg^{2+} adsorption by MerR-displaying yeast cells" section. The adsorption experiment procedure was the same as the "Hg^{2+} adsorption by MerR-displaying yeast cells" section. The Hg^{2+} concentration was measured by the hydride generation atomic absorption spectrometry method. Six parallel experiments were conducted for each type of yeast strain.

Effect of Cu^{2+} and Cd^{2+} on the Adsorption for Hg^{2+}

The solution (pH 7.0) with coexistence of Cu^{2+}, Cd^{2+} and Hg^{2+} was prepared before adsorption experiment. The yeast cells were collected, and the adsorption experiments were conducted according to the methods described in the "Hg^{2+} adsorption by MerR-displaying yeast cells" section. Six parallel experiments were conducted for each type of yeast strain.

The Ultra-Trace Adsorption Ability of Hg^{2+} by MerR-Displaying Yeast Cells

The yeast cells were harvested, and the adsorption experiment was conducted in 100 μg/L Hg^{2+} solution (pH 7.0). The rest method is similar to the "Hg^{2+} adsorption by MerR-displaying yeast cells" section. The Hg^{2+} concentration was measured by hydride generation atomic absorption spectrometry method. Six parallel experiments were conducted for each type of yeast strain.

Growth in Hg^{2+}-Containing Medium

The yeast strains were harvested at stationary phase and diluted by the SC medium (the final culture broth at 600 nm was 0.2) whose Hg^{2+} concentration was 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 mg/L, respectively. Then, they were cultivated at 30 °C and 160 rpm in shaking incubator. The cell growth condition was measured through the light absorbance value of the culture broth at 600 nm by NanoDrop 2000C Spectrophotometer. The experiment was conducted in triplicate.

Results

Plasmid Construction and Transformant Confirmation

The plasmid pYES2-*Tpi-α-merR-AG* constructed above was used for displaying MerR. The control plasmid lacking of *merR* was pYES2-*Tpi-α-AG*. The two types of plasmids were confirmed by sequencing, and the *Tpi* promoter, yeast secretion signal alpha factor, 3′ half sequence of anchoring protein-encoding gene *AG* and MerR-encoding gene were all in the right site of the plasmid (Figs. 1, 2).

The plasmids pYES2-*Tpi-α-merR-AG* and pYES2-*Tpi-α-AG* were transformed to *S. cerevisiae* through electrotransformation, respectively. The positive colony was reconfirmed by PCR (Fig. 3). The enlargement culturing of correct positive colony was carried out in SC medium and can be used for the next experiment.

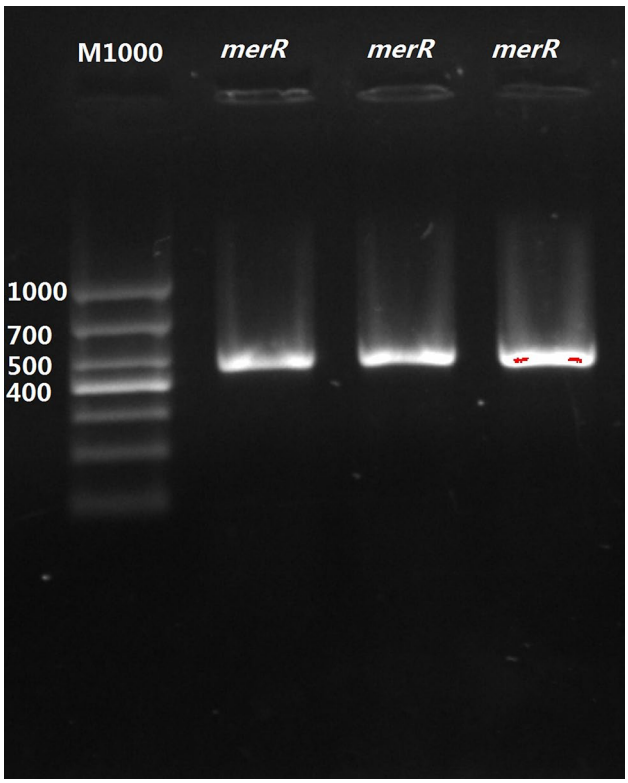

Fig. 3 Colony PCR of transformed yeast strain C5D-MerR

Table 1 Adsorption of Hg^{2+} by different types of yeast

Yeast strains	Hg^{2+} adsorption ability [mg/(g dry weight)]
C5D	3.06 ± 0.06
C5D-CT	3.13 ± 0.04
C5D-Mer R	8.42 ± 0.52

Adsorption ability of Hg^{2+} by Different Yeast Strains

The adsorption ability for Hg^{2+} by the three yeast strains (C5D, C5D-C and C5D-MerR) was tested in order to verify the effect of MerR display on the cell surface. The result showed that the display of MerR on the cell surface enhanced its adsorption ability for Hg^{2+} (Table 1). The enhancement of this ability indirectly indicted that the MerR was successfully displayed on the cell surface of *S. cerevisiae,* and the MerR protein was also functional as an Hg^{2+} chelator. The engineered *S. cerevisiae* that displaying MerR could adsorb Hg^{2+} effectively and could be used to purify Hg^{2+}-polluted water.

Hg^{2+} Tolerance Ability of Different Yeast Strains

The experiment showed that the Hg^{2+} tolerance ability of C5D-MerR was stronger than that of C5D and C5D-C. C5D and C5D-C could not grow in solution with Hg^{2+} of 40 mg/L, while the engineered yeast strain C5D-MerR could grow in solution not exceed 100 mg/L of Hg^{2+} (Fig. 4). The stronger tolerance ability might suggest that most of the Hg^{2+} was combined by MerR on the cell surface and little Hg^{2+} flowed into the cell. This character of the engineered yeast makes it can be used in purifying polluted water with high concentration of Hg^{2+}.

The Influence of pH on the Adsorption Ability for Hg^{2+} by Different Yeast Strains

The pH influence experiment indicted that the C5D-MerR could effectively adsorb Hg^{2+} under pH levels between 4 and 8. The influence of pH on the adsorption ability of C5D-MerR was lower than that of C5D and C5D-C. The highest adsorption ability appeared when the pH was about 7 (Fig. 5). The results of the experiment suggested that the C5D-MerR could be used to purify Hg^{2+}-polluted water with wide range of pH levels. This might be very useful for purifying Hg^{2+}-polluted water.

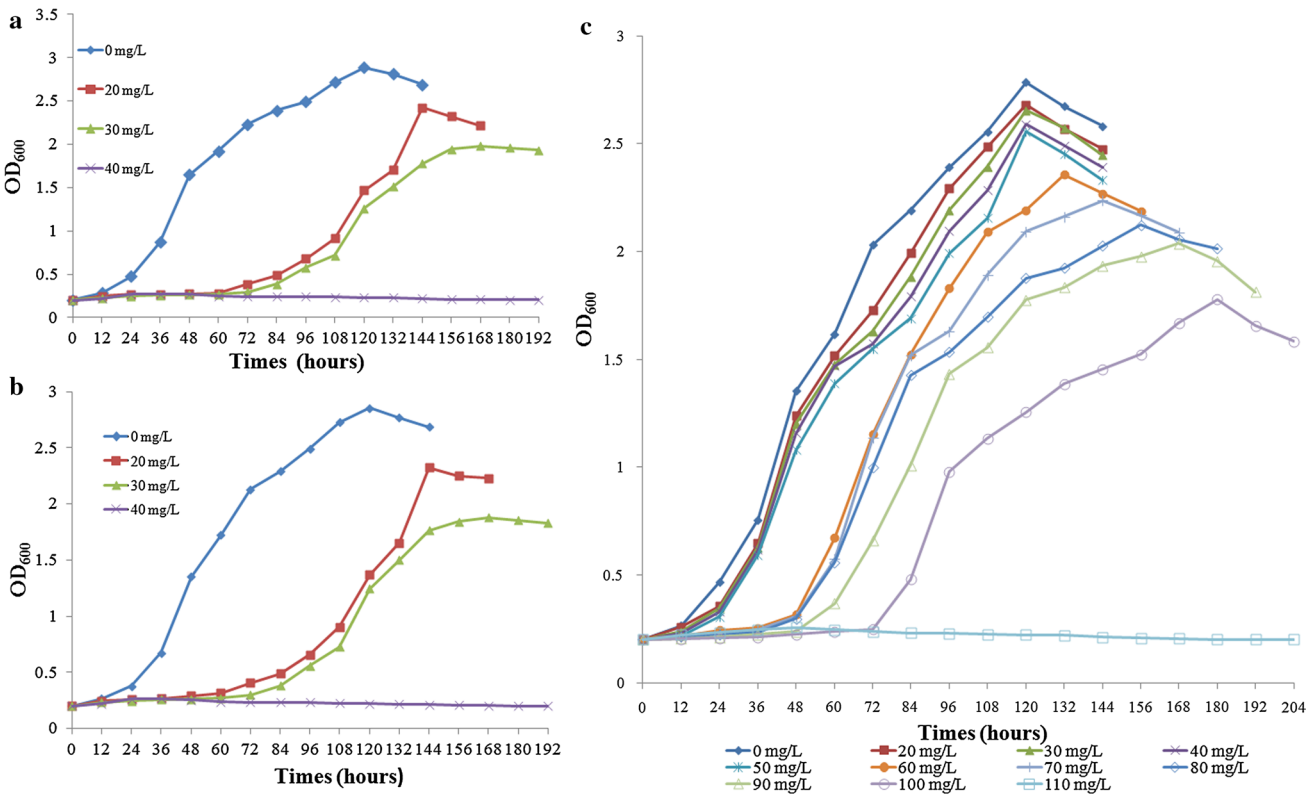

Fig. 4 Yeast strains' growth condition in different Hg^{2+} solutions. **a** C5D; **b** C5D-CT; **c** C5D-MerR

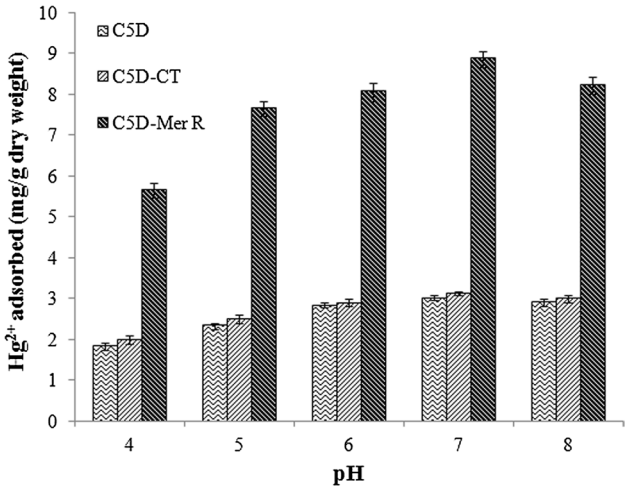

Fig. 5 pH influence on the adsorption ability for Hg^{2+} of different yeast strains. Error bars represented the standard deviation of the experiment

The Adsorption Ability for Hg^{2+} of Different Yeast Strains with Coexistence of Cu^{2+} and Cd^{2+}

The coexistence of heavy metal ions probably has effect on the cells' adsorption ability. The result of the experiment also supported our hypothesis. When the concentration of Cu^{2+} and Cd^{2+} equals to 20 mg/L, the adsorption ability of C5D-MerR for Hg^{2+} decreased 5.04%, while the adsorption ability of C5D and the C5D-C decreased 62.46 and 62.62%, respectively (Fig. 6). We inferred that the C5D-MerR could selectively adsorb Hg^{2+} with the coexistence

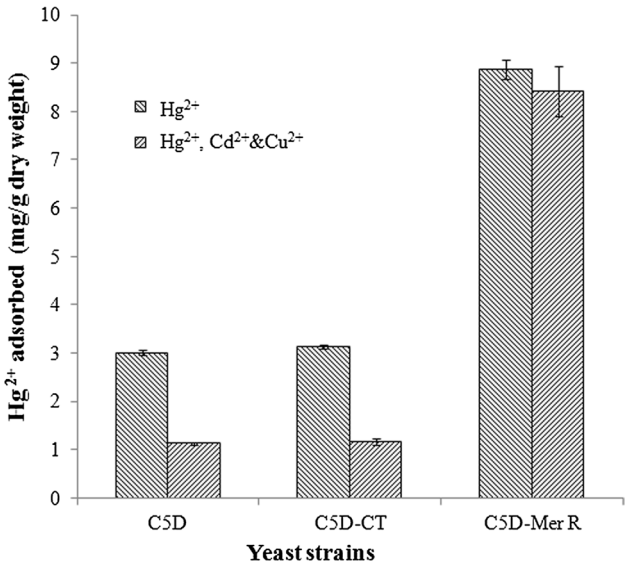

Fig. 6 Adsorption ability for Hg^{2+} of different yeast strains with coexistence of Cu^{2+} and Cd^{2+}. Error bars represented the standard deviation of the experiment

of Cu^{2+} and Cd^{2+}. Therefore, the C5D-MerR could be used to purify water that is polluted by different kinds of heavy metal ions simultaneously.

Adsorption Ability of Ultra-trace Hg^{2+} by Different Yeast Strains

The display of MerR exactly enhanced the adsorption ability of C5D-MerR for ultra-trace Hg^{2+}. We found that the adsorption ability of C5D-MerR was stronger than that of C5D and C5D-C (Table 2). It demonstrated that the C5D-MerR could adsorb the Hg^{2+} in the water much more thoroughly than that of C5D and C5D-C did. And we could obtain pure water at accepted level after its handling.

Discussion

Cell surface display system can display the metal binding protein on the cell surface. The cell surface-displayed engineered microbe cells have many advantages when compared with other engineered microbes that have to adsorb metal ions into cells. Firstly, the cells with surface metal binding protein on its surface can adsorb metal ions onto the cell surface rather than into the cell, which can alleviate their toxicity to the cell [41, 42]. On the other hand, the recovery of the metal ions from cell surface was much easier than that from the inside of the cell. We can easily recover the metal ions from the cell surface without disintegrating the cells, while the cells must be damaged when you recover metal ions adsorbed into the cells [17]. Therefore, the recovery of metal ions adsorbed on the cell surface is convenient and economic, which is very important in the practical application for purifying polluted water and recovering metal ions from the bioadsorbents. In contrast, it is not convenient and economic to recover heavy metal ions from intracellular bioremediation adsorbents [43]. Furthermore, the cell surface-engineered adsorbents can be reused because the cells did not damage during the adsorbing and recovering processes [33, 44]. We supposed that the cell surface-engineered yeast cells could be used to adsorb, recover and recycle of metal ions.

Table 2 Adsorption of ultra-trace Hg^{2+} by different types of yeast

Yeast strains	Original Hg^{2+} concentration (μg/L)	Yeast cells concentration (g/L)	Adsorption ratio (%)
C5D	100	10	2.90
C5D-CT	100	10	6.80
C5D-Mer R	100	10	97.15

The display of MerR on the yeast cell surface enhanced its adsorption ability for Hg^{2+}. This result and the colony PCR experiment suggested that MerR was expressed and successfully displayed on the cell surface of *S. cerevisiae.* The MerR displayed on the cell surface was active and could bind Hg^{2+} effectively. Our results showed that the engineered C5D-MerR could adsorb almost three times Hg^{2+} as much as that of C5D and C5D-C (Table 1). The enhancement of the adsorption ability makes C5D-MerR a potential bioadsorbents to purify Hg^{2+}-polluted water.

The tolerance ability for Hg^{2+} of C5D-MerR was much stronger than that of C5D and C5D-C. The C5D-MerR could be cultured in medium with Hg^{2+} under the concentration of 100 mg/L, while the C5D and C5D-C's growth could be inhibited by Hg^{2+} that is excess of 30 mg/L. The resistance character to heavy metal ions of C5D-MerR also found in other cell surface-displayed engineered yeast cells [45]. The strong resistance ability of C5D-MerR for Hg^{2+} mainly thanks to the successful display of MerR on its surface, which can inhibit the Hg^{2+} permeating into its cytosol. Therefore, it is a feasible way to enhance the resistance ability of yeast cells by displaying metal binding protein on their surface. On the other hand, the cell division period was lengthened by Hg^{2+} in the medium when compared with the cells growth in the medium without Hg^{2+}. However, the reason for this phenomenon is still unclear.

The pH levels influenced C5D-MerR less than that of C5D and C5D-C. The C5D-MerR has the highest adsorption ability at about pH 7, but it can effectively adsorb Hg^{2+} at a wide range of pH levels, which will be very useful in treating different types of water with different pH levels (Fig. 5). The mechanism for the relative stability of the C5D-MerR at a wide range of pH levels still needs much more investigation.

The coexistence of heavy metal ions Cu^{2+} and Cd^{2+} seriously affected the adsorption ability of C5D and C5D-C for Hg^{2+}. This indicted that these two yeast strains could not selectively adsorb Hg^{2+} from water polluted by Hg^{2+}, Cu^{2+} and Cd^{2+} at the same time. On the other hand, the adsorption ability of C5D-MerR was almost not influenced by the coexistence of Cu^{2+} and Cd^{2+}. This result demonstrated that C5D-MerR had higher affinity and selectivity toward Hg^{2+} than C5D and C5D-C. The MerR displayed on the cell surface was functional and could be used as an effective binder for Hg^{2+}. The engineered yeast strain C5D-MerR can be used to adsorb Hg^{2+} from mixed heavy metals ions, and this character will be very useful in the practical application.

The adsorption ability for ultra-trace Hg^{2+} of C5D-MerR was much stronger than that of C5D and C5D-C. We predicted that this enhancement was mainly attributed to the MerR displayed on the cell surface because it could easily detect the ultra-trace Hg^{2+} in the environment. The C5D and C5D-C do not have displayed protein on their surface, so they have much difficult to detect ultra-trace Hg^{2+} in the water.

The mechanisms of microorganism–metal interreaction were classified into active and passive types [46]. The active type adsorbs heavy metal ions by energy-dependent metabolism, while the other type is likely to adsorb heavy metal ions with surface binding mechanism without energy consuming [47]. Therefore, we inferred that the engineered C5D-MerR constructed in this study could be used as a functional bioadsorbents for Hg^{2+} under both living and non-living conductions.

The function of the engineered yeast mainly depends on the character of the protein displayed on the cell surface. Therefore, finding more proteins that have high affinity, capacity and selectivity for certain heavy metal ions should be the future research focus.

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Compliance with ethical standards

Conflict of interest All of the authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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