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Research Article

Mechanism of Cr (VI) reduction by *Pichia guilliermondii* ZJH-1

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Background: Chromium is one of the most used toxic heavy metals. A large amount of chromium waste is discharged into the environment every year, causing serious environmental pollution, especially the pollution of soil and water by hexavalent chromium. Eliminating hexavalent chromium is the primary challenge to achieve a pollution-free environment.
Objectives: This study aims to understand the mechanism of *Pichia guilliermondii*'s reduction of hexavalent chromium through enzymatic characteristic, oxidative stress response, and reduction product.
Material and Methods: The strain *Pichia guilliermondii* ZJH-1 was isolated and stored in our laboratory. The hexavalent chromium uses 1,5-diphenyl carbazide method (DPC) to measure. The UV spectrophotometer was used to measure the intracellular antioxidant enzyme activity, and the kit was used to measure the activity of catalase and glutathione reductase. The reduction products were analyzed by ultraviolet full-wavelength scanning and FTIR.
Results: The reduction of hexavalent chromium by ZJH-1 is accompanied by an increase in active oxygen and antioxidant levels. Chromate reductase mainly exists in the extracellular fluid, and the carboxyl, amide, hydroxide and other groups of the cell wall are involved in the bioremediation of Cr(VI) by complexing with Cr(VI) and Cr(III). After ZJH-1 was treated with different concentrations of Cr(VI), the expression of proteins with molecular weights of 15 kDa, 18 kDa, 35 kDa, 62 kDa, and 115 kDa increased significantly. This strain is the most suitable for chromate reductase (CChR). The optimum temperature is 40℃ and the optimum pH is 7.0. Cu^{2+} can enhance the activity of chromate reductase. At the optimum temperature and pH, the chromate reductase Km of this strain is 0.40 μmol and Vmax is 14.47 $\mu moL.L^{-1}\cdot min^{-1}$.
Conclusions: The bioremediation of Cr(VI) by *Pichia guilliermondii* ZJH-1 is attributable to the reduction product (Cr(III)) that can be removed in the precipitate and can be fixed on the cell surface and accumulated in the cell.

Keywords: Cr(VI); Chromate reductase; Enzyme dynamic curve; *Pichia guilliermondii*

1. Background

Chromium (Cr) is a toxic heavy metal widely distributed in the natural environment and exists in the form of oxides formed in different valence states. Due to the special characteristics of physics, chemistry and biology, the solubility of chromium in water is very high, and the most stable forms are Cr(VI) and Cr(III) (1). Both Cr(VI) and Cr(III) are harmful to human health, but the toxicity of Cr(VI) is about 100 times higher than that of Cr(III). Cr(VI) is a carcinogen of biological organisms and organs(2). The Cr(III) in the water body is mainly adsorbed on the solid matter and exists in the sediment, thereby purifying the water body. In addition, Cr(III) is considered to be a trace

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element essential to the normal operation of organisms, and low concentrations of Cr(III) are involved in the regulation and metabolism of insulin. Therefore, the elimination of chromium pollution in the environment can concentrate the reduction of highly toxic and easily soluble Cr(VI) into low-toxicity and insoluble Cr(III)(3).

In nature, organisms and toxic substances form a specific mechanism to help them resist toxic effects, so that they can live in a toxic environment(4). Studies have proven that using Cr(VI)-tolerant microbial species for bioremediation has a significant effect on the treatment of Cr(VI) pollution. There are two descriptions about the mechanism of microorganisms tolerating Cr(VI): (1) Some chromium ions flow out from the cytoplasm and are reduced to Cr(III). (2) Detoxification by free radicals, repair of DNA damage and enzymes involved in sulfur or iron metabolism are considered to be the key to the mechanism of microbial tolerance to Cr(VI). For example, chromium ions in Saccharomyces cerevisiae can tolerate Cr(VI) by affecting mitochondrial function, superoxide dismutase activity, protein oxidation and production(5). The enzymatic reduction reaction of chromium has been mainly studied in bacterial cells. The enzyme is related to soluble NAD(P)H- or NADH-dependent reductase and can transfer electrons to Cr(VI)(6). Martorell et al.(7) have demonstrated that the main resistance mechanism of yeast M9 and M10 is the reduction of Cr(VI) to Cr(III).

Yeast is becoming more and more popular in dealing with Cr(VI) water pollution. As a fungus, yeast can be cheaply and easily produced on a large scale from an industrial fermentation process(8, 9). The removal of metal ions from aqueous solutions by microorganisms usually involves an integrated mechanism of bioaccumulation, biosorption and reduction processes(10).

Although there have been many studies on the removal of Cr(VI) by Pichia gillis, the exact mechanism of its reduction of Cr(VI) to Cr(III) is still unclear. In addition, many studies have explained the oxidative stress induced by various heavy metals in plants, algae and bacteria. However, few literatures describe Cr(VI)-induced oxidative stress in Pichia pastoris. The purpose of this study is to study the mechanism of ZJH-1 reducing Cr(VI)(11).

2. Objective

This study aims to research the mechanism of *Pichia guilliermondii*'s reduction of hexavalent chromium through enzymatic characteristic, oxidative stress response, and reduction product.

3. Materials and Methods

3.1. Reagents and Cultivation of Microorganism

All chemicals used were analytically pure grade throughout this study and used without further purification.

The strain *Pichia guilliermondii* ZJH-1 was screened from the bacterial solution of *Plodia interpunctella* (Hübener) intestinal fluid treated with polyethylene film for 60 days and was isolated and stored in the laboratory of Northeast Forestry University of China. Details are presented in Supplementary Information. The cultures were grown in sterilized yeast extract peptone dextrose dedium (YPD) and yeast nitrogen base (YNB) liquid medium. GENBANK accession number is MT376978. The source of chromium is $K_2Cr_2O_7$, Cr (VI) was measured spectrophotometrically using 1,5-diphenyl carbazide method(12).

*3.2. Effect of Cr (*VI*) on ZJH-1 Antioxidant System*

Add 100 mL of culture medium to a 250 mL Erlenmeyer flask and add Cr (VI) to a final concentration of 0 $mg.L^{-1}$, 20 $mg.L^{-1}$, 40 $mg.L^{-1}$, 60 $mg.L^{-1}$, 80 $mg.L^{-1}$, and 100 $mg.L^{-1}$, 2% of the inoculum. Incubate in an incubator at 40 °C and 125 rpm for 48 hours for testing in the following experiments.

3.2.1. Detection of ROS in ZJH-1 Cells

The detection of intracellular ROS refers to Feng's *et al.*(13) method for detection.

3.2.2. Determination of Antioxidant Enzyme Activity in ZJH-1 Cells

The determination of T-SOD, POD，CAT，GR activities was conducted according to the instructions of total superoxide dismutase assay kit, peroxidase kit, catalase assay kit and glutathione reductase kit, respectively. All these kits are provided by SuZhou Grace Biotechnology Co.Ltd, China.

3.2.3. Determination of Malondialdehyde Content in ZJH-1 Cells

The content of MDA was determined by utilizing thiobarbituric acid method (14).

3.3 Analysis of Reduction Products

Ultraviolet full-wavelength scanning analysis: YNB was used as the culture medium, Cr (VI) concentration was 20 mg. L^{-1}, 8% inoculation amount, and the culture was performed at 40 °C with constant temperature shaking. Every 12 h, centrifuge at 5000 rpm for 10min at 4 °C and take the supernatant for full-wavelength scanning analysis under a UV spectrophotometer. Distilled water was blank, and no Cr (VI) was added as a negative control.

FTIR analysis: YNB was used as the medium, Cr (VI) concentration was 50 mg. L^{-1}, 8% inoculum, cultured at 40 °C for 48 h. Then refer to Gu's *et al*.(10) method for analysis.

3.4. Cr (VI) Reduction by Resting and Permeable Cells

Cells of ZJH-1 were grown in YPD liquid medium for 24 h and harvested by centrifugation as above. Resting and permeable cells were prepared according to Gu *et al*(10) method.

3.5. Chromate Reductase Localization

Intracellular fluid, cell membrane debris and extracellular fluid were used to reduce Cr (VI). Refer to Gu's et al(10) method to prepare Supernatant metabolites (A1), Cell-free extract (A2) and cell debris (A3).

3.6. Preparation of Chromate Reductase

8% of the inoculum was inoculated into YPD liquid medium, and the supernatant protein concentration was tested every 24 h. Determine the optimal extraction time for Chromate Reductase (CChR). After centrifugation at 5000 rmp for 10 min under the conditions of the optimal extraction time, the supernatant was obtained. The obtained supernatant was filtered through a 0.22 μm filter, and finally the filtrate was concentrated in an ultrafiltration centrifuge tube with a cut-off molecular weight of 10 kDa, and the crude chromate reductase was obtained by lyophilization at -40 °C for subsequent experiments.

3.7. Effects of Different Environmental Factors on Chromate Reductase Activity

3.7.1. Temperature

Investigate the change of CChR activity at 25-45 °C. The reaction system was 0.2 g enzyme powder, 1 mL of 10 mg. L^{-1} Cr (VI), 2 mL of PBS (pH 7.0), and 1 mL of NADH (0.2 mM). The reaction time is 30min, and the activity of CChR is judged by measuring the content of Cr(VI), the same below.

3.7.2. PH

The Britton-Robinson(B-R) buffer solution was prepared at different pH values, and the change of CChR activity was investigated at pH 3.0-11.0. The reaction system was 0.2 g enzyme powder, 1 mL of Cr (VI), and 2 mL of B-R and 1 mL NADH, the reaction was performed at the optimal temperature for 30 min.

3.7.3. Metal Ion

The effects of Pb (Ⅱ), Cu (Ⅱ), Mn (Ⅱ), Co (Ⅱ), Ag (Ⅰ), Ni (Ⅱ), Cd (Ⅱ) and Hg (Ⅱ) metal ions on the activity of CChR were investigated. The reaction system was 0.2 g enzyme powder, 1 mL of Cr (VI), 1 mL of Britton-Robinson buffer, 1 mL of NADH, and 1 mL of 10 mg. L^{-1} of different heavy metal ions. The reaction was performed at the optimal temperature for 30 min.

3.8. Kinetic Study of Chromate Reductase

Using Cr (VI) as the substrate to determine the kinetic parameters: take 0.2 g of crude enzyme powder, add 2 mL Britton-Robinson buffer, then add 1mL (2-10 mg. L^{-1}) Cr (VI), and finally add 1mLNADH, the reaction system totaled 4 mL. After 30 minutes of reaction at the optimal temperature, the reaction was stopped on ice. Cr (VI) content was measured at OD540 nm. Each group was repeated three times.

3.9. Statistical Analysis

The results shown in the figure represent the average of three independent replicates. Data are expressed as mean ± standard deviation. Statistical analysis was performed using IBM SPSS Statistics software to evaluate the statistical differences between treatments at $p<0.05$ significance level.

4. Results

4.1. Cr (VI) Induces ROS Production in ZJH-1 Cells

In this study, as the concentration of Cr (VI) increased, the fluorescence intensity gradually increased (**Fig. 1**). The stronger the fluorescence intensity, the higher the content of reactive oxygen species in the cell. It is shown

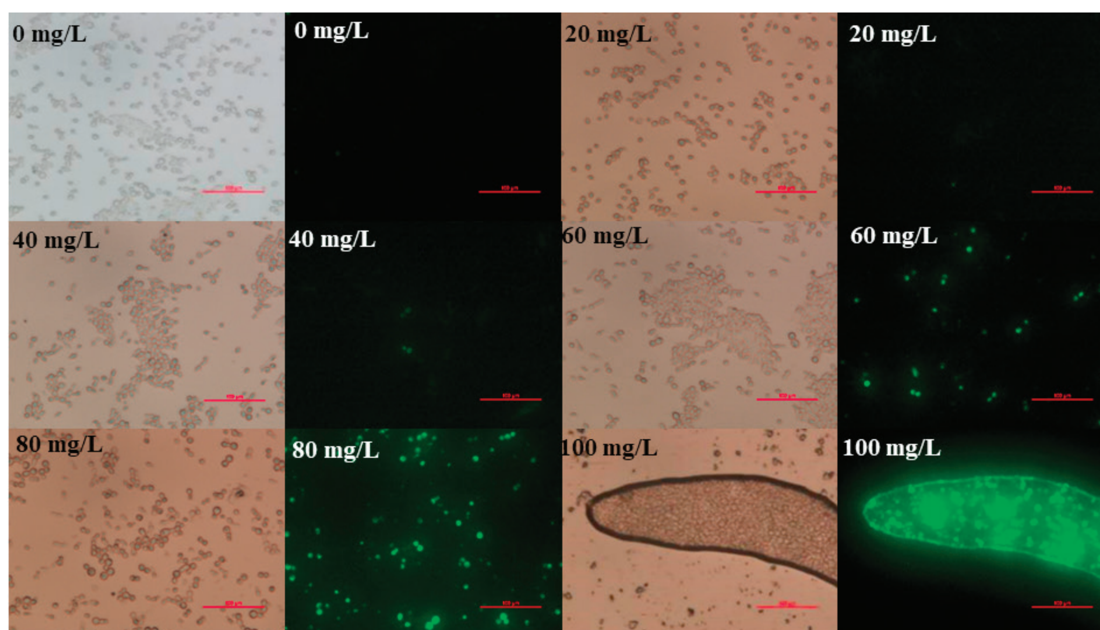

Figure 1. ROS fluorescence images after treatment of ZJH-1 with different concentrations of Cr (VI)

that the addition of Cr (VI) can induce the production of ROS in ZJH-1 cells. At the same time, it can be seen from the figure that when the Cr (VI) concentration is 40 mg. L^{-1}, fluorescence begins to appear in ZJH-1 cells, that is, the minimum concentration of Cr (VI) that induces ROS formation in ZJH-1 cells is 40 mg. L^{-1}.

4.2. Cr (VI) -Induced Changes in Antioxidant Enzyme Activity in ZJH-1 Cells

As shown in **Figure 2A**, the curve of Cr (VI) concentration and T-SOD activity is bell-shaped. When the Cr (VI) concentration range is 0-40 mg. L^{-1}, the activity of T-SOD increases with the increase of Cr (VI) concentration; when the concentration of Cr (VI) is in the range of 40-100 mg. L^{-1}, the activity of T-SOD decreases with the increase of Cr (VI) concentration.

The change in POD **Figure 2B** activity is like the change in T-SOD activity. With the increase of Cr (VI) concentration (0-40 mg. L^{-1}), POD activity increased, and then the POD activity decreased in the range of Cr (VI) concentration of 40-100 mg. L^{-1}.

CAT activity is shown in **Figure 2C**. With the increase of Cr (VI) concentration (0-40 mg. L^{-1}), the CAT activity was significantly enhanced. When the concentration of Cr (VI) is in the range of 80-100 mg. L^{-1}, the CAT activity decreases.

The GR activity is shown in **Figure 2D**. With the increase of Cr (VI) concentration (0-60 mg. L^{-1}), the GR activity is significantly enhanced; when the Cr (VI) concentration range is 60-80 mg. L^{-1}, the GR activity decreased; GR activity increased when Cr (VI) concentration range was 80-100 mg. L^{-1}.

4.3. Cr (VI) -Induced Changes in MDA Content in ZJH-1 Cells

Compared to the control group, the presence of Cr (VI) resulted in a significant increase in MDA content (**Fig. 2E**). When the Cr (VI) concentration range was 20-80 mg. L^{-1}, there was no significant difference in MDA content ($p>0.05$). When the Cr (VI) concentration was 100 mg. L^{-1}, the maximum MDA content was 4.84 nmoL.g^{-1}.

4.4. Analysis of Reduction Products

Figure 3A-3C shows the change in solution color during the reduction of Cr (VI) by ZJH-1. Compared with the control group, the color of the solution changed from yellow to green due to the addition of ZJH-1, and the color of the bacteria also changed to green.

It can be seen from **Figure 3D** that chromium peaks appear at 280 nm, 380 nm, and 390 nm; at 48h Figure 3e, the chromium peak is weakened by ZJH-1 treatment; at 120 h Figure 3f, the chromium peak disappears.

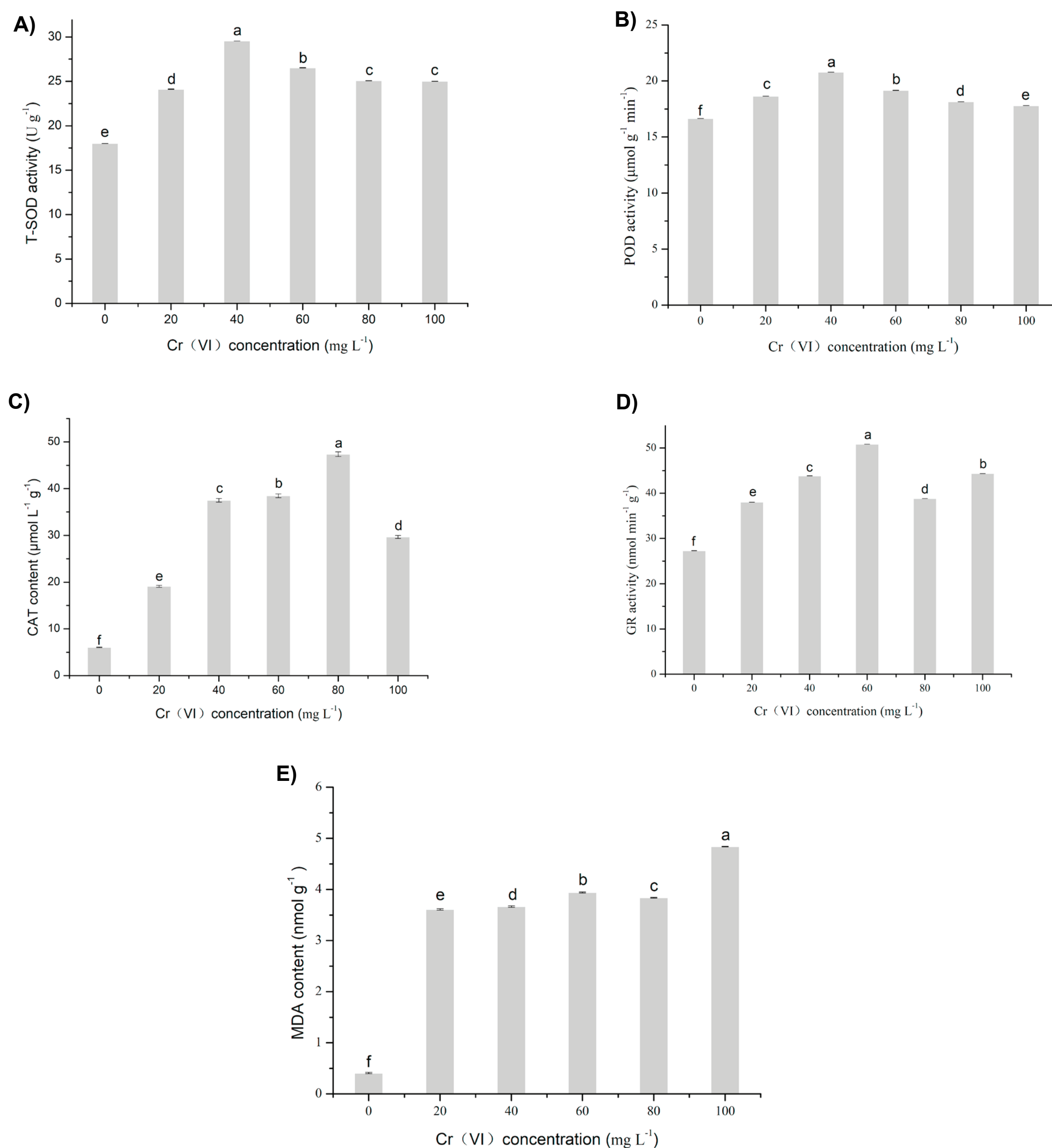

Figure 2. Cr(VI) induces the changes of antioxidant enzyme activity and MDA content in ZJH-1 cells. **A)** T-SOD activity; **B)** POD activity; **C)** CAT activity; **D)** GR activity; **E)** MDA content.
Note: Different lowercase letters represent significant differences in enzyme activity between different groups ($p<0.05$), the same below.

4.5. FTIR Analysis

Compared with the control spectrum, the offset at 3305 cm^{-1}, the peak enhancement at 3280 cm^{-1}, and the appearance of 1740 cm^{-1} indicate that the hydroxyl and carboxyl groups help cell adsorption; the peaks at 2363cm^{-1} and 1313cm^{-1} decreased significantly, indicating that the -NH and -CN groups of the protein are involved in the binding of chromium (VI); The decrease in peak intensity at 1035 cm^{-1} indicates that $-PO_4^{3-}$ composed of polysaccharides is involved in Cr (VI) binding; The shrinkage of the peak intensities at 1647 cm^{-1} and 1546 cm^{-1} indicates that amide I and amide II are involved in Cr (VI) binding in some way. Details are presented in Supplementary Information.

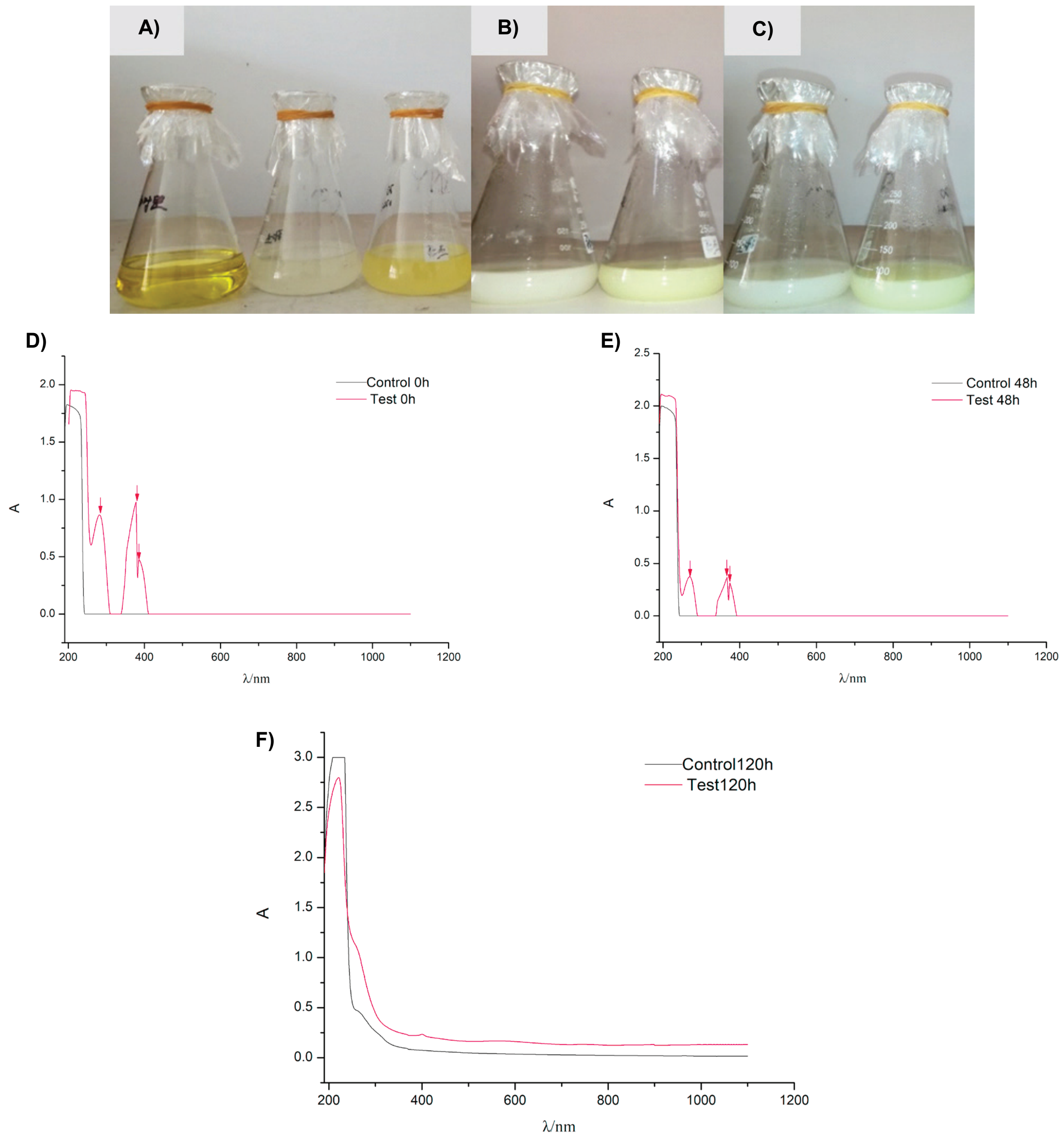

Figure 3. The color change of the solution during the reduction of Cr(VI) by ZJH-1 and the ultraviolet full-wavelength scanning of extracellular fluid. **A)** 0 h, from left to right, the control group, -Cr, +Cr; **B)** 48 h, from left to right, -Cr, +Cr; **C)** 120 h, from left to right, -Cr, +Cr; **D)** 0 h; **E)** 48 h; **F)** 120 h.

4.6. Characterization of Reduction of Cr (VI) by ZJH-1 Cells

The experiment showed that the reduction rate of 50 $mg \cdot L^{-1}$ Cr (VI) by resting cells reached 56.7%, while the reduction rate of Cr (VI) by permeable cells was 60.5%. Within 6 hours, the reduction rate of Cr (VI) of A1 reached 70%. The results show that the reduction of Cr (VI) by ZJH-1 mainly depends on A1. Unlike most fungus, the CChR activity of ZJH-1 is related to supernatant metabolites. The reduction rate of Cr (VI) by A2 is 10%, indicating that some substances in the cells can reduce a small amount of Cr (VI). The reduction rate of Cr (VI) by A3 is only 3.9%, which

indicates that cell debris cannot directly reduce Cr (VI), and the reduction rate of 3.9% may be caused by physical adsorption. It is worth mentioning that after NADH is added, the reduction rate of A1 to Cr (VI) reaches 100%, and the reduction rate of A2 to Cr (VI) is 15%, which is an increase of 5% compared with that without NADH. The reduction rate of Cr (VI) by A3 is only 3% higher than that without NADH. Details are presented in Supplementary Information.

4.7. Preparation of Chromate Reductase

Figure 4A shows the changes in protein content of ZJH-1 treated and untreated Cr (VI) extracellular fluid. Without the addition of Cr (VI), the extracellular fluid of the strain was less than 48 h, and the extracellular protein level became stable. After 48 h, the extracellular protein content began to decrease, because the strain entered a declining period after 48 h, and the cell viability decline. Under the condition of adding Cr (VI), the protein content of extracellular fluid was the same as that without Cr (VI). At 72 h, Cr (VI) was all reduced. At this time, 50 mg. L^{-1} Cr (VI) was added again, and it was found that the protein content increased sharply. The results indicate that the optimal extraction time for extracellular proteins is 48 h, and that Cr (VI) induces overexpression of a substance.

ZJH-1 treated, and untreated Cr (VI) extracellular fluids were concentrated and lyophilized, and then dissolved in a loading buffer to make the protein concentration of 20 µg. μL^{-1} and loaded. As shown in **Figure 4B**, compared with 1 band (without Cr (VI) added), a new band appears with 2, 3, and 4 (with Cr (VI) added) bands, with a relative molecular weight of 62 kDa and a relative molecular weight of 115 kDa, 35 kDa, 22 kDa, 18 kDa, and 15 kDa protein content increased.

4.8. Effects of Different Environmental Factors on Chromate Reductase Activity

4.8.1. Temperature

Experiments show that the optimal reaction temperature for CChR of this bacterium is 40 °C. Details are presented in Supplementary Information.

4.8.2. PH

To determine the optimal pH value of CChR, the activity of CChR was measured at different pH values using B-R buffer. CChR activity showed two peaks, pH 4.0 and pH 7.0, but the optimal pH of CChR was 7.0. When pH is 6.0-11.0, the enzyme activity is relatively stable. Details are presented in Supplementary Information.

4.8.3. Metal Ion

Experiments on the effect of metal ions on the CChR activity of ZJH-1 show that: Hg (Ⅱ) and Cd (Ⅱ) significantly inhibited the activity of CChR; Ag (Ⅰ),

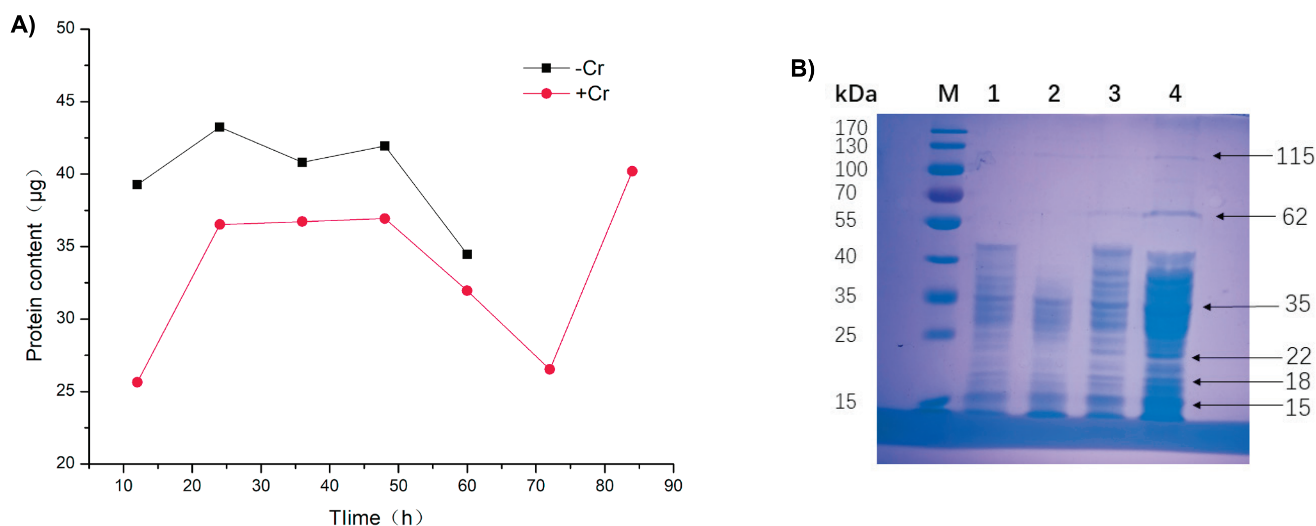

Figure 4. ZJH-1 treatment and untreated Cr (VI) extracellular fluid protein content changes and extracellular fluid SDS-PAGE results. **A)** Changes in protein content; **B)**: SDS-PAGE results, 1: Without Cr (VI); 2: 20 mg. L^{-1} Cr (VI); 3: 40 mg. L^{-1} Cr (VI); 4: 60 mg·L^{-1} Cr (VI)

Pb (Ⅱ), Ni (Ⅱ), Co (Ⅱ) and Mn (Ⅱ) all inhibited CChR, and the relative enzyme activity was 35%-55%; Cu (Ⅱ) significantly enhanced the activity of CChR, and the relative enzyme activity was 100%. Details are presented in Supplementary Information.

4.8.4. Kinetic Study of Chromate Reductase

The reduction kinetics of ZJH-1's CChR complies with the Lineweaver-Burk equation:

$$Y = Km/Vmax\ (x) + 1/Vmax$$

From this, Vmax is 14.47 μm.min^{-1}.mg^{-1} and Km is 0.40 μm·L^{-1}. R^2 is 0.99832, which indicates that the fitted curve has higher reliability. Details are presented in Supplementary Information.

5. Discussion

The reason that Cr (VI) induces intracellular ROS production is mainly because after the Cr (VI) ions enter the cell, the intracellular reduction of Cr (VI) starts to act as a detoxification mechanism (**Fig. 1**). However, bioreducing agents release ROS by reducing Cr (VI) to Cr (V) with a single electron provided by nicotinamide adenine dinucleotide (NADH) (15). Feng *et al*. (13) Reported that when P. *sanguineus* was below 10 mg. L^{-1}, Cr (VI) had no significant effect on active oxygen levels, but when the concentration of Cr (VI) increased from 20 mg. L^{-1} to 40 mg. L^{-1} There is an increasing trend over time. Shao *et al*. (15) Reported that Cr (VI) can induce the formation of ROS in *Bacillus* sp. Cells with a minimum induction concentration of 0.5 mmoL. L^{-1} and inhibit the growth of strains.

The color of ZJH-1 solution changes during the process of reducing Cr(VI) because Cr(VI) is reduced and transformed in the presence of ZJH-1(**Fig. 2**). In order to adapt to this toxic environment, ZJH-1 synthesizes the necessary enzymes needed to accumulate and reduce Cr(VI) to Cr(III). Amina *et al*. (16) reported a similar phenomenon. Due to the reduction effect of ZJH-1 on Cr(VI), the chromium form has changed.Research by Lotlikar *et al*. (17) also reported that the peak of chromium shifted and weakened due to bioreduction. Cr (VI) is a highly oxidizing agent, which is easily converted to Cr (III) in the presence of organic matter, so it may cause peak shifts and intensity changes.

Phospholipids, proteins and polysaccharides, as the main components of fungal cell membranes, play an important role in the adsorption of Cr (VI). Gu *et al*. (10) reported that the reduction rate of aniger's resting cells and permeable cells to 50 mg. L^{-1} Cr (VI) within 4 hours was 42.2% and 46%, respectively. The reduction rates in this study were 56.7% and 60.5%.

The chromate reductase of ZJH-1 is a NADH-dependent reductase. Ontañon *et al*. (6)believe that the main mechanism for Bacillus sp. To repair Cr (VI) pollution is the NADH-dependent chromate reductase secreted by cytosol to reduce Cr (VI) to Cr (Ⅲ). Studies by Suzuki *et al*. (18) show that after NADH is oxidized, the electrons are transferred to chromate reductase, and then the electrons are transferred to Cr (Ⅵ) to reduce it to the intermediate form of Cr (V). Each electron produces Cr (III).

Low molecular weight metallothionein (MTS) (16) is up-regulated in all experimental groups (**Fig. 4**), which is due to the phenomenon of fungal overexpression in response to metal oxidative stress, especially low molecular weight proteins with a relative molecular mass range of <20kda.

Research by Martorell *et a*l. (7) showed that P. *jadinii* M9 had the highest CChR activity at 60 °C and pH 6.0, and P. *anomala* M10 CChR had the highest activity at 50 °C and pH 7.0. The CChR of M9 has the highest chromate reductase activity in the presence of Cu^{2+} or Na^{+}; M10 CChR has the highest activity in the presence of Cu^{2+}. Research by Camargo *et al*. (19) also showed that the presence of Cu^{2+} promoted the activity of reducing Cr (Ⅵ) in cell-free extracts of Bacillus ES29. Cu (Ⅱ) can significantly enhance CChR activity, mainly because copper ions can serve as co-groups of many reductases. Whether as a redox center or as an electron transporter between protein subunits, its main function is related to electron transfer. The Vmax and Km of chromate reductases ChrR, Yief and NfsA using NADPH as electron donors were: 8.8, 5.0 and 0.25 μm·min^{-1}·mg^{-1} and 260, 200 and 36 μm, respectively (20). In this study, Vmax was higher than the identified chromate reductase, and Km was lower than the identified chromate reductase. Therefore, the chromate reductase in this study is more sensitive to Cr (VI) and decreases Cr (VI) faster.

6. Conclusion

Based on the above experiments, the mechanism of ZJH-1 for reducing Cr (VI) is shown in **Figure 5**. The mechanism of ZJH-1 to reduce Cr (VI) can be divided

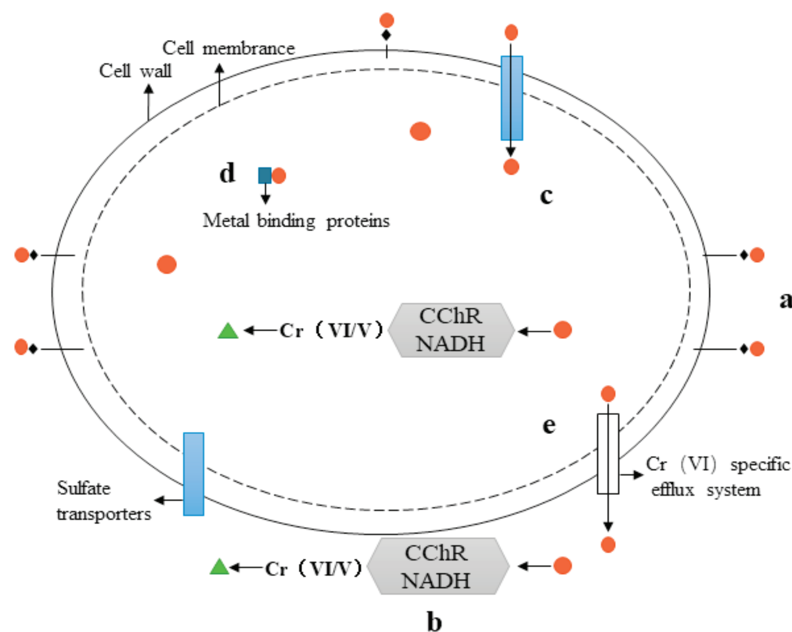

Figure 5. Mechanism of ZJH-1 reduction of Cr (VI). ● is Cr (VI); ▲ is Cr (III); ⬡ is chromate reductase; ◆ is functional group.

into three parts: (1) Cell adsorption. Cr (VI) forms a chemical bond with cell surface functional groups to fix on the cell surface. These functional groups include carboxyl, amide, hydroxide, hydroxyl, and phosphoric acid (**Fig. 5A**). (2) Extracellular reduction. Cr (VI) is reduced to Cr (III) by the action of chromate reductase and NADH to form a highly unstable intermediate Cr (V/IV) (**Fig. 5B**). In addition, some reducing substances in cell secretions can also reduce Cr (VI) in solution to Cr (III), such as reducing substances such as glutathione. (3) Intracellular accumulation. Cr (VI) enters the cell through the uptake system (sulfate transporter) and is further reduced to Cr (III) by the action of intracellular chromate reductase (**Fig. 5C**). A part of Cr (VI) undergoes intracellular translocation of Cr (VI) under the action of metal-binding proteins (**Fig. 5D**), and excess Cr (VI) in the cell is discharged outside the cell through the chromium-specific efflux system for further reduction (**Fig. 5E**).

Supplementary Information

S1 Figure. The phenomenon of the Plodia interpunctella (Hübener) biting PE.
S2 Figure. The SEM image of ZJH-1.
S3 Figure. FT-IR spectra before and after Cr (VI) treatment by ZJH-1.
S4 Figure. Effect of temperature on CChR activity.
S5 Figure. Effect of pH on CChR activity.
S6 Figure. Effect of different metal ions on CChR activity.
S7 Figure. CChR's kinetic curve.
S1 Table. The groups corresponding to each characteristic peak.
S2 Table. Reduction of 50 mg·L-1 Cr (VI) by various components of cells.

Conflict of interests

The authors have no conflicting interests, and all authors have approved the manuscript and agree with submission of its final version.

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