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Chromium (VI) tolerance and bioaccumulation by *Candida tropicalis* isolated from textile wastewater

Sidra Ilyas[1], Dilara A. Bukhari[2] and Abdul Rehman[1*]

Abstract

In the present study a yeast strain isolated from industrial wastewater, identified as *Candida tropicalis*, showed chromium (Cr) tolerance level up to 5 mM. Yeast grown in minimal salt medium containing Cr (VI) ions for 48 h and crude enzyme extracts were tested for chromate reductase activity. Optimum temperature and pH of chromate reductase were 30 °C and pH of 7. The enzyme activity was greatly enhanced in the presence of divalent metal cations. Total protein profile revealed some protein bands were present in hexavalent chromium [Cr (VI)] treated samples but were absent in non-treated samples, especially low molecular-weight protein bands in the mass range of < 25 kDa with greater intensity in Cr (VI) treated samples. Yeast cells were able to uptake Cr (VI) between 21 and 80 mg g^{-1} within 2–12-d of time, indicating yeast strain promising potential for Cr (VI) removal from the wastewater. The present study results suggest that *C. tropicalis* is a suitable candidate for bioremediating chromium ions from the contaminated-environment.

Keywords: *Candida tropicalis*, Cr (VI) resistance, Chromate reductase, Bioremediation

Introduction

One of the most abundant elements on earth is chromium (Cr) existing in various oxidation states ranging from 0 to + 6 [1]. Cr is used in leather tanning, textile dyeing, chrome electroplating and finishing, metal processing industries, wood treatment, mining equipment, corrosion inhibition in power plants, manufacturing of refractory materials, and pigments. This extensive anthropogenic use has increased its concentration in environment higher than the recommended into the environment [2, 3]. The United State Environmental Protection Agency has declared it as a priority pollutant [4]. Cr poses several health threats to humans and has been reported to link to mutagenicity, genotoxicity, carcinogenicity, and allergenicity [5–7]. Generally, industrial effluents contain multiple metals including chromium and chromium salts which have adverse effects on the microbial biota [8].

Chromium exists in several forms but trivalent Cr (III) and hexavalent Cr (VI) are the most stable forms [9]. Cr (VI) compounds are 100 times more toxic than Cr (III) due to having higher solubility, greater permeability through biological membrane systems and their successive interaction with intracellular macromolecules including nucleic acids and proteins [10, 11]. On the other hand, Cr (III) which is an essential trace element plays a significant role in glucose and fat metabolism by helping the smooth insulin functioning [12] in almost all types of living organisms. Cr (VI) inside the cells is partially reduced to highly unstable Cr (IV) and Cr(V) radicals that

* Correspondence: rehman.mmg@pu.edu.pk
[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan
Full list of author information is available at the end of the article

cause oxidative burden through the generation of reactive oxygen species, eventually leading to carcinogenicity [13, 14].

Textile industrial effluents usually contain a mixture of various toxic heavy metals such as chromium, cadmium, lead, arsenic and other chemicals like ammonia, salts etc. This industrial wastewater poses serious threat to the aquatic biota and environment. To decontaminate such wastewater generally two treatment strategies are being used; one is through chemicals and other is through living organisms. The chemical treatment is expensive and a source of addition of more chemicals into the environment while biological treatment is cost effective, benign and more reliable [15].

The microbial potential to resist and reduce Cr (VI) into Cr (III) is regarded as an important phenomenon [14, 15] to prevent chromium pollution. The ability of yeast biomass to uptake a variety of heavy metal ions in a wide range of external conditions has been reported by many workers [15–17]. When Cr (VI) enters into the cell via nonspecific sulfate transporters [18] through facilitated diffusion, a gradient by metabolically active cells is established between two sides of the cell membrane. This gradient helps in the enzymatic reduction of Cr (VI) to Cr (III) via flavoenzymes and non-enzymatic via ascorbate, reduced glutathione, and nicotinamide adenine dinucleotide phosphate [19]. The response of yeast cells to metal involves several cellular processes like redox reactions [17, 20], binding by cytosolic molecules, interactions with cellular organelles [21], and generation of protein-DNA and Cr-DNA adducts, DNA–DNA cross links, and DNA strand breaks [22, 23]. The induction of stress proteins, metal precipitation, metal chelation, Cr-entrapment into membranous organelles, and active efflux has been found in other living organisms in lieu of oxidative stress generated by Cr [14] that such processes are speculated to occur in yeast cells [24]. However, the exact mechanism of yeast cells-Cr interaction is not yet clear.

Therefore, in the present study a yeast strain isolated from industrial wastewater was evaluated for its tolerance against Cr (VI). Chromate reductase involved in Cr (VI) reduction in yeast cells was assayed and the potential of yeast for bioremediation of chromium was also studied. Proteins extracted from yeast strain were further explored through one dimensional gel electrophoresis.

Materials and methods

Yeast strain and culturing conditions

Yeast strain, *Candida tropicalis* [25], isolated from textile industrial wastewater from Sheikupura, Pakistan (Fig. S1), was cultured on yeast-peptone-dextrose (YPD) agar plates consisted of; [glucose (20 g L^{-1}), peptone (20 g L^{-1}), yeast extract (10 g L^{-1}), and agar (20 g L^{-1})]. The medium pH was maintained at 7. For each experiment yeast strain was grown anaerobically in minimal salt medium (MSM) containing: [glucose (10 g L^{-1}), $(NH_4)_2SO_4$ (1 g L^{-1}), 0.15 g L^{-1} KH_2PO_4 (0.15 g L^{-1}), K_2HPO_4, $MgSO_4{\cdot}7H_2O$ (0.1 g L^{-1}), $FeSO_4$ (0.026 g L^{-1}), and $CaCl_2$ (0.086 g L^{-1}) (pH = 7.0–7.2)] and was incubated for 24 h at 30 °C. The Cr (VI) was introduced into the yeast culture for 48 h at a concentration of 100 mg L^{-1}.

Effect of Cr (VI) exposure

The minimum inhibitory concentration was measured by culturing yeast cells in YPD agar plates containing different concentrations of K_2CrO_7 [26]. The yeast cells were successively changed from a given concentration to the next concentration and maximum resistance was determined until the yeast cells were unable to grow as colonies on agar plates containing chromium. The solicitous observations were made regarding any color change in yeast colonies in response to Cr-exposure.

Extraction of protein

For intracellular chromate reductase activity, yeast cells were obtained by centrifugation at 1500 *g* for 10 min, pellet was washed two times with sodium phosphate buffer (50 mM, pH 7.0) and was lysed by sonication for 15 s with an interval of 60 s (five times) at 4 °C. The yeast cells free extract obtained by centrifugation was used as a crude enzyme for relative chromate reductase activity. Bradford [27] assay was employed to measure protein concentration with the standard bovine serum albumin used.

Enzyme assay

Chromate reductase activity was measured through an enzyme assay consisting of crude enzyme, 20 μM Cr (VI), and 0.1 mM NADH in 50 mM potassium phosphate buffer (pH 6.0). The initiation of reaction was done by adding NADH as an electron donor and the reaction assay was incubated for 30 min at 30 °C [28] and relative activity (%) of crude chromate reductase was determined by any comparable change in absorbance with reference to control. An assay mixture having no enzyme or NADH was treated as control. The enzyme concentration required to reduce one μmole of Cr (VI) per min mL^{-1} at 30 °C is equal to one-unit enzyme activity under prescribed assay conditions.

Cr (VI) reduction

Diphenylcarbazide method [29] was used to measure the amount of Cr (VI) in the culture filtrate to further determine the Cr (IV) reduction potential of yeast strain at a given time. For this, the reaction mixture was kept for 10 min at room temperature to form a pink-violet colored complex and finally optical density was measured by a spectrophotometer at 540 nm.

Enzyme characteristics

Chromate reductase activity was determined by incubating the reaction mixture at 30, 40, 50, 70, and 90 °C through the standard enzyme assay procedure. Likewise, pH profiling of chromate reductase was performed at pH range of 5–9 and the reaction assays were kept at 30 °C for 30 min. For this, three buffer systems: sodium acetate buffer (pH 5.0–6.0), sodium phosphate buffer, (pH 7.0–8.0), and Tris-HCl buffer (pH 9.0) were used. The assay containing no enzyme was treated as control and was used to determine any optical density change in enzyme assays containing enzyme. For the metal ion effect on enzyme activity, various chloride salts ($CaCl_2$, $MgCl_2$, $HgCl_2$, $CoCl_2$, and NaCl) were used to determine their effects on chromate reductase activity. The reaction assay containing no metal ion was treated as control.

One dimensional gel electrophoresis

For total cell proteins, one dimensional gel electrophoresis was performed on 12% polyacrylamide gels. Then, 10 vol% chilled trichloroacetic acid was used to precipitate protein samples (20 μg μL^{-1}) and then centrifuged at 11,000 *g* at 4 °C for 15 min. Finally the gel was run at a constant voltage (120 V) to electrophorese the protein samples according to Laemmli [30].

Cr (IV) biosorption by yeast strain

Yeast cells were cultured in MSM medium containing Cr (VI) under optimum conditions. Then 5 mL sample from each flask were taken out after a regular interval of time 2, 4, 6, 8, 10, and 12 d under sterilized conditions. The pellets obtained by centrifugation at 4000 *g* for 10 min were weighted and washed three times with distilled water. Then pellets were divided into almost two equal portions: the first portion was again washed with 0.1 M EDTA three times for 10 min and metal associated with the yeast cell surface was removed as a soluble fraction. The second portion, treated with 0.2 N HNO_3 (1:1) for acid digestion, was placed on a hot plate for 30 min. The Atomic Absorption Spectrophotometer was employed to estimate the amount of chromium present in the medium or removed by yeast strain through the air-acetylene burner at 359.3 nm [15, 31, 32].

Statistical analysis

The observations were made and each experiment was run in triplicate. Usually, three separate flasks were maintained for each treatment. Every time three readings were taken from each sample, their mean, and standard error of the mean were calculated. Student's *t*-test was used for significance testing between the samples.

Results and discussion

Cr (VI) resistant yeast

In this study, Cr (VI) was tested for tolerance separately by 11 yeast isolates. *C. tropicalis* [25] exhibiting the highest ability to tolerate chromium effectively up to 5 mM was selected for further study. The most favorable temperature and pH required for yeast growth was found to be 30 °C and 7.0. The growth (no. of cells) was also decreased under Cr (VI) stress as compared to the control (Fig. 1).

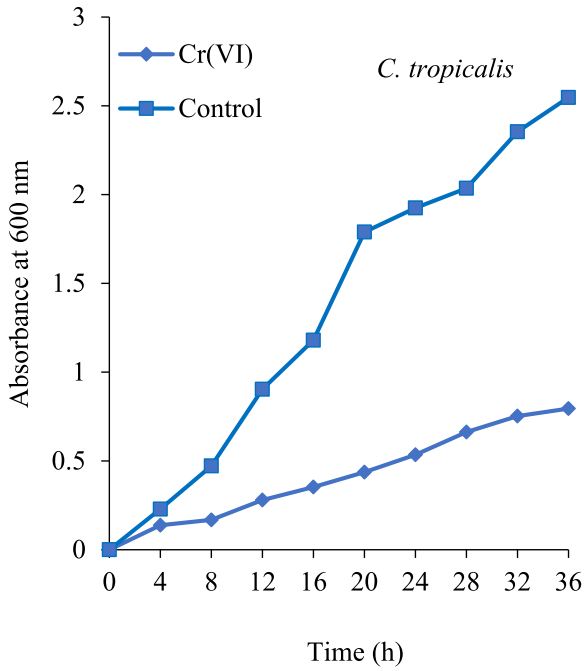

Fig. 1 Growth curve of *Candida tropicalis* without (control) and treated with 0.1 mM Cr (VI) at 30 °C and 7.0. Experiments were carried out in chemically-defined media with 1% glucose (carbon source)

The color of yeast isolate was affected and transformed to green in comparison with the control (Fig. S2), which was due to enzymatic reduction of Cr (VI) into Cr (III). The color change of yeast cells clearly indicates the synthesis of specific enzymes which convert Cr (VI) to Cr (III). Yeast biomass content was decreased in the presence of Cr (VI) as compared to the control (Table S1).

An increase in chromate reductase activity was determined in *Streptomyces* sp. M3 cells when exposed to Cr (VI) as reported by Das and Chandra [33]. Many researchers have reported enzymatic role in Cr (VI) reduction obtained from the yeast cells [25, 34, 35]. Similarly, chromate reductase role in Cr (VI) reduction by many bacterial genera *Escherichia*, *Pseudomonas*, *Bacillus*, and *Arthrobacter* has also been reported [36, 37]. Similar phenomenon was reported in *Candida utilis* [20], *Candida maltose* [38], and fungi, *Hypocrea tawa* [39], and *Aspergillus* sp. [40].

Present study has investigated a few aspects of Cr-interaction with yeast cells including resistance, uptake and reduction of Cr (VI) into Cr (III). This metal-microbe interaction is a very intricate process. When metal ions interact with cells then various mechanisms are activated at cellular, genomic, and proteomic level to combat this stress. The studies at molecular and proteomic level can help to understand such delicately regulated mechanisms.

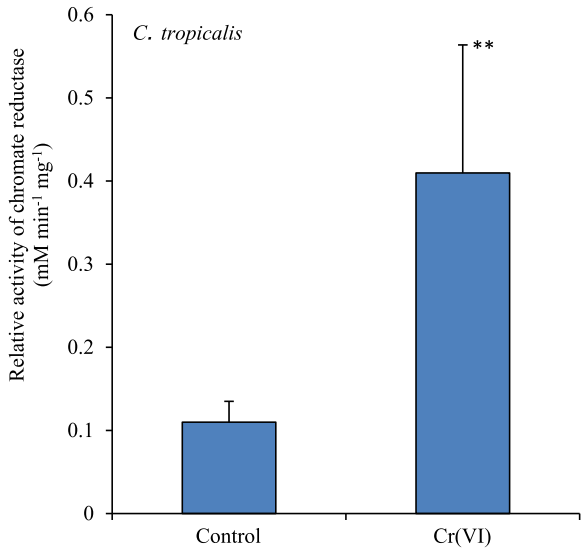

Fig. 2 Relative activity of chromate reductase isolated from *C. tropicalis*. Values were expressed as mean of ± SD where *$p = 0.05$ and **$p = 0.1$ and the experiments were performed in triplicate

Chromate reductase characteristics

The crude chromate reductase obtained from *C. tropicalis* was tested and induced by 4-fold under 20 μM Cr (VI) stress (Fig. 2). The maximum enzyme activity was determined at 30 °C and gradually decreased as the temperature increased (Fig. 3a). Similarly, pH experiments confirmed that chromate reductase activity was optimum at pH 7 as compared to the other pH (Fig. 3b). Our results are in good agreement with the findings of Martorell et al. [41]. A continuous decline was observed with an increase in temperature. Enzyme activity was lost to 21% by decreasing pH to 6 and 15% when increasing pH to 9. At pH value above or below the optimum, a marked decrease in chromate reductase activity was determined. Among bacterial chromate reductases, the optimal temperature varies in the range of 30–50 °C [25, 42, 43]. Elahi and Rehman [44] reported that optimum chromate reductase activity was achieved at pH 6 (50 °C) in *Trichosporon asahii* while *Rhodotorula mucilaginosa* showed maximum activity at pH 7 (30 °C).

The enzyme activity was also sensitive to the tested metal ions, relative activity was increased in the presence of Mg^{2+} (98%) and Na^{+} (75%) while inhibited to 81% by Hg^{2+} due to Hg^{2+} blocking active site of the enzyme (Fig. 3c). Microbial chromate reductase activity could potentially be decreased in the presence of heavy metal ions. In the current study, enzyme activity was highest in the presence of Mg^{2+} and other divalent metal ions also showed positive effect on chromate reductase activity indicating that the enzyme is not absolutely specific to a single metal. These results also agree with previous reports [25, 43, 45]. Elahi and Rehman [44] reported that activity of chromate reductase from both *T. asahii* and *R. mucilaginosa* was increased in the presence of Na, Ca, Mg, and Co but significantly decreased in the presence of Hg cations.

Total protein profile

Total protein profile of Cr-treated and untreated yeast cultures was obtained by one dimension gel electrophoresis. The low molecular-weight protein bands were found in Cr (VI) treated samples (Fig. 4) and it is possible that these low molecular-weight bands play key role in the protection and survival of microorganisms including yeasts against metal generated oxidative stress. The presence of lower molecular-weight proteins in the mass range of < 25 kDa with increased intensity in metal-treated samples indicates their involvement in metal sequestration which eventually leads to metal detoxification. Elahi and Rehman [44] reported that various proteins were overexpressed in *T. asahii* and *R.*

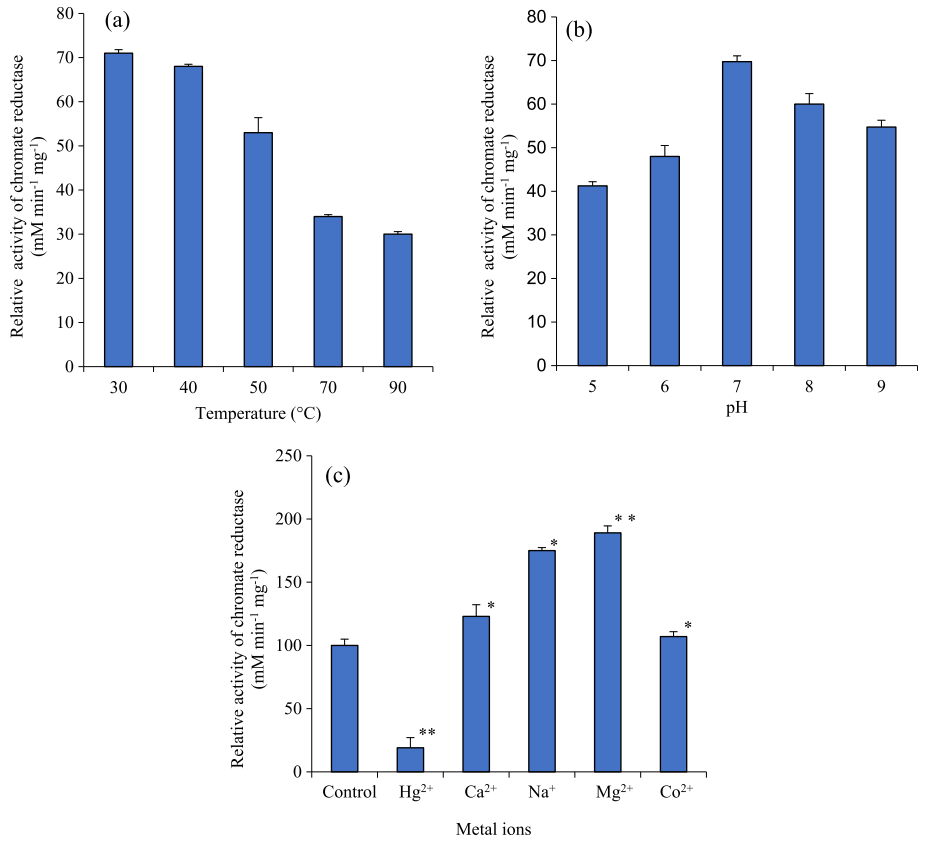

Fig. 3 **a** Effect of temperature on chromate reductase activity in *C. tropicalis*. **b** Effect of pH (5–9) on chromate reductase activity in *C. tropicalis*. **c** Metal ions effect on chromate reductase activity pattern of *C. tropicalis*. Concentration of 0.1 mM of each metal ion was used. Values were expressed as mean ± SD where $*p = 0.05$ and $**p = 0.1$ and all experiments were performed in triplicate

mucilaginosa under Cr (VI) stress indicating their possible role in cells protection against metal ions generated oxidative stress. Khan et al. [17] reported that besides cellular redox homeostasis, proteins involved in protein folding, cysteine biosynthesis, and cytoplasmic detoxification response elements were also upregulated in *C. tropicalis* 3Aer under cadmium stress.

Cr (VI) biosorption

The chromium in the medium processed by *C. tropicalis* was measured using atomic absorption spectroscopy. The highest uptake potential was 15, 21, 28, 35, 45 and 57 mg g^{-1} (Fig. 5a) and the cells adsorbed 6, 9, 13, 16, 20, and 23 mg g^{-1} after 2, 4, 6, 8, 10, and 12 d of incubation, respectively (Fig. 5a). In the present study, *C. tropicalis* efficiently removed 80% chromium from the medium within 12 d of incubation (Fig. 5b). The highest biosorption ability shown by a macro fungus, *Amanita rubescens*, was 27.3 mg Cd g^{-1} reported by Sari and Tuzen [46]. Elahi and Rehman [44] reported that *R. mucilaginosa* and *T. asahii* were able to accumulate 43–97% and 35–88% Cr (VI) from the medium, respectively.

Yeast cells have the ability to survive in heavy metal ions stress assuring their metabolic activities [47]. The yeast cells can uptake higher concentrations of heavy metals by utilizing the uptake process rather than using biosorption. The highest chromium adsorption by *C. tropicalis* cells reveals more meal-binding sites are present on the cell walls which reflect their ability to uptake metal ions from the medium and eventually remove Cr (VI) from the wastewater. *Rhodotorula* sp. Y11 was able to uptake 19.4 mg Cd g^{-1} from the medium as reported by Li and Yuan [48]. Mainly two processes i.e. biosorption (adsorption; ATP independent) and uptake (accumulation, ATP dependent) are involved to detoxify wastewater containing multiple metal ions. Both biosorption and uptake can be influenced in the presence of other metal ions. The use of microbes as compared to the chemical methods is less-expensive, environment-friendly, and can efficiently be used to remove dissolved toxic metal ions from the wastewater even at relatively high metal concentration. This

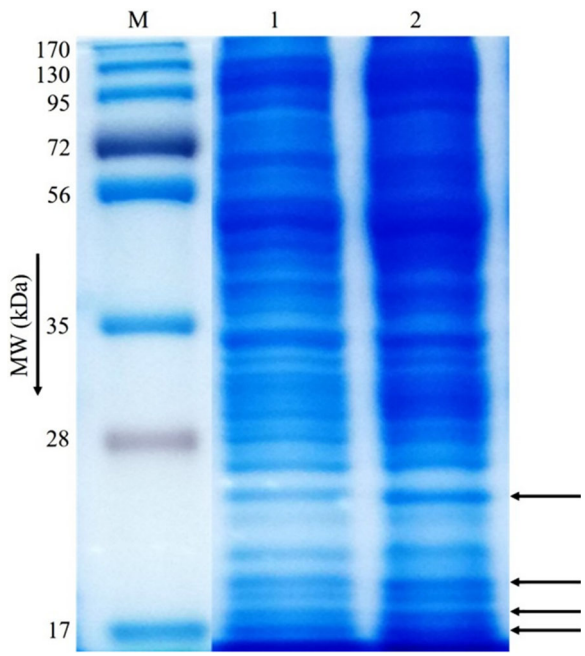

Fig. 4 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE analysis of total proteins extracted from *C. tropicalis* stained with Coomassie Blue R-250. Lane M, protein marker; lane 1 and 2 are showing control and Cr-treated *C. tropicalis*

microbially purified wastewater can at least be used for crops irrigation. In this study, *C. tropicalis* could efficiently remove chromium up to 80% within 12 d of incubation.

Conclusions

In the present study, *C. tropicalis* showed significant resistance against Cr (VI) and growth was slowed in Cr-treated cultures as compared to the control. The crude enzyme exhibited maximum activity at 30 °C (pH 7). The enzyme activity was not affected greatly in the presence of divalent metal ions. The gel electrophoresis revealed several low-molecular weight protein bands with greater intensity in Cr-treated samples as compared to the non-treated samples that may correspond to protein marker. Yeast cells were capable of uptaking noxious and soluble Cr (VI) and reduced it into less harmful and insoluble Cr (III) by chromate reductase. Hence, *C. tropicalis* can be used as biosorbent for Cr (VI) detoxification from the wastewater laden with toxic chromium ions. Further research work is needed to explore its molecular biology so that it can become an attractive environmental tool for green chemistry.

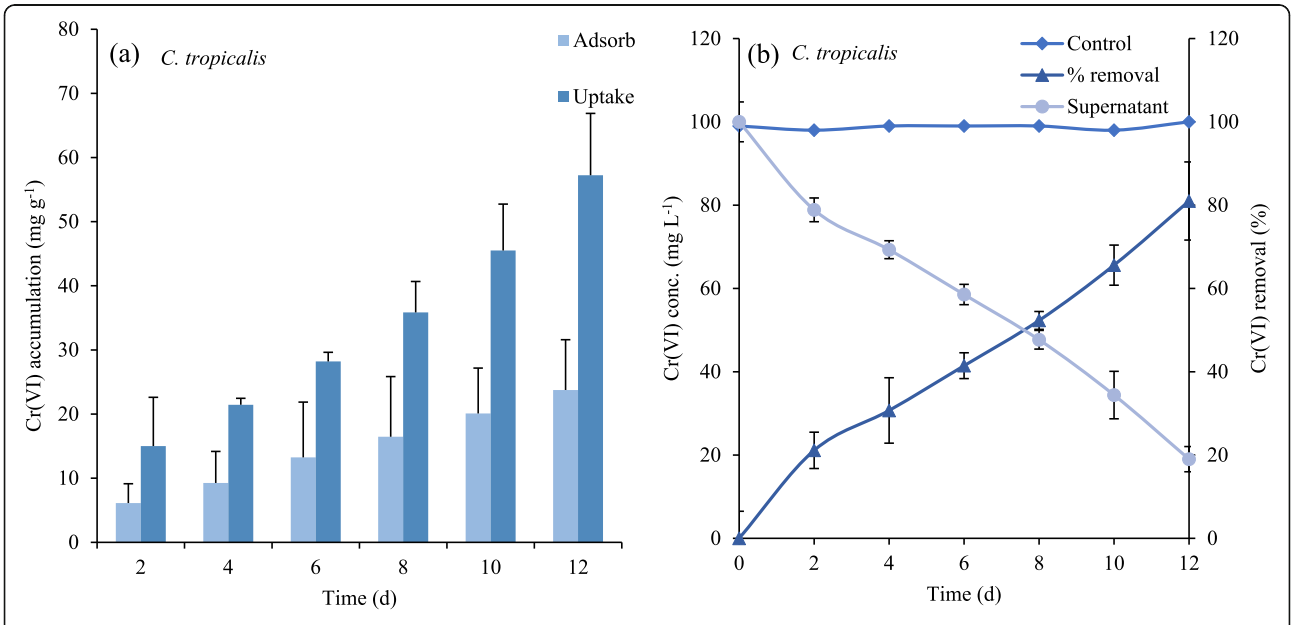

Fig. 5 a Uptake and adsorption of chromium metals ions from culture media by *C. tropicalis*. **b** Supernatants were taken out at regular intervals of 2, 4, 6, 8, 10 and 12 d of incubation (controls contained heavy metal ions but did not contain yeast cells) and percentage removal was calculated at each time-point

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42834-020-00069-1.

Additional file 1: Table S1. Gram fresh weight (biomass) of *Candida tropicalis* grown in MSM supplemented with and without chromium. Three biological replicates were used and an equal gram weight was taken for protein extraction. **Fig. S1** Geographical map of district Sheikhupura, Pakistan, from where wastewater samples were collected. **Fig. S2** Hexavalent chromium reduction by *C. tropicalis* cells augmented with chromium after 24 h of incubation. Control test tube (yellow in color) only contains chromium while *C. tropicalis* (green in color) indicates the ability of yeast strain to reduce the hexavalent chromium.

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Authors' contributions

SI performed experiments and wrote the article. DAB helped in data analysis and in manuscript editing. AR contributed in the design, write up, and final editing of the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Competing interests

The authors have declared that no competing interests exist.

Author details

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan. [2]Department of Zoology, Government College University Lahore, Lahore 54000, Pakistan.

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