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Removal Mechanism of Hexavalent Chromium by a Novel Strain of *Pichia anomala* Isolated from Industrial Effluents of Fez (Morocco)

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Abstract: Hexavalent chromium (Cr(VI)) pollution has become one of the most serious environmental problems today. One removal strategy comprises the microbial reduction of Cr(VI), is regarded as a cost-effective biotechnology for the treatment of high volume and low concentration complex wastewaters. In this work a yeast strain that exhibits high Cr(VI) resistance was isolated from soil sediment. The isolated yeast was identified as *Pichia anomala* by sequencing analysis. The yeast showed a remarkable capacity to completely reduce 25 and 50 mg/L of Cr(VI) in 48 h under aerobic conditions. The increase of initial Cr(VI) concentration influenced reduction, growth and specific growth rate. This strain also exhibited multiple heavy metal tolerance. The presence of anions and cations in the medium had a great influence on chromium reduction. Fractionation of the cells showed that the mechanism of Cr(VI) removal by this strain is "adsorption-coupled reduction" and the hexavalent chromate reductase activity was expressed constitutively. FTIR analysis of the biomass exposed to chromium showed that the binding process of the chromium ions involves the active participation of functional groups present in the external surface of biomass. High Cr(VI) concentration resistance and high Cr(VI) reducing ability of this strain make it a suitable candidate for bioremediation.

Key words: Bioremediation, Cr(VI) removal, Cr(VI) tolerant yeasts, *Pichia anomala*.

1. Introduction

Chromium toxicity is one of the major causes of environmental pollution; it is often discharged by a number of industries, such as petroleum refineries, iron and steel industries, textile dyeing and leather tanning. Chromium can exist in several states of oxidation, Cr(III) and Cr(VI) are the most stables [1]. Cr(III) is an essential trace element well known for its particular role in the maintenance of normal carbohydrate metabolism in mammals and yeasts [2]. It is generally accepted that Cr(III) species are not highly toxic and due to their limited solubility, they can be easily removed by chemical and microbial approaches. It has also been suggested that this ion is involved in the tertiary structure of proteins and the conformation of cell RNA and DNA [3, 4]. However, at high concentrations, Cr(III) showed negative effects on cellular structures. In contrast, Cr (VI) is always toxic and exhibits mutagenic and carcinogenic effects on biological systems [5, 6]. Thus, the development of efficient methods for detoxification and remediation of this hazardous chromium oxy-anion is of great importance. For this purpose, microbial systems seem to be the most promising, although genetic and biochemical aspects of this process are poorly studied. One of the most ubiquitous biomass types available for bioremediation of heavy metals at low pH is yeast, which can serve as a suitable model for studying physiological and molecular mechanisms of eukaryotic cell interactions with heavy metals. Yeasts are a better

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raw biosorbent material for the removal of reactive heavy metal ions due to their unicellular nature and high growth rate [7]. In particular, they are found effective in accumulation and/or biosorption of aggressive Cr compounds and are able to bioconvert them into stable and non-toxic forms [8]. General mechanism of chromium removal by yeast strains such as *Candida* and *Rhodosporidium* genera is related to limited ion uptake rather than to biological reduction of Cr(VI) to Cr(III) [9]. However, other yeasts such as *Candida utilis* [10] and *C. maltosa* [11] showed ability to partially reduce Cr(VI) to Cr(III) and a good efficiency to accumulate chromium in the biomass. Recent reports have also examined chromium removal by different species of *Pichia* [12] and demonstrated that *P. anomala* is capable of removing chromium, but the mechanism of its detoxification was not investigated.

In this work we describe the mechanism of Cr(VI) removal by a *P. anomala* strain isolated from chromium contaminated site. Also, in order to evaluate its potential in bioremediation of chromium contaminated environments, the influence of some factors (concentration of chromium, coexistent heavy metals and anions) on Cr(VI) reduction by the isolated yeast strain is studied.

2. Materials

2.1 Sampling, Isolation and Characterization of Selected Isolate

Soil sediment and wastewater samples were collected from chromium contaminated site located in oued fez and oued sebou, Fez province (city), Morocco, which was heavily contaminated with chemical industrial wastes including tanning processing.

Isolation of the yeasts strains was done by enrichment culture technique. A yeast medium (YPG) modified (0.2% yeast extract + 0.2% peptone + 2% glucose) prepared in sterile distilled water, which was amended with 100-300 mg/L of a sterile solution of Cr(VI) as $K_2Cr_2O_4$ and 2 g of soil (1 mL of wastewater) was incubated at 30 °C on a shaker incubator at 150 r/min. After 6 days enriched yeast strains were isolated by plating on YPG agar plate amended with 100-300 mg of Cr(VI)/L. The culture Y2, which showed high tolerance, was identified by 5.8S RNA analysis. Nucleotide sequence was obtained with Genetic analysis system model 3130. Comparison of this sequence with partial 5.8S rDNA sequence published in the GenBank was performed using the BLAST tool from the National Center for Biotechnology Information (NCBI) (http//:www.ncbi.nlm.nih.gov) to search similar sequences in public databases. All further studies were done using Y2.

2.2 Quantitative Cr(VI) Tolerance Analysis

Quantitative Cr(VI) tolerance assays were performed in 250 mL Erlenmeyer flasks containing 50 mL YPG-glucose medium separately supplemented with 25, 50 and 100 mg of Cr(VI)/L. Flasks were inoculated at a final concentration of 0.2 g biomass/L and incubated at 30 °C on a rotary shaker at 150 r/min. The initial pH of medium was 6. The growth was monitored by measuring absorbance of culture at 600 nm after 24, 48 and 72 h of incubation.

Media inoculated with the selected strain without Cr(VI) solution and non-inoculated medium with Cr(VI) were included as controls and incubated under identical conditions. Specific growth rates (h^{-1}) were calculated from the slope of the linear regression of the natural logarithm of culture biomass versus time.

2.3 Evaluation of Chromium Resistance and Other Metals Tolerance

Contaminated habitats are generally characterized by the co-existence of a large number of toxic cations and, therefore, it is necessary to study the multiple metal resistances of yeast. The minimum inhibitory concentration (MIC) was determined by plating 100 μL of culture on YPG agar amended with Cr(VI) concentrations ranging from 100 to 2000 mg/L. The minimum concentration of the metal inhibiting complete growth was taken as the MIC. The resistance

of the yeast isolate was also checked against salts of various other heavy metals such as $NiCl_2$, $CoCl_2$, $ZnSO_4$, $HgCl_2$, $CuSO_4$, $Pb(NO_3)_2$ amended in YPG agar at a concentration ranging from 100 to 4000 mg/L.

2.4 Chromium Removal Experiments

Reduction of chromium was determined by adding 25 mg of Cr(VI)/L to the yeast culture with cell concentration 2 g/L (an early exponential phase of the growth) and incubated with aeration at 30 °C under continuous shaking (150 r/min). Samples of 200 μL were taken every 24 h and centrifuged at 7000 r/min for 10 min, supernatants were kept at 4 °C for residual chromium concentration analyses. Reduction was estimated by measuring the decrease of Cr(VI) in the culture.

2.5 Effect of Initial Cr(VI) Concentration

The effects of initial concentration of chromium on reduction of Cr(VI) were investigated at 25, 50 and 100 mg of Cr(VI)/L in the medium.

2.6 Effect of Anions and Cations on Cr(VI) Reduction

Some studies indicated that cations and anions additional to the ions of interest have a generally detrimental impact on metal removal [13].

To determine the effects of various anions and cations on Cr(VI) reduction capacity of the yeast, the isolate was assessed for its ability to reduce Cr(VI) in the presence of anions such as carbonate, nitrate, chloride, sulphate, phosphate at 25 mg/L and cations such as Zn^{2+}, Ni^{2+}, Hg^{2+}, Cu^{2+}, Co^{2+}, and Pb^{2+} at equivalent concentrations.

2.7 Cell Fractionation

To determine the localization of chromate reductase activity, cells were disrupted and separated into various fractions. The cell extracts of isolate were prepared using the following protocol. A yeast suspension cultured for 48 h in 100 mL of YPG medium was recovered by centrifugation at 6,000 r/min for 20 min. The float (supernatant) or the extracellular fraction is collected and then sterilized by filtration through a nitrocellulose filter of 0.45 μm, while cells (pellet) were washed three times with 10 mM Tris-HCl (pH 7.2) and resuspended in 5 mL of Tris-HCl 25 mM (pH 7.2). These cell suspensions were placed at (-20 °C) and lysed using an ultrasonic probe (Elma sonic x-tra 50H) for 15 min at a temperature of 0 °C. The sonicate thus obtained is then centrifuged at 12,000 r/min for 40 min at 4 °C. The membrane fraction of sonicated cells was resuspended in the same volume of Tris-HCl (pH 7.2).

Extracellular fraction, cytosolic fraction and membrane fraction thus obtained are then tested for their ability to reduce Cr(VI) at a concentration of 10 mg/L, The reaction mixture was incubated for 4 h at 30 °C. After centrifugation, the residual concentration of Cr(VI) was determined by using method of diphenylcarbazide. All fractions that were heated at 100 °C for 30 min act as controls.

2.8 Chromium Reductase Induction in the Presence of Chromium

To study the constitutive or inducible nature of chromate reductase, strain was cultured in YPG medium with and without 50 mg/L of Cr(VI). Cells were harvested by centrifugation and lysed by sonication as described. The chromate reductase activity in different fractions was determined.

2.9 FTIR Analysis

Infrared spectra for *P. anomala* cells grown for 24 h in YPG broth supplemented with 50 mg/L of Cr(VI) and without Cr(VI) for 24 h were obtained using a Fourier Transform Infrared Spectrometer (Bruker Vertex 70 FT-IR Spectrometer). Control and test cells loaded with Cr(VI) were pelleted by centrifugation at 7,000 rpm, 4 °C for 30 min, displayed over glass slide and dried at 50 °C for 8 h. The FTIR spectra were collected at resolution of 4 cm^{-1} in the transmission mode (4,000-400 cm^{-1}).

2.10 Analytical Techniques

2.10.1 Cell Concentration

The cell concentration was estimated by dry cell measurements, cells from overnight grown culture were harvested by centrifugation 6000 r/min for 10 min and washed with distilled water and dried at 100 °C to find out the dry weight of cells. Corresponding absorbance was measured at 600 nm using a spectrophotometer and was converted to dry weight of cells.

2.10.2 Chromium Determinations

The assay of Cr(VI) concentration in a medium was carried out using hexavalent chromium specific colorimetric reagent S-diphenyl carbazide (DPC). The reaction mixture was set up in 10 mL volumetric flask as follows: 200 μL of supernatant followed by addition of 330 μL of 6M H_2SO_4 and 400 μL of DPC (0.25% (w/v) prepared in acetone) and final volume was made to 10 mL using distilled water [14]. Spectrophotometric measurements were made immediately at 540 nm.

2.10.3 Protein Estimation

Unit enzyme activity for chromate reductase was derived as amount of enzyme that reduces 1 μmole of Cr(VI)/min at 30 °C. Specific activity was defined as unit chromate reductase activity/mg protein concentration. Protein concentrations were estimated using Lowry method [15].

2.10.4 Statistical Analysis

All the experiments were carried out at least in duplicate, and in triplicate in some cases. The results were subjected to statistical analyzes and standard error of the means (S.E.M.) were calculated [16].

3. Results and Discussion

3.1 Isolation and Identification of Yeast Isolate

After a series of dilution and plating assays, four different pure isolates with colonies and cellular morphology typical to yeasts were obtained from tannery effluent and one from sediment samples from oued Fez. All further studies were done using Y2 since it showed maximum tolerance. After PCR amplification, the partially sequenced gene was uploaded to the National Center for Biotechnology Information (NCBI) website to search for similarity to known DNA sequences and to confirm the species of this local isolate. The BLAST query revealed that this gene is 100% homologous to *Pichia anomala*.

3.2 Effect of Cr(VI) on Growth of P. anomala

Batch cultures of *P. anomala* were performed in unbaffled flasks containing culture media with different initial Cr(VI) concentrations. The relationship between the growth and the initial concentration of Cr(VI) is shown in Fig. 1A. The growth of cells was heavily influenced by Cr(VI) at a concentration of 100 mg/L, while Cr(VI) at 25 mg/L had only slight effect on the growth. As the initial Cr(VI) concentration increased, the growth rate decreased (Fig. 1B). These results are in agreement with several authors. Stasinakis et al. [17] reported that Cr(VI) concentrations equal or higher than 10 mg/L caused a significant decrease in the growth rate values of activated sludge, as well as with the findings of Juvera-Espinosa et al. [18], who observed that Cr(VI) had an inhibitory effect on growth of *Candida* sp. LMB2 and cause a decrease in the specific growth rate values. A similar effect was observed for *C. utilis* [19], *Lecythophora* sp. NGV-1 and *A. pullulans* VR-8 [20]. These data prove that Cr(VI) affect negatively the metabolic activity and genetic material of yeast cells [21, 22].

However, it is important to take into account that toxicity of Cr(VI) is dependent on medium composition. The use of nutrient-rich media may have affected the Cr(VI) toxicity via a ‘masking’ effect [23, 24]. Laxman and More [25] studied Cr(VI) toxicity in *Streptomyces griseus* and found that tolerance was higher in nutrient-rich than in semi-synthetic medium, indicating that Chelation of Cr(VI) to the organic constituents of the medium, may cause, reduction of free Cr(VI) ions and/or inhibition in its availability.

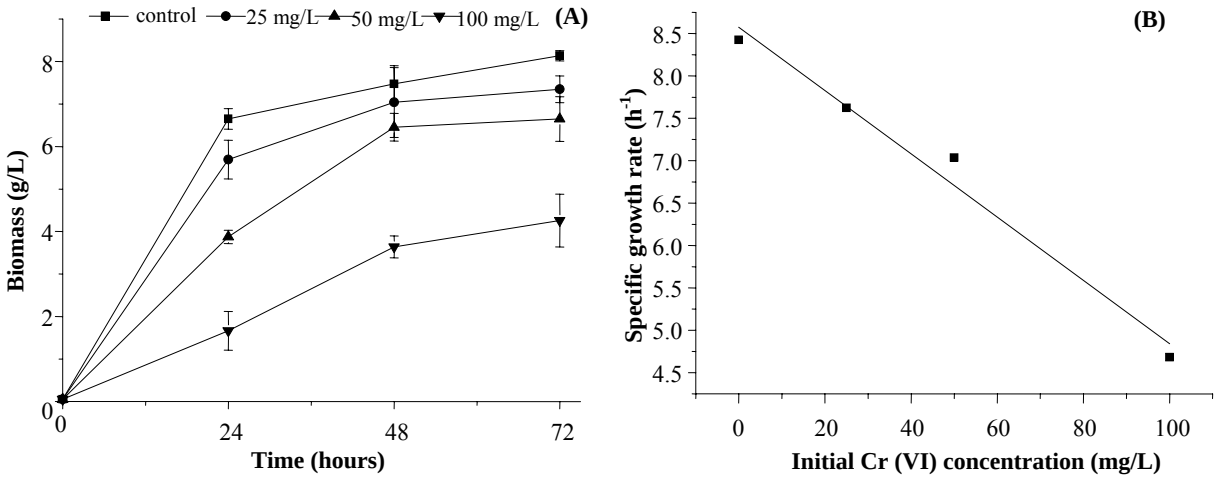

Fig. 1 Effect of different Cr(VI) concentrations (25, 50 and 100 mg/L) on cell growth (A) and specific growth rate of *P. anomala* (B). Error bars represent standard deviations.

3.3 Metals Yeast Resistance

The strain identified as *P. anomala* showed tolerance to various heavy metals. MIC of various metallic salts viz. $K_2Cr_2O_7$, $NiCl_2$, $CoCl_2$, $ZnSO_4$, $HgCl_2$, $Pb(NO_3)_2$ have been determined .

The yeast isolate was found to be resistant to Cr(VI) up to a concentration of 1500 mg/L. The MIC of various others heavy metals salts viz, Hg^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Pb^{2+}, was 100, 100, 200, 500, 1100, 4000 mg/L, respectively as shown in Fig. 2.

A similar level of metal resistance shown by filamentous fungi such as *Fusarium* sp. and *Penicillium* sp was also reported [26]. Viti et al. [27] have compared the MIC of the chromium resistant isolates to various heavy metals and reported that different isolates exhibited different level of metal tolerance.

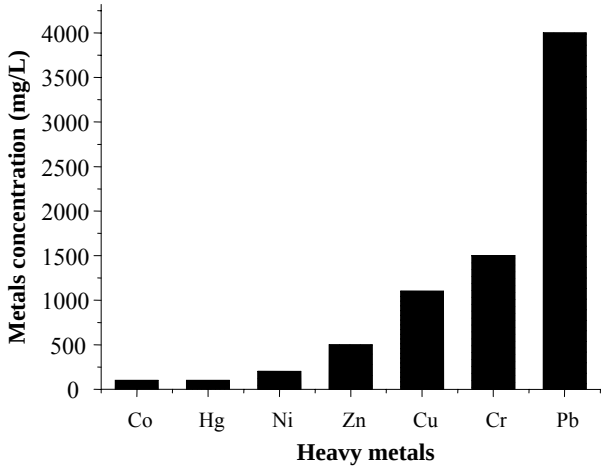

Fig. 2 Qualitative tolerance of *P. anomala* to various heavy metals, on solid medium.

Tolerance of *P. anomala* to other metals has an added advantage of withstanding the presence of different metallic ions while performing the Cr(VI) reduction.

Cr(VI) resistance mechanisms are not well known in yeasts [20]. Recent studies with some yeast genera showed that Cr(VI) reduction to Cr(III) and chromium bioaccumulation are related to chromate resistance [11, 19, 28-30]. However, studies in some species of *Candida* and *Rhodosporidium* demonstrated that chromate resistance was related to reduced ion uptake and not to biological reduction of Cr(VI) to Cr(III) [9, 31-32]. Other authors demonstrate that chromium resistance by yeast or filamentous fungus depended on accumulation of these metals [8].

3.4 Chromium Reduction Studies in Liquid Medium and Effect of Initial Cr(VI) Concentration

Chromium reducing capability of the yeast isolate was checked by adding 50 mg of Cr(VI)/L in the culture medium. Our strain could reduce 50% and 100%, from the medium after 24 and 48 h of incubation.

It can be observed that the capacity of Cr(VI) reduction by *P. anomala* in our experiments was much greater than those found with *Lecythophora* sp. NGV-1 and *Candida* sp. NGV-9 which reduced only 55% and 10% respectively of similar concentration of Cr(VI) after 100 h of incubation. However, it is important to take into account that the microbial chromate-reduction ability is correlated with the medium composition and the cell density [10, 33].

In order to determine the effect of initial Cr(VI) concentration on microbial Cr(VI) reduction and on the rate of Cr(VI) reduction, studies were carried out using yeast strain with different initial Cr(VI) concentrations (25-100 mg/L) under aerobic condition at 30 °C. Fig. 3A shows that 25 mg/L were reduced to zero in 48 h, and 50 mg/L were also reduced in same interval of time. These results showed that the yeast Cr(VI) reducing capacity did not appear to be affected for both Cr(VI) concentration 25 mg/L and 50 mg/L, which indicate that Cr(VI) concentrations up to 50 mg/L were not high enough to reduce Cr(VI) reduction capacity of *P. anomala*. Similar observations have already been reported by Juvera-Espinosa et al. [18] who indicate that Cr(VI) concentrations which inhibited multiplication of the strain *Candida* sp. LMB2 did not prevent Cr(VI) reduction. Juvera-Espinosa et al. [18] informed that this difference between Cr(VI) toxicity and Cr(VI) reducing capacity has been accredited to the different metabolic mechanisms implicated in both processes.

At 100 mg/L, 50% are reduced in 72 h. Megharaj et al. [34] also reported that the time taken for total reduction of Cr(VI) increased with increasing concentrations of Cr(VI). The lesser chromium reduction in *P. anomala* produced by higher initial Cr(VI) concentration may be due to the mutagenic and toxicity of chromium for culture metabolism [35].

On the other hand, Figs. 3B and 3C show that the rate of reduction (mg of Cr(VI) reduced/L/h) by *P. anomala* increased linearly with increasing Cr(VI) concentration but decreased with incubation time. Amoozegar et al. [36] also indicate that initial Cr(VI) concentration did not affect the reduction rates. These results are in agreement with those reported for *S. griseus* [37] and for *Candida* sp. NGV-9 [18].

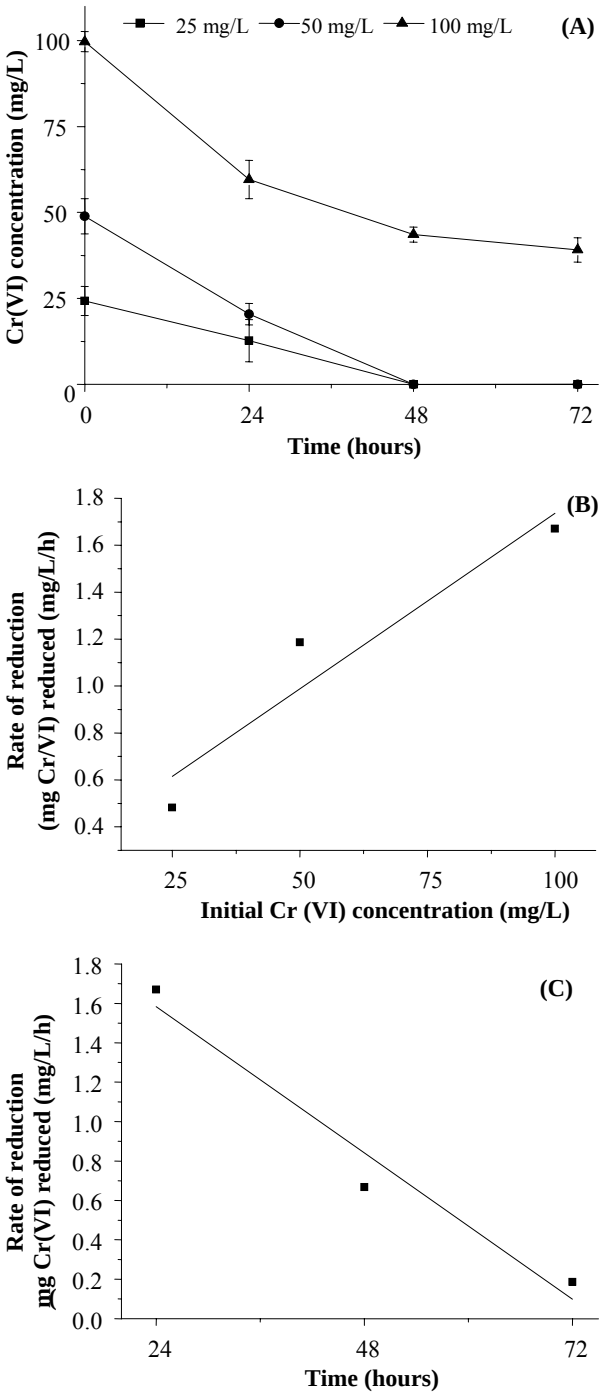

Fig. 3 Effect of initial Cr(VI) concentration (0.5-2 mM) on Cr(VI)-reduction (A), rate of Cr(VI) reduction (B) and effect of time incubation on rate of Cr(VI) reduction (C) by *P. anomala.* Error bars represent standard deviations.

3.5 Effect of Heavy Metals on Cr(VI) Reduction

In this work, effect of various heavy metals on Cr(VI)-reduction by *P. anomala* was also studied. As shown in Fig. 4, addition of different heavy metals at 25 mg/L in the reduction medium affects significantly reduction potential of this strain.

The presence of Cu^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+} increased Cr(VI) reduction while Zn^{2+} slightly inhibited Cr(VI) reduction by strain *P. anomala*.

Similar results are carried by Sultan et al. [38] who informed that chromate reduction by *Ochrobactrum intermedium* strain SDCr-5, was inhibited by the presence of Zn^{2+} while Cu^{2+} had a stimulatory effect on Cr(VI) reduction. Bae et al. [39] also found that 1 mmol/L Zn^{2+} caused a 32% decrease in Cr(VI) reduction by *E. coli*, while Desai et al. [40] demonstrated that the presence of Cu^{2+} stimulated the Cr(VI) reductase activity of *Pseudomonas* sp. G1DM21 by 33%.

Wang et al. [41] reported that the decrease of metal uptake in competitive conditions was thought to be a response to increased competition between same charged species for binding sites of the yeast cells. This indication allows us to suggest that *P. anomala* seems to have more affinity, higher selectivity and biosorption capacity to Zn^{2+} than to Cr(VI) in aqueous solutions, what explains the decrease of Cr(VI) reduction capacity by *P. anomala*, in the presence of Zn^{2+}.

The stimulatory mechanism of Cr(VI) reduction activity by Cu^{2+} and other metals is not clear. However, the main function of Cu^{2+} has been reported to be related to electron transport protection or to act as an electron redox center and, in some cases, as a shuttle for electrons between protein subunits [42].

3.6 Effect of Anions

The effect of ions on Cr(VI) reduction during reduction of Cr(VI) by *P. anomala* is presented in Fig. 5. Results revealed that anion like phosphate decrease Cr(VI) reduction by 17%. Whereas carbonate and nitrate increase Cr(VI) reduction by 18% and 27% respectively. No significant effect was observed by sulfate and chloride.

It is surprising to see that sulfate a known competitive inhibitor of chromate transport [43] did not inhibit Cr(VI) reduction in the present study. Similar results were reported by several authors, which find no inhibitory or stimulating effect of sulfate on Cr(VI) reduction in aerobic microbial cultures. Sulfate concentration of up to 1 mM was reported to have no effect on chromate reduction by *S. griseuse* [37] and *Pseudomonas putida* cells [44]. Also sulfate at 10 mM concentration did not affect Cr(VI) reduction by

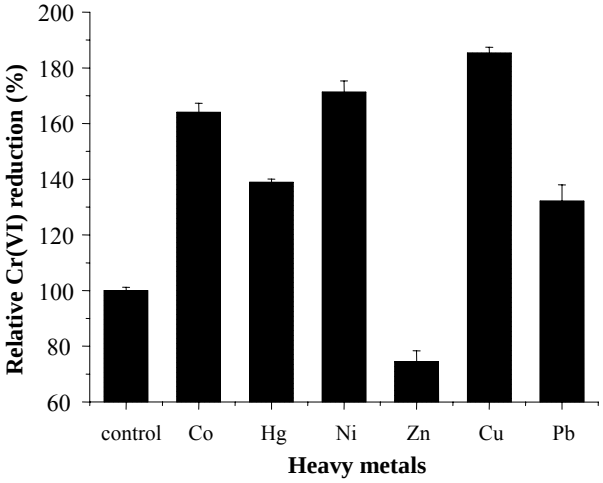

Fig. 4 Effect of heavy metals supplementation on Cr(VI) reduction by *P. anomala*. Final concentrations of heavy metals (25 mg/L). Error bars represent standard deviations.

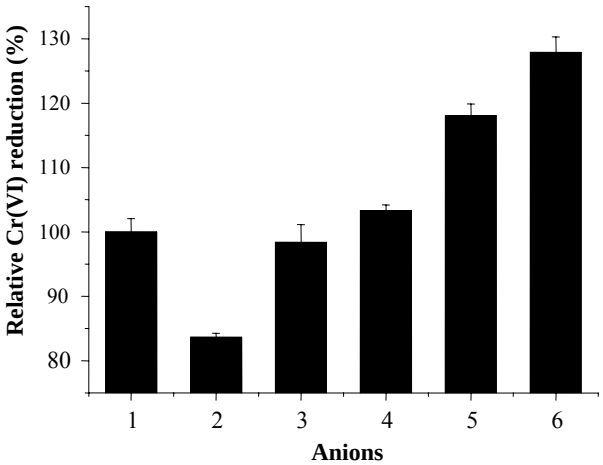

Fig. 5 Effect of anions on chromate reduction (1, none; 2, phosphate; 3, sulfate; 4, chloride; 5, carbonate; 6, nitrate). Error bars represent standard deviations.

Bacillus sp. [45] and *Candida* sp. FGSFEP [46]. It's possible that the effect of sulfate on Cr(VI) reduction might be problematic at higher concentrations.

On the other hand, Agarwal et al. [47] reported that in solution some inorganic anions existing in the solution may form complexes with metal ions, and therefore, affect adversely the adsorption process which can explain the inhibitory effect of phosphate. Also we can suggest that this inhibition may be to the competitive effect between the metal ion and this anion.

3.7 Localization of Chromate Reductase Activity

As the knowledge about the mechanisms of yeast chromium reduction, the localization of Cr(VI) reductase activity was made by performing the assays using the ultrasonicated sub-cellular fractions. The Cr(VI) reduction by extracellular and intracellular (the cell-free extracts) is shown in Fig. 6.

Results indicate that the intracellular fraction could reduce 100% of 10 mg of Cr(VI) in 4 hours. Whereas, a negligible Cr(VI) reductase activity was detected in the extracellular fractions. The intracellular fraction heated at 100 °C for 30 min did not cause a decrease in Cr(VI) concentration, indicating that the reducing activity associated to this fraction was heat labile, while as well for the membrane fraction (sonicated cell pellet) heated as for not heated fraction, a reduction of 100% of chromium was detected in 12 h, which explain that membrane fraction involve adsorption process rather than an enzymatic chromium reduction.

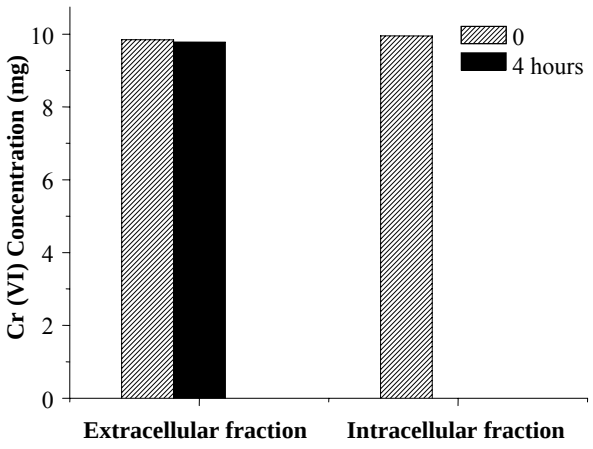

Fig. 6 Cr (VI) reduction in the intracellular and extracellular fractions of *P. anomala*.

These results indicate that the mechanism of Cr(VI) removal by *P. anomala* is "adsorption-coupled reduction" as clarified for *Termitomyces clypeatus* biomass [48], suggesting that *P. anomala* removes chromate ions from aqueous solution initially by adsorption, which consists of binding of anionic Cr(VI) ion species to the positively charged groups present on the biomass surface, followed by reduction into less toxic Cr(III) compound after transportation of Cr(VI) into the cellular organelle.

3.8 Chromium Reductase Induction in the Presence of Chromium

The chromate reductase activity in intracellular fraction from cells grown in the absence and presence of Cr(VI), were 1.078 ± 0.01 μmoles/min/mg and 1.089 ± 0.02 μmoles/min/mg protein, respectively (Table 1), which indicated the constitutive nature of the chromate reductases for this strain. It is apparent that the chromate reductase expression was not induced by addition of Cr(VI) in the medium. Similar constitutive chromate reductases have been demonstrated in other studies [49, 50].

3.9 FTIR Analysis

To confirm the Cr(VI) loaded biosorbent surface or possible metal–biosorbent interactions and to determine the main functional groups of *P. anomala* participate in chromium ions biosorption for assessment the sorption mechanism, IR spectra of yeast biomass free and after chromium ions loaded was carried out. Fig. 7 is FTIR profiles (of control and metal loaded cells) respectively.

The FTIR spectra of the control cells showed a number of peaks reflecting a complex nature of the yeast cell surfaces. It is apparent that there were some changes in the intensity of the bands at different regions after interaction with Cr(VI). A very strong

Table 1 The Cr(VI) reductase activity in the cytosolic and extracellular fractions of *P. anomala* in the absence and in the presence of 50 mg/L of Cr(VI).

	Cr(VI) reductase activity (μm/min/mg of protein)	
	Extracellular fraction	Intracellular fraction
Strain + 50 mg of Cr(VI)	0.020 ± 0.001	1.089 ± 0.02
Strain without Cr(VI)	0.017 ± 0.002	1.078 ± 0.01

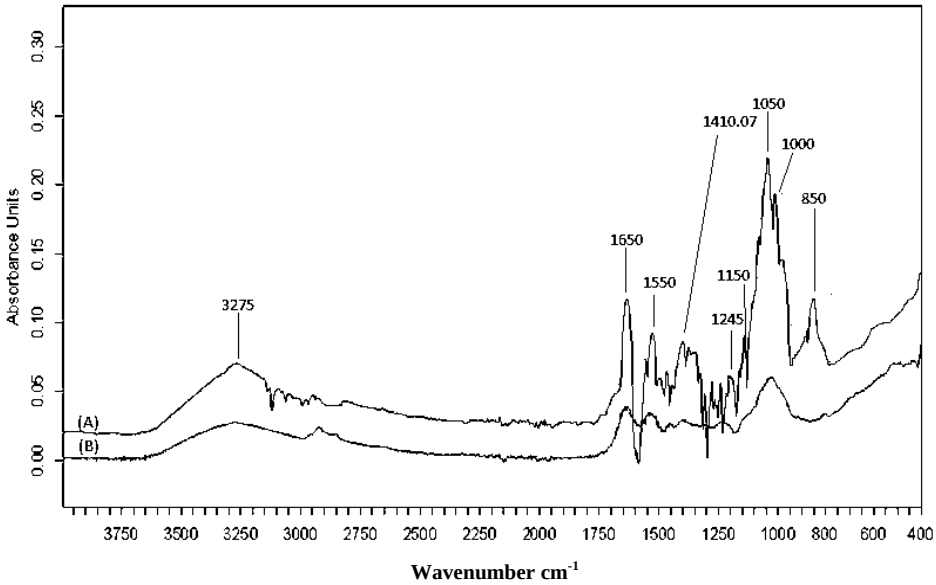

Fig. 7 FTIR spectra of *P. anomala* cells grown (A) without Cr(VI) (control) and (B) with 50 mg/L of chromium.

band in the 3200-3600 cm^{-1} rang corresponds to -OH of glucose and -NH stretching of the protein and acetamido group [51, 52]. The amide I band is primarily a C-O stretching mode and is characterized at 1,650.0 cm^{-1}, while the amide II band is a combination of N-H bending and C-N stretching and it absorb to 1,550.0 cm^{-1}. Peak at the proximity of 1,410.7 cm^{-1} can be attributed to the COO- of the carboxylate group of the biomass [53]. Other peaks located near 1,100-1,000 cm^{-1} are related to the C-O bond, which is characteristic for polysaccharides, it also relates to -CN stretching [54]. A distinct peak at 800-850 cm^{-1} suggests the presence of sulfonate group on the cell surface [53].

Changes were observed in the region of 1,655-750 and 3,450-2,800 cm^{-1}. These changes indicate that Cr(VI) was complexed by carboxyl, amide, polysaccharides and sulfonate functional groups in the external surface of the adsorbent. This observation implies chromium complexation with protein molecules [55, 56].

4. Conclusions

The present work demonstrates that *P. anomala*, isolated from effluent of tanneries, was able to efficiently reduce Cr(VI) with concentrations ranging between 25 and 100 mg/L under aerobic conditions. This strain showed very high-level resistance against a broad range of heavy metals such as Hg, Ni, Zn, Pb, Cu and Co. The presence of different heavy metals increased Cr(VI) reduction potential of this strain, while Zn^{2+} caused a slight decrease in Cr(VI) reduction.

The mechanism study of Cr(VI) removal by this strain reveals that the uptake of chromium by *P. anomala* cells involves initially adsorption on the cell surface followed by intracellular accumulation and reduction of Cr(VI) to Cr (III). Bonded carboxyl group, amide I, amide II, amide III, polysaccharide and

sulfonate were the functional groups for binding Cr(VI) by this strain.

The main mechanism of Cr(VI) removal by this strain is "adsorption-coupled reduction", which suggests that *P. anomala* has significant potential for bioremediation of Cr(VI) pollution.

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