Ann Microbiol (2011) 61:833–841
DOI 10.1007/s13213-011-0204-y

ORIGINAL PAPER

Biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp.

Kumar Saurav · Krishnan Kannabiran

Received: 13 September 2010 / Accepted: 10 January 2011 / Published online: 30 January 2011
© Springer-Verlag and the University of Milan 2011

Abstract The aim of the study was to evaluate the biosorption of heavy metals by actinomycetes isolated from marine sediment samples collected at the Bay of Bengal coast of Puducherry, India. The effect of initial metal ion concentration, pH and biomass dosage on biosorption of chromium ions was investigated. The isolate showed initial metal ion concentration of 80 and 100 mg l^{-1} for Cr(III) and Cr(VI), respectively, pH of 4.0 for Cr(III) and 7.0 for Cr(VI) with biosorption capacity of 76 and 84.27% for Cr(III) and Cr(VI), respectively. The biosorbent dosage was optimized as 3 g l^{-1} for both forms of chromium. The isolate showed the highest MTC (Maximum Tolerance Concentration) value of 1400 mg l^{-1} for Cr(VI) and 2,200 mg l^{-1} for Cr(III). The potential strain was characterized by polyphasic taxonomic approach and identified as a member of the genus *Streptomyces*. Based on the phenotypic and phylogenetic analysis, the strain was classified as a new species of the genus *Streptomyces* and designated as *Streptomyces* VITSVK9 sp. (HM137310). The BLAST search of 16S rDNA sequence of the strain showed highest similarity (95%) with *Streptomyces* sp. A515 Ydz-FQ (EU384279). The media and growth conditions were optimized for maximal growth under shake flask conditions by measuring the dry weight of mycelium. Maximal growth was seen with glucose as carbon source, peptone as nitrogen source and ammonium chloride as inorganic nitrogen. The growth of the isolate was maximal after 9 days of incubation at 35°C and pH of 7.0. Fourier transform infrared absorption spectrum results indicated that the chemical interactions between the functional groups hydroxyl (–OH), amine (–NH2), carboxyl (–COOH) and hydroxyl (–CHOH) of biomass and the metal ions. The results of our study revealed the biosorption property of metabolites produced by *Streptomyces* VITSVK9 spp. and this could be used as potential biosorbent for removal of heavy metal ions from aqueous environment.

Keywords Chromium · Biosorption · MTC (Maximum Tolerance Concentration) · *Streptomyces* VITSVK9 spp. · Biomass · Fourier transform infrared (FT-IR)

K. Saurav · K. Kannabiran (✉)
Biomolecules and Genetics Division,
School of Biosciences and Technology, VIT University,
Vellore 632014, Tamil Nadu, India
e-mail: kkb@vit.ac.in

K. Saurav
e-mail: sauravverma17@gmail.com

Introduction

Several industrial activities including electrolytic treatment, ceramic production, fertilizer production and pigments production can create severe heavy metal pollution. The heavy metals have high mobility in aquatic systems and in general may produce high toxicity (Zouboulis et al. 2004). Due to non-biodegradability, metal ions accumulate and their amounts are increased along the food chain. Hence, their toxic effects are more pronounced in the animals at higher trophic levels. Industrialization processes and continued release of emissions adversely affect the environment leading to the destruction of many agricultural lands and water bodies, thus becoming a matter of great concern. Metals occur naturally, and several of them are essential components of global ecosystems (Pinto et al. 2003). They are present in the environment in a wide range of oxidation states and coordination numbers, and these differences are related to their toxicity. Chromium is a common and very toxic pollutant introduced into natural

waters from a variety of industrial wastewaters particularly from tanneries. Chromium is widely used in many industrial operations such as chrome plating, wood preserving, textile dyeing, pigmenting, chromium chemical production, pulp and paper industries and leather tanning. The wastewater resulting from these processes contains high amounts of chromium metal, which is harmful for the environment and human health (Zayed and Terry 2003). In the last few decades, the amount of chromium in aquatic and terrestrial ecosystems has increased as a consequence of different human activities. Lead, cadmium and mercury are the major toxic metal series, and chromium can also be added in the list (Saha and Orvig 2010). Chromium exists in 11 valence states ranging from −IV to +VI (Fukai, 1967), of which only Cr(III) and Cr(VI) are significant. Hexavalent chromium compounds are approximately 1,000-fold more cytotoxic and mutagenic than trivalent chromium (Biedermann and Landolph 1990). Cr(VI) is highly water soluble and mobile, while Cr(III) shows poor solubility and is easily adsorbed on mineral surfaces. Differences in membrane transport may explain the abilities of these two speciations of chromium to induce the formation of reactive oxygen species and produce oxidative tissue damage. Cr(VI) to Cr(III) reduction, therefore, represents a significant immobilization mechanism (Bagchi et al. 2001).

Biosorption is an innovative technology that employs biological materials to accumulate heavy metals from wastewater through metabolically mediated or physicochemical pathways of uptake (Fourest and Roux 1992). Many microbes oxidize Cr(III) to Cr(VI) and reduce Cr(VI) to Cr(III) under aerobic and anaerobic conditions (Jeyasingh and Philip, 2005). Cr(III) is toxic to fish when its concentration in water exceeds 5 mg l^{-1} (Alloway and Ayres 1997). Cr(III) is an active allergic agent, and is also induced by Cr(VI) exposure. Kidney damage in long-term exposure of Cr(III) is of great concern. Cr(III) species normally carry a positive charge and therefore can be easily adsorbed on the negatively charged soil particles (Silva et al. 2008; Deng et al. 2006). This leads to pollution of the soil environment thereby entering the food chain.

Metal bioaccumulation by marine organisms has been the subject of considerable interest in recent years because of serious concern that high levels of metals may have detrimental effects on the marine organisms and may affect marine food and through them affect humans (El-Moselhy and Gabal 2004). Algae, bacteria and fungi have proved to be potential metal biosorbents (Volesky 1986). Generally, microorganisms interact with toxic metals by three processes including biosorption, bioaccumulation and enzymatical reduction. Actinomycetes constitute a significant component of the microbial population in most soils. Their metabolic diversity and particular growth characteristics, mycelial form and relatively rapid colonization of selective substrates indicate them as well suited to be agents for bioremediation of metal and organic compounds. However, there are very few studies on Cr(VI) resistance and bioreduction by actinomycetes. Amoroso et al. (1998) have reported that metal resistance and biosorption capability may be widespread among actinomycetes growing in contaminated environments. Richards et al. (2002) have studied the heavy-metal resistance patterns of *Frankia* strains. The first report on Cr(VI) reduction by *Streptomyces* was from Das and Chandra (1990). Later, Amoroso et al. (2001) reported Cr(VI) bioaccumulation by *Streptomyces* strains. Cr(VI) reduction by *Streptomyces griseus* was reported by Laxman and More (2002). The aim of the present study is to determine the Cr(III) and Cr(VI) resistance and removal by actinomycetes strains isolated from marine sediments collected at the Bay of Bengal coast of Puducherry and Marakkanam, Tamil Nadu, India.

Materials and methods

Strain isolation

Marine sediment samples (total 21) waere collected from Puducherry (11°56′N, 79°53′E) on the coast of the Bay of Bengal, India, at depths of 50–300 cm using a large sterile spatula. The sediments collected in sterile containers (covered with aluminium sheet) were maintained at ambient temperature with seawater and transported to the laboratory. The sediment samples were dried in laminar air flow for 8–12 h and then kept at 42°C for 10–30 days in a sterile Petri dish, and these preheated samples were used for isolation of actinomycetes. Collected samples were serially diluted and inoculated onto three culturing media, the International Streptomyces Project (ISP) No. 1 (Shirling and Gottlieb 1966), Starch casein agar (1% soluble starch, 0.1% casein, 0.05% KH_2PO_4, 0.05% $MgSO_4$, 3% NaCl, 2% agar) and Kuster's agar medium (1% glycerol, 0.003% casein, 0.02% KNO_3, 0.02% KH_2PO_4, 0.02% NaCl, 0.005% $MgSO_4$, 0.002% $CaCO_3$, 0.001% $FeSO_4$, 2% agar) (prepared with 25% sea water and 25% soil extract) for the isolation of actinomycetes (Kumar and Kannabiran 2010a). The medium was amended with 100 mg l^{-1} of chromium in the form of $K_2Cr_2O_7$ for Cr(VI) at pH 6.5 and Cr $(NO_3)_3{\cdot}9H_2O$ for Cr(III) at pH 3.5 and incubated at 30°C for 4–6 days under shaking (150 rpm) to enrich the chromium-tolerant actinobacterial populations, and the growth media were supplemented with the antibiotics cycloheximide (25 mg ml^{-1}) and nalidixic acid (25 mg ml^{-1}) (Himedia, India). The soil extract was prepared by mixing 400 g of sediments with 1,000 ml of distilled water and centrifuged. The supernatant was filtered through 0.45-μm membrane filters (Millipore, India). The clear supernatant obtained was adjusted to a pH of

7–9 and used as a soil extract. The colonies were recognized according to their cultural and biochemical characteristics and then transferred to slant culture at 4°C as well as at 20% (v/v) glycerol stock at –80°C.

Physicochemical properties and trace metal detection

The physicochemical properties of sediments were analyzed and recorded. The physicochemical parameters such as pH, electrical conductivity, nitrogen, phosphorus, potassium, soil texture, lime status, ferrous, manganese, zinc and copper (Jackson 1973; Lindsay and Norwell 1978) were determined. The collected sediment samples were also processed and analyzed for trace metal concentration. The estimation was done using Atomic Absorption Spectrophotometer (AAS) with Graphite Furnace, Inductively Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) and also by the colorimetric method.

Evaluation of tolerance to Cr(III) and Cr(VI)

The maximum tolerance concentration (MTC) for the selectively isolated strains were determined by the well diffusion and broth dilution methods in ISP No.1 medium with Cr(III) and Cr(VI) concentrations ranging from 100 to 4,000 mg l^{-1}. The maximum concentration of chromium in which the bacterial growth was found has been recorded as the MTC value of those bacteria.

Characterization and optimization of nutritional and culturing conditions of resistant strain

The morphological and biochemical characterization of the resistant isolate was carried out as described in International *Streptomyces* project (ISP). Antibiotic susceptibility test for the isolate was performed using disc diffusion assay, where different antibiotics discs were used at a concentration of 10 μg/ml. The morphology of the spore-bearing hyphae with the entire spore chain, substrate and aerial mycelium of the strain was examined by light microscope as well as by scanning electron microscope (Hitachi, S 3400 N). To determine the optimal nutritional and cultural conditions and to identify the suitable media for growth, the isolate was inoculated in different culture media (SCA, ISP #1, ISP #2, ISP #3, ISP #4, ISP #5, ISP #6, ISP #7 and Kuster's agar) and the growth was investigated by determining the weight of the biomass (Shirling and Gottlieb 1966; Kumar and Kannabiran 2010b). Different nutritional amendments (carbon, nitrogen, amino acid amendents and NaCl concentration) and culturing conditions (incubation temperature, pH and incubation time) were optimized for the maximal growth of the strain by inoculating it in basal medium with variable amounts.

Isolation of chromosomal DNA

Culture broth (1.5 ml) was taken in a sterile microfuge tube and vortexed for 20 s. The broth was centrifuged at 8,000 rpm for 5 min at room temperature and the supernatant was discarded. The process was repeated to get enough cell mass. The genomic DNA was isolated using a modified version of the procedure of Kutchma et al. (1998) and purified by phenol/chloroform extraction. DNA concentration was evaluating using 0.8% (w/v) agarose gel electrophoresis stained with ethidium bromide and then visualized using a Image Analyzer Gel Doc 2000 (Bio-Rad Laboratories, Hercules, CA, USA). Lambda DNA was used as control.

PCR amplification of the 16S rDNA

The sequencing templates of 16S ribosomal DNA (rDNA) was amplified from genomic DNA by PCR as described previously (Stach et al. 2003). Actinomycete-specific primers FC27 (AGAGTTTGATCCTGGCTCAG) and RC1492 (TACGGCTACCTTGTTACGACTT) were used. All sequencing reactions were carried out with an ABI PRISM 377TM DNA sequencer at the Invitrogen laboratory, Bangalore, India. The 16S rDNA sequences obtained were used to search the GenBank database by using BlastN algorithm to identify the closest matches among the known species. Sequences were aligned with representative actinomycetes 16S rDNA sequences and a phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0 (Kumar et al. 2001).

Biosorption experiments

Biosorption experiments were carried out at different pH values (3.0, 4.0, 5.0 and 6.0), varying initial metal ion concentration (10–100 mg l^{-1}) and different biomass dosage to optimize the biosorption efficiency. A fully-grown culture was centrifuged and different weights of the biomass ranging from 0.5 to 3.0 g obtained. This biomass of varying weight from *Streptomyces* strain was used for removal of Cr(III) and Cr(VI) ions and was optimized. A stoppard conical flask (100 ml) containing 25 ml of varying metal ion solution with appropriate biomass concentration and pH was kept for shaking in an electrically thermostatic reciprocating shaker at 100 rpm for 1 week. The contents of the flask were filtered through filter paper and the filtrate was analyzed for metal concentration by using a flame atomic absorption spectrophotometer (Kumar and Kannabiran 2011). The percentage biosorption of metal ions was calculated as follows:

$$\text{Biosorption}\,(\%) = \frac{(Ci - Cf)}{Ci} \times 100$$

Ci and *Cf* are the initial and final metal ion concentrations, respectively.

FT-IR analysis

In order to investigate the involvement of various functional groups in biosorption of metals and possible metal binding sites of functional groups present in the biomass of the isolate, infrared spectra of Cr(III) and Cr(VI) loaded and metal non-loaded samples were obtained using a Fourier transform infrared spectrometer (FT/IR-AVATAR 330).

Statistical Analysis

All the experiments were performed in triplicate and the data obtained were expressed as mean ± standard error. One-way ANOVA was used to calculate significant differences between the samples at a confidence level of a=0.05. All the statistical analysis was done using Graph Pad Prism software version 5.02.

Results and discussion

Physicochemical properties and trace metal detection

The analysis of physiochemical properties of sediments showed insignificant variation in dissolved oxygen, nitrate nitrogen, total phosphate and dissolved organic phosphate with significant variation in total hardness (Ca^{++} and Mg), pH, salinity and temperature for the observed period.

Analysis of sediments for trace metal concentration showed the presence of lead as 13±2.1 μg l^{-1}, chromium as 7.4±0.8 μg l^{-1}, cadmium as 3.1±0.3 μg l^{-1}, zinc as 8.4±2.6 μg l^{-1} and copper as 0.3±0.1 μg l^{-1} , whereas mercury was below the detection limit. All the samples was found to be statistically significant at $p<0.05$. The results showed the high percentage of lead in the sediment, well above the permissible limit. A prolonged exposure to heavy metals exerts a highly selective pressure on the microbial community, which could lead to the appearance of metal-resistant strains (Saxena and Bhattacharya 2006). These results indicate that the adaptation might be a common phenomenon of environmental isolates that could occur when the environment is contaminated with higher concentrations of pollutants.

Strain isolation and tolerance to chromium

The selective isolation process resulted in the isolation of 94 actinomycetes strains from 21 sediment samples. Different medias were used for the selective isolation of the actinomycetes with the preheated sediment samples and the use of metal solution in the medium resulted in the isolation of a metal-tolerant *Streptomyces* species. MTC for the isolates were estimated based on their growth on various concentration of chromium ranging from 100 to 4,000 mg l^{-1} amended in ISP No.1 medium by agar diffusion and broth dilution methods. The majority of the isolates showed the MTC range of 100–500 mg l^{-1} to Cr(VI) and Cr(III). The highest MTC value found for the strain VITSVK9 was 1,400 mg l^{-1} for Cr(VI) and 2,200 mg l^{-1} for Cr(III). Ksheminska et al. (2005) have reported that the tolerance of yeast was up to 5 mM concentration of Cr(III). Our results were found to be in accordance with Amoroso et al. (2001) who has isolated two *Streptomyces* strains, able to grow up to 2 mM Cr(VI) concentration in MM agar medium with efficient Cr(VI) biosorption capability. Similar Cr(VI) biosorption value was reported with *Bacillus* sp. (Nurbap Nourbakhsh et al. 2002).

Characterisation and optimization of nutritional and culturing conditions of resistant strain

The biochemical, morphological and cultural characteristics of the strain revealed that the strain is a Gram-positive, non-acid fast, non-motile, aerobic actinomycetes. The susceptibility of the isolate to various antibiotics was checked by the disc diffusion technique and the strain VITSVK9 was sensitive to cefixime and neomycin but resistant to ciprofloxacin, gentamycin, tetracycline and penicillin G (Table 1). The aerial mycelium of the strain was branched, white in color and the substrate mycelium was also branched and produced a powdery colony on optimized medium and optimized culturing conditions. But both the aerial and substrate mycelium were found to be medium dependent. A long chain of spores were oval to cylindrical in shape, arranged in long chains and each contained more than 10–25 spores. The mature spores were 0.5–1.0 mm in diameter and the length was between 0.8 and 1.0 mm (Fig. 1). Spacial morphology such as sporangia and sclerotia was not observed. Scanning electron microscopy revealed the presence of spiral arrangement of spores which confirmed that isolate belongs to the genus *Streptomyces*.

Cultural characteristics and media composition were optimized by a systematic study and the suitabilities of various carbon and nitrogen sources were evaluated and correlated. Optimization for the growth of VITSVK9 isolate was carried out in batch culture. The strain was cultured on the basal medium with different parameters and their effect on the growth was studied in terms of their biomass. Strains were capable of growing on all the media used. However, VITSVK9 showed the highest biomass (6.86±0.23 mg ml^{-1}) when grown onto ISP No. 1 medium. Concentration of glucose in the medium influenced the growth of the isolate.

Table 1 Biochemical characteristics of *Streptomyces* VITSVK9 spp.

Tests	Results
Gram's stain	+
Aerial mycelium	White
Substrate mycelium	White
Motility	−
Endospore staining	−
Colony color	White
Spores	Short, oval
Catalase	+
Oxidase	+
Indole	−
Methyl red	+
Voges proskeur	−
Citrate utilization	+
Starch hydrolysis	+
Gelatin liquefaction	+
H_2S	+
Resistance to antibiotic	
Cefixime10	+
Gentamycin10	−
Tetracycline10	−
Ciprofloxacin10	−
Neomycin10	+
Penicillin G10	−

+ Positive; − negative

Glucose at 1% (w/v) provided the highest growth (4.9± 0.46 mg ml^{-1}) for the strain when used as carbon source. In contrast, growth was decreased with either increase or decrease of glucose concentration. High concentration of glucose is generally considered as repressor of secondary metabolisms (Demain 1989) and maximum cell growth rates can inhibit antimicrobial agent production (Gallo and Katz 1972). Of all the nitrogen sources tested, maximal growth (6.86±0.23 mg ml^{-1}) was seen with peptone. Among inorganic nitrogen sources, ammonium chloride showed a moderate effect on the growth of the isolate. It is clear from the results that the growth was greatly influenced by the nature and type of the nitrogen source supplied in the culture medium. The optimized nutritional and culturing conditions for the growth by the strain VITSVK9 are presented in Table 2.

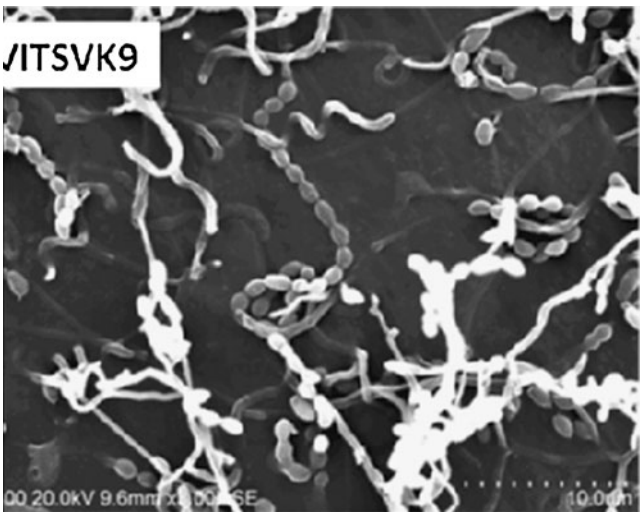

Fig. 1 Scanning electron micrograph of *Streptomyces* VITSVK9 spp. grown in optimized medium at 30°C for 9 days; *bar* 10 μm

Phylogenetic analyses

The partial sequencing of 16S rRNA gene of the strain VITSVK9 on both directions yielded 16S rDNA nucleotide sequence with 1,532 base pairs. The 16S rDNA sequence of the strain was deposited in the GenBank (NCBI, USA) under the accession number HM137310. The BLAST search of 16S rDNA sequence of the strain showed highest similarity (95%) with *Streptomyces* sp. A515 Ydz-FQ (EU384279). A neighbor-joining tree based on 16S rDNA sequences showed that the isolate occupies a distinct phylogenetic position within the radiation including representatives of the *Streptomycetes* family (Fig. 2). The phylogenetic tree based on the maximum-parsimony method also showed that the isolate forms a separate clade. Based on the molecular taxonomy and phylogeny, the strain was identified as *Streptomyces* species and designated as *Streptomyces* VITSVK9 spp.

Effect of wet biomass dosage on biosorption

Initially, a highest metal ion concentration of 100 mg l^{-1} was taken for the biosorption experiment. The biosorption efficiency for Cr(III) and Cr(VI) ions as a function of biomass dosage showed that the biosorption efficacy

Table 2 Optimal nutritional and culturing conditions for the growth of *Streptomyces* VITSVK9 spp.

Parameters	*Streptomyces* VITSVK9 spp.	
	Optimum value	Biomass (mg/ml)
Carbon source	Glucose (1%)	4.9±0.46
Nitrogen source	Yeast extract	6.8±0.23
NaCl	5%	7.03±0.39
Amino acid	Methionine	6.86±0.23
Temperature	30°C	6.9±0.43
pH	7	7.03±0.43
Incubation time	9 days	6.9±0.08

Fig. 2 The phylogram showing the position of the strain VITSVK9 with other *Streptomyces* species based on 16S rDNA sequence. Phylogenetic tree based on neighbor-joining analysis of 1,000 replicated data. *Numbers* at *nodes* indicates the percent level of bootstrap support. *Score bar* 1 nucleotide substitution per 100 nucleotides. Bootstrap values of 50 and above are shown

increased gradually with increase in biosorbent concentration (0.5 to 3 g l^{-1}) (Fig. 3). The maximum biosorption was found to be 83.24% for Cr(III) and 71.24% for Cr(VI) at the biomass dosage of 3 g l^{-1}. The optimum condition identified for one parameter was used for optimizing other parameters one by one. Earlier studies of Yao and Ye (2009) and Acharya et al. (2009) have indicated that the biosorbent dose was also an important parameter affecting biosorption capacity as well as removal efficiency.

Effect of initial metal ion concentration on biosorption

The initial metal ion concentration remarkably influenced the percentage metal biosorption (Fig. 4). The initial Cr(III) concentration increased from 10 to 100 mg l^{-1}, the biosorption capacity increased to 80 mg l^{-1}, but the biosorption ability drastically decreased thereafter with increase of metal ion concentration. On the other hand, the biosorption capability for Cr(VI) was found to be at a maximum at 100 mg l^{-1} metal ion concentration. For Cr(VI), the biosorption ability increased with the increase of metal ion concentration. The higher biosorption ability with increased metal ion concentration could be attributed to higher probability of interaction between metal ions and biosorbents.

Effect of pH on biosorption

The effect of pH on the uptake of Cr(III) and Cr(VI) ions by *Streptomyces* VITSVK9 spp. is shown in the Fig. 5. The maximum uptake of Cr(III) was observed at pH 4.0, and Cr (VI) ion at 7.0. At higher pH values, the biosorption was dramatically decreased. The increased biosorption at pH 4.0 may be due to more of negatively charged groups in biomass surface capable of binding positively charged metal ions. Decrease in biosorption at higher pH (pH>4) is may be due to the formation of soluble hydroxylated complexes of the metal ions and their competition with the active sites, and as a consequence, the retention would decrease. The metal-binding properties of Gram-positive

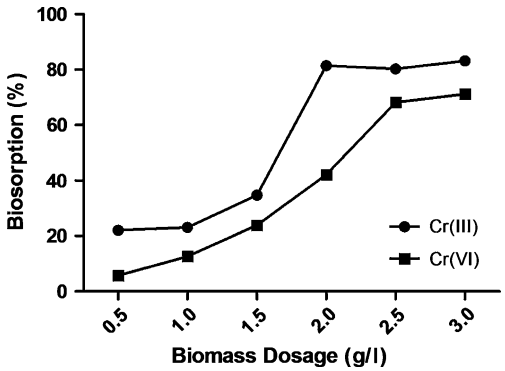

Fig. 3 Effect of biomass dosage on biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp. biomass

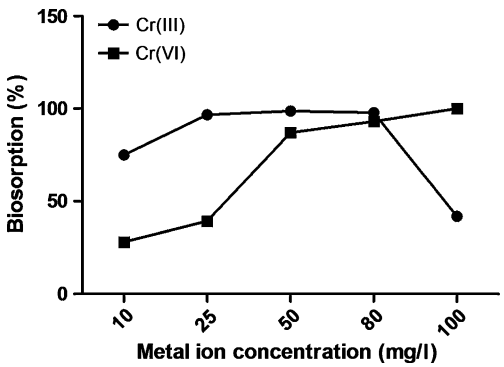

Fig. 4 Effect of metal ion concentration on biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp. biomass (3 g l^{-1})

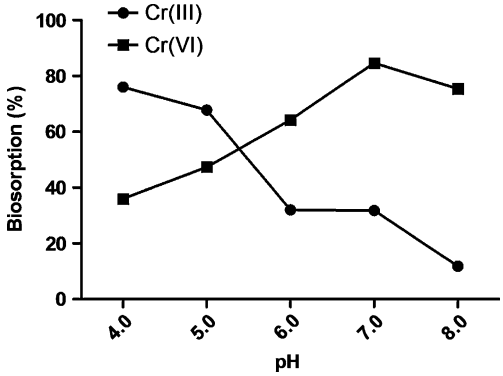

Fig. 5 Effect of pH on biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp. biomass

bacteria, such as actinomycetes (*Streptomyces*), are largely due to the existence of specific anionic polymers in the cell wall structure, consisting mainly of peptidoglycan, teichoic or teichuronic acids (Yee et al. 2004). Earlier studies on heavy metal biosorption have shown that pH was the single most important parameter affecting the biosorption process (Aksu et al. 1991; Donmez et al. 1999).

The biosorption of Cr(III) was studied by Prigione et al. (2009) with 5 different fungal species; maximum removal observed was 38% from real tanning effluents. Bioaccumulation of Cr(III) was studied in yeast (Kaszycki et al. 2004), lichens (Ksheminska et al. 2005), and in *Parmelina tiliaceae* (Uluozlu et al. 2008). But not much reports are available on Cr(III) bioaccumulation by bacteria. Different types of pure and mixed microbial cultures isolated from chromium-polluted areas have been investigated for their ability to bioaccumulate chromium (VI) (Zouboulis et al.

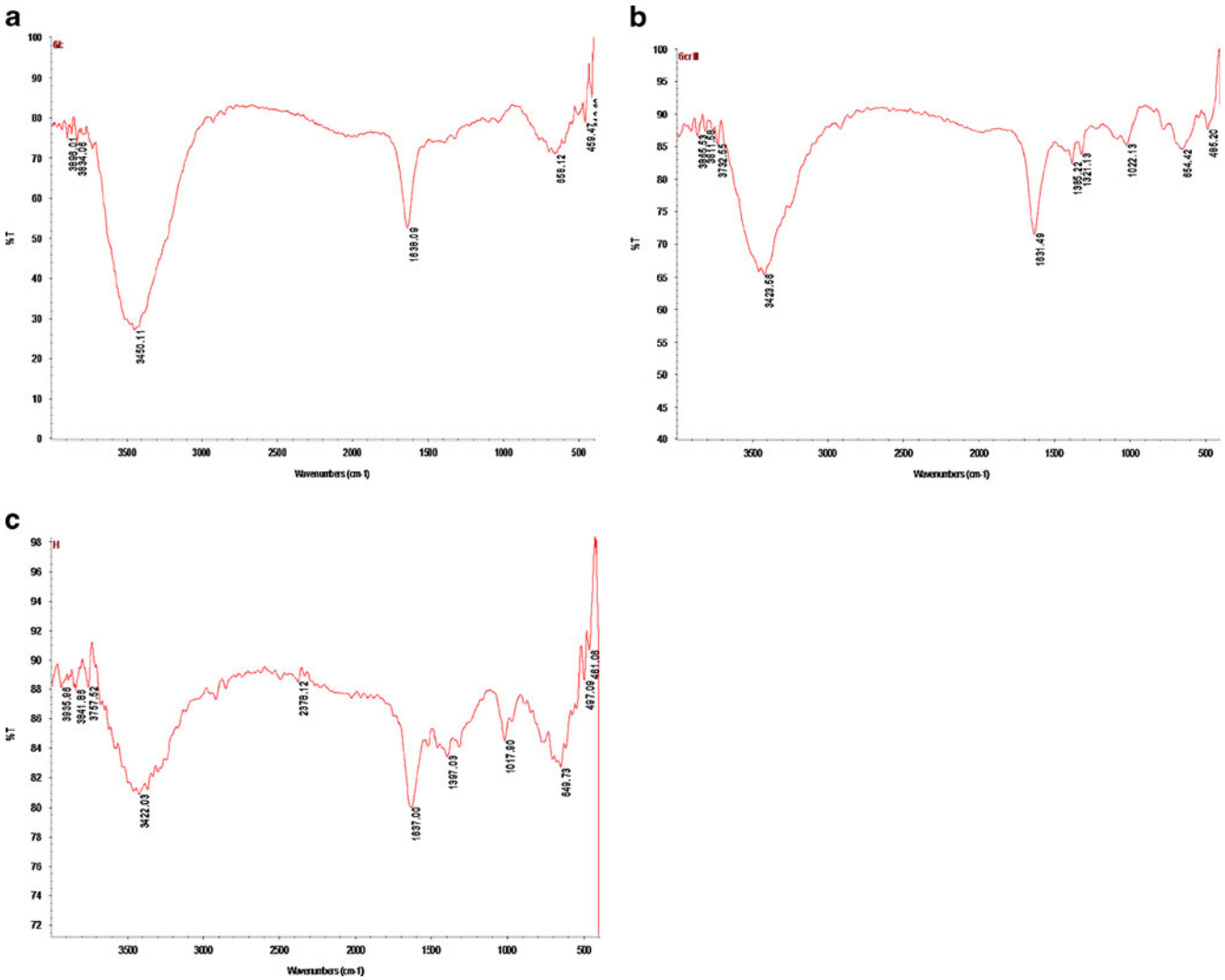

Fig. 6 Fourier transform infrared absorption spectrum of biosorption of Cr(III) and Cr(VI) by *Streptomyces* VITSVK9 spp. **a** Native biomass of *Streptomyces* VITSVK9 spp. **b** Cr(III)-treated biomass (3 g l^{-1}) of *Streptomyces* VITSVK9 spp., and **c** Cr(VI)-treated biomass (3 g l^{-1}) of *Streptomyces* VITSVK9 spp.

2004; Srinath et al. 2002; Badar et al. 2000; Saxena et al. 2001). Metal bioremoval by living cells include two processes, one is active metabolism in which metals accumulate inside the cell, and another of passive metabolism in which metals adhere to surface molecules such as the S-layer proteins (Velásquez and Dussan 2009). If an adsorption process is taking place, surface molecules such as the S-layer proteins can have a saturation level in which no more metallic ions can adhere. If the process is metal accumulation, an efflux mechanism can be functioning up to certain metal concentration preventing more metal accumulation. Similar processes have been reported with *Pseudomonas aeruginosa* and *Cupriavidus metallidurans* by Ramírez et al. (2008).

FT-IR analysis

The possible interactions between the functional groups of *Streptomyces* VITSVK9 spp. biomass with chromium were evaluated by FT-IR spectroscopy. The FTIR spectra of lyophilized native culture biomass are shown in Fig. 6a. The broad band spectra at 3,150 cm^{-1} was due to bounded hydroxyl (–OH) or amine (–NH2) groups in the native culture. This band was slightly shifted to 3,123 cm^{-1} in Cr(III)-treated culture (Fig. 6b). The peaks observed between 1,623 and 1,638 cm^{-1} were attributed to stretching vibration of C = C group. The Cr(III)-treated *Streptomyces* VITSVK9 spp. showed the presence of polysaccharides which were indicated by peaks in the range of 1,020 cm^{-1}. The FTIR spectra of lyophilized Cr(VI- loaded cell biomass are shown in Fig. 6c. The band at 3,150 cm^{-1} in native culture was slightly shifted to 3,122 cm^{-1} in Cr(VI- treated *Streptomyces* VITSVK9 spp. The peaks observed between 1,623 and 1,638 cm^{-1} were attributed to stretching vibration of C = C group. This might be due to the secretion of high molecular mass polymers, which can either be released into the environment or remain attached to cell surfaces. The extensive heavy metal binding capacities of polysaccharides are recommended as a surface-active decontamination agent.

The microbial cell wall is known to be rich in polysaccharides and glycoproteins such as glucans, chitin, mannans and phospho-mannans. These polymers form abundant sources of metal binding ligands. The cell wall of *Streptomyces* generally contains three components, namely peptidoglycan, teichoic acid and surface protein. These compounds may contain several functional groups, such as amino, carboxyl, sulphate, hydroxyl, etc., which could play an important role in the biosorption of metal ions. The biosorption of metal ions by microbial biomass has several advantages over other conventional methods used to remove heavy metals. It has been reported that metabolism-independent metal binding to the cell walls and external surfaces is the only mechanism present in the case of non-living biomass (Ahluwalia and Goyal 2003). FTIR spectroscopy was also more often used to study the biosorption of metal ions (Ahluwalia and Goyal 2005). Bacteria may uptake and accumulate a significant amount of metal ions, resulting in transfer of metals to a contaminated matrix of biomass (Smith et al. 1994). The removal of chromium, cadmium and copper from dilute aqueous solution using dead polysaccharide-producing *Ochrobactrum anthropi* isolated from activated sludge has already been reported (Ozdemir et al. 2003).

Conclusion

Based on results of this study, the wet biomass and aqueous solution of *Streptomyces* VITSVK9 spp. exhibited concentration- and pH-dependent biosorption of heavy metal ions Cr(III) and Cr(VI).

Acknowledgement Authors thank the management of VIT University for proving facilities to carry out this study.

Conflicts of interest The authors declare that they have no conflicts of interest.

References

Acharya J, Sahu JN, Mohanty CR (2009) Removal of lead(II) from wastewater by activated carbon developed from Tamarind wood by zinc chloride activation. Chem Eng J 149:249–262

Ahluwalia SS, Goyal D (2003) Removal of lead from aqueous solution by different fungi. Ind J Microbiol 43:237–241

Ahluwalia SS, Goyal D (2005) Removal of heavy metals by waste tealeaves from aqueous solution. Eng Life Sci 5:158–162

Aksu Z, Kutsal T, Gun S, Haciosmanoglu N, Gholaminejad M (1991) Investigation of biosorption of Cu (II), Ni (II) and Cr (IV) ions to activated ions bacteria. Environ Technol 12:915–921

Alloway BJ, Ayres AK (1997) Chemical principles of environmental pollution, 2nd edn. Blackie, London

Amoroso MJ, Castro G, Carlino Federico J, Romero NC, Hill R, Oliver G (1998) Screening of heavy metal-tolerant actinomycetes isolated from the Sali River. J Gen Appl Microbiol 44:29–32

Amoroso MJ, Castro GR, Duran A, Peraud O, Oliver G, Hill RT (2001) Chromium accumulation by two Streptomyces spp. isolated from riverine sediments. J Ind Microbiol Biotechnol 26:210–215

Badar U, Ahmed N, Beswick AJ, Pattanapipitpaisal P, Macaskie LE (2000) Reduction of chromate by microorganisms isolated from metal contaminated sites of Karachi, Pakistan. Biotechnol Lett 22:829–836

Bagchi D, Bagchi M, Stohs SJ (2001) Chromium (VI)-induced oxidative stress, apoptotic cell death and modulation of p53 tumor suppressor gene. Mol Cell Biochem 222:149–158

Biedermann KA, Landolph JR (1990) Role of valence state and solubility of chromium compounds on induction of cytotoxicity, mutagenesis, and anchorage independence in diploid human fibroblasts. Cancer Res 50:7835–7842

Das S, Chandra AL (1990) Chromate reduction in Streptomyces. Experientia 46:731–733

Demain AL (1989) Carbon source regulation of idiolite biosynthesis in actinomycetes. In: Shapiro S (ed) Regulation of actinomycetes. CRC Press, Boca Raton, FL, pp 127–134

Deng H, Ye ZH, Wong MH (2006) Lead and zinc accumulation and tolerance in populations of six wetland plants. Environ Pollut 141:69–80

Donmez G, Aksu Z, Ozturk A, Kutsal T (1999) A comparative study on heavy metal biosorption characteristics of some algae. Process Biochem 34:885

El-Moselhy KM, Gabal MN (2004) Trace metals in water, sediments and marine organisms from the northern part of the Gulf of Suez, Red Sea. J Mar Syst 46:39–46

Fourest E, Roux JC (1992) Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. Appl Microbiol Biotechnol 3:399–403

Fukai R (1967) Valency state of chromium in seawater. Nature 213:901

Gallo M, Katz E (1972) Regulation of secondary metabolite biosynthesis: Catabolite repression of phenoxazinone synthase and actinomycin formation by glucose. J Bacteriol 109:659–667

Jackson ML (1973) Soil chemical analysis. Prentice Hall, New Delhi

Jeyasingh J, Philip L (2005) Bioremediation of chromium contaminated soil: optimization of operating parameters under laboratory conditions. J Hazard Mater 118:113–120

Kaszyckia P, Fedorovychb D, Ksheminskab H, Babyaka L, Wojcika D, Koloczeka H (2004) Chromium accumulation by living yeast at various environmental conditions. Microbiol Res 159:11–17

Ksheminska H, Fedorovych D, Babyak L, Yanovych D, Kaszycki P, Koloczek H (2005) Chromium(III) and (VI) tolerance and bioaccumulation in yeast: a survey of cellular chromium content in selected strains of representative genera. Process Biochem 40:1565–1572

Kumar S, Kannabiran K (2010a) Antifungal activity of *Streptomyces* VITSVK5 spp. against drug resistant *Aspergillus* clinical isolates from pulmonary tuberculosis patients. J Mycol Med 20:101–107

Kumar S, Kannabiran K (2010b) Diversity and Optimization of process parameters for the growth of *Streptomyces* VITSVK9 spp. isolated from Bay of Bengal, India. J Nat Environ Sci 2:56–65

Kumar S, Kannabiran K (2011) Biosorption of Cd(II) and Pb(II) ions by aqueous solutions of novel alkalophilic *Streptomyces* VITSVK5 spp. biomass. J Ocean Univ China 10:365–370

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245

Kutchma AJ, Roberts MA, Knaebel DB, Crawford DL (1998) Small-scale isolation of genomic DNA from *Streptomyces mycelia* or spores. Biotechniques 24:452–456

Laxman SR, More S (2002) Reduction of hexavalent chromium by *Streptomyces griseus*. Miner Eng 15:831–837

Lindsay WC, Norwell A (1978) Development of a DTPA soil test for zinc, iron, manganese and copper. Proc Soil Sci Soc Am 42:421–428

Nurbap Nourbakhsh M, Kilicarslan S, Ilhan S, Ozdag H (2002) Biosorption of Cr6+, Pb2+ and Cu2+ ions in industrial waste water on *Bacillus* sp. Chem Eng J 85:351–355

Ozdemir G, Ozturk T, Ceyhan N, Isler R, Cosar T (2003) Heavy metal biosorption by biomass of *Ochrobactrum anthropi* producing exopolysaccharide in activated sludge. Bioresour Technol 90:71–74

Pinto E, Teresa CS, Maria ASL, Oswaldo KO, Morse D, Pio C (2003) Heavy metal induced oxidative stress in seaweeds. J Phycol 39:1008–1018

Prigione V, Zerlottin M, Refosco D, Tigini V, Anastasi A, Varese GC (2009) Chromium removal from a real tanning effluent by autochthonous and allochthonous fungi. Bioresour Technol 100:2770–2776

Ramírez M, Diaz C, Vargas E, Riveros H, Campos J, Cervantes C (2008) Mechanisms of bacterial resistance to chromium compounds. Biometals 21:321–332

Richards JW, Krumholz GD, Chval MS, Tisa LS (2002) Heavy metal resistance patterns of Frankia strains. Appl Environ Microbiol 68:923–927

Saha B, Orvig C (2010) Biosorbents for hexavalent chromium elimination from industrial and municipal effluents. Coord Chem Rev. doi:10.1016/j.ccr.2010.06.005

Saxena D, Levin R, Firer MA (2001) Removal of chromate from industrial effluent by a new isolate of *Staphylococcus cohnii*. Water Sci Technol 42:93–98

Saxena P, Bhattacharyya (2006) Nickel tolerance and accumulation by filamentous fungi from sludge of metal finishing industry. Geomicrobiol J 23:333–340

Shirling EB, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:312–340

Silva B, Figueiredo H, Quintelas C, Neves IC, Tavares T (2008) Zeolites as supports for the biorecovery of hexavalent and trivalent chromium. Microporous Mesoporous Mater 116:555–560

Smith LA, Alleman BC, Copley-Graves L (1994) Biological treatment options. In: Means JL, Hinchee RE (eds) Emerging technology for bioremediation of metals. CRC Press, Boca Raton, FL

Srinath T, Verma T, Ramteke PW, Garg SK (2002) Chromium (VI) biosorption and bioaccumulation by chromate resistant bacteria. Chemosphere 48:427–435

Stach JE, Maldonado LA, Ward AC, Goodfellow M, Bull AT (2003) New primers for the class actinobacteria: application to marine and terrestrial environments. Environ Microbiol 5:828–841

Uluozlu OD, Sari A, Tuzen M, Soylak M (2008) Biosorption of Pb (II) and Cr (III) from aqueous solution by lichen (*Parmelina tiliaceae*) biomass. Bioresour Technol 99:2972–2980

Velásquez L, Dussan J (2009) Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*. J Hazard Mater 167:713–716

Volesky B (1986) Biosorbent materials. Biotechnol Bioeng Symp Ser 16:121–126

Yao L, Ye ZF (2009) Characteristics of Pb^{2+} biosorption with aerobic granular biomass. Chin Sci Bull 53:948–953

Yee N, Benning LG, Phoenix VR, Ferris FG (2004) Characterization of metal–cyanobacteria sorption reactions: a combined macroscopic and infrared spectroscopic investigation. Environ Sci Technol 38:775–782

Zayed AM, Terry N (2003) Chromium in environment: Factors affecting biological remediation. Plant Soil 249:139–156

Zouboulis AI, Loukidou MX, Matis KA (2004) Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. Process Biochem 39:909–916