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## Bioremediation of Chromium (VI) by *Bacillus aerophilus* VIT SN Strain

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### ABSTRACT

Bacterial strain (CDB1) which is more efficient to transform/ reduce Cr (VI) to Cr (III) was isolated from Common Effluent Treatment Plant of Ranipet, Tamil Nadu. Based on biochemical characterization, it was identified as *Bacillus* sp. and after sequencing, it showed 99% similarities with *Bacillus aerophilus* and thus named as *Bacillus aerophilus* VIT SN strain. Optimum pH and temperature for the growth of bacterial isolate were found to be 7.5 and 37°C respectively. The isolated bacteria showed maximum growth on media containing 2% NaCl and they were able to survive in media containing  $K_2Cr_2O_7$  as source of Cr (VI) up to 500 ppm concentration. Although slight decrease of growth was also noticed when it was incubated in LB broth containing 100 ppm  $K_2Cr_2O_7$  in comparison to control (absence of  $K_2Cr_2O_7$ ). The ability to reduce hexavalent chromium to trivalent form by *Bacillus aerophilus* VIT SN strain was estimated by using Atomic Absorption Spectroscopy (AAS). It showed 95%, 94%, 90%, 85% and 73% degradation ability for 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm concentration of  $K_2Cr_2O_7$  respectively as source of Cr (VI). The bacterial strain showed maximum growth in culture broth containing Dextrose (DEX) and Yeast Extract (YE) supplemented as carbon and nitrogen sources respectively. Apart from Chromium, *Bacillus aerophilus* VIT SN strain also showed resistance to other heavy metals such as Cadmium (500 ppm), Lead (500 ppm), Zinc (500 ppm) and Copper (300 ppm) up to certain concentration.

**Key words:** *Bacillus aerophilus*, Chromium, AAS, Dextrose, Yeast Extract

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## INTRODUCTION

Metals play an important role in the metabolic processes of the organisms. Some of the heavy metals such as cobalt, chromium, nickel, iron, manganese, zinc etc. are essential and required by the living beings as micronutrients, also called as 'trace elements'. They are involved in redox processes, catalysis of enzymatic reaction and regulating the osmotic balance. Another side some other heavy metals such as cadmium, mercury, lead etc. have no biological role and are harmful to the living beings even at very low concentration but at high concentration both the essential and nonessential metals become toxic to the living beings. Heavy metals can influence the microbial population by affecting their growth, morphology, biochemical activities. This eventually results in decreased biomass and diversity. Heavy metals can also damage the cell membranes, alter enzyme specificity, disrupt cellular functions and damage the structure of DNA [1].

Industries liberate large amount of heavy metals in the form of waste or by-products. This type of industrial waste has permanent toxic effect on human health and also possesses threat to environment. Accumulation of toxic metals like Cd, Cr, Cu, Hg, Zn etc. above threshold level, in humans has several consequences such as growth and developmental abnormalities, carcinogenesis, neuromuscular control defects, mental retardation, renal malfunction and a wide range of other illnesses. Chromium is one of the toxic heavy metals that is widely used in electroplating, leather tanning, textile dyeing and metal processing industries. In nature chromium exists either as Cr (VI) which is soluble and highly toxic or as Cr (III) which is less soluble and less toxic. The conventional method to detoxify and remove chromium (VI) from the environment involves chemical reduction of chromium (VI) followed by precipitation, ion exchange and adsorption on coal, activated carbon, alum, kaolinite and flyash. But application of such traditional treatment techniques is very costly and requires continuous input of chemicals which makes this method highly impractical and uneconomical. Use of large quantity of chemicals poses another threat to environment.

Hence an easy, economic and eco-friendly technique is required for fine tuning of effluent treatment. This problem has led us to look for some biological or microbiological methods for metal decontamination as alternatives to conventional methods. Biological methods of reduction of Cr (VI) using indigenous microorganisms offers an environmentally compatible and cost effective solution compared to traditional methods [2, 3 and 4].

## MATERIALS AND METHODS

### Sample collection

The treated tannery effluent was collected in sterile glass bottles from Common Effluent Treatment Plant (CETP), Ranipet (Latitude: 12.9275° N and longitude: 79.3302° E), Tamil Nadu, India. It was transported to laboratory and analyzed within 6 hours of collection.

## Physico-chemical analysis of tannery effluent

The effluent was analyzed for different physico-chemical properties such as colour, odour, temperature, pH, electrical conductivity, BOD (Biological Oxygen Demand), COD (Chemical Oxygen Demand), concentration of Cr (VI).

## Isolation and screening of Cr (VI) resistant bacteria

Tannery effluent was serially diluted up to  $10^{-6}$ . Samples from  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  were spread on nutrient agar (NA) plate. Plates were incubated at 37°C for 24 hours. After 24 hours of incubation colony morphology was recorded with respect to shape, size, colour etc. Further purification was done by streak plate method on nutrient agar plate (four isolates). The Cr(VI) resistant bacterial isolates (four isolates) were screened on nutrient agar enriched with different concentrations of  $K_2Cr_2O_7$  (100 ppm to 600 ppm) as a source of Cr(VI) and incubated at 37°C for 24 hours. After incubation plates were observed.

## Bio-removal of hexavalent chromium

In order to determine the ability of bacterial isolates to reduce Cr (VI) to Cr (III), atomic absorption spectroscopy (AAS) was used. 100 mL LB broth was prepared in different 250 mL conical flasks and inoculated with 1 mL of selected bacterial isolates (two isolates) which were obtained after screening from each plate (100 ppm to 500 ppm concentration of  $K_2Cr_2O_7$ ). The flasks were incubated at orbital shaker at 120 rpm for 24 hours at 37°C. After 24 hour of incubation 10 mL aliquot sample were centrifuged at 2500 rpm for 15 minutes. The clear supernatant was used for AAS analysis directly, and acid digestion was performed with the pellet for AAS analysis.

Among the two bacterial isolates, only one isolate was selected for further studies which had highest capability of degrading Cr (VI).

## Identification of bacterial isolate

The selected bacterial isolate was identified by Gram staining, Endospore staining (**Schaeffer-Fulton method**), Hanging Drop method and some biochemical tests such as Indole test, Methyl Red (MR) and Voges- Proskauer (VP) test, Starch hydrolysis test, Urease production, Citrate utilization test, Catalase activity, Oxidase test, Nitrate reduction test, Triple sugar iron test, Gelatin hydrolysis, Sugar fermentation test.

The molecular characterization of selected bacterial isolate was done by 16S rRNA sequence analysis. The bacterial genomic DNA was extracted using standard protocol of Sambrook et al., (1989) [5]. The 16S rRNA gene was amplified by PCR using two bacterial 16S rRNA primers, 5'- CWG RCC TAN CAC ATG SAA GTC- 3' for forward sequencing and 5'- GRC GGW GTG TAC NAG GC – 3' for reverse sequencing. Sequencing was carried out by ABI 3730x1 genetic analyser Sanger Dideoxy Sequencing/chemistry BDT version 3.1. The 16S rRNA sequence

was deposited to GenBank database to get the accession number, and identified most similar sequence alignment using [www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST) [6]. The nucleotide sequences were aligned with Clustal W, and phylogenetic tree was constructed by the help of TREE VIEW software.

### **Determination of optimum growth conditions for the bacterial isolate**

To determine the optimum temperature for growth of the bacterial isolate, 5 mL LB broth was added into five sets, each set consisted of three test tubes. The tubes were autoclaved and inoculated with 20  $\mu$ L of freshly prepared overnight culture. The 5 sets of tubes were incubated at 4°C, 25°C, 37°C, 40°C and 45°C. After 12 hours incubation, their absorbance was measured at 600 nm by using colorimeter.

For determination of the optimum pH, 5 ml LB broth was added into nine sets, each set consisted of three test tubes and their pH was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 and then autoclaved. These tubes were inoculated with 20  $\mu$ L of overnight culture. After incubation for 12 hours their absorbance was measured at 600 nm [2].

### **Effect of chromium on bacterial growth**

To study the effect of chromium on the growth of bacterial isolate, two 250 mL of side arm Erlenmeyer flasks were taken and 100 mL of LB broth was prepared for each flask. 100 ppm of  $K_2Cr_2O_7$  was added as a source of Cr (VI), into only one flask and both the flasks were autoclaved. After autoclave, flasks were allowed to cool and then 1mL culture was added into the both flasks, with chromium (test) and without chromium (control). Growth was measured as optical density at 600 nm in every one hour of interval up to 32 hours.

### **NaCl tolerance of the isolated strain**

5 mL of LB broth was added into 7 sets, each set consists of 3 test tubes containing different concentration of NaCl adjusted to 1%, 2%, 4%, 6%, 8% and 10% and then autoclaved. After that 20  $\mu$ L of fresh overnight culture was added and incubated at 37°C for 12 hour. After 12 hours of incubation absorbance was measured at 600 nm by using colorimeter.

### **Optimization of carbon and nitrogen sources of the culture medium**

Sugars are the main sources of carbon for the microorganisms. So, to check the best carbon source for the growth of isolated strain, 10% of different sugars such as Glucose (GLU), Sucrose (SUC), Lactose (LAC), Fructose (FRU) and Dextrose (DEX) were added into the nutrient broth in different conical flasks and culture was inoculated into it and incubated for 24 hours at 37°C. After 24 hours O.D. was checked at 600 nm by using colorimeter.

Nitrogen is an important nutrient factor for the growth of microorganism. For determination of the best nitrogen source of the isolated strain, 10% of different nitrogen

sources such as Meat Extract (ME), Beef Extract (BE), Malt Extract (MtE), Yeast Extract (YE) and Peptone (Pe) were added into the nutrient broth in different conical flasks and the isolated bacteria was inoculated into it. After 24 hours incubation at 37°C, optical density (O.D.) was checked by colorimeter at 600 nm.

### **Resistance to different heavy metals**

The heavy metal resistance of the bacterial isolate was determined by using different metal salts (lead nitrate, cadmium chloride, copper sulphate and zinc sulphate). This was checked by increasing the concentration of the respective metals. Culture flasks containing 50 mL of medium and metal salts were inoculated with 20µl of the bacterial culture and incubated at 37°C for 12 hours. After incubation the O.D. was measured at 600 nm.

### **Statistical Analysis**

All experiments were performed in triplicate. The statistical calculations were done based on the standard method [7]. The results are given as mean  $\pm$  SD values.

## **RESULTS AND DISCUSSION**

### **Physicochemical analysis of tannery effluent**

The physicochemical properties of treated tannery effluent were summarized in Table 1. Variation of physical properties of test effluent was also found in comparison with the standard value of the tannery effluent. The colour of the test effluent was found as light brown while the standard colour of tannery effluent in general is found to be colourless. Foul smell is common for all the tannery effluent. Temperature of the test effluent was found as  $37 \pm 0.5^\circ\text{C}$  while the standard value shows  $<35^\circ\text{C}$  but this parameter may vary from sample to sample due to difference in regional average temperature or seasonal variations. pH of the test effluent was  $6.8 \pm 0.28$  while the standard pH value of tannery effluent is 5.5-9.0. Electrical conductivity of the test effluent was found to be  $59.8 \pm 0.75$  mS/cm. Chemical oxygen demand of the test effluent was found to be  $278 \pm 0.5$  mg/L while the standard value shows 250 mg/L. The BOD and chromium (VI) level of the test effluent were found to be  $95.7 \pm 0.15$  mg/L and  $1.38 \pm 0.02$  mg/L respectively which crossed the standard value of the tannery effluent. As the physicochemical properties of treated tannery effluent was not satisfactory, so the release of the effluent will have harmful effect on environment. The high values of BOD and COD indicate concentration of organic compounds are high in effluent while being discharged into the fresh water bodies lead to eutrophication and thus negatively affect aquatic organisms. Exposure of animals and humans to Cr(VI) in drinking water induced tumours in the alimentary tract and the most abundant form of DNA damage (Cr-DNA adducts), which cause mutations and chromosomal breakage [8].

**Table 1: Physicochemical analysis of tannery effluent**

Parameters	Test	Standard <sup>#</sup>
Colour	Very light brown in colour	Colourless
Odour	Foul smell	Foul smell
Temperature (°C)	37 ± 0.5	<35°
pH	6.8 ± 0.28	5.5- 9.0
Electrical conductivity (mS/cm)	59.8 ± 0.75	-
BOD (mg/L)	95.7 ± 0.15	30
COD (mg/L)	278 ± 0.5	250
Chromium(VI) (mg/L)	1.38± 0.02	0.1

# Standards given by Ministry of Environment and Forest and United States of Environment Protection Agency

### Isolation and screening of Cr(VI) resistant bacteria

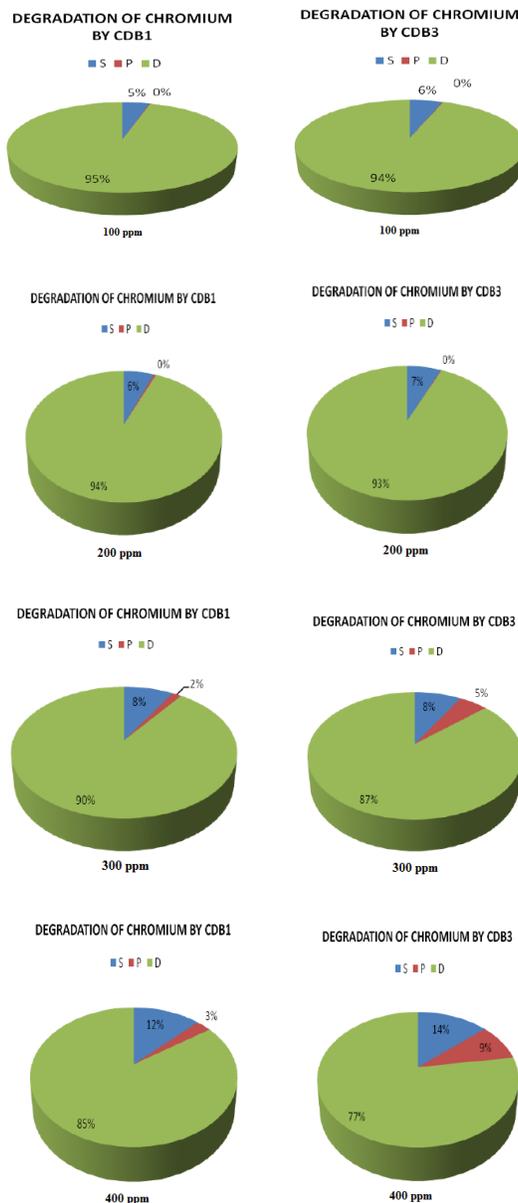
After serial dilution of the tannery effluent, four different bacterial isolates were selected based on colony morphology and pigment production. Further purification was done by streak plate method. Bacterial isolates were marked as CDB1, CDB2, CDB3 and CDB4 (CDB stands for Chromium Degrading Bacteria). All four bacterial isolates showed resistance against chromium in 100 to 400 ppm range, but in 500 ppm only two bacterial isolates (CDB1 and CDB3) showed resistance. In 600 ppm there was no sign of growth of bacterial isolates. So, CDB1 and CDB3 were selected for further studies. Srivastava et al. (2007) reported that *Acinetobacter* sp. isolated from pulp and paper mill effluent could tolerate maximum Cr(VI) up to 500 µg/mL [9]. According to the study of Hemambika B and Kannan VR (2012); the Cr(VI)-resistant plant growth promoting bacteria (*Bacillus* sp. VRK 1) was isolated from soil samples which were collected from an electroplating industry at Coimbatore, India, and it showed tolerance to chromium (VI) concentrations up to 500 mg/L in Luria-Bertani medium [10].

### Bio-removal of hexavalent chromium

The percentage of degradation of chromium by CDB1 and CDB3 isolates was represented in figure 1 (S=% of Cr present in supernatant, P=% of Cr present in pellet and D=% of degradation of Cr). Supernatant and pellet of CDB1 and CDB3 were examined by atomic absorption spectroscopy to determine the amount of chromium absorbed by the bacterial cell. Two bacterial isolates were examined with different concentration of chromium (100ppm to 500 ppm). After atomic absorption spectroscopy analysis, it was observed that among two isolates only CDB1 was showing greatest percentage of degradation, so CDB1 was selected for further sequencing and optimization studies.

In 100 ppm CDB1 and CDB3 degraded chromium 95% and 94 % respectively; in 200 ppm CDB1 and CDB3, 94% and 93% respectively; in 300 ppm CDB1 and CDB3 90% and 87% respectively; in 400 ppm CDB1 and CDB3, 85% and 77% respectively and 500 ppm CDB1 and CDB3 degraded 73% and 72% respectively. Since, CDB1 showed highest percentage of degradation than CDB3 isolate. So, initially only CDB1 was selected for further studies. Studies

are also going on CDB3 isolate that will be revealed in future. Apart from probiotic and antioxidant activity [11], *Lactobacillus* spp. also have the ability to biodegrade Cr(VI) to Cr(III). According to the study of Mishra et al. (2012) the chromium resistant *Lactobacillus* strains would be useful for chromium detoxification from gastrointestinal tract as well as for bioremediation of hexavalent chromium from polluted environment. Cr-resistant *L. acidophilus*, *L. rhamnosus*, *L. casei* and mixed rat gut *Lactobacillus* sp. showed 15-30 % reduction of Cr (VI) in 30 minutes and 100% reduction were observed during 6 to 8 hour. AAS data showed negligible concentration of chromium inside the cells [12].



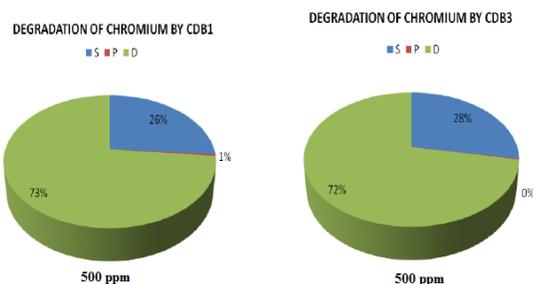


Figure 1: Percentage of degradation of chromium [Cr(VI) to Cr(III)] by CDB1 and CDB3 isolates (S=% of Cr present in supernatant, P=% of Cr present in pellet and D=% of degradation of Cr)

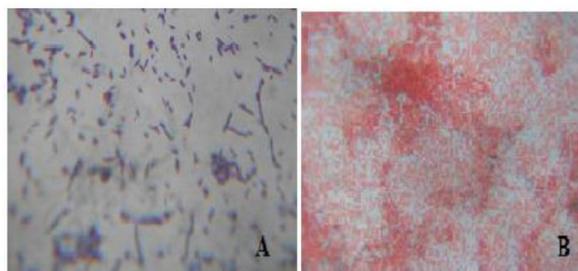


Figure 2: (A) Gram positive, rod shaped, (B) Endospore positive

### Identification of selected bacterial isolate

The selected bacterial isolate was gram positive, rod shaped, spore forming and non-motile. It showed positive result in Methyl Red (MR) test, Starch hydrolysis test, Citrate utilization test, Catalase activity test, Oxidase test, Nitrate reduction test, Gelatin hydrolysis test and negative result in Indole test, Voges- Proskauer (VP) test, Urease production, Triple sugar iron test. The selected bacterial isolate was unable to ferment D-mannitol, fructose, lactose, sucrose but showed positive result in glucose and dextrose fermentation. Based on microscopic visualization and biochemical characteristics, the isolate was identified as *Bacillus* sp. (Table 2 and Figure 2).

Table 2: Identification of the isolate CDB1

Characteristics	Remark
Gram stain	Gram positive, rod shaped
Endospore	+
Motility test	-
Indole test	-
Methyl Red (MR) test	+
Voges- Proskauer (VP) test	-
Starch hydrolysis	+
Urease production	-
Citrate utilization test	+
Catalase activity	+
Oxidase test	+

Nitrate reduction test	+
Triple sugar iron test	-
Gelatin hydrolysis test	+
D-Mannitol	-
Fructose	-
Lactose	-
Dextrose	+ Acid produce but no gas
Sucrose	-
Glucose	+ Acid produce but no gas

\*\* '+'=Positive, '-'= Negative

The molecular characterization of selected bacterial isolate was done by 16S rRNA sequence analysis which revealed about the bacterial strain. After sequencing 99% similarities were found with *Bacillus aerophilus* strain by BLAST tool ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)). After that isolated strain was named as *Bacillus aerophilus* VIT SN and submitted to GenBank database to get accession number and FASTA sequence (Figure 3). Phylogenetic tree was made using 'TREE VIEW' software which is illustrated in Figure 4, where isolated new strain was named in bold letters with accession number, *Bacillus aerophilus* VIT SN (KC986388).

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>gi|508083306|gb|KC986388.1| Bacillus aerophilus strain VITSN 16S ribosomal RNA
gene, partial sequence
AAAGACCAGGGGGCTTGCTCCGGATGTTAGCGGGCGGACGGGTGAGTAACACGTGGGTAACTGCGCTGTA
AGACTGGGATAACTCCGGGAACCGGAGCTAATACCGGATAGTTCCCTGAACCGCATGGTTCAGGATGA
AAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCATTAGCTAGTTGGTGAGGTAACGGCTCAC
CAAGGCCACGATGCGTAGCCGACTGAGAGGGTATCGGCCACACTGGGACTGAGACCGGCCAGACTC
CTACGGGAGGAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACCGCGCTGAGTGA
TGAAGTTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAAGAACAGTGCAAGAGTAACTGCTGCACCTTG
ACGGTACCTAACCGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGT
TGTCCGGAATTATGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTAAAGCCCCCGGCTCA
ACCGGGAGGGTCATTGGAACTGGGAACTTGAAGTGCAGAAAGGAGAGAGTGGAAATCCACGTGTAGCGG
TGAATGCGTAGAGATGTGGAGGAACACCAAGTGGCGAAGCGGACTCTCTGGTCTGTAAGTACGCTGAGG
AGCGAAACGGTGGGAGCGAACAGGATTAGATACCTGGTGTCCACGCGGTAACAGATGAGTGTAAAGT
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AGACTGAAACTCAAAGGAATTGACGGGGCCCGCACAGCGGTGGAGCATGTGGTTAAATCGAAGCAAC
GCCAAGAACCTTACCAGGCTTACATCCTCTGACACCCCTAGAGATAGGGCTTTCCCTTCGGGGACAGA
GTGACAGGTGGTGCATGGTGTGCTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCTTGTATCTTAGTGGCCAGTTCAGTTGGGCACCTTAAGGTGACTGCCGCTGACAAACCGGGAGGAG
GTGGGGATGACGCAATCATCATGCCCTTATGACCTGGGCTACACACGTGCTCAATGGACAGAACAA
AGGGCTGCGAGACCGCAAGGTTTAGCCAATCCACAAATCTGTTCTCAGTTCGGATCGCATCTGCAACT
CGACTGCGTGAAGCTGGAATCGTAGTAAATCGCGGATCAGCATTCCCGCCGATCCCCC
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Figure 3: *Bacillus aerophilus* strain VITSN 16S r RNA gene, partial sequence

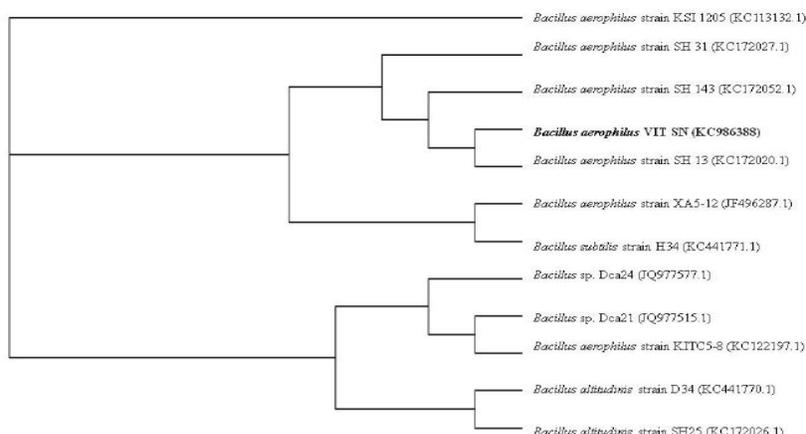


Figure 4: Phylogenetic tree of the isolate CDB1 (*Bacillus aerophilus* VITSN strain)

### Determination of optimum growth conditions

The bacterial isolate *Bacillus aerophilus* VIT SN strain was incubated at different temperature (4°C, 25°C, 37°C, 40°C and 45°C ) and maximum growth was shown at 37°C. So 37°C is the optimum temperature for the growth of the isolated bacteria (Figure 5). The isolated strain was incubated at different pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0) and maximum growth was found at pH 7.5 in the medium. So, 7.5 is the optimum pH for the growth of *Bacillus aerophilus* VIT SN (Figure 6). The optimum pH and temperature not only influence the growth of bacteria but also contribute to the efficiency of bio-removal properties for heavy metals. According to the study of Hossain (2005), maximum biosorption of chromium by *Bacillus subtilis* is found up to 94.25 % with optimum pH of 3.5 and temperature 40°C [13]. Congeevaram et al. (2007) reported that *Micrococcus* sp. shows maximum removal of Cr (VI) (90%) at pH 7.0. At higher alkaline pH values (8 and above), a reduction in the solubility of metals contributes to lower uptake rates. Bioaccumulation of chromium by bacterial species is also depend on temperature. Maximum removal of Cr (VI) was observed at 30°C for the isolated *Micrococcus* sp. Temperature also affects the stability of the cell wall, its configuration and can cause ionization of chemical moieties. These factors may simultaneously affect the binding sites on isolated bacterial species causing reduction in heavy metal removal. The range of optimal temperature values were comparable to the range of room temperature that was used when isolating the microorganisms, suggesting that the selection of these isolates might have been influenced not only with the heavy metal but also with the temperature used in the isolation process [14].

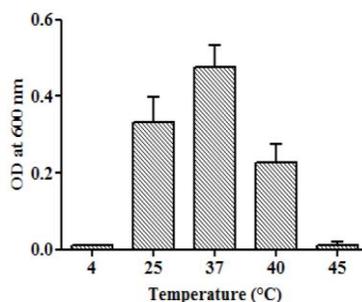


Figure 5: Determination of optimum temperature for the growth of *Bacillus aerophilus* VIT SN strain

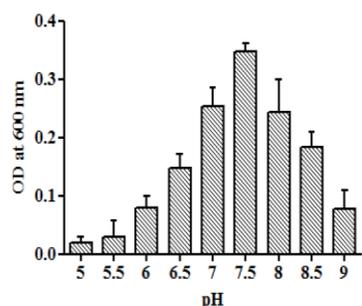


Figure 6: Determination of optimum pH for the growth of *Bacillus aerophilus* VIT SN strain

### Effect of chromium on *Bacillus aerophilus* VIT SN strain's growth

The selected bacterial strain without chromium as a control was grown well in the media and the growth curve was observed normal. But growth was little decreased compared to the control when it was incubated in the media containing 100 ppm  $K_2Cr_2O_7$  as source of Cr (VI) (Figure 7). This result can be compared with the study of Zahoor et al. (2009) and clearly indicates that chromium has toxic effect to the cell [2].

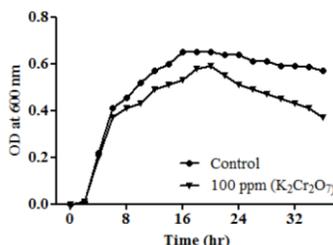


Figure 7: Effect of chromium on *Bacillus aerophilus* VIT SN strain's growth

### NaCl tolerance of *Bacillus aerophilus* VIT SN strain

The bacterial strain *Bacillus aerophilus* VIT SN strain was able to survive up to 6% salt concentration in the media, but maximum growth of the isolate was observed in 2% salt concentration (Figure 8).

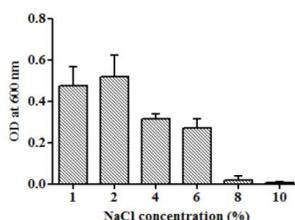


Figure 8: NaCl tolerance of *Bacillus aerophilus* VIT SN strain

### Optimization of carbon and nitrogen sources of the culture medium

Glucose, Sucrose, lactose, Fructose and Dextrose are most common carbon source for the bacterial growth. Maximum growth of *Bacillus aerophilus* VIT SN strain was observed in medium containing dextrose (Figure 9), so dextrose is the good carbon source for isolated bacterium. Maximum growth of *Bacillus aerophilus* VIT SN strain was also recorded in medium containing Yeast Extract as a nitrogen source than other nitrogen sources (Figure 10). So, Yeast Extract is the best nitrogen source for the growth of isolated bacteria.

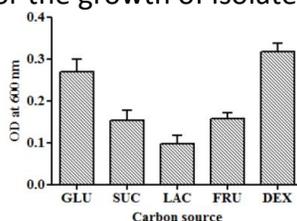


Figure 9: Influence of sugar as a carbon source of *Bacillus aerophilus* VIT SN strain

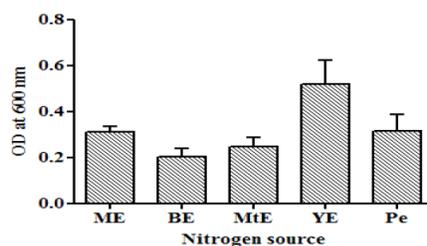


Figure 10: Determination of nitrogen source of *Bacillus aerophilus* VIT SN strain

### Resistance to the different metal ions

Growth of *Bacillus aerophilus* VIT SN strain was recorded in different concentrations of four metals, i.e. cadmium, Lead, Copper and Zinc. But as the concentration of metals increase, the growth rate is decreased. In case of copper minimum growth was recorded up to 300 ppm concentration but at 400 ppm and 500 ppm bacterial growth was inhibited (Figure 11). All the heavy metals are toxic for bacteria as concentration increases but *Bacillus aerophilus* VIT SN strain can tolerate all the heavy metal till 500 ppm except copper. Copper metal was found to be more toxic to this organism. This result can be compared with the study of Faisal and Hasnain (2004) [15]. Mohammad et al. (2011) also investigated the effects of different heavy metals (Cu, Zn, Ni, Cd and Hg) on the growth of bacterial isolates [16].

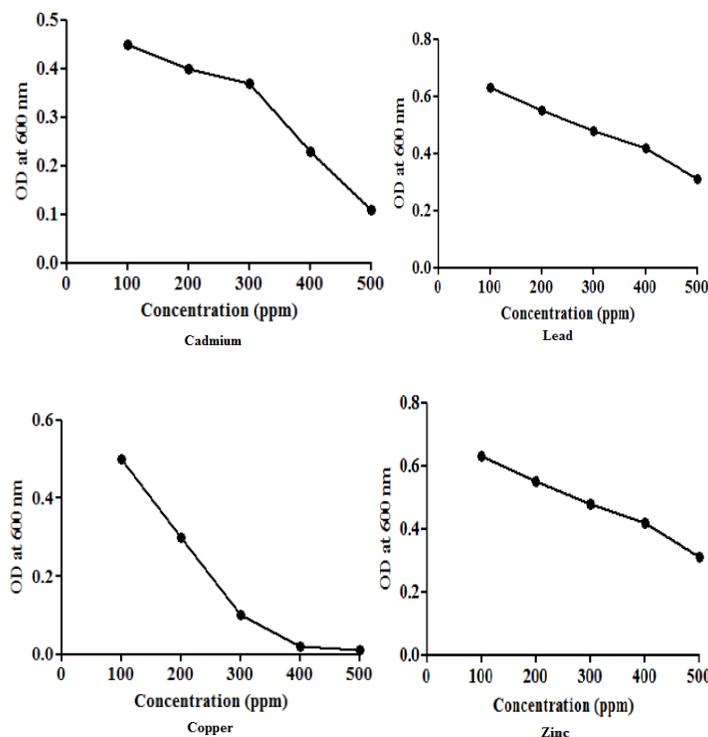


Figure 11: Resistance to different metal ions of *Bacillus aerophilus* VIT SN strain



## CONCLUSION

The extent of ability to reduce hexavalent chromium by bacteria, isolated from tannery effluent has been evaluated. *Bacillus aerophilus* VIT SN strain is a potential microorganism to degrade hexavalent chromium to nontoxic trivalent chromium. The bacterial isolate can be used for the bioremediation of hexavalent chromium containing wastes, since it has potential to reduce the toxic hexavalent form to its nontoxic trivalent form.

## ACKNOWLEDGEMENT

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