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Removal of chromium by a Bacterial consortium isolated from Kolar gold fields and chromium contaminated sites

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Abstract

Pollutants from mining and tannery industries adversely affect the natural ecosystem and pose harmful effect to the human beings once it enters the food chain. They also deteriorate the soil fertility and the quality of the ground water. Bioremediation is being viewed as a clean technology for the removal of chromium from tannery effluent. This study was conducted to isolate an efficient bacterial consortium from Kolar Gold Fields and from tannery effluent contaminated site which could remove Chromium. The isolated bacterial consortium could remove chromium at the concentrations of 10 mg/L to 50 mg/L concentration. The optimum concentration found to be 50 mg/L removal up to 96.77 % by the end of 5th day. The isolated bacterial consortium consisted of three strains, which were identified through biochemical tests and 16s rRNA sequencing as *Catellicoccus sp.*, *Bacillus safensis strain FFA35*, and *Pseudomonas stutzeri* strain AO 0002. In the present study the isolated bacterial consortium could remove chromium at pH 7 at 37°C up to 96.77 %. The removal of chromium by bacterial consortium was found to be maximum at 25°C up to 97.92 %. The bacterial consortium was supplemented with carbon sources like glucose, lactose, mannitol and fructose. The bacterial consortium could grow their best and remove chromium in the media supplemented with 1 % of fructose showing removal up to 97.85 %. Among nitrogen sources used in the present study, yeast extract could enhance the growth of the organism and the removal reached maximum up to 96.77 %, followed by ammonium nitrate and potassium nitrate showing removal up to 96.08 and 95.12 %. Sodium nitrate could enhance only 93.28 % of removal. Thus, our isolated consortium appears to have great potential for simultaneous bioremediation hexavalent chromium from the contaminated sites.

Keywords: Bacterial Consortium, chromium removal, gold fields.

Introduction

Effluents from mining areas, tannery, dyes etc possess considerable amount of toxic metal ions. These ions from mining pose problems to the land environment by discharging mine soil from underground and open pit mines. These toxic metal ions not only cause potential human health hazards but also affect other life forms. Chromium is a toxic, carcinogenic and mutagenic agent to humans and animals as well as plants, which changes the morphology of plant. Chromium polluted soils and sediments are usually the result of sewage sludge disposal or dumping of chromate wastes from industrial and manufacturing activities. It is released into environment by a large number of industrial operations such as electroplating, chromate manufacturing, dyes and pigment manufacturing, wood preservation, leather tanning inhibitor in conventional and nuclear power plants Tanneries are a major source of chromium pollution and release Cr (VI) ranging from 40 – 25000 mg/L of waste water (Cheung and Gu, 2007). The concentrations range in soil is between 1 and 300 mg/kg, in sea water 5 to 800 µg/liter, and in rivers and lakes 26 µg/liter to 5.2 mg/liter. According to World Health Organization the allowable concentration of chromium in drinking water is 0.05 g/L. Thus it is essential to reduce Cr (VI) concentrations from water/waste water to acceptable levels (Ozturk et al., 2009). Pollution of chromium is of considerable concern as the metal has found widespread use in electroplating, leather tanning, metal finishing and chromate preparation. Various physico chemical methods such as oxidation / reduction, chemical precipitation, filtration, ion exchange, electrochemical treatment, reverse osmosis, evaporation recovery and membrane technologies are used for removal of chromium (Ahluwalia and Goyal, 2007; Zahoor and Rehman, 2009). The large scale applications of these methods are consuming energy excessively and utilize huge amounts of reagents in addition to their high cost. Biodegradation is the process of

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decaying or reduction of different organic materials and toxic metals to their non toxic form with the help of microorganisms. In this process complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds takes place. In the mixed culture of microorganisms phenol degrading organisms utilizes phenol as sole source of carbon and produce energy, metabolites, electron donor which is used by the chromium degrading organisms to reduce chromium. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulphur, calcium, magnesium, and several metals are the micronutrients that also show a significant impact on degradation behaviour is reported. There are various advantages associated with biodegradation such as the process is simple. It is an eco-friendly and cost effective process that requires low capital and operating cost. Being environmentally friendly process it produces no harmful end products. Many bacterial species surviving in presence of chromium for years in contaminated sites are found to be highly resistant to chromium and are considered important for removal of chromium (Pinon-Castillo et al., 2010)

Tannery effluents are the major source of contamination of soil. One such compound is chromium which is highly toxic. The chromium concentration range in soil is between 1 and 300 mg/kg (World Health Organization, 1988). Hence it is mandatory to remove chromium from soil to avoid soil pollution. The present study focuses on the removal of chromium using bacterial consortium. The bacterial consortium which was enriched from chromium contaminated soil was used in the removal of chromium at different concentrations, their growth parameters was optimized. The bacterial strains present in the consortium was biochemically characterized and identified by 16s rRNA sequencing. Such enriched bacterial consortium could be used cost effectively to treat chromium contaminated soil.

Materials and Method

Soil samples were collected from a mining industry, Kolar Gold Fields, Karnataka and tannery industry situated in Pallavaram, Tamil Nadu. Soil samples were collected and transferred to sterile zip lock covers and immediately transported to the laboratory. The bacterial consortium was enriched in MSM amended with 50 mg/L of potassium-di-chromate. The composition of the MSM used for enrichment and degradation was as follows: Sucrose 5.0 g, Di sodium hydrogen phosphate 2.0g, Magnesium sulphate 0.5 g, Calcium carbonate 0.1 g, Ferric chloride 5-10 drops, Hydrogen molybdate 0.002 g with $_P$H 7.0 (Hu et al., 2010).The medium was autoclaved, cooled, and then amended with 100 mg/L of filter sterilized in a 250 mL Erlenmeyer flask. An amount of 10 g of soil sample was aseptically inoculated into the medium. Individual bacterial isolates were obtained from the enriched culture by plating on nutrient agar medium containing 10-50 mg/L of potassium-di-chromate. The selected isolates were then purified by streaking on nutrient agar added with 10 mg/L of potassium –di-chromate. The single colony pure cultures were stored in 15 % glycerol at 20 °C. Sub culturing was done at every 24 hours of interval. Pure cultures were isolated by plating on the nutrient agar medium containing 10 ppm of potassium-di-chromate. Pour plate was done on sterilized petri plate and incubated for 24 h under room temperature. Based on the morphology of the organisms, individual organisms were isolated. Pure cultures were isolated by plating on the nutrient agar medium containing 10 ppm of potassium-di-chromate. Pour plate was done on sterilized petri plate and incubated for 24 hours under room temperature. Based on the morphology of the organisms, individual organisms were isolated.

Total protein content of bacterial consortium

For analysis of total cell protein, samples were centrifuged at 12,000 rpm for 10 mins and washed with fresh (substrate-free) mineral medium, then centrifuged and washed few times to remove the substrate. The pellet from each sample was then disrupted by sonication at 30 % amplitude for a total of 3 minutes (1.5 min x 2) on an ice-water bath. Sample (0.5 ml) was added to 0.5 mL Coomassie Blue protein dye and the absorbance were measured at 595 nm. The total protein concentration was determined by calibration with bovine serum albumin standard according to Bradford (1976).

Measurement of removal of chromium by bacterial consortium

Removal of chromium by bacterial consortium was observed till 72 h. Estimation of chromium removal by Diphenyl carbazide method: The reagent used for the estimation of chromium was 1, 5-Diphenyl Carbazide – 500 mg of 1, 5-Diphenyl Carbazide was mixed in 100 ml of acetone and the pH was adjusted to 2 ± 0.5 by adding 10% H_2SO_4. To obtain a standard graph 10 ppm to 100 ppm of potassium-di-chromate was taken in a 100 ml volumetric flask. A flask with no chromium was served as blank. 10 ml of 5% H_2SO_4 was added and diluted to 40ml. 4 ml of Diphenyl carbazide was added to this and diluted to mark with 5% H_2SO_4. Absorbance was measured after 5 minutes at 540 nm. 5 ml of culture (0 to 72 hours) was taken in a sterilized 1.5 ml vials and it was centrifuged at 10,000 rpm for 5 minutes. 1 ml of supernatant was taken in a sterilized test tube and 9 ml of distilled water was added to it. To this 1 ml of 1, 5 Diphenyl carbazide was added and shaken immediately. The absorbance was measured at 540 nm (Calomiris et al., 1984).

Optimization of growth condition for removal of chromium by bacterial consortium

Effect of pH and temperature on the removal of chromium

In order to study the effect of pH and temperature, the **s**terilized MSM was amended with 50 mg/L of potassium-di-chromate.The medium was maintained at different pH from pH 5 to pH 7.5. A volume of 1 mL of overnight culture was inoculated in the flasks and incubated in a shaker at 37 °C. The effect of temperature was studied by inoculating overnight culture and incubating in a shaker at 25 °C, 35 °C, and 45 °C. The medium was maintained at pH 7. The measurement of degradation of chromium concentration was performed at an interval of 24 h for 5 days. Total protein estimation was done at 595 nm from 0^{th} day till 5^{th} day.

Effect of carbon and nitrogen sources on the decolorization of mixed dyes

The effect of carbon sources were studied using various compounds, such as fructose, lactose, and mannitol, at a concentration of 1 % and they were added individually as a supplement to MSM for the removal of chromium. A volume of 1 mL of the overnight culture was inoculated in the flasks and incubated in a shaker at 37 °C. Nitrogen sources, such as sodium nitrate, potassium nitrate and ammonium nitrate were added to MSM at a concentration of 0.5 %; and 1 mL of overnight culture was incubated at 37 °C. In order to study the effect of efficient carbon and nitrogen sources, the optimum carbon and nitrogen sources, i.e., sucrose and yeast extract, were added to MSM at a concentration of 1 % and 0.5 %, respectively, and the removal of chromium was measured by the diphenyl carbozide assay. Total Protein estimation was done at 540 nm from 0^{th} day till 5^{th} day.

Identification of chromium removing bacterial strains in the consortium

The individual bacterial strains were separated from the consortium which was used for the removal of the chromium. The bacterial strains present in the consortium were initially examined using conventional biochemical tests. The molecular identification of the bacterial strains was performed by 16S rDNA sequencing. The bacterial strains present in the consortium were isolated and grown separately. Initially, Gram staining and motility tests were performed and then the biochemical characterization was carried out for different parameters (Catalase, Oxidase, Indole Production, Citrate Utilization, Methyl Red, Voges Proskauer, Triple Sugar Iron Agar, And Urease) using 24 h old culture of individual the individual bacterial strains. After 24 h of incubation at 37 °C, the color change observed was accounted for a positive/negative result. The genus level identification of the unknown bacterial strains was carried out using Bergey's Manual of Systematic Bacteriology (2005) to ascertain the existence of variable biochemical test results for each strain.

16S rRNA Partial Gene Sequencing

Chromosomal DNA was isolated from the pure strains of the consortium by the standard phenol/chloroform extraction method (Sambrook et al., 1989). The 1.5 kilo base partial sequence of the 16S rRNA gene was amplified from the chromosomal DNA using polymerase chain reaction (PCR) with universal Eubacteria- specific primers 16F27 (5'-CCA GAG TTT GAT CMT GGC TCA G-3') and 16R1525XP (5'-TTC TGCAGT CTA GAA GGA GGT GWT CCA GCC-3') (Pidiyar et al., 2002). The PCR

conditions used were an initial denaturation at 94°C for two minutes, followed by 35 cycles of denaturation at 95°C for one minute, annealing at 55°C for one minute, and extension at 72°C for one minute, and a final extension at 72°C for 10 minutes and sequenced on an ABI310 automated DNA sequencer using the Big Dye terminator kit (Applied Biosystems 3730 x l DNA Analyzer). The amplified 16S rRNA gene PCR products from these isolates were directly sequenced after purification by precipitation with polyethylene glycol and NaCl. The primers used to obtain the complete sequence of 16S rRNA gene of the isolates were the same as for PCR amplification (16F27N and 16R1525XP). Sequence data analysis was done using ChromasPro and Sequencing Analysis software. The output file of sequence alignment was used to compute phylogenetic trees for aligned sequences of 16s rRNA sequencing results of the four bacterial strains (AM1, AM2 and AM3). Neighbour joining method was used for tree building with MEGA 6 software. To access the reliability of the phylogenetic tree, MEGA provides bootstrap test which used the bootstrap resampling strategy. The user has to input the number of replicates. In this experiment, 500 replicates were used.

Results and Discussion

Screening and isolation of bacterial consortium

Soil samples were collected from mining and tannery affected area, which were enriched with chromium to isolate chromium utilizing bacterial consortium. During isolation period, several (five) bacterial strains co-existed in the consortium which could remove chromium (10 mg/L). After successive transfer during enrichment period, only three bacterial strains sustained and were isolated (AM1, AM2, AM3), which could survive and remove chromium as a sole source of carbon and energy. Fig 1 shows the bacterial strains utilizing chromium on nutrient agar. The Fig 2 shows that there was an increase in total protein from 0^{th} day (4.6 mg/ml) and by the end of 4^{th} day, there was decrease in total protein (2.8 mg/ml) indicating that bacterial consortium could not survive after 5^{th} day. Hence for all the experiments the total protein and removal of chromium was checked up to 5^{th} day.

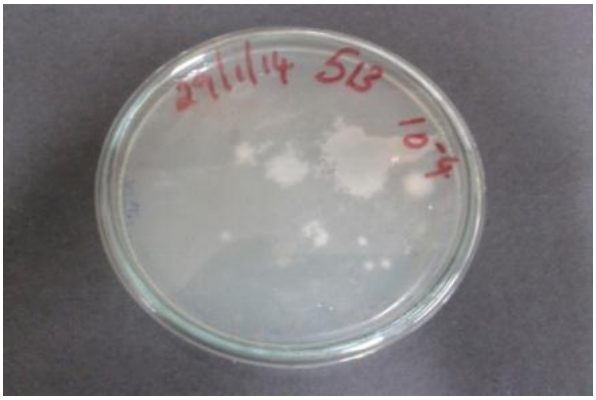

Fig 1 Enriched chromium utilizing bacterial isolates on nutrient agar plate

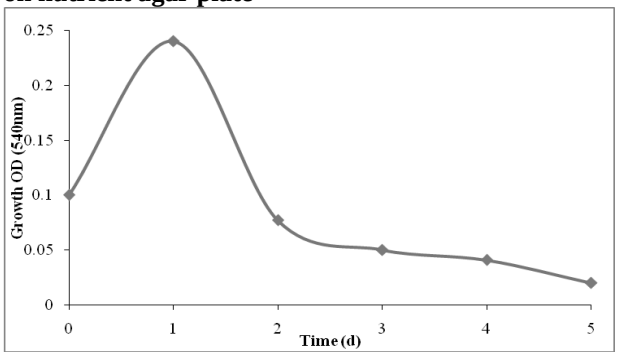

Fig 2 Growth pattern of the bacterial consortium on the removal of chromium

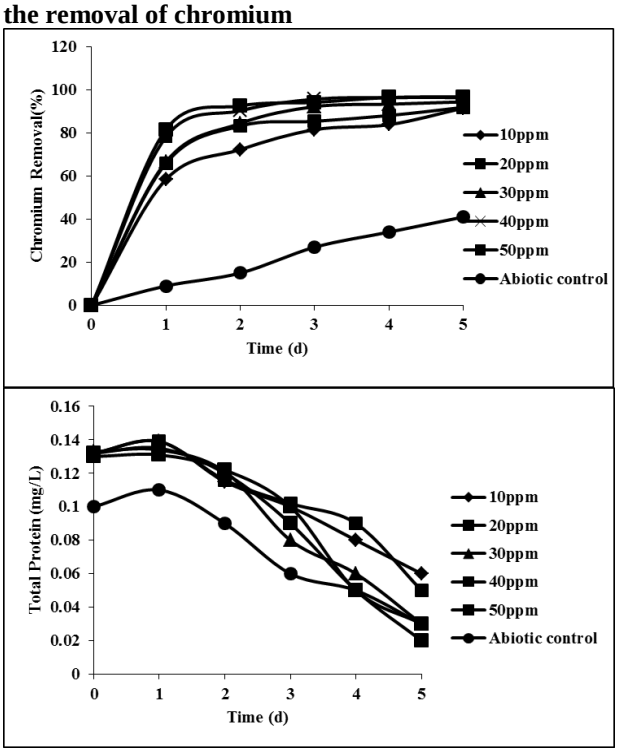

Fig 3 Removal of Different concentrations of chromium and Total protein content by the Bacterial Consortium

Removal of various concentrations of chromium by the bacterial consortium

The ability of the bacterial consortium to use chromium as sole source of carbon and energy was studied at 10 mg/L to 50 mg/L (10 mg/L, 20 mg/L, 30 mg/L, 40 mg/L and 50 mg/L) of chromium. Chromium at 10 mg/L of concentration showed maximum removal i.e. up to 58.46 % by the end of 24 hours and the final degradation being 91.53 % by the end of 5th day and maximum protein content was also observed in this concentration during 24 hours (4.6 mg/ml) to 48 hours (4.8 mg/ml) of incubation. This was followed by 20 mg/L of chromium concentration showing degradation up to 91.89 % by the end of 5th day (Fig 3). When the concentration was increased to 30 mg/L, the removal of chromium was up to 94.44 %. The chromium removal was up to 96.45 % at the concentration of 40 mg/L and the maximum chromium removal was high at 50 mg/L concentration with 96.77 % and there was corresponding decrease in the protein content of organism by the end of 5th day (2.0 mg/ml). As the concentrations of chromium were increased there is decrease in protein content of the bacterial consortium. Therefore bacterial consortium used in the present study could utilize chromium up to 50 mg/L with optimum degradation at 50 mg/L in 4 days.

Optimization of growth conditions on the removal of chromium

Effect of pH on the removal of chromium

The study was carried out to determine the effect of pH (pH 5.5 to pH 7.5) on the removal of chromium at optimum concentration i.e. 50 mg/L. Fig 4 predicts the protein content of bacterial consortium at different pH from pH 5 to pH 7.5, where maximum removal was achieved at pH 7 showing up to 96.77 % at the end of 5th day and the total protein was found to be 4.4 mg/ml and 4.6 mg/ml by the end of 2nd day. The bacterial consortium was able to remove chromium at optimum pH 7.

Effect of temperature on the removal of chromium

To determine the effect of temperature on the growth of bacterial consortium a study was conducted at different temperatures (25°C, 35°C and 45°C) at optimum concentration of 50 mg/L and pH 7. Fig 5 predicts there was maximum removal of chromium achieved at 25°C which showed maximum removal of 97.92 % by the end of 5th day and the total protein was found to be 6.0 mg/ml by the end of 2nd day. There was no marginal variation in the temperature used 25°C, 35°C and 45°C. There was only 1% difference in the removal of chromium. The bacterial consortium was able to grow at lower temperature.

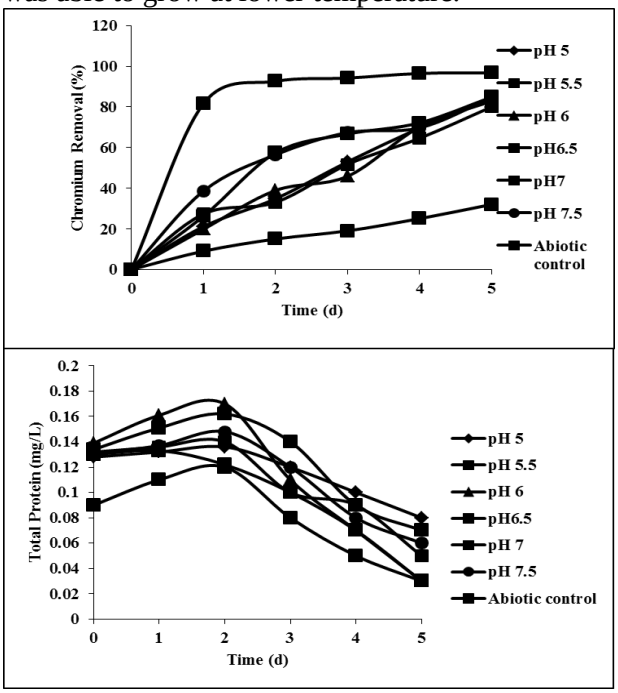

Fig 4 Effect of pH on removal of chromium and total protein by the bacterial consortium

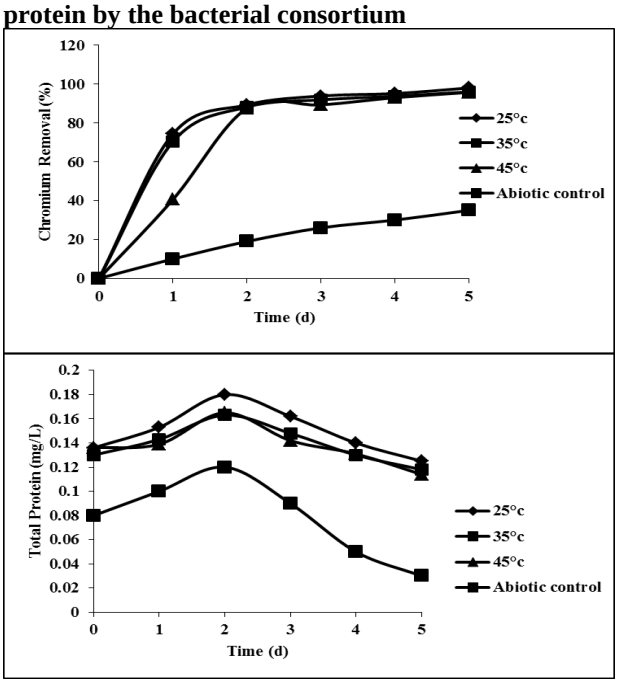

Fig 5 Effect of Temperature on removal of chromium and total protein by the bacterial consortium

Effect of carbon sources on the removal of chromium

To examine the influence of carbon sources on the removal of chromium at optimum concentration, carbon sources like glucose, lactose, mannitol and fructose were supplemented along with chromium. Fig 6 shows that, almost all the carbon sources were able to enhance the removal of chromium. While in presence of fructose as carbon sources the chromium removal was maximum up to 97.85 %. This was followed by mannitol and glucose having 97.70 and 96.77 % of removal. Maximum total protein was observed in mannitol (5.5 mg/ml) supplemented media followed by fructose (4.8 mg/ml) and lactose (4.6 mg/ml). Lactose as a carbon source showed a maximum removal of chromium up to 96.45 %. The total protein of bacterial consortium in glucose (4.4 mg/ml) supplemented media was less when compared to other carbon sources used in the study.

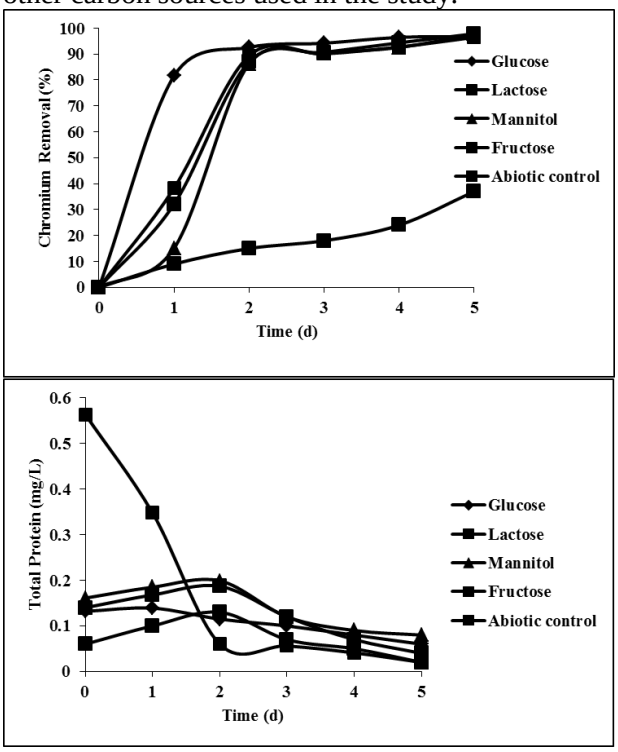

Fig 6 Effect of carbon sources on removal of chromium and total protein by the bacterial consortium

Effect of nitrogen sources on the removal of chromium

To examine the influence of nitrogen sources on the removal of chromium at optimum concentration, various nitrogen sources like yeast extract, sodium nitrate, potassium nitrate and ammonium nitrate were supplemented in medium along with chromium. Fig 7 shows that sodium nitrate, potassium nitrate and ammonium nitrate played an important role in enhancing the total protein but the removal of chromium was maximum in yeast extract showing maximum removal up to 96.77 % by the end of 5th day. This was followed by ammonium nitrate and potassium nitrate showing 96.08 and 95.12 % of removal respectively by the end of 5th day. Maximum total protein was observed in the ammonium nitrate (4.6 mg/ml) supplemented media. The removal of chromium up to 93.28 % was observed in sodium nitrate as nitrogen source.

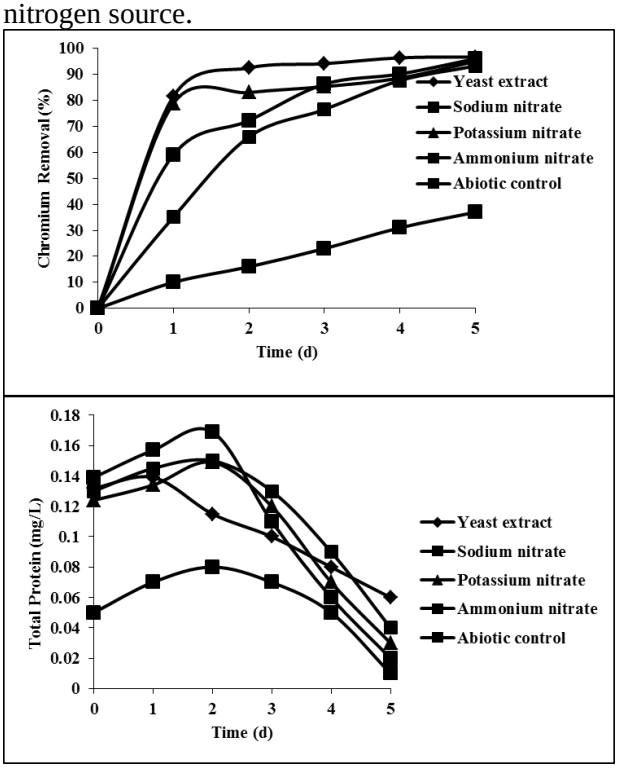

Fig 7 Effect of nitrogen sources on removal of chromium and total protein by the bacterial consortium

Morphological and Biochemical Characterization

The bacterial consortium consisted of three bacterial strains, the AM1 and AM3 where shown as Gram negative while AM2 showed Gram positive result. The biochemical characteristics of three bacterial strains showed that they belong to the phyla Firmicutes. These groups of bacteria are

commonly present in the contaminant soil, water and wastewater. Table 1 summarizes the results on the biochemical tests of the isolated bacterial strains. All the three strains were round in shape with smooth and had irregular morphology on the nutrient agar plate. AM1 showed translucent raised colony with irregular edges. AM2 was yellow mucoid colony with smooth edges and AM3 was small transparent flat colony with smooth edges.

Table 2 Bacterial strains identified by 16s rRNA sequencing

CHARACTERISTICS	AM1	AM2	AM3
Gram staining	-	+	-
Motility	+	+	+
Catalase	-	+	+
Urease	-	-	-
Indole	-	-	-
Methyl red	-	+	-
Voges proskauer	-	-	-
Citrate utilization	-	+	-
Triple Sugar Iron Agar	Alkaline slant and Alkaline butt	Alkaline slant and Alkaline butt	Alkaline slant and Alkaline butt

Genomic DNA extraction and PCR amplification

The genomic DNA was isolated from each bacterial isolates. PCR amplification was performed and the unknown bacterial strains were identified through 16S rRNA sequencing. The bacterial isolates were identified from the sequence using BLAST tool.

Phylogenetic analysis

Table 2 summarizes the nearest neighborhood analysis of the Bacterial strains identified by 16s rRNA sequencing. Through 16s rRNA sequencing results, the strains were identified as *Catellicoccus sp,* (AM1), *Bacillus safensis* strain FFA35 (AM2), and *Pseudomonas stutzeri* strain AO 0002 (AM3).

Phylogenetic tree for three strains was constructed from 16s rRNA of *Catellicoccus sp,* (AM1), *Bacillus safensis* strain FFA35 (AM2), and *Pseudomonas stutzeri* strain AO 0002 (AM3) and the evolutionary history was inferred using the Neighbor-Joining method. Fig 8 represents the phylogenetic tree computed based on 16s rRNA sequencing results of the three bacterial strains. AM1, AM2 and AM3 were identified as *Catellicoccus* sp., *Bacillus safensis* strain FFA35 and *Pseudomonas stutzeri* strain AO 0002 respectively.

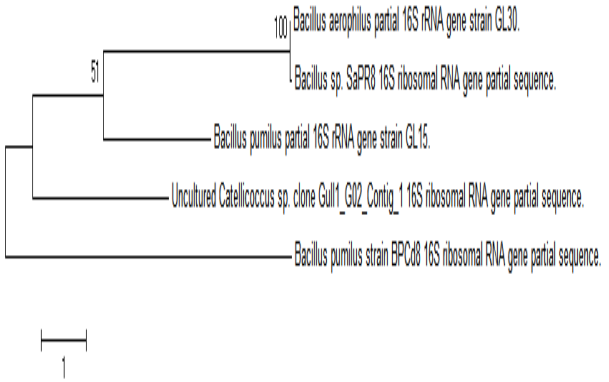

Fig 8 Phylogenetic analysis of *Catellicoccus* sp. (AM1) with its nearby substrains

Biodegradation is adjudged as an important mechanism of organic chemical removal in the natural system, owing to its environment compatibility. Aromatic compounds are important group of contaminants, for which efficient treatment methods are required. Biodegradation is an emerging technology, which utilize the potential of microorganisms to degrade the chemical compounds which are toxic in nature. Tanneries are responsible for the release of huge amounts of chromium into the environment. Bacterial consortium and bacterial strains are used for the removal of chromium from industrial effluents (Thakur, 2003). Cyanobacterial strains *Nostoc linckia* HH-203 and *Nostoc spongiaeforme* HH-204 that were isolated from chromium contaminated sites were used to study their chromium tolerance and metal removal efficiency in saline environment (Bala et al., 2004). In the present study, bacterial strains were isolated from mining and tannery industries and through 16s rRNA sequencing results, they were identified as *Catellicoccus sp,* (AM1), *Bacillus safensis* strain FFA35 (AM2), and *Pseudomonas stutzeri* strain AO 0002 (AM3).

Fathima Benazir et al., (2009) isolated *Bacillus* sp. and *Pseudomonas* from tannery effluent which showed an efficiency of 99.6 % with a reduction rate of 1.565 mg/l per hour. The initial concentration of chromium was found to be 570 mg/L, after the removal of chromium by the

bacterial consortium the concentration of the chromium was found to be 2 mg/L.

The chromium removal using microbial waste biomass corresponding to different initial metal concentrations (5-50 mg/L) was done. This was because at high initial concentrations the number of moles of Cr (III) available to the surface area were high, so functional adsorption becomes dependent on initial concentration. This sorption characteristic indicated that surface saturation was dependent on the initial metal ion concentrations (Bhatti et al., 2007). In the present study the bacterial consortium could utilize the chromium up to 50 ppm with a maximum removal of 96.77 % by the end of 5th day. Through biochemical characterization and 16s rRNA sequencing results, they were identified as *Catellicoccus sp,* (AM1), *Bacillus safensis* strain FFA35 (AM2), and *Pseudomonas stutzeri* strain AO 0002 (AM3). Previous reports also suggests that removal of Cr (III) is dependent on concentration of chromium because the increase in the initial chromium concentration (50 -300 mg/L) increased the amount of Cr (III) adsorbed (Fahim et al., 2006).

Table 2 Bacterial strains identified by 16s rRNA sequencing

Isolate No.	Phylum	Nearest phylogenetic neighbor (Accession no.)	Affiliation	Similarity (%)
AM1	Firmicutes	*Catellicoccus* sp *(*KF250993.1)	*Catellicoccus*	99
AM2	Firmicutes	*Bacillus safensis* strain FFA35 (JN092817.1)	*Bacillus safensis*	99
AM3	Proteobacteria	*Pseudomonas stutzeri* strain AO 0002 (KF984304.1)	*Pseudomonas stutzeri*	99

Poornima (2010) isolated *Pseudomonas putida*, where chromium removal occurred to maximum at pH 7 up to 97.92 % in 12 hours culture, but in 24 hours chromium removal was high at pH 9 (99.62 %), where the initial concentration of chromium was 100 µg/mL and after 24 hours the concentration of chromium was found to be 2.22 µg/mL at pH 7 and 0.38 µg/mL at pH 9. In the present study bacterial consortium (AM1, AM2 and AM3) could remove chromium at pH 7 up to 96.77 %. As the pH of the medium was increased there is a decline in the growth of bacterial consortium and also there is a decrease in the chromium removal, which shows that the neutral pH was optimum for removal of chromium.Okeke (2008) isolated *Pseudomonas maritimus* VITP21 which could survive and remove chromium at optimum temperature of 35°C up to 98 %. The initial concentration of chromium is 200 mg/L beyond this temperature, there is decrease in growth and removal of chromium. In the present study the removal of chromium by bacterial consortium could remove chromium at optimum temperature of 25°C with a maximum removal of chromium up to 97.92 %. As the temperature increased there is a decrease in the percentage of chromium removal.

He et al., (2009) reported on the effect of carbon source on the removal of chromium, where the removal drastically increased by addition of 1 to 1.5 % glucose to the culture medium (65.7%). At higher concentration, there was no further increase in the removal of chromium. Glucose has been reported as an electron donor and demonstrated to increase chromium removal by *Bacillus* sp. Tripathi, (2010) studied on the effect of carbon sources on the removal of chromium by Bacterial isolates. 0.2 to 1 % of glucose, sucrose and maltose were supplemented with mineral salts medium along with 200 mg/L of chromium. Sucrose and maltose supported the growth of *Bacillus* sp. while glucose was a better additional source of carbon and energy. In the present study the bacterial consortium could grow their best and remove chromium up to 97.85 % in the media supplemented with 1 % of fructose. This was followed by mannitol and glucose having 97.70 and 96.77 % of removal of chromium by the bacterial consortium respectively. Lactose as a carbon source showed a less removal of chromium up to 96.45%. Among nitrogen sources used in the present study for the optimization, yeast extract could enhance the growth of the organisms and the removal of

chromium reached maximum up to 96.77 % followed by Ammonium Nitrate and Potassium Nitrate showing degradation up to 96.08 and 95.12 % respectively.

Conclusion

Mining and tannery industries are the major source of contamination of soil. One such compound is chromium which is highly toxic. They pose harmful effects to the environment and their effects to biological system are very severe. Hence an efficient treatment method is required for the removal of chromium from contaminated soil. From this present study, it can be concluded that enriched bacterial consortium could be used for the removal of chromium at an optimum concentration of 50 mg/L at pH 7 and temperature 25°C. Supplementing the media with fructose as carbon source and yeast extract as nitrogen source could greatly enhance the growth of bacterial consortium and removal of chromium by the bacterial consortium. On the basis of these results, such isolated bacterial consortium could be effectively applied in removal of chromium in chromium contaminated environment.

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