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ISOLATION AND CHARACTERIZATION OF CHROMIUM REDUCING BACILLUS SP FROM SOIL SAMPLES OF TANNERY INDUSTRIES AT RANIPET, VELLORE DISTRICT

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ABSTRACT

Place Ranipet is famous for tannery industries. All the industries release more than 10000 litre of purified, partially purifies and polluted effluents into the nearby water bodies. The chromium present in this effluent contaminates the quality of both surface and ground water and affects the aquatic organisms in various ways. Hence, in the present investigation, the chromium contaminated soils were screened for the isolation of chromium reducing bacteria to evaluate its efficacy. According to the obtained results, the *Bacillus sp* obtained from this study can tolerate chromium concentration up to 500ppm. The chromium degrading efficiencies were bound to be 80%, 65%, 44% and 18.4% at Cr concentrations 50,100,150 and 250ppm respectively (pH-7). The degrading efficiencies were bound to be 80%, 65%,44% and 18.4% at Cr concentrations 50,100,150 and 250ppm respectively.

KEYWORDS: Ranipet, chromium, Bacillus sp , isolation, characterization, soil and tannery industry.

INTRODUCTION

In the recent years, most of the rivers in the developing countries are contaminated by heavy metal pollution, because, most of the industrial waste waters contain heavy metals like cadmium, lead, zinc, cobalt and chromium. They are well known for their toxicities, mutagenic, and carcinogenic impact on human beings and other living system especially those metals classified under priority list of pollutants[1] and have deleterious effect on the environment at high concentration.[2] Among all heavy metals, chromium is the one of the

important pollutant released into the environment by a large number of industrial operations such as textile dyeing, chromate manufacturing, chemicals and pigments production, wood preservation, tanning activity, paints, pigments, and electroplating for surface treatment.[3-5] It is the major pollutant in the leather tanning industry and is toxic to plants and animals around the environment.[6] Tannery waste waters are mainly characterized by high salinity, high organic loading and specific pollutants such as chromium.[7] All the heavy metals including chromium are biologically transformed to more or less toxic products and hence persist in the environment indefinitely.[8] Generally, various conventional methods such as electrochemical treatment, coagulation, cementation, elctro-dialysis, elctro-winning, elctro-coagulation, ion exchange, evaporation, chemical precipitation, reverse osmosis, and sorption[9], evaporation, sol- vent extraction and membrane separation[5] were used to remove the heavy metals from the contaminated effluents. All these conventional methods have technical and economical constrains like high cost of operation, present some technological problems, mainly when applied to diluted metal solution and release of chemical and huge sludge to the environment as by-product therefore the need to replace them with cost effective and environmental friendly biological method of treatments.[10] Among these, the use of bacteria in removal of chromium under both aerobic and anaerobic conditions e.g. *Pseudomonas fluorescens* LB 300[11], *Enterobacter cloacae* HO1[12], *Bacillus sp* [13] is one of the emerging research area in the present decades. Hence, in the present study, the soil samples were collected from six selected sites in the Ranipet tannery industrial area to screen, isolate and identify the high efficient chromium degrading bacteria and its efficacy in chromium degradation were analyzed to get new novel bacterial strains.

Study site related information

MATERIALS AND METHODS

Collection of soil samples

The six sites at Ranipet, Vellore district of Tamilnadu were selected for soil sample collection. From all the sample sites, the chromium contaminated soils were collected in screw type glass container (50ml). The soil samples were serially diluted with the help of sterile double distilled water (Fig 1- 3).

Isolation of chromium resistant bacteria

For isolation of chromium resistant bacteria, 1mg of the chromium soil is dissolved in 5ml of water. The microbes in the contaminated soil were washed in this way and washed water was

transferred to 10ml glass test tube. This is known as stock contaminated water. From this water different serial dilution were made using double distilled water. The serial dilutions were carried out up to 10^{-5}in the following sequence 1/10, 2/10, 3/10, 4/10 and 5/10. All the dilutions were done only in small glass sterile test tubes to minimize the risk of contamination. The serial dilution is only to reduce a dense culture of cells to more usable concentration. The water from each dilution was spread on the PYE (Peptone, Yeast extract) agar plates containing 100 Tµg of Cr^{6}+/ml supplemented as K2Cr2O7 to the medium. PYE agar plates were prepared by dissolving 0.1 g NaCl, 1 g Peptone and 0.5 g yeast extract in 100 ml distilled water. The pH was maintained at 7±0.2 by using HCl or NaOH. The medium was autoclaved under 15 Lb pressure for 20 minutes at 120c. The nutrient medium containing agar plates were inoculated with soil water. The bacterial colonies on the plate were observed at 24h of incubation at room temperature (Fiure .4).

Broth culture of chromium degrading dominant colonies

The agar plate containing bacterial colonies were carefully observed to identify the dominant colonies. The PYE agar plate containing three dominant colonies were selected. These three colonies were inoculated into the sterile broth medium containing 100 ml conical flask for 48hours at room temperature. The cultured colonies were identified by conducting usual biochemical tests followed Bergy s manual of Systematic Bacteriology.[14]

Detection of minimum inhibitory concentration of chromium

To detect the minimum inhibitory concentration of chromium for chromium resistant bacteria, the bacterial isolates were again incubated at 37^{0}C for 24 h. This process was repeated with successive higher concentrations (100, 150,200, 250, 300, 350, 400, 450, 500,600, 700, 800,900 and 1000 ppm) of Cr6+ until the minimum inhibitory concentration (MIC) of bacterial isolate was obtained. Significant growth and rapid Cr (VI) degradation kinetics of the specific bacterial species in the presence of 50, 100, 150, 250 and 500 ppm of Cr (VI) during twenty four hours of incubation at 30°C were considered as Cr (VI) resistant. There was no any microbial growth in the chromium concentrations above 500 ppm. Hence, the 500ppm 0f chromium is considered as MIC for present isolate *Bacillus sp*. A single strain capable of growing at this condition was selected for further experiments.

Methods to evaluate the chromium degrading capacity of bacterial species

Below to the 500 µg/ml MIC, different concentrations of potassium chromium (K2Cr2O7), such as 50, 100, 250 and 300 ppm were prepared by dissolving required amount of potassium

chromate. Addition to these, the standard nutrient broth was prepared and autoclaved at121°C for 15 minutes and was cooled in a water bath. In 100 ml of conical flask 20 ml of nutrients were taken along with the above potassium chromium concentrations. In this experiment, the 25ppm of chromium concentration with nutrient broth Is alone prepared in double the number. In all flasks, including one 25ppm of chromium concentration cum nutrient broth flask, under aseptic condition, the chosen high chromium resistant single species of bacteria was inoculated with 0.1ml cells. The flasks were incubated at shaking incubator 140 rpm, 370C temperatures. Another 25 ppm of chromium concentration with nutrient broth was not inoculated with any bacterial species is considered as control. All the broth culture flasks were incubated up to 48 hours (Figure-5).

Detection of chromium reduction in the broth by Di-phenyl carbazide method

The content of the broth after 48 hours incubation were transferred to centrifuge tube and centrifuged at 8,000 rpm for fifteen minutes. The chromium reduction in the supernatants were analysed with the help of spectrophotometer by following diphenyl carbazide (DPC) For the chromium estimation, the colorimetric reagent, 250mg of 1, 5-diphenyl carbazide dissolved in 50 mL of acetone. Along with this, required amount of 10% H2SO4 was added to maintain the pH 2 ± 0.5. The total Volume was made up to 100 mL in borosil volumetric flasks. Method. In this method, the Chromium (VI) present in the supernatant reacts with DPC to form reddish violet complex. The reaction is selective for chromium and very sensitive. The chromium present in the reddish violet solution was estimated by taking the absorbance at 540nm on UV-VIS spectrometer (make Systronics). Before that, the standard graph was prepared by using different concentration of chromium 20ppm to 120ppm by using DPC. Percentage of reduction in chromium concentration was calculated for each control and experimental broth based on the initial and final readings.

The metal removal efficiency the of isolates were calculated by using the formula,

$$\% \text{ Removal} = (Ci-Cf)/Ci \times 100$$

Where,

Ci = Initial concentration of the metal in the liquid broth before bacterial inoculation (ppm),

Cf = Final concentration of the metal in the culture broth after filtration (ppm).

RESULTS AND DISCUSSION

Rivers and lakes in India is highly polluted by the discharge of industrial effluents, domestic and agricultural wastes, sewage consisting of varying hazardous chemicals and heavy

metals.[15] The tannery industries alone in India released about 2000-3000 tons of chromium into the environment annually with chromium concentrations ranging between 2000 and 5000 mg/ L in the aqueous effluent compared to the recommended permissible limits of 2 mg/L.[16]The tannery industries located in the Ranipet, Vellore District are releasing huge amount of chromium rich effluents into the nearby fresh water bodies every day. These effluents affect the ground water quality and the health of aquatic organisms in this area. Hence, proper remediation measures are essential to remove all these pollutants. The researcher throughout the world is indulged in various researches to discover the correct remedial methods to eliminate all these pollutants. Worldwide, various numbers of conventional methods such as chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery were using to remove the chromium like various types of toxic metals.[9] These processes may be ineffective or extremely expensive especially when the metals in solution are in the range of 1–100 mg/L.[17] Hence, it is important to develop an innovative, low cost, and eco-friendly method for of toxic heavy metal removal from the wastewater. In recent days, worldwide the researchers are Screening different types of environment to isolate the high efficient chromium degrading microorganisms to remove the chromium. In the present investigation, the *Bacillus sp* isolated from the soil on tannery effluent discharging channel in the Ranipet, Vellore District was bound to be highly resistant to chromium. The degrading efficiencies were bound to be 80%, 65%, 44% and 18.4% at Cr concentrations 50,100,150 and 250ppm respectively. Hence, the present isolate *Bacillus sp* can remove the maximum amount of chromium at 50ppm. These result has a strong agreement with the on *Bacillus sp* and *Staphylococcus* spp possessing high chromium resistant properties and remove up to 86% and 74% of chromium.[15] Similar observation made in *Bacillus sp*. JDM-2-1 and *Staphylococcus capitis* because both species could reduce 85% and 81% of hexavalent chromium from the medium after 96 h and were also capable of reducing hexavalent chromium 86% a nd 89%, respectively, from the industrial effluents after 144 h.[18] Isolation of *Acinetobacter sp*. from pulp-paper industry was simultaneously tolerant to only 50 mg/ L PCP and 500 mg/ L chromate.[19] Bacteria isolated from tannery effluent which was simultaneously tolerant to 500 mg/ L PCP and 200 mg/ L Cr(VI) concentrations.[20] *Bacillus pumilus*, Staphylococcus species and *Alcaligenes faecalis* reduces Cr_6 95%, 91% and 97% within 24 h from the medium containing 100µg/ml chromium .[21] *Pseudomonus* species from Lonar Lake and revealed that isolates reduces chromium 65.38% to 64.88% in 96 hrs.[22] Two different bacterial strains such as *Pseudomonus sp* and *Bacillus*

sp. from waste water, among these two species, *Bacillus sp*. was has high chromium degradation capacity than *Pseudomonus sp*.[23]

Table: 1. Chromium degrading efficiency of bacterial isolates Bacillus spp from the soil of tannery effluent channel at Ranipet.

Experiments	Concentration of chromium in ppm		
	Initial concentration	Final concentration	Percentage
Control	100	100	0
1	50	10	80
2	100	35	65
3	150	84	44
4	250	240	18.4

Fig 1. The effluents from the tannery industry discharged into the nearby water bodies.

Fig- 2 and 3. The chromium contaminated effluents from tannery industry and screw container containing effluent contaminated soil sample

Figure: 4.

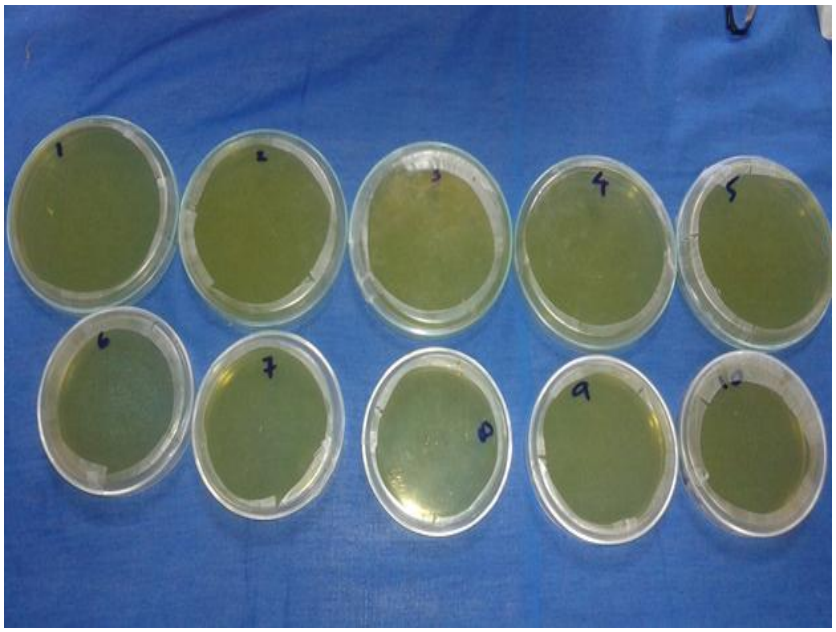

Figure-5. Serial dilution culture of extracts of contaminated soils on PYE agar plate

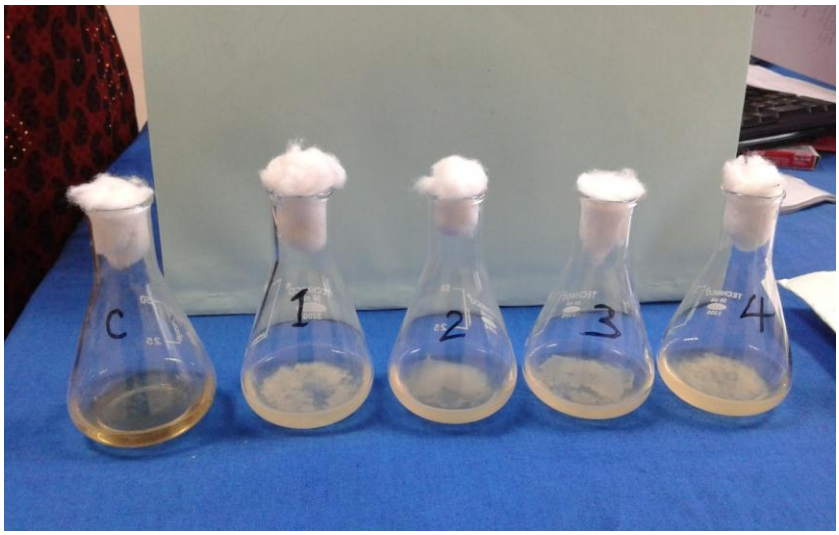

Fig.6. Culture of chromium degrading bacterial in different concentration of potassium chromate mixed broth medium.

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