

Nazeema & Nirmala, 2017

Volume 3 Issue 1, pp. 35 - 48

Date of Publication: 15th March, 2017

DOI-<https://dx.doi.org/10.20319/lijhls.2017.31.3548>

This paper can be cited as: Nazeema, M. & Nirmala, T. (2017). Isolation and Characterization of Chromium Tolerant Bacteria from Tannery Effluent at Dindigul District, Tamilnadu, India. LIFE: International Journal of Health and Life-Sciences, 3(1), 35 - 48.

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ISOLATION AND CHARACTERIZATION OF CHROMIUM TOLERANT BACTERIA FROM TANNERY EFFLUENT AT DINDIGUL DISTRICT, TAMILNADU, INDIA

Nazeema. M

Research Scholar, PG & Research Centre of Zoology, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Tamil Nadu, India
nazee.banuu@gmail.com

Nirmala. T

Principal, PG & Research Centre of Zoology, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Tamil Nadu, India
principal@annejac.com

Abstract

*Hexavalent chromium contamination in environment became a serious problem in all over the world. The chromium, a toxic heavy metal, is a major contaminant in tannery wastes and their accumulation into the soil and water is an environmental issue particularly in Tamilnadu. Dindigul is the headquarters of leather tannery industry. Tannery effluent was collected from leather industry located at Dindigul district. Bacteria having tolerance to chromium has been isolated. A total of six species of chromium tolerant bacteria were isolated and their minimum inhibitory concentration of all the six isolates against hexavalent chromium was determined on nutrient agar supplemented with varying concentration of chromium from 100 ppm to 600 ppm. The bacterial strains were characterized by morphological, cultural and biochemical analysis. They were identified as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus* species, *Bacillus cereus* and *Bacillus licheniformis*. The analysis of the results*

concluded that all the bacteria showed resistance against chromium with Minimum Inhibitory Concentration (MIC) values up to 100 mg/L.

Keywords

Chromium Tolerant Bacteria, Hexavalent Chromium, MIC, Tannery Effluent, Dindigul

1. Introduction

Dindigul is situated in South Indian state of Tamil Nadu. Dindigul is located 420 km (260 miles) southwest of the state capital, Chennai. Industries in Dindigul mainly focusing on lock making, leather, textile spinning, administrative services, agricultural trading, banking, agricultural machinery and educational services.

Leather industries are one of the major industrial sectors of India but are recognized as one of the highly polluting industries leading to soil and water pollution (Verma *et al.*, 2004 & 2008). Near about 170,000 tones of Chromium waste are discharged into the environment annually based on its consequences of industrial and manufacturing activities (Kamaludeen *et al.*, 2003).

Chromium generally exists in oxidation states (I–VI), more stable as Chromium (III) and Chromium (VI). Chromium (VI) is the toxic form of the element which causes severe diseases in human beings like diarrhea, ulcers, eye and skin irritation, kidney dysfunction and probably lung carcinoma (Malaviya and Singh, 2011). Tannery wastewaters contain large quantities of Chemical Oxygen Demand (COD), color, sodium sulphide, nitrate, chloride, chromium and suspended solids (Sharma and Malaviya, 2014).

Application of chromium in various industries such as electroplating, leather tanning, stainlesssteel, metal processing, and textile has caused a widespread environmental contamination (Shukla *et al.*, 2007). Chromium generally exists in two forms; Chromium (III) and Chromium (VI) (Kaur and Kumar, 2014). Tanneries are mainly responsible for the release of huge amount of toxic hexavalent chromium [Cr (VI), chromate] through their solid waste into the environment.

A number of heavy metals released by industrial sectors are major pollutants in marine, ground, industrial and even treated wastewaters (Martins *et al.*, 2006). Heavy metals can be extremely toxic as they damage and cause several disorders and also block functional groups of vital enzymes in humans. Two stable oxidation states of Chromium persist in the environment,

Chromium (III) and Chromium (VI), which having contrasting toxicities, mobility and bioavailability. Beside the above Chromium (III) is essential in human nutrition especially in glucose metabolism.

Chromium contamination of the environment especially by hexavalent chromium has become a major area of concern. Towards this direction, several conventional wastewater treatment technologies were developed and are used successfully at large scale, to reduce the hazardous compounds concentration in effluents from higher to lower levels (Verma and Rahal, 1996).

Despite the fact that heavy metals are acutely toxic to most microbes, there are metal tolerant bacteria. Proliferation of microbes occurs due to long term exposure of metals and it favors the bacteria tolerant to metals. Hutchinson and Symington, 1997 has been investigated by assaying habitats exposed to anthropogenic or natural metal contamination.

Microbial tolerance to hexavalent chromium has practical importance because it can serve as a basis for selecting organism that can be used to detoxify chromium in the environment (Ganguli and Tripathi, 2002). Bacteria have certain concentration where it totally cannot live (Mythili and Karthikeyan, 2011) and that concentration was known as minimum inhibitory Concentration (MIC). MIC will be usually checked before applying bioremediation to know whether Bacteria used are alive or not (Pavel *et al.*, 2012).

Easy, economic and eco-friendly techniques are required for fine tanning of effluent wastewater treatment. Biological reduction of Chromium (VI) using indigenous microorganism offer a new cost-effective and environmentally compatible technology (Camargo *et al.*, 2005 and Liu *et al.*, 2006). Chromium (VI) reducing bacteria reduce chromium under both by aerobic and anaerobic microorganisms (Wang and Shen, 1995; Philip *et al.*, 1998; Pal and Paul, 2004; Ma *et al.*, 2007; Farag and Zaki, 2010; Sharma and Adholeya, 2012). On the other hand, bioremediation, which uses indigenous microorganisms, is an ecofriendly alternative for the detoxification and removal of Cr-pollutants (Dey *et al.* 2014).

Thus this study focused on isolating hexavalent chromium tolerated bacteria which could be used for remediation of the polluted environment.

2. Objectives

The objectives of the study is to isolate the chromium tolerant bacterial strains from the tannery effluent, and to measure minimum inhibitory concentration of six bacterial strains that can be used for bioremediation process.



Figure 1: Study Area

3. Materials and Methods

3.1. Method of Sample Collection

Tannery effluent was collected, using 500ml sampling bottles. The bottle containing the sample was capped tightly and kept in an ice cold clean box to protect them from direct sunlight. The sample was transported immediately to the microbiological laboratory for further analysis.

3.2. Isolation of Microorganisms

Microbial assembly of the sample collected from the tannery effluent tank was studied and were selectively isolated, enumerated and characterized. Microorganisms were isolated by serial dilution method. The plates were observed periodically for the growth of microbes. The

pure colonies developed on plates were selected, isolated and maintained in nutrient agar slants at 4°C for the further studies.

3.3. Characterization of Isolates

Morphological characters of the isolates were studied. The macroscopic studies of microorganisms growing on agar medium were useful for rapid identification of their respective genus, which includes characters such as colony characteristics (configuration, margin, elevation, surface, pigment, shape, colour and arrangement), absence or presence of aerial mycelium and extent of spore formation. Bergey's Manual of Determinative Bacteriology (1930) was followed for the structure resemblance and comparison for genus identification of purified isolates.

3.4. Biochemical Tests

Biochemical tests, which encompasses Indole utilization, Methyl red, Voges Proskauer, and Citrate utilization tests were carried out to identify the isolates up to their species level. Pure cultures of bacteria isolated were presumptively identified on the basis of their morphological and biochemical characteristics. Species were identified as described by Buchanan and Gibbons (1974).

3.5. Minimum Inhibitory Concentration (MIC)

A method used for minimum inhibitory concentration measurement was streaked nutrient agar method adopted from Mgbemena *et al.*, (2012). The medium used was Nutrient Agar containing Chromium (VI) with varying concentration of 100, 200, 300, 400, 500 and 600 ppm. The sterile 15mL nutrient agar medium containing Chromium (VI) poured into the sterile petri dish plate. After the medium become solid, bacteria streaked at the surface of the medium using sterile loop in aseptic condition. The plate then checked for growth after 24 hours incubation in incubator at 37°C (Mgbemena *et al.*, 2012). After 24 hour observation, scoring was conducted to the streaked plate by quantitative indicator from good growth (+++) to poor growth (+).

4. Results and Discussion

4.1 Isolation of Bacterial Strains

An investigation on bacteriological quality assessment of effluent discharged from Dindigul tannery effluent was carried out with a view to isolate and identifies the bacterial species present in the tannery effluent, and to compare the level of microbial inhibition concentration of tannery effluents. The isolated chromium tolerant strains are characterized by

colony characteristics, morphologically, physiologically and biochemically. From the results it was observed that total of six isolates were found in the treated samples, they were *pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus*, *Bacillus cereus* and *Bacillus licheniformis* (plate 1).

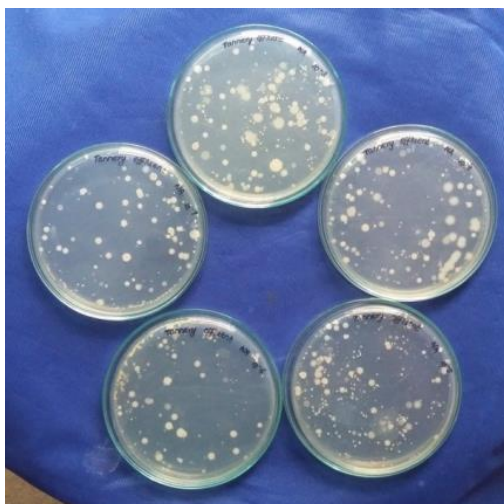


Figure 2: Isolation of Microorganisms from Tannery Effluent

Table 1: Total Number of Colonies in Tannery Effluent

Dilutions	Colony count
10^{-3}	244
10^{-4}	136
10^{-5}	108
10^{-6}	84
10^{-7}	48

Table 1 shows the growth of colonies of bacteria in serial diluted test tubes. A total of 244 colonies were found at lower concentration and the colonies decreased to 48 at higher concentration of chromium.

Table 2: Biochemical Characterization of the Strains

Tests	Strain 1	Strain 2	Strain 3	Strain 4	Strain 5	Strain 6
Gram staining	+	+	+	+	+	+
Spore staining	Absent	present	present	Absent	present	present
Motility	Non Motile	Motile	Motile	Motile	Motile	Motile
Indole Production	-	-	-	-	-	-
Methyl Red	+	-	+	-	-	-
VogesProskauer	-	-	-	+	-	-
Citrate Utilization	-	+	+	+	+	-
Gelatin liquefaction	-	+	+	+	+	+
Starch hydrolysis	-	+	+	-	+	+
Nitrate reduction	-	-	+	+	+	-
Identified as	<i>Pseudomonas aeruginosa</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Micrococcus sp</i>	<i>Bacillus cereus</i>	<i>Bacillus licheniformis</i>

In the present investigation, the *Bacillus* species isolated from the tannery effluent was found to be highly resistant to chromium discharging channel in the Dindigul District. In India tannery industries 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 which is compared to the recommended permissible limits of 2 mg/L (Chandra and Kulshreshtha 2004).

Morphological characterization of all the six isolates revealed that all above six isolates were gram-positive in nature. Gram-positive bacteria were reported previously by several workers (Ellis *et al*, 2003, Chovanova *et al*, 2004 and Karellova *et al.*, 2011). The gram-negative bacteria are considered to be more metal tolerant as compared to gram-positive bacteria.

4.2. Minimum Inhibitory Concentration (MIC)

The bacterial isolates obtained from nutrient agar with or without chromium were tested for their ability to tolerate different concentrations of Chromium (VI) incorporated into Lysogeny broth medium. The effects of different concentrations of Chromium (VI) on the growth of the isolates were determined by incubating the isolates in 50 ml liquid broth contained 250 ml Erlenmeyer flasks. The medium was amended with different concentrations of Chromium (VI), namely, 100, 200, 300, 400, 500 and 600 ppm.

Table 3: Score of Bacteria on Minimum Inhibitory Concentration of Chromium (VI) Test

Bacteria	Concentration of Cr ⁺⁶ in ppm					
	100	200	300	400	500	600
<i>Pseudomonas aeruginosa</i>	+++	+++	+++	++	++	+
<i>Bacillus subtilis</i>	+++	+++	+++	++	++	-
<i>Staphylococcus aureus</i>	+++	+++	+++	++	++	-
<i>Micrococcus</i>	+++	+++	+++	++	++	-
<i>Bacillus cereus</i>	+++	+++	+++	++	++	+
<i>Bacillus licheniformis</i>	+++	+++	+++	++	++	+

The growth of bacteria decreases with increasing concentration of chromium. At 100 to 300 ppm there was a tremendous growth of the bacteria observed. Poor growth was noticed at 400 and 500 ppm concentration of chromium level (Table 3). *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus* were absent at 600 ppm (plate2) as they can only resist under 600 ppm of hexavalent chromium. Purwanti *et al.*, 2015 reported that excessive amount of chemical which bound between the medium that will affect the bacterial growth at higher concentration of chromium.

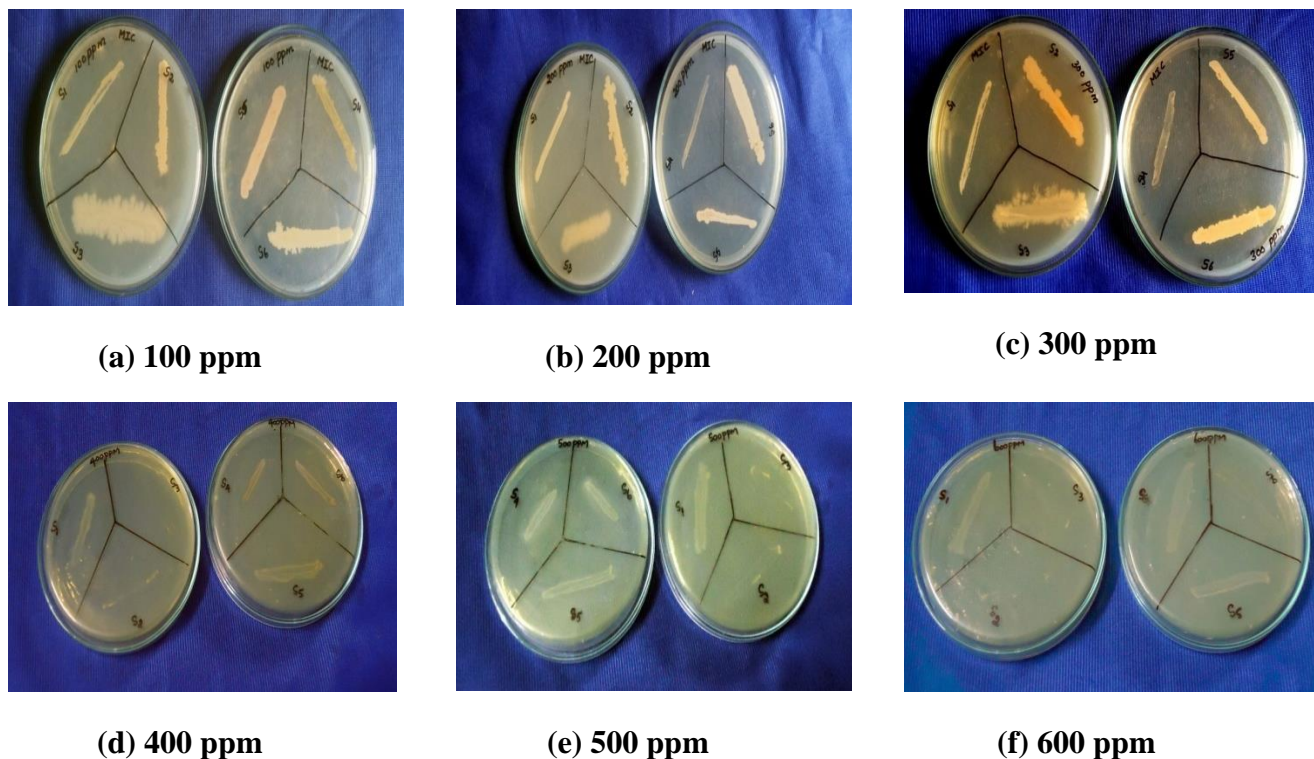


Figure 3: Determination of Minimum Inhibitory Concentration of Chromium Tolerant Strains in Lysogenic Broth Medium Supplemented with Various Concentration of Hexavalent Chromium (100, 200, 300, 400, 500 & 600 ppm)

Minimum inhibitory concentration is the concentration at which no or very less growth of bacterium were observed. Out of six isolates *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus licheniformis* showed minimum Chromium (VI) tolerant ability of hexavalent chromium. Whereas *Pseudomonas aeruginosa*, *Micrococcus sp* and *Bacillus cereus* was exhibited maximum Chromium (VI) tolerant ability at 600 ppm of hexavalent chromium.

Ganguli and Tripathi (2002) has pointed out that microbial tolerance to hexavalent chromium has practical importance because it could serve as a basis for selecting organism that can be used to detoxify chromium in the environment. Gupta and Balomajumder (2015) reported that for reduction of Chromium (VI) accumulation of mixed culture of *Escherichia coli* and *Bacillus* species was more efficient than pure culture of *Bacillus* species.

Anyanwu and Ezaka (2011) have identified their resistance potential of the bacterial isolates and the evaluation of their ability to detoxify hexavalent chromium in the environment.

Growth of the staphylococcus species increased with increase in incubation time at the concentrations of 50, 100, 150 and 200µg/ml while at 500 µg/ml in the study of sewage

treatment oxidation pond, Nigeria the growth was decreased as reported by Parameswari *et al.* (2009). The chromium (VI) resistance above 2500 mg/l has also been reported by Shakoori *et al.* (1999). The native isolates, which tolerated high concentration by of 500µg/ml of Cr (VI), can be effective in remediation strategies for ecosystem polluted with hexavalent chromium (Ezaka and Anyanwu 2011).

Bacteria isolated from tannery effluent which was simultaneously tolerant to 500 mg/ L PCP and 200 mg/ L Chromium(VI) concentrations (Tripathi and Garg 2010). (Muhammad 2013) observed and investigated that *Bacillus* species has high chromium degradation capacity than *Pseudomonas* species.

5. Conclusion

The lowest concentrations of hexavalent chromium that completely inhibits the growth of the bacteria were tested. Microorganisms which have the ability to tolerate and reduce Chromium can be used for detoxification of environments contaminated with Chromium. Therefore chromium tolerant bacterial strains were isolated and can be use to remediate the present study site which was contaminated by the tannery effluent in Dindigul District.

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