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Testing of Bacterial Population Isolated from Tannery effluent and its Chromium reduction efficiency

Nilofer Nisha H.M*[1] and Kavitha P[2]
[1,2]**Department of Microbiology, Srimad Andavan College of Arts and Science (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamilnadu, India**

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***Corresponding Author Email:** nishumphil@mail.com

Abstract
The present study reveals isolation, identification and characterization of chromium heavy metal resistant bacteria from tannery effluent collected in and around Tiruchirappalli. Initially, a total of 11 distinct isolates were screened from three different tannery effluent samples. Based on morphologicaland biochemical analysis revealed that, the isolates were authentically identified. The six isolates were selected based on the level of heavy metal resistances and among the four isolates respectively, *Pseudomonas fluorescens, Bacillus subtilis, Pseudomonas putida* and *Bacillus licheniformis* were found to be tolerate 1000 ppm Cr. Isolate designated as SII3 belongs to *Pseudomonas putida* showed maximum absorption of chromium (70%) and the chromium reductase assay was 1.18 units. The results on pH and nitrogen indicate that medium with peptone and yeast extract with pH 5-7 given significant growth and absorption of chromium under static condition.

Keywords
Heavy metal, Biosorption, NADH, Bioremediation, Effluent.

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INTRODUCTION
Heavy metal pollution is a risk for human health. The industrial disposal of the heavy metals is a major concern of eco toxicity caused by chemical manufacturing, painting and coating, mining, extractive metallurgy, nuclear and other industries. The metal causes a deleterious effect on the flora and fauna that grow in lakes and streams (Sayari *et al*. 2005). Compare to conventional methods, biosorption of metal ions by fungi at different concentrations found to be efficient method. Developing knowledge on biosorption pathway mechanism is important to the design and optimization of the heavy metal removal. Metal removal from industrial effluents and subsequent recovery is widely studied by fungal biosorption but less applied. The biosorption method consists of two phases, solid phase (biological material used as biosorbent) and a liquid phase (solvent is used in liquid phase such as water) containing a metal to be sorbed. Many bacteria, fungi and yeasts were studied and reported as potential source of biosorbents (Quintelas et al. 2008). The heavy metals are removed by biological materials and the process is known as biosorption and material used is abiosorbents. Different types of biosorbents re used

it includes, bacteria, fungi, yeasts and agricultural products has been used for biosorption. *Pseudomonas fluorescens, Enterobacter cloacae* and *Acinetobacter* sp. are resistant to chromium from tannery effluent (Srivastava and Thakur 2007). Research studies evidenced theCr^{6+}biosorptionability of different bacterial isolates such as *Bacillus megaterium*, *Bacillus coagulans*, *Acinetobacter sp* and *Pseudomonas sp* (Sukop *et al*. 2009). Therefore, microbes have high surface area and the volume ratio permits to interact with metals from the environment (Zhu *et al*. 2008). Functional groups such as -NH_2, -COOH, -SH and –OH, present on the microbial surface mediate physicochemical interaction with metal and the thereby regulate the uptake of metal ions (Kotas and Stasicka, 2000). Application of fungi as bio absorbant is highly selective, efficient, easy to maintain and cost effective for the treatment of large volumes of wastewaters. Bioaccumulation or Biosorption is a process that use the functional groups to form complex with metal ions and play a vital role in removing heavy metals (Sahi *et al*. 2006). Metal ions entered into the cytoplasm through a specific carrier system and the transport processes in live bacteria cell is studied other than accumulation (Costa *et al*. 2001). Chromate reducing microbes detoxify chromium via valence transformation ($Cr^{6+}$to Cr^{3+}) by reductase is considered to be an ideal onsite and in situ bioremediation (Mangaiyarkarasi and Geetha ramanai, 2014).

MATERIAL AND METHOD

Sample Collection

Chromium contain effluent was collected from KMM Tannery during March 2018 located at Airport (Fig.1), Tiruchirappalli in a sterile bottle and processed for microbial studies.

Figure1: Sampling site

Isolation of Bacteria

Samples were serially diluted and plated on Luria–Bertani (LB) agar (tryptone: 10 g l-1; yeast extract: 5 g l-1; NaCl: 10 g l-1; glucose: 0.1 g l-1) adjusted at normal pH value (7.0). The molten medium was amended with Cr (VI) as K_2 Cr_2 O_7 to final concentration 100 ppm using sterile filtered Cr stock solutions. Plates were incubated at 37°C for 48 h. Subsequently, isolates were selected according to growth and tested up to 1000 ppm for further studies.

Growth phase studies

Growth patterns of isolated strains were measured by growing approximately equal number of cells (1×10^{-7} cells) in minimal broth contains 100 to 1000 ppm potassium dichromate. Growth rate was measured at 610 nm. In case of resistant strain, growth measurements were carried out in the absence and presence of chromium in the media.

Determination of Cr (VI) reduction

Cells grown on minimal medium with 1000 ppm Cr were harvested by centrifugation at 15° C for 10 min and the supernatant was used for chromium estimation. The hexavalent chromium in the culture supernatants was measured by the addition of 1, 5-diphenyl carbazide (DPC) reagent. One mL of the supernatant, 9 mL of 0.2 M sulphuric acid and 0.2 mL of 0.25% diphenyl carbazide in acetone were added, and the absorbance of the pink colour developed was read at 540 nm using distilled water as blank. Chromium reduction was also tested at pH 2, 3, 5, 7, 9 and 10. Effect of nitrogen such as yeast extract, peptone and ammonium sulphate also tested at 0.01% level with 2% fixed con of glucose.

The chromium removal percentage was calculated using the following formula:

E = (Ci - Cf / Ci) × 100

Where, E = Percentage removal of heavy metal; Ci = initial metal ion concentration, mg/L; Cf = final metal ion concentration, mg/L

Chromate reductase assay (Sau *et al.* 2010)

The CFE was fractionated with ammonium sulfate at 30 % (w/v) and protein precipitate was collected and dialyzed. The assay mixture was prepared using 0.2mM NADH and 4.8µM Cr (VI) in 50 mM potassium phosphate buffer (pH 6.0) and protein0.1(10 mg/ml) mL of partially purified enzyme in a total volume of five mL. Assay mixtures except enzyme or NADH were used as respective controls. The assay mixtures were incubated at 30° C for 30 min. The amount of hexavalent chromium was measured using 1, 5-(DPC) reagent. One unit of enzyme is defined as the amount that catalyzes the conversion of 1 µM of substrate Cr (VI) per minute per mg.

RESULT AND DISCUSSION

Isolation of chromium resistant bacteria

Three different samples were collected from tannery and the effluent was dark green in color. The pH of samples was 5.48, 10.50 and 10.28 respectively for

sample I, II and III. The CFU of SI is $38x10^5$and isolates designated as SI1 to SI5. Colonies from sample II were designated as SII1 to SII3 and sample III as SIII1 to SIII3. The pH of the untreated tannery effluent was acidic (SI-5.48) in nature and other two samples II and III are alkaline in nature (10.50, 10.28), with an unpleasant odor and color. Unpleasant odor may be due to the deposition of organic substances present in the effluent. Out of 11, the predominate isolate was *Bacillus* (27 %), *Pseudomonas* (18 %) and other genera (9 %). Among the isolates the frequency of Gram positive is 63 % and Gram negative (36 %).Based on biochemical character isolated strains were identified as *Pseudomonas fluorescens, Escherichia coli, Alcaligenes* sp, *Micrococcus* sp, *Bacillus methylotrophicus, Bacillus subtilis, E.faecalis, Pseudomonas putida, Streptococcus Anaerobius, Ruminococcus albus, Bacillus licheniformis* were isolated from different samples.

Testing of chromium resistance

Out of 11, 6 were grown well under 100 ppm Cr (table 1). The growth rate of isolate at 100 ppm Cr was given in figure 3. The maximum growth was observed in isolate SI1 followed by SII1 respectively comes under Genera of *Pseudomonas* and *Bacillus*. Figure 2 reveals the growth rate of *Pseudomonas fluorescens*, *Bacillus subtilis, Pseudomonas putida* and *Bacillus licheniformis*at 1000 ppm. The maximum growth rate was observed in isolate SI1 followed by SII1 respectively comes under Genera of *Pseudomonas* and *Bacillus*. The table-2 represents, the percentage of chromium absorption by *Pseudomonas fluorescens* was 74% with chromium reductase (Fig-3) 1.26 U/mg. 70% of chromium absorption was carried out by *Pseudomonas putida* and chromium reductase rate was 1.18 U/mg. Chromium absorption by *Bacillus licheniformis* was 68% and *Bacillus subtilis* (62%). The percentage of chromium absorption and chromium reductase rate was high by *Pseudomonas fluorescens* when compared to other isolates.

The potential of isolated bacterial species heavy metals from solutions by biosorption was effective in Gram negative than Gram positive. The growth rate of *Pseudomonas fluorescens* and *Bacillus subtilis* at 100 and 1000 ppm chromium was comparatively higher than *P.putida* and *B.licheniformis*. Even though the growth rate of *B.licheniformis* was moderate, the chromium absorption was high when compared to *B.subtilis*. Totally 11 strains and four were selected for chromium absorption studies due to its tolerance at 100 and 1000 ppm. Among the two genera *Pseudomonas* and *Bacillus, Pseudomonas fluorescens* and *Pseudomonas putida* shows maximum chromium absorption respectively followed by *Bacillus licheniformis* and *Bacillus subtilis.* The types of mechanism to remove heavy metals from effluent is varies among bacteria, fungi, ciliates, algae, mosses, macrophytes and higher plants (Sultan and Hasnain,2007). *Bacillus subtilis* are Gram positive bacteria and the walls of the bacteria contain glutamic acid act as efficient chelating agents to remove metal.

Effect of pH and Nitrogen on Chromium absorbance

Based on reductase assay *Pseudomonas fluorescens* was tested for its efficiency on Cr reduction under nitrogen and pH (Table 3). The best pH was 7 showed 72% of reduction followed by 68% at pH 5. Last acidic and basic was not found to be significant. 92 % chromium reduction by a Gram-negative strain that reduce chromium at 500 mg/L respectively at pH 7 and found as *Pseudomonas* family reported as potent candidate for bioremediation (Rosa *et al.* 2018). Wani *et al.*(2015) isolated a bacterial strains *Klebsiella* sp. were resistance to Cr (VI) and found tolerance to Chromium (VI).

Chromium reduction was carried out by supplying various nitrogen sources like peptone, yeast extract and ammonium sulphate. Among the three nitrogen sources *Pseudomonas fluorescens* utilized yeast extract and chromium biosorption was 68% when compared to peptone (60%) and ammonium sulphate (56%). Plumi *et al*. (2012) reported that yeast extract (65%) were act as a best nitrogen source for biosorption of chromium when compared to peptone (61%), beef extract (58%), ammonium sulfate (35%) and ammonium carbonate (27%). Ammonium is a source of nitrogen when it is given at a suitable for *A. junii* for chromium biosorption compared to peptone and yeast extract (Somasundaram *et al*. 2011). In previous studies reported that, *Bacillus methylotrophicus* was isolated and tested for its effectiveness against chromium. The strain shows maximum reduction of 95M to 7.14M Cr (VI) in a complete synthetic medium formulation within 48 hrs. Polti *et al.* (2011) have suggested that through adsorption process chromium is reduced its form from hexavalent to trivalent. *Streptomyces* sp. are identified as resistant strain. *Cellulosimicrobium sp*. (KX710177) was isolated and identified from tannery effluent based on 16S rRNA gene sequence analysis. Chromium tolerance were tested at various concentration level such as 50, 100,200 and 300 mg/L. *Cellulosimicrobium* it persists up to 300 mg/L of chromium. However, when the chromium level decrease below 100mg/L the reduction rate was 99.33% (Bharagava and Mishra, 2018). *Pseudomonas putida* and *Pseudomonas plecolglossicide* are a novel strain and these strains were checked for its degrading efficiency. *Pseudomonas putida* and

Pseudomonas plecolglossicide shows 98.4% and 98.3% (Poornima *et al*. 2010). These two isolates are able to breakdown the chemical compounds (aliphatic and aromatic hydrocarbons, fatty acids, insecticides) and it promote maximum bioremediation properties.

CONCLUSION

Heavy metal resistant *Pseudomonas fluorescens* isolated from tannery effluent was found to be potent biological agent for bioremediation of chromium.

Table 1: Growth of bacterial isolates among chromium supplemented medium

Strain code	Genera of isolates	Growth at 100 ppm Chromium	Growth at 1000 ppm Chromium
SI1	*Pseudomonas fluorescens*	+	+
SI2	*Escherichia coli*	-	-
SI3	*Bacillus methylotrophicus*	-	-
SI4	*Alcaligenes*sp	+	-
SI5	*Micrococcus* sp	-	-
SII1	*Bacillus subtilis*	+	+
SII2	*E. faecalis*	+	-
SII3	*Pseudomonas putida*	+	+
SIII1	*Streptococcus Anaerobius*	-	-
SIII2	*Ruminococcusalbus*	-	-
SIII3	*Bacillus licheniformis*	+	+

Table 2: Chromium absorption and enzyme assay of selected bacterial isolates

Strain code	Genera of isolates	Percentage of chromium absorption	Chromium reductase U/mg
SI1	*Pseudomonas fluorescens*	74	1.26
SII1	*Bacillus subtilis*	62	0.7
SII3	*Pseudomonas putida*	70	1.18
SIII3	*Bacillus licheniformis*	68	0.9

Table 3. Effect of Nitrogen and pH on chromium absorption by *Pseudomonas fluorescens*

Nutrient	Biosorption percentage
pH 2	34
pH 3	36
pH 5	68
pH 7	72
pH 9	28
pH 10	18
peptone	60
Yeast extract	68
Ammonium sulphate	56

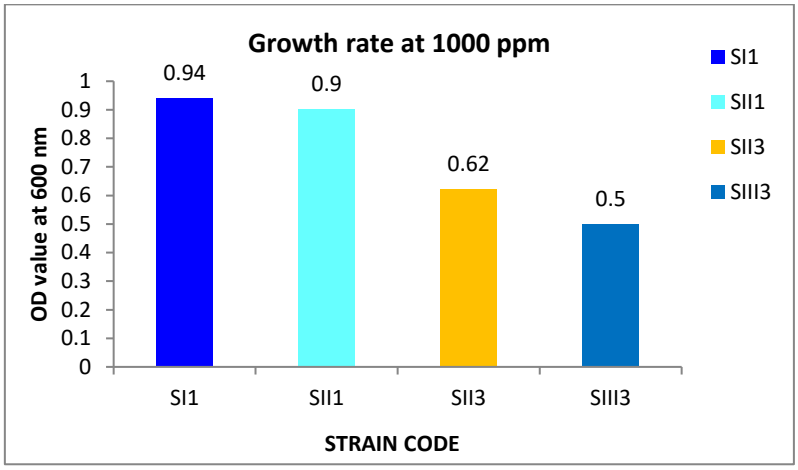

Figure 2. Growth rate of bacteria at 100 ppm

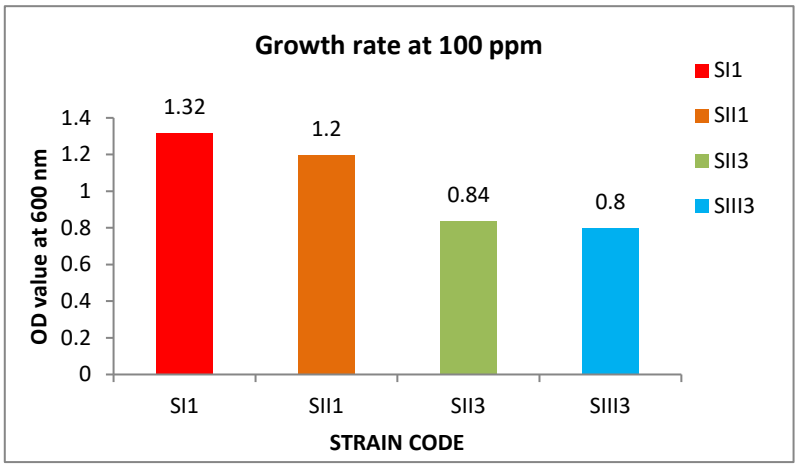

Figure 3. Growth rate of bacteria at 1000 ppm

REFERENCES

1. Sayari, A., Hamoudi, S. and Yang,Y.(2005). Applications of pore-expanded mesoporous silica removal of heavy metal cations and organic pollutants from wastewater. *Chemistry of Materials*17: 212-216.
2. Quintelas, C., Fernandes, B., Castro, J., Figueiredo, H. and Tavares, T. (2008). Biosorption of Cr (VI) by three different bacterial species supported on granular activated carbon-A comparative study. *J. Hazard Mat*153: 799-809.
3. Srivastava, S. and Thakur, I.S. (2007). Evaluation of biosorption potency of *Acinetobacter* sp. for removal of hexavalent chromium from tannery effluent. *Biodegradation*18: 637-646.
4. Sukop, H., Blume, H.P. and Kunick,W.(2009). The soil, flora and vegetation of Berlin's waste land, Nature in cities- The natural environment in the design and development of urban green space. *John Wiley and Sons Chichester*2: 115-132.
5. Zhu, D., Asnani, P.U., Zurbrugg, C., Anapolsky, S and Mani, S. (2008). Improving Solid Waste Management in India. A Sourcebook for Policy Makers and Practitioners. *The World Bank,Washington* 27(153): 799-809.
6. Kotas, J. andStasicka, Z. (2000). Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution*107: 263-283.
7. Sahi, S.V., Israr, M., Srivastava, A.K., Gardea, J.L., Torresdey, J.G. and Parsons (2006). Accumulation, speciation and cellular localization of copper in Sesbania drummondii. *Chemosphere* 67: 2257-2266.
8. Costa, A.D., Carlos, A. and Pereira, F. (2001). Bioaccumulation of copper, zinc, cadmium and lead by *Bacillus* sp., *Bacillus cereus, Bacillus sphaericus* and *Bacillus subtilis.Brazilian Journal of Microbiology*32: 1-5.
9. Mangaiyarkarasi and Geetharamanai, D. (2014).Bioabsorption of chromium employing microorganism isolated from tannery effluent. *Scrutiny International Research Journal of Biological and Environmental Science* 1(9): 29-36.
10. Sau, G.B., Chatterjee, S and Mukherjee,S.K. (2010). Chromate reduction by cell free extract of *Bacillus firmus* KUCr1. *Polish Journal of Microbiology*59:185-90.

11. Sultan, S. and Hasnain, S. (2007). Reduction of toxic hexavalent chromium by *Ochrobactrium intermedium* strain SDCr-5 stimulated by heavy metals. *Bioresource Technology*98: 340-344.
12. Polti, M.A., Amoroso, M.J. and Abate, C.M. (2011). Intracellular chromium accumulation by *Streptomyces* sp MC1.*Water, Air and Soil Pollution*214:49-57.
13. Bharagava, R.N. and Mishra, S. (2018). Hexavalent chromium reduction potential of *Cellulosimicrobium* sp. isolated from common effluent treatment plant of tannery industries. *Ecotox. Environ. Saf*147:102-109.
14. Rosa Baldiris, Natali Acosta Tapia, Alfredo Montes, Jennifer Hernandez and Ricardo Vivas Reyes. (2018). Reduction of Hexavalent Chromium and Detection of Chromate Reductase (ChrR) in *Stenotrophomonas maltophilia*. *Molecules*23: 406.
15. Wani, P.A., Omozele, I.A., Wasiu, K.O. and Jamiu. (2015). Cr (VI) reduction by indigenous Bacillus species PB5 isolated from contaminated soil of Abeokuta ogun state, Nigeria. *Int. J. Soil Sci* 10(4): 203-210.
16. Poornima, K., Karthik, L., Swadhini, S.P., Mythili, S and Sathiavelu, A. (2010). Degrading of chromium using a novel strains of *Pseudomonas* species. *Journal of Microbial and Biochemical Technology* 2(4): 095-099.
17. Pulimi, Mrudula, SubikaJamwal, Jastin Samuel, Natarajan Chandra Sekaran, and Amitava Mukherjee. (2012). Enhancing the Hexavalent Chromium Bioremediation Potential of *Acinetobacterjunii* VITSUKMW2 Using Statistical Design Experiments. *J. Microbiol. Biotechnol* 22(12):1758–1766.
18. Somasundaram, V., Philip L and Bhallamudi, S.M. (2011). Laboratory scale column studies on transport and biotransformation of Cr (VI) through porous media in presence of CRB, SRB and IRB. *Chem. Eng. J.*171: 572-581.