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In-Vivo Toxicity Study of Heavy Metal Contaminated Industrial Effluent with Murrel Fish and Removal of Cadmium by Indigenous Bacterial Isolates

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Abstract

Heavy metals are toxic to living organisms when exceeding a certain concentration and may pose a risk to human health when transferred to the food chain. The problems associated with heavy metals are very difficult to solve. The physico-chemical methods for heavy metal removal are expensive and have like incomplete metal removal, higher reagents, energy requirements and generation of toxic sludge. Biological approach decreases the cost of remediating contaminated sites. Microbial reduction of these metals is an important aspect of biological remediation. In view of the potential applications of microorganisms in metal reduction, the present investigation was aimed to isolate and characterize the heavy metal resistant strains from the tannery effluent. Presence of toxic heavy metals in the effluent was tested using *Channa marulius* (Murrel fish) by *in-vivo* test, and the result was found to be highly toxic for the aquatic organisms. The efficiency of heavy metal reduction by the isolates was determined by Atomic Adsorption Spectroscopy showed complete removal of cadmium from the effluent.

Keywords

Heavy metals, Cadmium, *In- vivo* toxicity, Bioremediation.

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INTRODUCTION

The chemical industry is of importance in terms of its impact on the environment. The wastewaters from this industry are generally strong and may contain toxic pollutants. Chemical industrial wastes usually contain organic and inorganic matter in varying degrees of concentration. It contains acids, bases, toxic materials, and matter high in biological oxygen demand, color, and low in suspended solids. Many materials in the chemical industry are toxic, mutagenic, carcinogenic or simply hardly biodegradable (EPA, 1998).

Cadmium is a toxic and non-essential metal for an aquatic environment, which is released into and distributed in by industrial sources such as mining, refining of ores, and the plating process (Vido *et al.,* 2001). When Cd contaminates the aquatic ecosystem, it can enter the aquatic food chain through direct consumption of water or biota; and through non-

dietary routes such as absorption through epithelia. They may be concentrated from the water and sediments into aquatic mammalas (Henkel *et al*., 2004).

Cadmium may easily move from soil to food plants through root absorption, and fairly large amounts can accumulate in their tissues without showing stress (Oliver 1997).

The best strategy to clean the heavy metal contaminated site is in general to treat them at the source (Peringer 1997) and sometimes by applying onsite treatment within the production lines with recycling of treated effluent (Hu *et al*., 1999). Since these wastes differ from domestic sewage in general characteristics, pretreatment is required to produce an equivalent effluent (Meric *et al*., 1999).

Treatment of this wastewater is ineffective especially with the use of chemical and physical methods. Therefore the need calls for biological method. Bioremediation according to (Bako *et al*., 2008) is a new method more effective than the chemical and physical methods, and involves the use of microorganisms (petrophiles) to breakdown complex and clean up polluted environment.

The aim of the study is to isolate and identify the cadmium removing bacteria from industrial waste water.

MATERIALS AND METHODS

Collection of sample

The tannery effluent was collected from the release point of the effluent treatment plant (CEPT) of tanneries located at Ranipet in sterile containers, transported in an ice box to the laboratory and processed for bacterial analyses within 6-8 hrs of collection. The sample was further stored at 4° C for physio-chemical and heavy metal analyses in the laboratory.

Physicochemical analysis

Physicochemical analysis is an important tool in conducting research. The collected effluent was analyzed for physicochemical properties like colour, odour, pH, total suspended solids, total dissolved salts, biological oxygen demand (BOD) and chemical oxygen demand (COD).

The pH was determined by direct measurement with a pH meter while electrical conductivity (EC) and total dissolved solids (TDS) were determined by using Elumech EC-TDS meter while total solids were estimated by gravimetric method. Total Suspended Solids (TSS) was determined by the equation, $TSS^{1/4}$TS-TDS. Biological oxygen demand (BOD) was estimated by preparing required volume of dilution water with the addition of nutrients and incubation periods of five days at 20° C while chemical oxygen demand (COD) determination was based on rapid dichromate oxidation method. .

Isolation of heavy metal resistant bacterial isolates from tannery effluent

1ml of the sample was serially diluted in sterile distilled water and plated onto various Nutrient agar (NA) plates containing lead acetate, cadmium sulphate and mercury chloride individually and incubated at 37°C for 24hrs.

Determination of heavy metal resistance by Diffusion method

Heavy metal resistance of the isolates was determined by well diffusion method (Hassen *et al.,* 1998). Heavy metal salt solutions were prepared in different concentrations (10, 20, 40, 60, 80 and 100 mg/L). The plates were spread with overnight cultures of appropriate organisms. To each of the plate, 100 µl of appropriate metal salt solutions were added in each wells of 10 mm in diameter and 4 mm in depth. NA plates were incubated at 37°C for 24 hrs. After incubation, the zone of inhibition was measured. A zone size less than 1 mm was considered as resistance strain.

Determination of minimum inhibitory concentration

The Maximum Tolerance Limit of heavy metals resistant bacterial isolates were determined by broth dilution method (Calomoria *et al.,* 1984) in Muller Hinton Broth (MHB) with gradually increasing concentrations ranging from 100-600 mgl^{-1}. The minimum concentration of heavy metals in the medium inhibiting the complete growth of the isolates was taken as the MIC. Three extremely tolerant isolates were selected for further analysis.

Atomic adsorption spectroscopy

100ml of effluent was taken in three conical flasks and incubated with the heavy metal resistant bacterial isolates. Media without bacterial inoculation was considered as control. The conical flasks were subjected to centrifugation at 130 rpm at 37° C for 10 days. Bacterial biomass in the supernatant were separated and subjected for AAS (Farang and Zaki,

2010). The percentage of reduction was determined by the following equation.

% of reduction = (A-B) / A×100

A=Concentration in control

B=Concentration in Test sample

Characterization of the heavy metal resistant isolates

Morphology, physiological and biochemical characteristics of the isolates were determined by adopting standard methods (Cuppucino and Sherman, 1983).

RESULTS

Physico-Chemical analysis of effluent

The average temperature at the sampling site was around 35° C at day time. The physico-chemical characteristics of the treated tannery effluent sample were shown in the Table.1. The level of DO, BOD, COD, TDS and TSS were well above the permissible limits which needed to be treated.

Physico-Chemical analyses revealed the composition and strength of the tannery effluent. The highly colored effluent might hinder the penetration of sunlight causing the depletion in the rate of oxidation process and thus contributing to the anaerobic oxidation which can be sensed from the putrefying odour of the receiving water bodies (Hemapriya *et al*., 2013).

Table.1 Physico-chemical characteristics of tannery effluent

Parameter	Effluent	Permissible limit
PH	8.8	6.0-8.0
Conductivity (moles/cm)	12,560	850
Alkalinity (mg/L)	620	500
Total solids (TS) (mg/L)	2,400	2,200
Total dissolved solids (TDS) (mg/L)	2,300	2,100
Total (suspended solids (TSS) (mg/L)	281	100
DO (mg/L)	2.8	4.0-6.0
BOD (mg/L)	240	30
COD (mg/L)	460	250

Heavy metal resistance efficiency

Heavy metal resistance of the bacterial strains was investigated by well diffusion method, results of zone formation indicates the heavy metal resistant or sensitive. Heavy metal resistant isolates such as SL1, SZ2 and SM1 showed no inhibition of growth for higher concentration of heavy metals (Lead, Cadmium and Mercury respectively) The metal resistant bacterial strains such as SL1, SC2 and SM1 were selected for further studies (Fig.1a, 1b & 1c)

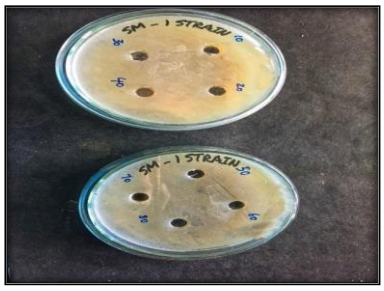


Fig.1a, Heavy metal resistant SL-1 strain

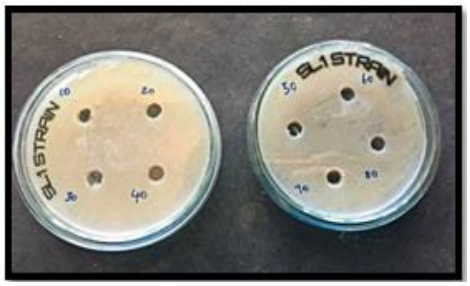
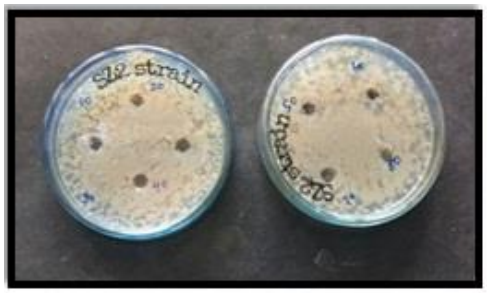


Fig. 1b, Heavy metal resistant SM-1 strain



Fig, 1c, Heavy metal resistant SZ-2 strain

Minimum inhibitory concentration

MIC of the bacterial isolates towards the heavy metals was evaluated to determine their maximum tolerance limit by broth dilution methods. SL1, SZ2, SM1 showed remarkable tolerance (upto 300 mg/L) towards lead, zinc and mercury respectively. Microbial load decreased with increase in heavy metals concentration (Fig.4, 5 & 6).

Fig.2 MIC concentration of SL-1 strain

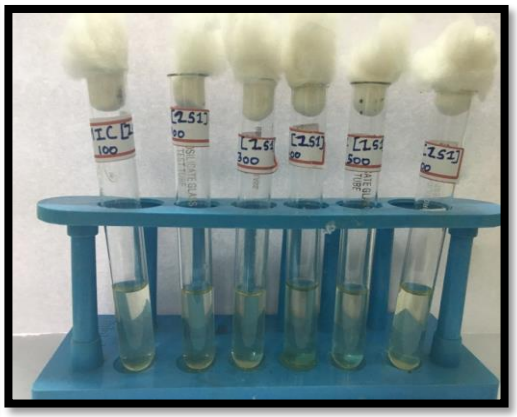


Fig.3 MIC concentration of SZ-2 strain

Minimum Inhibitory concentration of SZ-2 strain

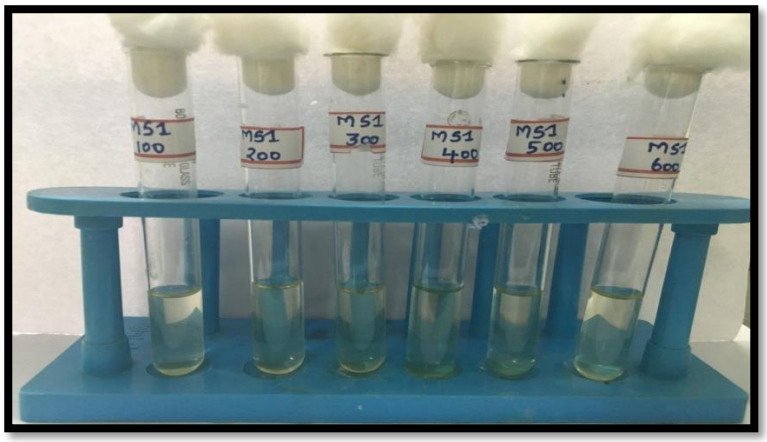


Fig. 4 Minimum Inhibitory concentration of SM-1 strain

Identification of heavy metal resistant bacteria

The morphological and cultural characteristics of the isolates were investigated to identify the bacterial isolates. SL1 strain was found to be Gram negative, pleomorphic rods producing smooth, large, translucent, greenish blue pigmentation on nutrient agar plates. SZ2 strain was found to be Gram positive cocci, smooth, pale white, non-motile entire colonies on nutrient agar plate. SM1 strain was found to be Gram positive, rods producing round, smooth, entire, mucoidal colonies on nutrient agar plates (Table 2). On the basis of the morphological, cultural, biochemical

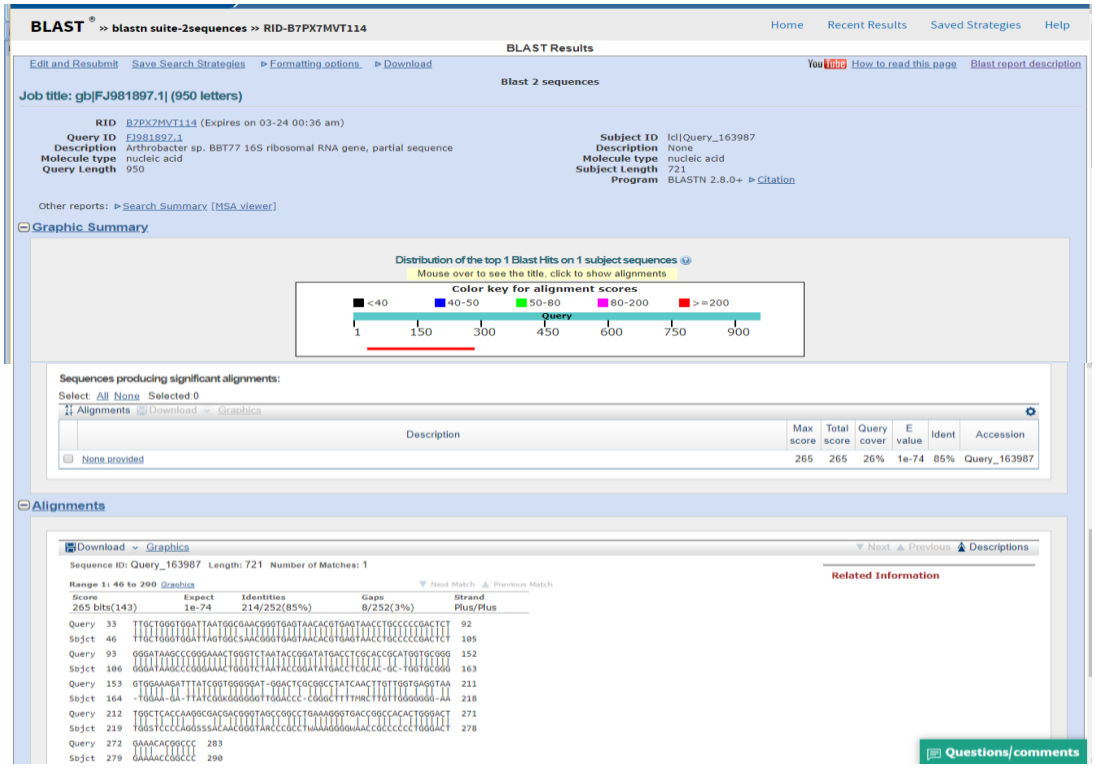
characteristics and by 16SrRNA analysis of the isolates, they were identified as *Pseudomonas* sp., *Arthrobacter* sp., and *Bacillus* sp. (Fig.5)

Table.2 Morphological,Cultural & Biochemical characteristics of heavy metal resistance strains

Characteristics	Bacterial Isolate (SL1)	Bacterial Isolate (SZ2)	Bacterial Isolate (SM1)
Colony morphology	Smooth, large, translucent, Greenish blue pigment	Smooth, coccoid, pale white	Smooth, large, translucent
Cell morphology	Rod shape	Coccoid	Rod shape
Gram reaction	-ve	+ve	+ve
Motility	+ve	-ve	+ve
Catalase	+ve	+ve	+ve
Oxidase	+ve	-ve	-ve
Indole	-ve	-ve	-ve
Methyl red	-ve	-ve	+ve
VP test	-ve	-ve	+ve
Citrate	+ve	+ve	+ve
Triple Sugar Iron test	AK/AK, no H_2S & no gas	-	AK/AK, no gas & no H_2S
Urease test	-ve	-	+ve
Nitrate reduction test	+ve	-	-ve
Identification	***Pseudomonas* sp.**	***Arthrobacter* sp.**	***Bacillus* sp**

Fig 5. 16s rRNA sequencing of SZ-2 isolate

16s rRNA sequence result of isolate 2 showing 85% similarity with *Arthrobacter*



Heavy metal reduction analysis

The heavy metal cadmium reduction efficacy of the isolates was investigated by Atomic Adsorption Spectroscopy. From the results of ASS, it was found that all the three heavy metal resistant isolates (SL1, SZ2 and SM1) were found to completely achieved the removal of the heavy metal in the effluent sample (Fig. 6).

Fig. 6 Atomic Adsorption Spectroscopy of treated effluent and control

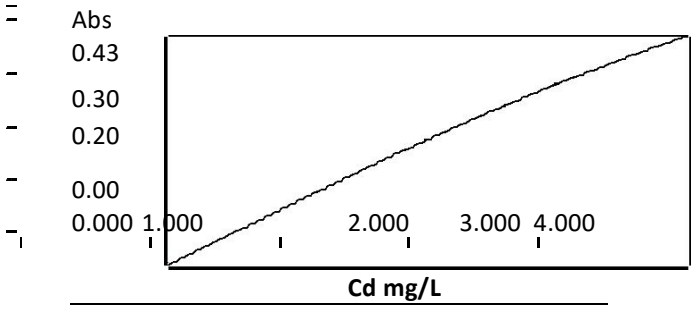
SpectrAA Report. 9:29 AM 12/28/2017 Page 1 of 1

Analyst	
Date Started	9:24 AM 12/28/2017
Worksheet	Cd 28
Comment	
Methods	Cd
Computer name	VIT

Method: Cd (Flame)

Sample ID	Conc mg/L	Mean Abs
CAL ZERO	0.000	0.0000
STANDARD 1	2.000	0.2334
STANDARD 2	3.000	0.3377
STANDARD 3	4.000	0.4265

New Rational - Cal. Set 1



CON	0.032	0.0037
T1	0.018	0.0021
T2	0.011	0.0013
T3	0.000	0.0000

***In-vivo* test of the Effluent**

The effluent water was tested with live Murrel fishes. The Murrel fishes were introduced into water and effluent sample respectively, one of the Murrel fishes which introduced into effluent died within 24 hours while the second one died after ten (10) days (Fig.7).

Fig. 7 *In- vivo* test of the effluent with the Murrel fish

CONCLUSION

Biodegradation of wastewater is reliable and cost-effective method of treatment. Identification of microorganisms using biochemical tests revealed the presence of *Arthrobacter* and *Bacillus* species. This preliminary study indicates that these microorganisms can be successfully used in bioremediation process for the removal of industrial effluents. This lab scale study is being extended to the bench top bioreactor so that it can further be scaled up to the industrial level. From the result of AAS it can be concluded that the isolated strains are well efficient and useful for bioremediation of cadmium contaminated waste water. The present study is an evident that the *Bacillus subtilis* is a promising candidate for biosorption which can eventually lead to their bioremediation and detoxification of cadmium. Genetic improvement may help to develop the field of existing methodologies to decontamination processes.

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