



Screening and Characterization of Chromium Tolerant Bacterial Strains from Electroplating Effluent Contaminated Soil

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Abstract

The activities of electroplating and metal processing industries are regarded as one of the major sources of heavy metal pollution. Removal of heavy metals from contaminated sites using microorganisms is a cheaper alternative technology. This present study is focused on screening and characterization of chromium tolerant bacterial strains from the electroplating effluent contaminated soil sample. The chromium containing electroplating effluent sample was collected and analyzed for various physicochemical parameters. Sixteen bacterial strains were isolated and identified based on morphological and biochemical characteristics. All the sixteen bacterial strains were screened for metal tolerance using nutrient agar medium incorporated with chromium metal (100 mg/L). Based on the screening study, only six bacterial strains were selected as potential metal tolerant strains and they were characterized with the various environmental conditions viz., pH (pH 5, pH 7 and pH 9), temperatures (5°C 28°C, 37°C and 45°C) and with different metal concentrations (100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L). Among the six bacterial isolates characterized, *Pseudomonas* spp strain 1 was found to be better chromium resistant organism.

Keywords: Electroplating effluents, heavy metals, chromium, *Pseudomonas* spp1

1. Introduction

The environmental pollution by heavy metals comes from anthropogenic sources such as smelters, mining, power stations and the application of pesticides containing metal, fertilizer and sewage sludge and the irresponsible disposal of wastes by various industries [1]. The presence of heavy metals in industrial effluents is known to have major hazard to natural water, animal and human health. High concentrations of heavy metals have deleterious effect on the environment [2]. Untreated industrial wastewater discharged to the nearby water bodies may cause severe ground water pollution [3]. Electroplating industry is one of the industries where various heavy metals are used for cleaning technique as a result of which the excess of metals released into the environment [4]. Therefore, the removal of excesses of heavy metal ions from the wastewaters is essential due to their extreme toxicity towards aquatic life and humans [5]. Heavy metal ions is not easy to remove in the environment that are already contaminated, contrary to many other organic pollutants, heavy metals cannot be biologically or chemically degraded and are therefore difficult to control [6, 7]. Bioremediation is an emerging technology suitable for the removal of heavy metals employing biological systems including microorganisms, plants, animal and

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their bioproducts. Furthermore, it is cost effective and economical to the environment [8]. Bioremediation process includes detoxification and/or transforms pollutants from toxic to their non-toxic forms. The end products of a successful bioremediation are nontoxic and can be fit in without damage to the environment and living organisms [6, 7]. The removal of metal by microorganisms is a complex process that depends upon several factors such as the chemistry of metal ions, cell wall composition of microorganisms, cell physiology and physico-chemical factors such as pH, temperature, contact time, ionic strength and metal concentration [9]. Bacteria could tolerate to grow in electroplating effluent and could be used effectively in bioremediation process [4]. Bacteria have the capability to grow in condition with high concentration of heavy metals and maintain a homeostasis within the cell that keeps the heavy metals an optimal sub-toxic level [10]. To survive under metal-stressed conditions, bacteria have evolved several types of adaptation mechanisms to tolerate the uptake of heavy metal ions. These mechanisms include the efflux of metal ions outside the cell, accumulation and complexation of the metal ions inside the cell, and the reduction of the heavy metal ions to a less toxic state [11].

Therefore, the present study focused on screening and characterization of chromium tolerant bacterial strains from the electroplating effluent contaminated soil.

2. Materials and Methods

2.1. Collection of Sample

The chromium electroplating effluent was collected from the direct outlet of Meena Electroplating Industry, Madurai, Tamil Nadu, India and the soil samples were collected from the agricultural field contaminated with the electroplating effluent. The sample was immediately transported to the laboratory, Department of Biology, GRI, Gandhigram for further analysis.

2.2. Physicochemical Characteristics of the Electroplating Effluent

The physicochemical parameters of the electroplating effluents such as pH, temperature, electrical conductivity, total solids, total dissolved solids, chloride, sodium, calcium, potassium, biological oxygen demand and chemical oxygen demand were carried out as per the method recommended by APHA [12] and the values are compared with the maximum permissible limit prescribed by BIS for effluent standard [13] and chromium content of the electroplating industrial effluent was analyzed using standard methods [14].

2.3. Screening of Selected Bacterial Isolates for Chromium Resistance

Sixteen predominant bacterial strains were isolated from the electroplating effluent contaminated soil sample and were screened for their potential to tolerate chromium metal [15, 16]. Nutrient agar medium was prepared with pH 7 and incorporated with chromium metal (100 mg/L). The medium was sterilized at 121°C with 15 lbs for 20 min. All the sixteen bacterial isolates were streaked on the chromium containing agar medium and incubated at 37°C for 5 days. The growth performance of all the sixteen bacterial isolates were observed and recorded. Out of sixteen bacterial isolates, only six bacterial strains via *Pseudomonas* spp 1, *Escherichia coli*, *Proteus* spp 2, *Staphylococcus* spp 1, *Salmonella* spp 2 and *Shigella* spp 2 were selected as potential strains for treatment of chromium metal.

2.4. Characterization of Chromium Tolerant Bacterial Strains

The six chromium tolerant bacterial strains were characterized as per the method of Margeay *et al.* [17]. Six potential metal (chromium) tolerant bacterial strains, *Pseudomonas* spp 1, *Escherichia coli*, *Proteus* spp 2, *Staphylococcus* spp 1, *Salmonella* spp 2 and *Shigella* spp 2 were characterized by growing them in the metal - based nutrient agar medium with various environmental conditions like different pH (pH 5, pH 7 & pH 9), temperatures (5°C, 28°C, 37°C and 45°C) and metal concentrations (100 mg/L, 200 mg/L, 300 mg/L and 400 mg/L) in various treatments for 5 days.

3. Results and Discussions

The electroplating industrial effluent is the serious cause of ground water and soil contamination in vicinity area which pose significant threat to human health and ecology [18]. The presence of metal tolerant bacterium in the environment may be an indication that such area is affected by heavy metals which foster adaptation and selection for heavy metal resistant organisms [19]. In this study, an investigation was made to study the physicochemical characteristics of chromium-based electroplating effluent and to screen and characterize chromium tolerant bacterial strains from the electroplating effluent contaminated soil sample. The physicochemical parameters of electroplating industrial effluent sample were determined and the values are presented in Table 1. The physicochemical analysis revealed that the electroplating effluent has rich of

various physicochemical constituents above the permissible limits and the effluent found to contain higher amount of chromium (Table 1).

Table 1. Physicochemical Characteristics of Chromium Electroplating Effluent

S/N	Physicochemical parameters	Results
1	Temperature (°C)	33±1.8
2	pH	6.8±0.7
3	Total solids (mg/L)	4242±1.113
4	Total dissolved solids (mg/L)	2219±11.031
5	Calcium (mg/L)	9.21±0.545
6	Sodium (mg/L)	14.17±0.986
7	Potassium (mg/L)	0.64±0.113
8	Chloride (mg/L)	3300±9.839
9	Biological oxygen demand (g/L)	255±5.767
10	Chemical oxygen demand (mg/L)	4135.22±4.254
11	Chromium (mg/L)	4.21

(Values are mean of three replicates ± standard error)

Isolation of bacteria group from the metal polluted environment would represent an appropriate practice to select metal resistant strains that could be used for heavy metal removal and bioremediation purposes [3, 20, 21, 22]. Among the sixteen isolates, only six bacterial strains *Pseudomonas* spp 1, *Escherichia coli*, *Proteus* spp 2, *Staphylococcus* spp 1, *Salmonella* spp 2 and *Shigella* spp 2 were found to be potential metal tolerant strains by having better growth performance in the screening medium supplemented with chromium metal (Table 2).

Table 2. Growth Performance of Sixteen Bacterial Isolates in Chromium Containing Nutrient Agar Medium on 24 hr

Name of the isolates	Strain type	Growth performance scale
<i>Staphylococcus</i> spp 1	Positive	GG
<i>Staphylococcus</i> spp 2	Positive	MG
<i>Bacillus</i> spp 1	Positive	PG
<i>Bacillus</i> spp 2	Positive	GG
<i>Micrococcus</i> sp	Positive	MG
<i>Pseudomonas</i> spp 1	Negative	EG
<i>Pseudomonas</i> spp 2	Negative	MG
<i>Pseudomonas</i> spp 3	Negative	PG
<i>Pseudomonas</i> spp 4	Negative	GG
<i>Escherichia coli</i>	Negative	MG
<i>Proteus</i> spp 1	Negative	PG
<i>Proteus</i> spp 2	Negative	GG
<i>Salmonella</i> spp 1	Negative	PG
<i>Salmonella</i> spp 2	Negative	GG
<i>Shigella</i> spp 1	Negative	MG
<i>Shigella</i> spp 2	Negative	GG

BIS - Bacterial Isolate **EG**: Excellent Growth **GG**: Good Growth **MG**: Moderate Growth **PG**: Poor Growth

The six selected metal tolerant bacterial strains were characterized by growing them in the metal-based nutrient medium in different environmental conditions and the result reveals that, *Pseudomonas* spp 1 was found to be better metal tolerant organism which exhibit better growth performance in the 300 mg/L of chromium based medium with pH 7 at temperature 37°C and the results are presented in Table 3. Similarly, Mahalingam *et al.* [4] undertaken the studies on partial characterization of metal resistant *Bacillus* spp their results revealed that *Bacillus* spp exhibited better growth in metal containing electroplating industrial effluent in pH 7 at 30°C.

Table 3. Growth Performance of Six Potential Bacterial Isolates in Chromium Enriched Nutrient Agar Medium with Various Environmental Conditions and Metal Concentration

pH	Bacterial Isolates	Growth performance scale against chromium Metal concentrations (mg/L)															
		100				200				300				400			
		Temperature (°C)															
		5	28	37	45	5	28	37	45	5	28	37	45	5	28	37	45
5	<i>Pseudomonas</i> spp 1	NG	MG	GG	PG	NG	MG	GG	PG	NG	MG	GG	PG	NG	MG	GG	PG
	<i>Escherichia coli</i>	NG	GG	GG	MG	NG	GG	GG	MG	NG	MG	GG	GG	NG	GG	GG	GG
	<i>Proteus</i> spp 2	NG	PG	PG	PG	NG	MG	PG	PG	NG	MG	MG	PG	NG	MG	MG	MG

	<i>Staphylococcus</i> spp 1	NG	MG	MG	MG	NG	MG	MG	PG	NG	MG	MG	MG	NG	MG	MG	PG
	<i>Salmonella</i> spp 1	NG	GG	EG	MG	NG	GG	EG	MG	NG	MG	EG	MG	NG	GG	EG	GG
	<i>Shigella</i> spp 2	NG	MG	PG	PG	NG	PG	PG	MG	NG	MG	MG	PG	NG	PG	MG	MG
7	<i>Pseudomonas</i> spp 1	NG	MG	GG	PG	NG	MG	GG	PG	NG	GG	EG	PG	NG	MG	MG	PG
	<i>Escherichia coli</i>	NG	MG	GG	MG	NG	GG	GG	MG	NG	MG	MG	MG	NG	MG	MG	MG
	<i>Proteus</i> spp 2	NG	PG	GG	PG	NG	PG	PG	PG	NG	MG	MG	MG	NG	PG	PG	PG
	<i>Staphylococcus</i> spp 1	NG	GG	GG	MG	NG	MG	MG	PG	NG	MG	MG	MG	NG	MG	MG	MG
	<i>Salmonella</i> spp 1	NG	PG	EG	PG	NG	PG	PG	PG	NG	MG	MG	MG	NG	MG	MG	MG
	<i>Shigella</i> spp 2	NG	GG	GG	MG	NG	PG	PG	PG	NG	PG	PG	PG	NG	PG	MG	MG
9	<i>Pseudomonas</i> spp 1	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	GG	MG	NG	MG	MG	PG
	<i>Escherichia coli</i>	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	PG	PG
	<i>Proteus</i> spp 2	NG	PG	MG	PG	NG	PG	MG	PG	NG	MG	PG	PG	NG	MG	PG	PG
	<i>Staphylococcus</i> spp 1	NG	PG	MG	PG	NG	PG	MG	PG	NG	MG	MG	MG	NG	MG	PG	PG
	<i>Salmonella</i> spp 1	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	PG	MG	NG	MG	PG	PG
	<i>Shigella</i> spp 2	NG	GG	MG	PG	NG	PG	MG	PG	NG	MG	MG	PG	NG	MG	PG	PG

EG - Excellent Growth GG - Good Growth MG - Moderate Growth PG - Poor Growth

4. Conclusions

The study on screening and characterization of chromium tolerant bacterial strains clearly revealed that bacterial isolate *Pseudomonas* spp 1 was found to be a potential metal tolerant strain, therefore, bacterial strain, *Pseudomonas* spp 1 would be a better choice for the removal of chromium metals from the electroplating effluents before discharged into agricultural land/water bodies. Thus, it minimizes water/soil pollution.

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