Isolation and Characterization of Nickel Tolerant Bacterial Strains from Electroplating Effluent Sediments

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Abstract**—** *In the present study, an attempt was made to isolate and characterize nickel tolerant bacterial strains from the electroplating effluent contaminated soil. The effluent sample was collected at the direct outlet of electroplating industry and analyzed for physico-chemical characteristics such as pH (6.5), temperature (33), electrical conductivity (15.1 ms/cm), total solids (2309mg/l), total dissolved solids (5573 mg/l), chloride (0.20mg/l), sodium (0.13ppm), calcium (2.23ppm), potassium (0.20ppm), Biological Oxygen Demand (4200mg/l), Chemical Oxygen Demand (5243 mg/l) and nickel (4.063ppm). Enumeration of total bacterial population from the electroplating effluent contaminated soil sample was made in nutrient agar medium. Sixteen bacterial colonies were selected based on their abundance growth all of them were identified through morphological and biochemical characteristics. All the sixteen bacterial isolates were screened for its metal tolerance using nutrient agar medium incorporated with nickel metal. Based on the better growth performance, six bacterial strains were selected as potential metal tolerant organism. The selected metal tolerant bacterial strains were further characterized in the various environmental conditions such as pH (5, 7 & 9) temperature (5°C, 28°C, 37°C & 45°C) and concentration of metal ions (100ppm, 200ppm, 300ppm & 400ppm) for 5 days. The result reveals that one bacterial strain, Pseudomonas sp 1 was showed better growth in nickel metal based medium with pH 7 at 37°C temperature.*

Keywords**—** ***Electroplating effluents, Heavy metals, Nickel, Biosorption, Pseudomonas sp 1.***

I. INTRODUCTION

In this wake of industrialization, consequent urbanization and over increasing population, the basic amenities of life viz, air, water and land are being polluted continuously. Industrial complexes have become the focus of environmental pollution (Shukla *et al.,* 2007). Water, the most vital resource of all kinds of life on this planet and it is adversely affected both qualitatively and quantitatively by all kinds of human activities (Reddy *et al.,* 2001).

Disposal of industrial and urban wastes to soil and water bodies has led to disastrous consequences in the ecosystem (Smith, 1974). Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing, and chemical processing and fertilizer applications release alarmingly higher amounts of metals into the natural environment (Zoubolis *et al.,* 2004).

The increase in industrial activity during recent years is greatly contributing to the increase of heavy metals in the environment, mainly in the aquatic systems (Marques *et al.,* 2000). Water pollution due to heavy metals is an issue of great environmental concern (Vasudevan *et al.,* 2003).

Various sources of pollutants industrial effluents containing heavy metals pose a threat to ecosystem. There metals present in the wastewater of different industries such as metal cleaning, plating baths, refineries, mining, electroplating, paper and pulp, paint, textile and tanneries (Mistry *et al.,* 2010).

Release of heavy metals without proper treatment poses a significant threat to public health because of its persistence, biomagnifications and accumulation in food chain (Rajendran *et al.,* 2003).

Electroplating is the most commonly adopted metal finishing process, these result in the generation of heavy metal pollutants, which are toxic and non biodegradable. Heavy metals are generally deposited in liver, muscles, kidneys, spleen, skin, bone and soft tissues of human beings. Heavy metals pollution is one of great environmental concerns, because the heavy metals are non degradable and persistent in water. Heavy metals in the environment may also change plant diversity (Zafar *et al.,* 2007).

Heavy metals may disrupt the normal function of the central nervous system and cause changes in the blood content, and adversely affect the function of lungs, kidneys, liver and other organs. The long-term action of heavy metals may cause the development of cancer, allergy, dystrophy, physical and neurological degenerative processes, Alzheimer's and Parkinson's diseases. However, in small amounts, heavy metals are indispensable for many organisms, but their enhanced doses induce acute or chronic poisoning (Kvesitadze *et al.,* 2006).

Nickel is generated from batteries, wire and electrical parts. Steady ingestion (exposure to nickel) can lead to cancer especially of the lungs and nasal sinus (Hauser and Hauser, 2008). Nickel (Ni) is the 24th most abundant element in the ealih crust and has been detected in different media in all parts of the biosphere. Ni is classified as the borderline metal ion because it has both soft and hard metal properties and can bind to sulfur, nitrogen and oxygen groups. Ni has been implicated as an embryotoxin and teratogen (Weber and Digiano, 1996).

Conventional techniques for removing heavy metals from industrial effluents include chemical precipitation, chemical reduction, adsorption; ion exchange, evaporation and membrane processes. Compared with conventional methods for the removal of toxic metals from wastewater, the biosorption process offers potential advantages such as low operating cost, minimization of chemical or biological sludge, high efficiency of heavy metal removal from diluted solutions, regeneration of biosorbents, possibility of metal recovery and environmental friendly (Ahluwalia and Goyal, 2007).

Most of the industrial techniques are ineffective and excessively expensive at the metal concentration less than l mg/ml (Alloway, 1995). Microbial metal bioremediation is an efficient strategy due to its low cost, high efficiency and ecofriendly nature (Rajendran *et al.,* 2003).

In recent years, the biotechnology applied to control and remove metal pollution has received much attention, and gradually, becomes a hot topic in the field of metal pollution control because of its potential application. For heavy metal removal, an alternative process is biosorption, which utilizes certain natural materials of biological origin, including bacteria, fungi, yeast, and algae (Alloway, 1995). With a view of this, the present study is focused an isolation and characterization of nickel tolerant bacterial strains from the electroplating effluent contaminated soil.

II. MATERIALS AND METHODS

2.1 Collection of sample

The nickel electroplating effluent was collected from the direct outlet of Meena electroplating industry and the soil sample was collected from effluent contaminated land near Madurai, Tamil Nadu, India and the samples were immediately transported to the laboratory, Department of Biology, GRI, Gandhigram, for further analysis.

2.2 Physicochemical characteristics of the electroplating effluent

The physicochemical parameters such as Temperature, pH, Electrical Conductivity, Total Dissolved Solids, Total Solids, Sodium, Potassium, Calcium, Chloride, Biological Oxygen Demand And Chemical Oxygen Demand were analyzed from electroplating industrial effluent using standard methods (APHA, 1998) and concentration of nickel metal was estimated by Atomic Adsorption Spectrophotometer (Konstantinos *et al.,* 2011).

2.3 Isolation and Identification of metal tolerant Bacterial strains

The electroplating effluent contaminated soil sample was serially diluted with normal saline (85% Nacl) up to 10^{-9} dilutions. Sample (0.1ml) from 10^{-5} and 10^{-6} dilutions were taken and spread on the nutrient agar plates and incubated at 37°C for 24 hrs. The sixteen predominant colonies grown on nutrient agar medium were selected and maintained as pure cultures.

All the sixteen bacterial strains were identified through morphological and biochemical characteristics through Gram's staining and Motility Test, Indole Production, Methyl Red reaction, Voges Proskauver reaction, Citrate Utilization test, Urease test, Starch Hydrolysis, Catalase test. All the strains were authenticated by Bergey's manual of determinative bacteriology (Holt *et al.,* 1984).

2.4 Screening of selected bacterial isolated for nickel resistance

All the sixteen bacterial isolates were screened for its potential to tolerate heavy metal using nutrient agar medium incorporated with nickel metal (100ppm). All the plates were incubated at 37°C at 5 days. The comparative growth performance of all bacterial isolates were observed and recorded (Jamaluddin *et al.,* 2012; Pandit *et al.,* 2013).

2.5 Characterization of nickel tolerant bacterial strains

Six potential metal (Ni) 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 such as pH (pH 5, pH 7 & pH 9), temperatures (5°C, 28°C, 37°C & 45°C) and metal concentrations (100ppm, 200ppm, 300ppm & 400ppm) in various treatments for 5 days (Margeay *et al.*,1985).

III. RESULTS AND DISCUSSION

Microbes particularly bacteria and fungi are excellent agents for removal of heavy metals with lieu to production of metal chelators or enzymes which convert the metals to lesser toxic forms. The ability to tolerate heavy metals depends largely on the adaptability of the strains in a polluted environment or due to continuous exposure to the heavy metals (Volesky, 1990).

In this study, an investigation was made to study the physicochemical characteristics of nickel-based electroplating effluent and to isolate and characterize nickel tolerant bacterial isolates from the electroplating effluent contaminated soil sample.

Bacteria have evolved several types of mechanisms to tolerate and to the uptake of heavy metal ions include the efflux of metal ions outside the cell, accumulation and complexation of the metals inside the cell and reduction of the heavy metal ions to a less toxic state (Nies, 1999).

The physicochemical characteristics of nickel electroplating effluent was determined and was found to contain pH (6.5), temperature (33°C), electrical conductivity (15.1ms), total solids (2309mg/l), total dissolved solids (5573 mg/l), chloride (36mg/I), sodium (138ppm), calcium (220ppm), potassium (2.09ppm), Biological Oxygen Demand(42mg/I) and Chemical Oxygen Demand (5243mg/l). The nickel (1.03ppm) contents in the electroplating effluent were determined by Atomic Adsorption Spectrophotometer.

3.1 Isolation and identification of metal tolerant bacterial strains

Sixteen bacterial colonies shown the predominant growth on nutrient agar plates were selected and maintained as pure culture in agar slants. All the sixteen bacterial isolates were identified through morphological characteristics by staining techniques and biochemical characteristics and the results were compared with Bergy's manual of Determination Bacteriology (Holt *et al.,* 1994).

3.2 Screening of selected bacterial isolated for nickel resistance

All the sixteen bacterial isolates were further screened for its potential to tolerate heavy metal. Nutrient agar medium was prepared with pH and incorporated with nickel metal (100ppm). The medium was sterilized at 121°C with 15 lbs for 20 minutes. The results were recorded in Table 3.

All the sixteen bacterial isolates were streaked on the nickel containing agar medium and incubated at 37°C at 5 days. 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 potential metal tolerant strains by having better growth performance in the screening medium (Table 1).

3.3 Characterization of nickel tolerant bacterial strains

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 ppm of nickel based medium with pH 7 at temperature 37°C (Table 2).

These metal tolerant strains derive their nutritional requirement from the effluent waste. They are able to synthesize their enzymes, metabolic intermediates, structural proteins, lipids and nucleic acids from carbon compound in the feed, together with other elements. They derive their energy from oxidizing either organic compounds (chemoorganotrophic metabolism), or inorganic compounds (chemolithotrophic metabolism), such as reduced sulfur or nitrogen compounds. They use the energy for their bodily functions, reproduction and growth. Many research reported that a large number of bacterial species isolated from different industrial effluents (Zafar *et al.,* 2007).

TABLE 1
GROWTH PERFORMANCE OF SIXTEEN BACTERIAL ISOLATES IN NICKEL CONTAINING NUTRIENT AGAR MEDIUM ON 5 DAYS

Bacterial Isolate No.	Strain Type	Name of the Isolates	Growth Characterization
BIS-1	Positive	*Staphylococcus* spp 1	GG
BIS-2	Positive	*Staphylococcus* spp 2	MG
BIS-3	Positive	*Bacillus* spp 1	PG
BIS-4	Positive	*Bacillus* spp 2	GG
BIS-5	Positive	*Micrococcus* sp	MG
BIS-6	Negative	*Pseudomonas* spp 1	EG
BIS-7	Negative	*Pseudomonas* spp 2	MG
BIS-8	Negative	*Pseudomonas* spp 3	PG
BIS-9	Negative	*Pseudomonas* spp 4	GG
BIS-10	Negative	*Escherichia coli*	MG
BIS-11	Negative	*Proteus* spp 1	PG
BIS-12	Negative	*Proteus* spp 2	GG
BIS-13	Negative	*Salmonella* spp 1	PG
BIS-14	Negative	*Salmonella* spp 2	GG
BIS-15	Negative	*Shigella* spp 1	MG
BIS-16	Negative	*Shigella* spp 2	GG

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

TABLE 2
GROWTH PERFORMANCE OF SIX POTENTIAL BACTERIAL ISOLATES IN NICKEL ENRICHED NUTRIENT AGAR MEDIUM WITH VARIOUS ENVIRONMENTAL CONDITIONS AND METAL CONCENTRATION

pH	Bacterial Isolates	Metal concentration (ppm)															
		100				200				300				400			
		Temperature (°C)															
		5	28	37	45	5	28	37	45	5	28	37	45	5	28	37	45
5	*Pseudomonas* spp 1	NG	MG	GG	PG	NG	MG	GG	PG	NG	MG	GG	PG	NG	MG	GG	PG
	Escherichia coli	NG	GG	GG	MG	NG	GG	GG	MG	NG	MG	GG	GG	NG	GG	GG	GG
	Proteus spp 2	NG	PG	PG	PG	NG	MG	PG	PG	NG	MG	MG	PG	NG	MG	MG	MG
	Staphylococcus spp 1	NG	MG	MG	MG	NG	MG	MG	PG	NG	MG	MG	MG	NG	MG	MG	PG
	Salmonella spp 1	NG	GG	EG	MG	NG	GG	EG	MG	NG	MG	EG	MG	NG	GG	EG	GG
	Shigella spp 2	NG	MG	PG	PG	NG	PG	PG	MG	NG	MG	MG	PG	NG	PG	MG	MG
7	*Pseudomonas* spp 1	NG	MG	GG	PG	NG	MG	GG	PG	NG	GG	EG	PG	NG	MG	MG	PG
	Escherichia coli	NG	MG	GG	MG	NG	GG	GG	MG	NG	MG	MG	MG	NG	MG	MG	MG
	Proteus spp 2	NG	PG	GG	PG	NG	PG	PG	PG	NG	MG	MG	MG	NG	PG	PG	PG
	Staphylococcus spp 1	NG	GG	GG	MG	NG	MG	MG	PG	NG	MG	MG	MG	NG	MG	MG	MG
	Salmonella spp 1	NG	PG	EG	PG	NG	PG	PG	PG	NG	MG	MG	MG	NG	MG	MG	MG
	Shigella spp 2	NG	GG	GG	MG	NG	PG	PG	PG	NG	PG	PG	PG	NG	PG	MG	MG
9	*Pseudomonas* spp 1	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	GG	MG	NG	MG	MG	PG
	Escherichia coli	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	PG	PG
	Proteus spp 2	NG	PG	MG	PG	NG	PG	MG	PG	NG	MG	PG	PG	NG	MG	PG	PG
	Staphylococcus spp 1	NG	PG	MG	PG	NG	PG	MG	PG	NG	MG	MG	MG	NG	MG	PG	PG
	Salmonella spp 1	NG	MG	MG	MG	NG	MG	MG	MG	NG	MG	PG	MG	NG	MG	PG	PG
	Shigella 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

IV. CONCLUSION

The study on isolation and characterization of nickel tolerant bacterial strains reveals that, *Pseudomonas* spp 1 found to be the potential nickel tolerant strains and it would be better choice for removal of heavy metals from the electroplating effluents before discharged into agricultural land/water bodies. Thus, it minimizes water/soil pollution.

ACKNOWLEDGEMENTS

The authors wish to thank the Meena Electroplating Industry, Madurai, Tamil Nadu, India for supporting this study.

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