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Research Article

CHARACTERIZATION OF ZINC TOLERANT BACTERIAL STRAINS FROM THE ELECTROPLATING EFFLUENT CONTAMINATED SOIL

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ABSTRACT

Toxic heavy metal pollution is expanding throughout the world as a result of industrial progress. This work focuses on the characterization of zinc tolerant bacterial strains from an electroplating effluent polluted soil sample in order to minimize/control metal pollution. pH, Temperature, Electrical Conductivity, Total Solids, Total Dissolved Solids, Total Suspended Solids, Chloride, Sodium, Calcium, Potassium, Biological Oxygen Demand, and Chemical Oxygen Demand were all measured and evaluated in the zinc-containing electroplating effluent sample. The sixteen bacterial strains were isolated from a polluted soil sample with electroplating effluent and identified using morphological and biochemical features. Using nutritional agar medium enriched with zinc metal, all of the chosen strains were evaluated for metal tolerance. Only six bacterial strains were chosen as potential metal tolerant strains based on the screening study, and these strains were characterized under various environmental conditions such as different pH (pH 5, pH 7, and pH 9), different temperatures (5°C, 28°C, 37°C, and 45°C), and different metal concentrations (100ppm, 200ppm, 300ppm and 400ppm). Pseudomonas sp strain 1 was shown to be a better zinc resistant organism, according to the findings.

Keywords: Electroplating effluents, heavy metals, Zinc, Biosorption, *Pseudomonas* sp strain 1.

INTRODUCTION

With the advancement of technology, pollution from toxic heavy metals is spreading around the world. The most common heavy metals released from industries such as electroplating, metal finishing, tanning, and so on are copper, cadmium, chromium, and nickel(Aksu, 1998; APHA, 1998; Avery & Tobin, 1993; Brady *et al*., 1994). Toxic metals (Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn), precious metals (Pd, Ag, Au, Ru), and radionuclides (Wang & Chen, 2006) are the three types of heavy metals that are of importance to the environment, according to (Wang & Chen, 2006) (U, Th, Ra, Am). Because of their toxicity and threat to human life and the environment, electroplating industries are a key contributor to the large influx of chromium into the biosphere(Dönmez & Aksu, 1999). As a result, effluent processes are designed to ensure that when wastewater is discharged into natural water sources, it meets varying effluent discharge criteria depending on where it is deposited, as well as reducing or preventing detrimental effects(Holt *et al*., 1994).

Microbial biomass can operate as effective metal scavengers in both the bioaccumulation and biosorption processes. Bioaccumulation relies on metals being incorporated into living biomass, whereas biosorption is a metabolism-independent process in which metallic ions remain at the cellular surface. Complex formation, ion exchange, coordination, adsorption, and chelation are some of the mechanisms that cause biosorption (Hu *et al*., 1996). In general, the mechanism has been relied on ion interaction at the biomass/aqueous medium interface (Huang *et al*., 1990). Heavy metal traces are required as co-factors in enzyme reactions, but large quantities can be extremely harmful to living organisms due to metabolic reaction inhibition. Transport across the cell membrane, biosorption to the cell walls and trapping in extracellular capsules, precipitation, complexation, and oxidation-reduction reactions are some of the ways microorganisms respond to heavy metals(Dermentzis *et al*., 2011; Jamaluddin *et al*., 2012; Pandian *et al*., 2014).

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Zinc is a trace element that has a role in the formation of complexes (such as zinc fingers in DNA) and as a component of biological enzymes. Bacterial organisms collect zinc by an unspecific, quick absorption process, and it is typically found in larger amounts (but is less hazardous) than other heavy metals (Mahalingam *et al*., 2014). Zinc ion uptake is often linked to magnesium ion uptake, and the two ions may be transported by comparable methods in bacteria(Mergeay *et al*., 1985). Some bacterial species have high tolerance and resistance to heavy metals in high concentrations. Because of the advent of resistant species (Nies, 1999), the reasons for microorganisms' resistance and tolerance behaviour require further investigation. As a result, in this study, an attempt was made to investigate zinc-tolerant bacterial strains.

MATERIALS AND METHODS

Collection of sample

The zinc electroplating effluent was collected from the direct outlet of Meena Electroplating Industry, Madurai, Tamil Nadu, India and the sample was immediately transported to the laboratory, Department of Biology, GRI, Gandhigram for further analysis.

Physicochemical characteristics of the electroplating effluent

The physicochemical parameters of 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 measured using the method recommended by APHA, 1998 (Nies & Silver, 1995), and the results were compared to the maximum permissible limit prescribed by BIS for effluent standard(Pandit *et al*., 2013).

Isolation and Identification of metal tolerant Bacterial strains

The soil sample contaminated by electroplating effluent was collected in pre-cleaned polythene bags and brought to the laboratory for bacterial strain isolation. The sample was diluted up to 10-9 dilutions with normal saline (0.85 percent NaCl). 10-5 and 10-6 dilution samples (0.1ml) were obtained and distributed on nutrient agar plates, which were then incubated at 37°C for 24 hours. The sixteen most common colonies were chosen and kept as pure cultures on nutrient agar medium. Gram's staining, Motility test, Indole production, Methyl Red reaction, Voges Proskauer reaction, Citrate utilisation, Urease reaction, Starch hydrolysis, and Catalase reaction (Demirbaş, 2003) were used to identify the sixteen bacterial strains.

Screening of selected bacterial isolate for heavy metal zinc resistance

All sixteen bacterial isolates were then tested for their ability to withstand heavy metal. A nutrient agar medium with a pH of 7 was created and zinc metal was added (100ppm). The medium was sterilised at 121°C for 20 minutes with 15 lbs(Lyons & Genc, 2016; Rai *et al*., 1981). All sixteen bacterial isolates were streaked on zinc-containing agar medium and cultured for five days at 37°C. All of the bacterial isolates' growth performance was observed and documented.

Characterization of zinc tolerant bacterial strains

Six bacterial species that may be metal (Zn) tolerant *Pseudomonas* spp., *Escherichia coli*, *Proteus* spp., *Staphylococcus* spp., *Salmonell*a spp. 2 and *Shigella* spp. 2 were characterised by cultivating them for 5 days in a metal-based nutrient agar medium with varied environmental conditions such as pH (pH 5, pH 7 & pH 9), temperature (5°C, 28°C, 37°C & 45°)

RESULT AND DISCUSSION

The electroplating industry is a major user of water, it contains high amounts of heavy metal ions will be released. This heavy metal in industrial wastewaters is a serious concern because they are highly toxic, non-biodegradable, a carcinogen and continuous deposition into receiving water lakes, streams and other water sources within vicinity cause bioaccumulation in the living organisms(Sivasubramanian, 2006). Therefore, in the present work an attempt was made up to study on isolation and characterization of zinc tolerant bacterial isolates. In this present study, zinc containing electroplating effluent sample was collected from the direct outlet and analyzed for various physicochemical parameters and found to containing higher concentrations physicochemical components. The zinc containing electroplating effluent posses all the physicochemical components in higher concentration than the permissible level prescribed by BIS. The total bacterial population in the soil contaminated by electroplating effluent was counted. The sixteen most common bacterial strains were isolated and identified based on their morphological and biochemical properties. Bergey's Manual of Determinative Bacteriology was used to verify all of the strains. Tables 1 and 2 show the characteristics of gramme positive (5 strains) and gramme negative (11 strains) bacterial isolates. Metal-tolerant bacterial species such as *Staphylococcus* sp, *Bacillus* sp, and *Pseudomonas aeruginosa* have been found in electroplating effluent contaminated soil samples by several authors (Hansford & Vargas, 1999; Sugumaran *et al*., 2014; Veglio & Beolchini, 1997).

By growing all sixteen bacterial isolates in nutritional agar medium containing zinc metal, they were tested for their ability to withstand heavy metal, and the results were documented in Table 3. Only six bacterial strains, *Pseudomonas* spp. 1, *Escherichia coli*, *Proteus* spp. 2, *Staphylococcus* spp. 1, *Salmonella* spp. 2, and *Shigella* spp. 2, were identified as prospective metal tolerant strains based on their growth performance in the screening medium (Table 3). According to (Madigan & Pidcoe, 2003), the majority of metal-tolerant bacterial strains belong to the Gram negative bacterial group rather than the Gram positive bacterial group. This is due to the gramme negative cell wall's composition and interaction with certain metal ions. Some Gram-positive strains, on the other hand, have been demonstrated to be extremely resistant to high concentrations of heavy metals.

Table 1. Morphological and biochemical characteristics of Gram negative bacterial isolates.

		Biochemical characteristics													
Bacterial isolates No	Cultural characteristics	Simple staining	Motility test	Gram's reaction	Indole production	Methyl red reaction	Voges Proskauer reaction	Catalase activity	Starch hydrolysis	Citrate utilization test	Urease reaction	Glucose fermentation	Gelatin liquefaction	Name of the bacterial strain
BIS-1	WC	C	M	+	+	-	-	+	+	+	-	-	-	*Staphylococcus* spp 1
BIS-2	GBC	C	M	+	+	-	-	+	+	+	-	-	-	*Staphylococcus* spp 2
BIS-3	LYMC	R	M	+	-	-	+	-	-	-	-	+	-	*Bacillus* spp 1
BIS-4	WWC	R	M	+	-	-	+	-	-	-	-	+	-	*Bacillus* spp 2
BIS-5	BMC	C	M	+	+	+	-	-	-	+	-	+	+	*Micrococcus* sp

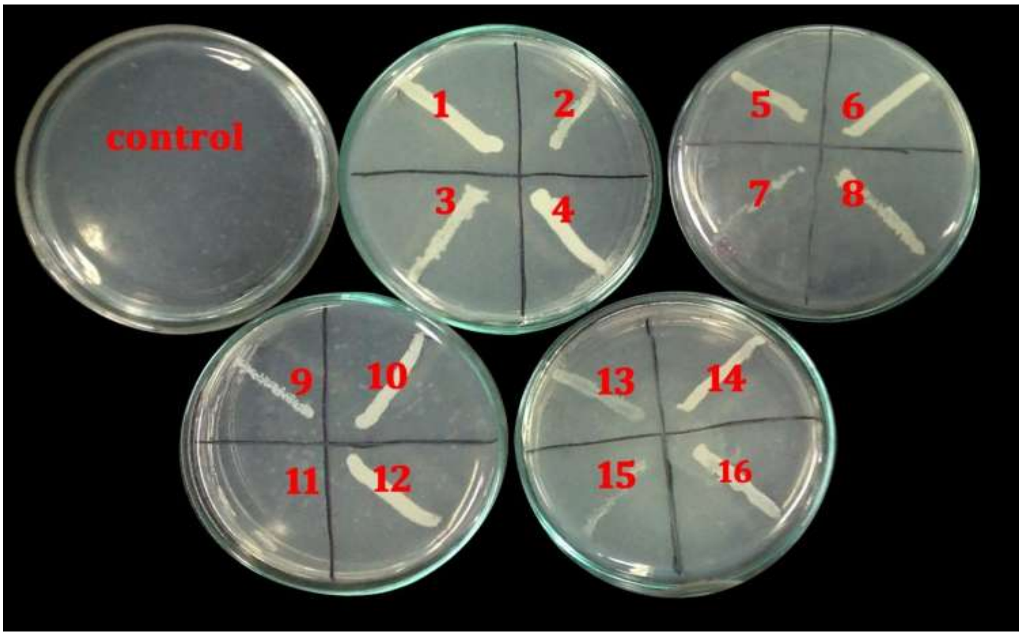
BIS - Bacterial Isolate, WC - Whitish Colony, GBC - Golden Brown Colony, LYMC - Light Yellow Mucoid Colony, WWC - White Waxy Colony, BMC - Brown color Mucoid Colony, C - Cocci, R - Rod, M - Motile, + - Positive, – - Negative.

Table 2. Morphological and biochemical characteristics of Gram positive bacterial isolates.

		Biochemical characteristics												
Bacterial isolates No	Cultural characteristics	Simple staining	Motility test	Gram's reaction	Indole production	Methyl red reaction	Voges Proskauer reaction	Catalase activity	Starch hydrolysis	Citrate utilization test	Urease reaction	Glucose fermentation	Gelatin liquefaction	Name of the bacterial strain
BIS-6	OSC	R	M	-	-	-	-	+	-	+	-	+	+	*Pseudomonas* spp 1
BIS-7	GMC	R	M	-	-	-	-	+	-	+	-	+	+	*Pseudomonas* spp 2
BIS-8	DWC	R	M	-	-	-	-	+	-	+	-	+	+	*Pseudomonas* spp 3
BIS-9	OCC	R	M	-	-	-	-	+	-	+	-	+	+	*Pseudomonas* spp 4
BIS-10	CMC	R	M	-	+	+	-	+	-	-	-	+	-	*Escherichia coli*
BIS-11	TGC	R	M	-	-	-	+	+	+	-	-	+	-	*Proteus* spp 1
BIS-12	WYC	R	M	-	-	-	+	+	+	-	-	+	-	*Proteus* spp 2
BIS-13	WPC	R	M	-	-	+	-	+	-	-	-	+	+	*Salmonella* spp 1
BIS-14	WOC	R	M	-	-	+	-	+	-	-	-	+	+	*Salmonella* spp 2
BIS-15	YC	R	M	-	-	-	-	-	+	-	-	-	-	*Shigella* spp 1
BIS-16	WMC	R	M	-	+	-	-	-	+	-	-	-	-	*Shigella* spp 2

BIS - Bacterial Isolate, OSC - Orange, Small Colony, GMC - Green color Mucoid Colony, DWC - Dusty White Colony, OCC - Orange color Creamy Colony, CMC - Colorless Mucoid Colony, TGC - Thin, Grey Colony, WYC - Whitish Yellow Colony, WPC - Whitish Pink Colony, WOC - Whitish Orange Colony,YC - Yellow Colony, WMC - Whitish Mucoid Colony, C - Cocci, R - Rod, M - Motile, + - Positive, – - Negative

Figure 1. Growth performance of 16 bacterial isolates in zinc containing nutrient agar medium on 5 days.



1.Staphylococcus spp 1
2.Staphylococcus spp 2
3.Bacillus spp 1
4.Bacillus spp 2
5.Micrococcus spp
6.Pseudomonas spp 1
7.Pseudomonas spp 2
8.Pseudomonas spp 3
9.Pseudomonas spp 4
10.Escherichia coli
11.Proteus spp 1
12.Proteus spp 2
13.Salmonella spp 1
14.Salmonella spp 2
15.Shigella spp 1
16. Shigella spp 2

Table 3. Growth performance of 16 bacterial isolates in zinc containing nutrient agar medium on 5 days.

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

BIS - Bacterial Isolates, EG - Excellent Growth, GG - Good Growth , MG - Moderate Growth ,PG - Poor Growth.

Six potential bacterial isolates, *Pseudomonas* spp. 1, *Escherichia coli*, *Proteus* spp. 2, *Staphylococcus* spp. 1, *Salmonella* spp. 2 and *Shigella* spp. 2, were characterised by growing them for 5 days in zinc-containing nutrient agar medium with various environmental conditions such as pH (pH 5, pH 7 & pH 9), temperature (5°C, 28°C, 37°)Table 4 contains all of the results.

Table 4. Growth performance of six potential bacterial isolates in zinc 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	PG	GG	NG	NG	MG	GG	NG	NG	MG	GG	PG	NG	PG	GG	MG
	Staphylococcus spp 1	NG	MG	MG	NG	NG	PG	MG	PG	NG	MG	GG	MG	NG	PG	PG	NG
	Escherichia coli	NG	PG	GG	NG	NG	MG	GG	MG	NG	PG	MG	PG	NG	PG	MG	PG
	Proteus spp 2	NG	PG	MG	PG	NG	MG	MG	MG	NG	PG	MG	PG	NG	PG	PG	MG
	Salmonella spp 1	NG	PG	MG	MG	NG	MG	EG	MG	NG	MG	PG	NG	NG	MG	MG	MG
	Shigella spp 2	NG	MG	MG	NG	NG	PG	MG	PG	NG	MG	MG	PG	NG	MG	PG	PG
7	*Pseudomonas* spp 1	NG	MG	GG	PG	NG	MG	GG	PG	NG	GG	EG	PG	NG	MG	GG	NG
	Staphylococcus spp 1	NG	PG	MG	MG	NG	MG	PG	NG	NG	PG	MG	PG	NG	NG	MG	PG
	Escherichia coli	NG	MG	GG	PG	NG	MG	MG	MG	NG	MG	GG	PG	NG	PG	MG	PG
	Proteus spp 2	NG	MG	MG	NG	NG	MG	MG	PG	NG	MG	MG	MG	NG	PG	PG	MG
	Salmonella spp 1	NG	PG	GG	PG	NG	PG	PG	NG	NG	PG	PG	PG	NG	PG	PG	PG
	Shigella spp 2	NG	MG	MG	NG	NG	PG	MG	NG	NG	PG	MG	PG	NG	MG	PG	PG
9	*Pseudomonas* spp 1	NG	MG	MG	PG	NG	MG	MG	PG	NG	MG	PG	PG	NG	NG	PG	NG
	Staphylococcus spp 1	NG	PG	MG	NG	NG	PG	MG	NG	NG	NG	PG	NG	NG	PG	PG	NG
	Escherichia coli	NG	PG	PG	NG	NG	PG	PG	PG	NG	NG	PG	PG	NG	PG	NG	NG
	Proteus spp 2	NG	PG	MG	NG	NG	PG	MG	NG	NG	PG	NG	NG	NG	NG	NG	NG
	Salmonella spp 1	NG	PG	PG	PG	NG	MG	PG	PG	NG	PG	NG	NG	NG	NG	NG	NG
	Shigella spp 2	NG	MG	MG	NG	NG	PG	MG	PG	NG	PG	PG	NG	NG	NG	NG	NG

EG - Excellent Growth, GG - Good Growth,MG - Moderate Growth,PG - Poor Growth ,NG - No Growth.

Pseudomonas spp strain 1 was identified as a potential zinc tolerant organism by displaying superior growth in zinc containing medium with pH 7, temperature 37°C, and 300 ppm concentration on the fifth day (Table 4). pH, temperature, and moisture all have an impact on microbial growth and activity. Although microorganisms have been isolated from extreme environments, most of them grow optimally across a restricted range, therefore achieving optimal circumstances is critical. Various environmental conditions were used to cultivate Staphylococcus spp. in chrome electroplating effluent medium. *Staphylococcus* spp grew best in a 20% electroplating effluent sample with pH 7 at 30°C28, according to the findings.

CONCLUSION

The investigation on the isolation and characterisation of zinc-tolerant bacterial strains indicated that Pseudomonas sp 1 is a potential metal-tolerant strain that would be a better alternative for removing zinc metals from electroplating effluents before they are released onto agricultural land/water bodies. As a result, it reduces water and soil contamination.

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