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Research Article
Biosorption of Zinc from Aqueous Solution by the Bacterial Strain, *Morganella morganii* ACZ05

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Abstract

Background and Objective: Many industries discharge their effluents in to the environment without treatment. Heavy metals present in these effluents cause damage to organisms present in polluted habitats. Microorganisms play an important role in bioremediation of heavy metal polluted soil and wastewater. This study is focused on studying removal of zinc from aqueous solution by bacterial biosorbent. **Methodology:** Bacterial strain was isolated from electroplating industrial effluent contaminated soil. The isolate was identified by biochemical tests and 16S rRNA sequencing analysis. Various parameters which influence metal removal like pH, biomass and percent removal were investigated. **Results:** Isolated bacterial strain was identified as *Morganella morganii* ACZ05. It was exposed to 250, 500, 750 and 1000 ppm concentrations of zinc for 4 days and pH, biomass and percent removal of zinc were observed. Maximum zinc removal was found at 500 ppm concentration on fourth day. The pH, biomass and percent removal were found increased. The highest biosorption capacity (72.6%) was observed at pH 6.8-7.8. The results indicated that *M. morganii* ACZ05 can be an attractive choice for the removal of zinc. **Conclusion:** Compared to other biosorbents reported in the literature, the *M. morganii* ACZ05 tested in this study showed very good promise for practical applicability.

Key words: Zinc, biosorbent, *Morganella morganii* ACZ05, 16S rRNA sequence, pH, biomass, biosorption

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Heavy metal pollution is a severe environmental problem worldwide, which is mainly induced by industrial development and financial advancement[1]. Zinc is the 23rd most plentiful element in the earth's crust[2]. It serves as an enzyme activator in humans and present at trace amounts but can be always lethal while present at elevated concentrations[3]. Excessive zinc stimulates obesity and associated illness in teenagers and makes diabetic patients more vulnerable, by an enhanced glycosylated hemoglobin level in the blood[4].

Simmons *et al.*[5] observed the pollution of rice paddy and soil by heavy metals like zinc and cadmium. Such pollution of water and soil leads to the aggregation of heavy metals in plants and aquatic animals[6]. Physical and chemical approaches such as ion-exchange, filtration and chemical precipitation are extensively employed to remove heavy metals from the atmosphere[7]. Still, the majority of these techniques are costly, nonspecific and of limited success, particularly while the concentrations of contaminating metals are below 100 mg L^{-1}. Besides, they frequently produce toxic secondary wastes[8].

In contrast to conventional treatment techniques, biosorption has numerous advantages that consist of elevated removal efficiency, capability to be renewed, inexpensive and chance of metal recovery. Biosorption requires biological materials that are used to form complexes with heavy metals using their ligands or functional groups[9]. The bacterial cell wall is the initial successful compartment for adsorbing heavy metals because it contains many anionic functional groups capable of binding to heavy metals, such as peptidoglycan, teichoic acids, lipopolysaccharides and phospholipids. So microorganisms have a highest potential for use in biosorption and bioaccumulation processes to remove heavy metals from contaminated environments[10].

Madurai city has a large number of electroplating industries and their effluents not only cause damage to environment but also have a harmful effect on living organisms. Therefore it is important to develop a low cost and eco-friendly method for the removal of toxic heavy metal ions from the wastewater. The aim of this present study was to investigate the ability of the zinc-resistant bacterium, *Morganella morganii* ACZ05 isolated from electroplating effluent contaminated soil to biosorb zinc from aqueous solution. Experiments have also been designed to study the changes in pH, biomass and bioremoval of zinc ions.

MATERIALS AND METHODS

Period of study: The study was carried out between September, 2011 and August, 2012 in the PG and Research Department of Zoology, The American College, Madurai.

Collection of soil samples: The soil samples were collected from electroplating industries located in Jaihindpuram area in Madurai city. Samples were collected in presterilized, polyethylene containers and brought to the laboratory immediately.

Isolation of bacteria: The collected soil samples were serially diluted up to 10^{-6} and 0.1 mL was taken from 10^{-6} dilution and plated onto the nutrient agar plates using spread plate technique[11]. The plates were then incubated at 37°C for 24 h.

Isolation of heavy metal resistant bacteria: The grown bacterial colonies were tested with different concentrations (100, 200, 400, 600, 800, 1000 and 2000 ppm) of zinc prepared from zinc sulphate for their resistance. From the resistant colonies one zinc resistant strain was selected[12].

Biochemical tests: The selected bacterial strain was subjected to the biochemical tests such as Lactose, Xylose, Maltose, Fructose, Dextrose, Galactose, Raffinose, Trehalose, Melibiose, Sucrose, L-Arabinose, Mannose, Inulin, Sodium gluconate, Glycerol, Salicin, Dulcitol, Inositol, Sorbitol, Mannitol, Adonitol, Arabitol, Erythritol, α-methyl-D-glucoside, Rhamnose, Cellobiose, Melezitose, α-methyl-D-mannoside, Xylitol, ONPG, Esculin hydrolysis, D-Arabinose, Citrate utilization, Malonate utilization and Sorbose tests by Rapid ID32E strip[13].

16S rRNA-based identification: A genomic DNA extraction from the bacterial strain was carried out as described by Ausubel *et al.*[14]. The PCR primers used to amplify 16S rRNA fragments were 5′-CCGAATTCGTCGACAACAGAGTTTG ATCCTGGCTCAG-3′ and the reverse primer 3′-CCCGGGATC CAAGCTTACGGCTACCTTGTTACGACTT-5′. A total of 25 µL of reaction mixture consisted of ten pmol of each primer, 5 µL from colony suspension as template DNA and 12.5 µL of Master Mix. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the following programme: denaturation at 98°C for 30 sec, followed by 40 cycles of 5 sec of denaturation at 98°C, 10 sec annealing at 68°C and 15 sec of

elongation at 72°C with final extension at 72°C for 10 min for the first set. The PCR product (1271bp) was cleaned by using NucleoSpin® Plant II Kit (Macherey-Nagel) in accordance with the direction of the manufacturer. Sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Phylogenetic analysis: The16S rRNA sequences were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.org) using BLAST tool and the corresponding sequences were downloaded. Evolutionary history was inferred using the Neighbour-joining method with the MEGA package. The sequence was deposited at GenBank[15].

Preparation of metal stock solutions: Stock solution of zinc was prepared by dissolving specific quantities of zinc sulphate in double distilled water. Broad range of zinc concentrations such as 400, 500, 1000, 1500, 2000 and 2500 ppm were prepared and sterile paper discs were dipped in respective concentrations.

Antibiotic sensitivity test: Muller Hinton agar plates were prepared and *M. morganii* ACZ05 was swabbed on the plates using a sterile cotton swab[16]. After 15 min, discs containing zinc concentrations and antibiotic were placed on the plates using a sterile forceps in such a way that the distances between two discs were at least 20 mm. The plates were incubated at 37°C for 24 h. The presence and size of inhibitory zones were observed[17].

Bioremoval of nickel: From the overnight culture maintained in nutrient broth, the organism was inoculated (1 mL) into 300 mL nutrient broth in 500 mL Erlenmeyer flasks containing the selected concentrations of zinc (250, 500, 750 and 1000 ppm). After 1, 2, 3 and 4 days of treatment, 10 mL of the sample was centrifuged at 2500 rpm. The pH of the medium was determined every day using pH meter for the 4 days. Pellet from the above step was collected and poured in a Petri dish. It was dried in a hot air oven at 80°C for 3 h and the final dried biomass was weighed. The clear supernatant was used for AAS analysis of zinc. The values so obtained by AAS analysis represent the residual concentration of zinc in the solutions. From this, percentage of removal of zinc was calculated[18].

Statistical analysis: Two-way analysis of variance (ANOVA) was performed to identify the percentage of removal of zinc, pH and biomass using MS Excel. Variability was considered only when the calculated F-value was greater than the tabulated F-value at P is less than or equal[19] to 0.05.

RESULTS

The morphological and biochemical tests were carried out for the identification of zinc tolerant bacterial strain and confirmed as *Morganella morganii* (Plate 1 and Table 1).

Table 1: Biochemical tests employed for the identification of *Morganella morganii* ACZ05

Tests	Results
Colony character	Off-white and opaque in color
Colony size	Medium
Cell type	Short, straight rod
Mac Conkey agar	+
Motility	+
Growth in KCN	+
Gram reaction	-
Lactose	-
Xylose	-
Maltose	-
Fructose	-
Dextrose	-
Galactose	+
Raffinose	-
Trehalose	-
Melibiose	-
Sucrose	-
L-Arabinose	-
Mannose	+
Inulin	-
Sodium gluconate	-
Glycerol	+
Salicin	-
Dulcitol	-
Inositol	-
Sorbitol	-
Mannitol	-
Adonitol	-
Arabitol	-
Erythritol	-
α-methyl-D-glucoside	-
Rhamnose	-
Cellobiose	-
Melezitose	-
α-methyl-D-mannoside	-
Xylitol	-
ONPG	-
Esculin hydrolysis	-
D-Arabinose	-
Citrate utilization	-
Malonate utilization	-
Sorbose	-
Indole	+
MR-VP	+/-
Catalase	+
Oxidase	-

+: Positive, -: Negative

Phylogenetic tree was constructed by using MEGA4 software and the evolutionary history was inferred by neighbour-joining method (NJ) (Fig. 1).

The *M. morganii* ACZ05 exhibited high MIC values for zinc, indicating its resistance to zinc. This bacterial strain was also resistant against the antibiotic, Ticarcillin alone (Table 2). Figure 2 shows the changes in pH which was recorded up to 4 days of treatment with *M. morganii* ACZ05. The pH showed gradual increase with the increase in treatment period and the highest pH was observed at 250, 500 and 1000 ppm of zinc concentration after 4 days.

Figure 3 illustrates the biomass of *M. morganii* ACZ05 during zinc removal. Maximum biomass was obtained at 1000 ppm concentration of zinc and minimum at 250 ppm.

Figure 4 depicts the percentage of removal of zinc after its treatment with *M. morganii* ACZ05. Among the zinc concentrations, maximum percentage of removal was observed at 500 and 750 ppm concentrations of zinc throughout the treatment period.

Table 3 shows the two way ANOVA for percentage of removal, pH and biomass with the two variables, treatment period and zinc concentration. The variations due to zinc concentration and treatment period were statistically significant at 5% level for all the three factors.

Table 2: Inhibition zone details of *Morganella morganii* ACZ05 exposed to zinc and antibiotics

Zn/Antibiotics	Diameter of inhibition zone (cm)
Zn concentration (ppm)	
3000	1.5
2500	1.4
2000	1.2
1500	R
1000	R
Antibiotics	
Levofloxacin	3.8
Ciprofloxacin	3.3
Nitrofuracin	1.8
Cefoperazone	2.5
Ampicillin	1.5
Cephtaxime	1.2
Chloramphenicol	1.0
Amoxicillin	1.0
Piperacillin	1.0
Ticarcillin	R

R: Resistant

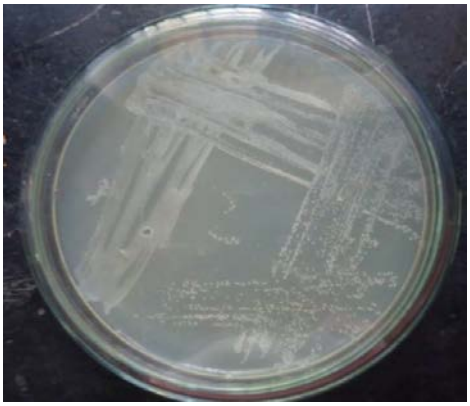

Plate 1: Growth of *Morganella morganii* ACZ05

DISCUSSION

Based on the assessment of biochemical characteristics with the standard description in Bergey's Manual of Determinative Bacteriology[20], the isolate was identified as

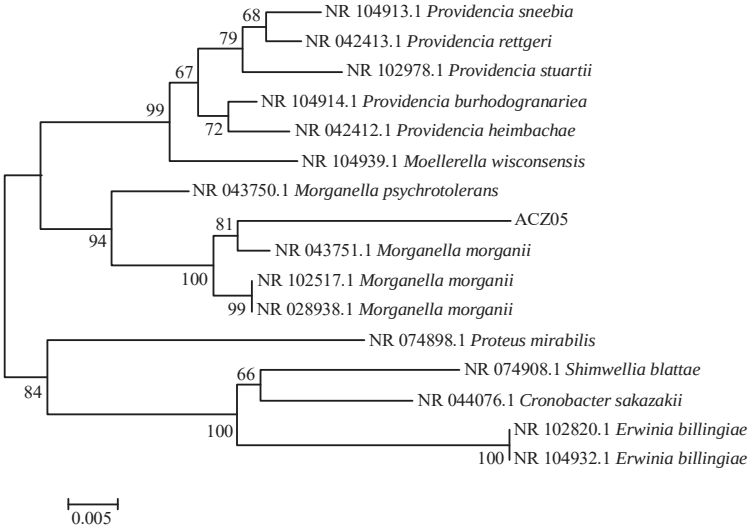

Fig. 1: Phylogenetic tree based on 16S rRNA gene sequence comparisons over 1436 bases of *Morganella morganii* ACZ05

Table 3: Two way analysis of variance (ANOVA) for the factors with the variables, treatment period and zinc concentration

Factors	Source of variation	df	MS	Calculated F-value	Table F-value	Level of significance at 5%
Removal of zinc (%)	Treatment period	3	2087.7060	35.9191	3.8625	Significant
	Zinc concentration	3	258.8320	4.4532	3.8625	Significant
pH	Treatment period	3	0.0706	5.2694	3.8625	Significant
	Zinc concentration	3	0.1822	13.6010	3.8625	Significant
Biomass	Treatment period	3	2.6431	6.3234	3.8625	Significant
	Zinc concentration	3	1.9784	4.7332	3.8625	Significant

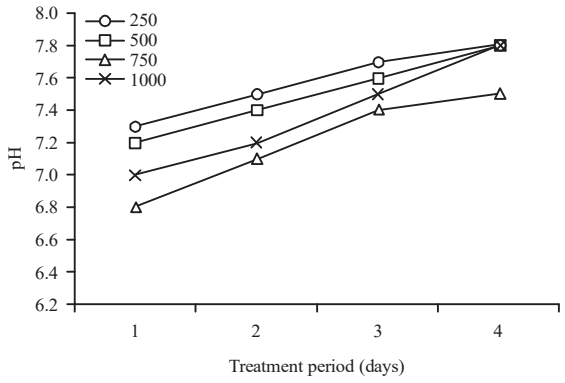

Fig. 2: Changes in pH during zinc removal by *Morganella morganii* ACZ05

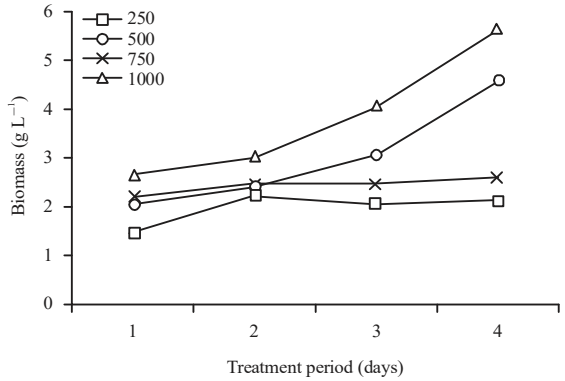

Fig. 3: Biomass of *Morganella morganii* ACZ05 during zinc removal

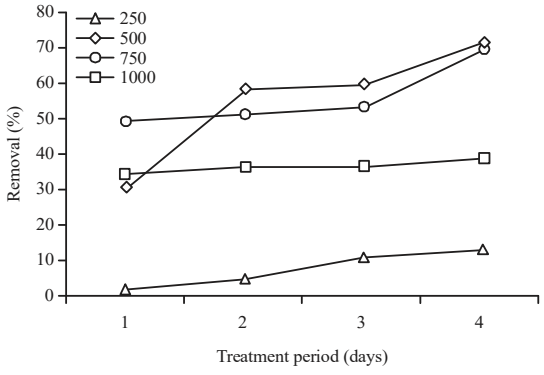

Fig. 4: Removal of zinc (%) by *Morganella morganii* ACZ05

Morganella morganii ACZ05. It was confirmed by 16S rRNA sequence. The sequence was deposited at GenBank accession No. KJ830754.1. Phylogenetic relationship was illustrated based on the alignments of 16S rRNA from *M. morganii* ACZ05. The bootstrap consensus was inferred from 1000 replicates.

Zinc and antibiotics resistance of the *M. morganii* ACZ05: Several bacteria have precise genetic mechanisms of resistance to poisonous metals[21]. Really, it is comparatively frequent the relationship of metal and antimicrobial resistance, because both resistance genes are commonly situated on similar mobile genetic elements. Therefore, it can be understood that the selective pressure exerted by heavy metals lead to the indirect co-selection of antibiotic resistance, mainly in environments polluted with the two elements[22].

Heavy metal resistant strains demonstrate no inhibition of growth at high concentrations of heavy metals but heavy metal susceptible strains confirm the inhibition of growth at high concentrations of heavy metals[23]. Based on this idea, *M. morganii* ACZ05 was recognized as a competent isolate that is resistant to zinc.

Removal of zinc from aqueous solutions using *M. morganii* ACZ05: pH is one of the most significant parameters that influence the solubility of metal ions and the functional groups on cell walls of the biomass[24]. Olaniran *et al.*[25] discovered that metal biosorption is strongly pH dependent and disclosed its result on the nature of biomass attaching sites, the activity of the functional groups in the biomass, the metal solubility and the competition of metallic ions.

Functional groups like carboxylate on bacterial cell wall had earlier been revealed to be[26] protonated under pH 4. Reduced metal removal at low pH might be attributed to competition of protons (H^+) and metal cations for nonspecific elimination sites on the biomass, resulting in a raise in protonated sites. This forms an objectionable ionic environment, resulting in decreased binding of cations, therefore diminished uptake of zinc by the biomass[27].

At pH 6.0, the biomass exterior will be highly negatively charged and promote the uptake of positively[28] charged Cd^{2+}.

The functional groups like carboxyl, phosphate and amino groups on the cell surface are deprotonated due to increase in hydronium ion concentration[29,30]. Bulgariu and Bulgariu[31] reported that alkaline pH reduces biosorption due to secondary hydrolysis, speciation and precipitation of Cd^{2+} as hydroxides. At pH 8.0, insoluble cadmium hydroxide precipitates from solution as hydroxyl species of $Cd(OH)_2$, $Cd(OH)_3$ and biosorption is not feasible[29,30,32-34]. Similarly, several studies reveal that biosorption is strongly influenced by the solution's preliminary pH. For *M. morganii* ACZ05, the efficient removal of zinc was noted at pH 6.8-7.8.

Lim *et al.*[35] noted that biosorption incorporated an amount of passive accretion action like surface adsorption, ion exchange, coordination, chelation and microprecipitation. The type and quantity of functional groups on bacterial cell wall really exaggerated the biosorption competence. Consequently, biomass concentration is a vital parameter which decides the adsorption rate and ability for a known initial concentration[36]. The adsorption rate considerably augmented in the starting which might be due to the augment in the availability of surface sites with the rising biomass concentration[37]. Then the adsorption rate attained the utmost (93.77%) when biomass concentration was 4.0 g L^{-1}, demonstrating that the adsorption process tended to equilibrium. however, the additional increase in biomass concentration lowered the adsorption rate, which was in agreement with the investigations of Ekmekyapar *et al.*[38] and Bueno *et al.*[39].

Factors such as pH, ionic strength, temperature and metal ion concentration in solution have been identified to be responsible for the decreased adsorption ability at increasing biomass[40]. In the present study, the biosorption rate attained the utmost (72.6%) when biomass concentration was 4.6 g L^{-1}. Further raise in biomass concentration lowered the biosorption rate, which was in concurrence with the study of Ekmekyapar *et al.*[38] and Bueno *et al.*[39].

Rathinam *et al.*[33] reported that the result of dissimilar initial cadmium concentrations of 25, 50, 150, 250 and 500 mg L^{-1} on its removal using *H. valentiae* biomass. The percentage removal of cadmium has been found to be higher at lower concentration of cadmium solution (25 mg L^{-1}), a maximum cadmium removal of 86.8% has been obtained for *H. valentiae* biomass. The percentage removal of zinc has been found to be elevated at decreased concentration of zinc solution (500 ppm), a highest zinc removal of 72.6% has been found for *M. morganii* ACZ05 biomass.

CONCLUSION

A zinc resistant isolate *M. morganii* ACZ05 was isolated from the electroplating industry soil in this study. The most favourable environment for both the cell growth and heavy metal removal were determined. In mid-log phase the cellular growth and biosorption capacity increased due to abundant availability of active binding sites on the biomass. The highest biosorption of zinc (72.6%) was noted in the optimal conditions as: pH 6.8-7.8 and with initial metal concentration of 250 ppm zinc. Hence this experiment suggests *M. morganii* ACZ05 might be effective, promising and possible biosorbent for the removal of zinc from industrial effluents.

SIGNIFICANCE STATEMENT

This study discovered that the isolated bacterial strain can be beneficial in the removal of zinc from aqueous solution and this study will help the researchers to uncover the critical areas of biosorption technology that many researchers were not able to explore. Thus a new theory on microbial cells can substitute expensive methods to remove harmful heavy metals.

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