Isolation and Characterization of Cadmium Tolerant *Cupriavidus spp.* from Mine Spoil of Zawar Mines, Udaipur (India)

Monica Sen[1] and Harshada Joshi[2]

[1,2]Department of Biotechnology, Vigyan Bhawan, Block B, Mohanlal Sukhadia University Udaipur, Rajasthan, India
E-mail: [1]hjbiotech@gmail.com, [2]hjbiotech@gmail.com

Abstract—*The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial progress. Cadmium, zinc, lead and nickel are known to be the most commonly heavy metals used and the more widespread contaminants of the environment. The present study is aimed to isolate and characterize cadmium tolerant bacteria from rhizospheric soil of plants growing in tailing dams of Zawar mines, Udaipur. A total of 15 cadmium tolerant bacterial strains were isolated on cadmium supplemented nutrient agar. The minimum inhibitory concentration (MIC) of all the 15 isolates for cadmium was determined on nutrient agar supplemented with varying concentrations of cadmium. The isolate HMT36 was found to tolerate high concentration of cadmium (7mM) and was selected for further characterization based on its cultural, morphological and biochemical characteristics. The isolate was tentatively identified as Cupriavidus gillardii HMT36. The isolation and characterization of cadmium tolerant bacteria will provide valuable information and opens a way to investigate further about the possible use of this strain for the purpose of bioremediation.*

Keywords: *Cadmium tolerance; Cupriavidus; heavy metals; MIC; zawar mines.*

1. INTRODUCTION

A large number of sites worldwide are polluted with heavy metals as a result of human activities such as mining and smelting industries, fertilizers and pesticide application and waste disposal, fuel production etc [4]. Mining activities generate a large amount of waste rocks and tailings which become source of metal pollution during course of time [2].Heavy metals are stable and persistent environmental contaminants since they cannot be degraded or destroyed. Various metals present in mine waste can be drained off during rain, which result into metal leaching and traced into watersheds downstream. These metals may be transferred and accumulated in the bodies of animals or human beings through food chain, which will probably cause DNA damage and carcinogenic effects by their mutagenic ability [9].

Cadmium (Cd) is among the top ten in the black list of agreements on environment protection and the World Health Organization (WHO) [17]. Cadmium is a toxic pollutant and has bioaccumulation and biomagnification properties. The microorganisms residing in such contaminated tailings and polluted lands have evolved mechanisms to tolerate the presence of cadmium/heavy metals by either efflux, complexation, or reduction of metal ions or to use them as terminal acceptors in aerobic respiration [7].Despite the toxic stress, micro-organisms that tolerate high metal concentrations and more rapidly decompose pollutants are more likely to survive. Therefore, microbial–based remediation technique is a better alternative to reduce metal pollution of such contaminated sites.

The aim of this study was to isolate and characterize indigenous *Cupriavidus* strain with high cadmium tolerance in the tailing site and to determine minimum inhibitory concentration of the strain against cadmium. In metal contaminated environments, the vitrification of metal by tolerant strains could be an efficient bioremediation process.

2. MATERIALS AND METHODS

1. Study area

The present study area is Zawar mines situated about 45 km away from Udaipur, Rajasthan ,India ; located at 24° 22´north and 73°43´ east.

2. Sampling

Soil samples were collected from rhizosphere of the plants growing in tailing dam of Zawar mines, Udaipur (India).The samples were kept in sterile zipper plastic bags in refrigerated conditions till transferred to laboratory for further studies.

3. Isolation of cadmium tolerant bacteria

Cadmium tolerant bacteria were isolated on nutrient agar supplemented with 0.5mM of cadmium chloride ($CdCl_2$) by standard pour plate method. Plates were incubated at 37°C for 48 h.

4. Determination of Minimum Inhibitory Concentration (MIC)

For determination of MIC, preliminary isolated cadmium tolerant bacteria were grown on nutrient agar plates supplemented with various concentrations of cadmium chloride ranging from 0.5mM to 10mM with a difference of 0.5mM. The petri plates were inoculated with the test organism and incubated at 37°C for 48 h.

5. Characterization of the isolates

a. Morphological characterization: Morphological characterization of the isolates was done by gram staining and by studying shape of the cell under microscope.

b. Cultural characterization: Isolated colonies of purified strains grown on solidified agar plates were observed and data was recorded regarding the form, elevation, margin and optical feature of the colonies

c. Biochemical characterization: Biochemical characterization of isolates was done by studying biochemical characteristics like catalase activity, oxidase activity, acid production from glucose, carbohydrate fermentation (using ten sugar discs namely glucose, sucrose, rhamnose, galactose, maltose mannose, inositol, fructose, cellibiose, lactose),oxidation fermentation reaction (OF), nitrate reduction , citrate utilization ,starch hydrolysis ,gelatin hydrolysis, arginine hydrolysis and esculin hydrolysis. The results obtained for biochemical characteristics were matched with Bergey's Manual of Systematics Bacteriology for identification of bacteria.[1]

3. RESULTS

A total of 12 rhizospheric soil samples were collected from contaminated sites of Zawar mines (Udaipur), Rajasthan, India and 15 indigenous cadmium tolerant bacteria were recovered on nutrient agar supplemented with 0.5mM of cadmium chloride by standard pour plate method. The minimum inhibitory concentration (MIC) of all the 15 isolates for cadmium was determined on nutrient agar supplemented with varying concentrations of cadmium ranging from 0.5mM to 10mM with a difference of 0.5mM.The well defined colonies of isolate HMT 36 were observed after 72 h of incubation in the nutrient agar medium up to 7mM concentration of cadmium. The isolate HMT36 didn't show any growth on high concentrations of cadmium (7.5 and 8 mM). Therefore the MIC of cadmium for HMT36 was found to be 7.5mM (Table 1).Rest of the 14 isolates exhibited lower MICs than 7.5 mM hence HMT 36 was selected for further studies.

The isolate HMT36 was selected and further characterized by cultural, morphological and biochemical characteristics .Cellular morphology such as arrangement, shape and Gram reaction were observed during Gram staining of the strain.

The results indicated that the isolate was gram- negative and rod shaped .The colonies of HMT 36 appeared as off-white, glistening , smooth colonies convex with entire edges. The isolate gave well-defined colonies at 42°C. The isolate was found to be non-fermentative as it did not ferment any of the sugars used in the study. The isolate showed positive reaction for oxidation fermentation test. The isolate also gave positive results for catalase activity and oxidase activity. It reduced nitrate, utilized citrate while gave negative reaction for gelatin, starch, arginine and esculin hydrolysis (Table 2).The above results obtained for morphological and biochemical characteristics were further matched with Bergey's Manual of Systematics Bacteriology [1]. On the basis of the above results the isolate HMT36 was tentatively identified as *Cupriavidus spp.* HMT36.

4. DISCUSSION

Sampling environments that contains elevated concentrations of heavy metals are a potential source of metal-tolerant bacteria [5]. For the present study samples were collected from rhizosphere of plants growing in tailing dam of Zawar mines, Udaipur. These tailings possesses high concentration of heavy metals mainly zinc, cadmium, lead etc. A total of 15 bacterial isolates were recovered on nutrient agar supplemented with 0.5mM concentration of cadmium chloride from 12 soil samples. Several workers in past have reported the cadmium tolerance in bacteria which were isolated from mine spoil [19, 22,23].They included bacteria of different genus namely *Pseudomonas ,Bacillus, Cupriavidus* etc. Cadmium tolerant bacteria from Zawar can be used as a suitable candidate for bioremediation of cadmium contaminated tailing sites.

Metal tolerance reflects the ability of an organism to survive in an environment with high concentration of metals or to accumulate high concentration of metal without dying. In the present study, all the 15 isolates can tolerate low concentration of cadmium but as the concentration increases majority of isolates were unable to grow. The maximum tolerant isolate with MIC 7.5 mM was selected and further identified on the basis of morphological and biochemical characteristics. The characteristics were in accordance to that of Bergey's Manual of Systematics Bacteriology [1] and hence the isolate is tentatively identified as *Cupriavidus spp.* HMT36. Our results are consistent with Makkar and Casida [10] who reported *Cupriavidus* for the first time and described its biochemical characteristics [20].

The MIC of 0.5mM as reported by Siripornadulsil *et al* [19] in past for *Cupriavidus* is very low as compared to the present study. The above results are in accordance to that of Xie *et al* (2010) [22] where MIC of 10 mM was reported for *Cupriavidus* (*Ralstonia spp* strain DX-T3-01).

These resistances may be attributed to the system which not only protect the organism in a harsh environment, but they also play an important role in the cycling of toxic metals in the biosphere. In some cases bacterial metal resistances have been shown to be due to differences in uptake or transport of the toxic metal, while in other the metal is enzymaticaly transformed, by oxidation, reduction etc. into chemical species which is either less toxic or more volatile than the parent compound [21].

Heavy metal tolerance of bacteria can play an important role in the bioremediation of metal pollution in the environment by either reducing the more toxic forms or by accumulating the metal ions thus removing it from the effluent or areas containing concentration of particular metals.

5. CONCLUSION

Cadmium tolerant *Cupriavidus gillardii* HMT36 isolated in the present study would enrich the study on diversity of cadmium tolerance and can be efficiently used for bioremediation and removal of cadmium from cadmium dumping sites.

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Table 1: MIC of cadmium tolerant isolates

S. No.	Name of the isolates	MIC of the isolates (mM)
1.	HMT24	5
2.	HMT25	4
3.	HMT26	6
4.	HMT27	5
5.	HMT28	2
6.	HMT29	4
7	HMT30	6
8	HMT31	6.5
9	HMT32	6.5
10	HMT33	6
11	HMT34	5
12	HMT35	7
13	HMT36	7.5
14	HMT37	5
15	HMT38	6.5

Table 2: Biochemical characterization of the isolate HMT36

S. No	Biochemical test	Isolate HMT 36
1.	Growth at 42 °C	+
2.	Catalase acitivity	+
3.	Oxidase acitivity	+
4.	Carbohydrate fermentation	-
5.	Oxidation/fermentation test(using H&L media)	+
6.	Nitrate reduction	+
7.	Citrate utilization	+
8.	Gelatin hydrolysis	-
9.	Arginine hydrolysis	-
10	Starch hydrolysis	-
11	Esculin hydrolysis	-
(+ = positive reaction ; - = negative reaction)		

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