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Evaluation of the genetic basis of heavy metal resistance in an isolate from electronic industry effluent

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Abstract *Halomonas BVR 1* isolated from an electronic industry effluent had high level of resistance to heavy metals like cadmium, lead, zinc and to various antibiotics. Minimum Inhibitory Concentration (MIC) of the strain toward cadmium and lead was found to be 200 mg L^{-1} and 400 mg L^{-1} respectively, while it could tolerate zinc up to 250 mg L^{-1} and chromium up to 150 mg L^{-1}. The present study proved the genetic contribution of heavy metal resistance in this strain to be plasmid mediated. Isolation of the plasmid from *Halomonas BVR 1* and its subsequent linearization with *Bam H1* confirmed the presence of a plasmid of size >10 kb. Plasmid curing experiments affirmed plasmid mediated heavy metal resistance. Additionally, genetic transformation of a non metal resistant lab strain *Escherichia coli* and the cured strain of *Halomonas BVR 1* with the isolated plasmid increased their metal tolerance level by 50% confirming the genetic determinant to be present in the plasmid.

1. Introduction

Pollution is escalating with time due to rapid industrialization leading to an increase in various hazardous compounds liberated into the environment. Environmental pollution by heavy metals from electronic industrial effluents is a global problem [13]. Heavy metals are the main group of inorganic pollutants which are continuously accumulating in our environment [15]. This is a worldwide predicament which disturbs the environmental equilibrium by gaining entry into the ecosystem due to their small size and bioaccumulation tendency [14]. Metals in higher concentrations displace the essential nutritional minerals in the living systems and prove deleterious to them by disrupting the functioning of vital organs [11,12], making it a global environmental concern.

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Electronic effluents loaded with metals and salts harbor microorganisms that have an inherent resistance to survive in such an adverse habitat. These bacterial strains may contain genetic determinants contributing to the resistance against heavy metals and are often classified to be found on plasmids, transposons or chromosomal DNA [4,5]. Recently, these innate resistance mechanisms that contribute to enhanced metal adsorption capacity by microorganisms have been exploited to carry out bioremediation. Our earlier papers have reported one such strain, *Halomonas BVR 1* isolated from a heavy metal rich electronic industry effluent and it has been clearly manifested that it is efficient in remediation of metals like lead, cadmium and zinc [7,8].

Resistance in bacteria against these toxic metals reflects the threshold of environmental contamination and the direct or indirect exposure of these bacteria to the toxic compounds. This adaptation to the adverse habitat can be natural or acquired through plasmids and the incidence of the plasmid bearing metal tolerant strains is more in a polluted site rather than an unpolluted site [6].

Organisms characterized are of interest for biotechnological purposes mainly due to production of exopolysaccharides along with their metal and antibiotic resistance. Some halotolerant bacteria are important candidates in the field of metal remediation, owing to their exceptional properties like high number of anionic and cationic functional groups on their cell surface that make them reliable for metal adsorption and intracellular accumulation processes [3].

Halomonas BVR 1 is a novel metallophilic bacteria isolated from electronic industry effluent which grows best at an optimum salt concentration of 3–15%. Several plasmids have been identified in halophilic bacteria specifically *Halomonas* genus which infer resistance to various heavy metals. However, the genetic basis of metal resistance in our isolated strain has not been elucidated. Hence, it would be interesting to understand if the inherent metal resistance is plasmid or chromosomal mediated? Our study aims to delineate the plasmid mediated heavy metal resistance in *Halomonas BVR 1* and evaluate the increase in metal tolerance levels in non metal resistant strains by transformation of the plasmid. The plasmid mediated metal resistance found in our strain was proved by experiments like plasmid curing and genetic transformations.

Existing methods for metal remediation have certain limitations that hinder their use [3]. Hence, there is an imperative need to explore new and effective techniques for their removal. Genetic engineering technology is an alternate method to develop novel biosorbents that has the potential to improve or redesign microorganisms pertaining to their selectivity and accumulating properties of the organisms [3]. The plasmids from these metal tolerant strains may therefore be used for the genetic transformation of the lower resistant strain to boost up its metal resistance. Data from this study, can be exploited to genetically transform lower metal resistant strains contributing novel organisms to the microbial culture collection.

2. Materials and methods

All the experiments were carried out after approval from the Institutional Biosafety Committee (IBSC).

2.1. Sampling site, characterization and selection of the organism

The electronic industry effluent was characterized to identify the microbial population. The detailed procedure and the outcome of this characterization have already been reported in our earlier publication [7]. Out of the ten strains isolated, three strains belonged to the genus *Halomonas*. The organisms belonging to this genus has not been exploited as a potential biosorbent for metal remediation. Among the three strains belonging to *Halomonas* genus, *Halomonas BVR 1* was selected for further studies owing to its high tolerance level to metals and antibiotics [7].

2.2. Determination of the Minimum Inhibitory Concentrations (MIC)

The Minimum Inhibitory Concentration (MIC) of this selected microbe was tested against various heavy metals like cadmium, lead and Zinc. A fixed inoculum volume of 10 µl (1.3×10^{-7} - cells) was inoculated into Luria Bertani medium with varied concentrations of heavy metals. Analytical grade heavy metal salts ($CdCl_2{\cdot}8H_2O$, $ZnSO_4{\cdot}7H_2O$, $Pb(NO_3)_2$) were used to prepare 1000 mg L^{-1} stock solutions. Each of these solutions were autoclaved separately and added to LB medium at a concentration of 50–400 mg L^{-1}. The growth of *Halomonas BVR 1* in this medium was analyzed by measuring the Optical Density (OD) at 600 nm [2]. This species was found to be highly resistant to cadmium with a minimal inhibitory concentration of 200 mg L^{-1}. The detailed MIC of *Halomonas BVR 1* against cadmium has already been reported earlier [7].

2.3. Plasmid DNA isolation and digestion

Plasmid DNA was isolated using the standard alkaline lysis method proposed by Sambrook [9]. The isolated product was detected by an agarose gel (0.8%) run. The product was visualized and compared with a standard 1 kb ladder. Single digestion of the plasmid was carried out using *BamH1* with the conditions recommended by the manufacturer (New England Biolabs). The digested products were separated using 0.8% agarose gel electrophoresis. A 10 kb standard DNA ladder was run along with the digested product to assess the size of the isolated plasmid.

2.4. Plasmid curing experiments

To determine if the heavy metal resistance genes are encoded by the plasmid, plasmid curing experiments were carried out with ethidium bromide as the curing agent. The colonies from the highest concentration of ethidium bromide (100 µg/ml) were selected for testing its plasmid curing efficiency. Appropriate dilutions of the inoculum were plated onto LB agar plates. Colonies from this master plate were picked up by the process of replica plating. Accordingly, a sterilized whatman filter paper was placed upon the master plate and subsequently transferred to a selective medium of LB with optimal concentration (200 mg L^{-1}) of heavy metal lead (secondary plate). The master plate and secondary plate were compared to assess the plasmid curing efficiency. Isolation of plasmid