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# MERCURY, ZINC, CADMIUM UPTAKE ABILITY AND POLYPEPTIDE PROFILE OF Cr-RESISTANT PSEUDOMONAS Spp UNDER THESE STRESSES

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**Abstract-**Three chromium resistant strains Pseudomonas CrT-8, Pseudomonas Crm-8, and Pseudomonas S-3, isolated by [1]Faisal and Hasnain (1998) were used to check their resistance against mercury, zinc and cadmium and their uptake ability of these metals was evaluated. Mercury, zinc and cadmium was estimated in pellet (take up/accumulation), washing of pellet (loosely bound/adsorbed) and supernatant (left over/unavailable) of these strains. Mercury, zinc and cadmium uptake ability of these strains was checked. Mercury uptake ability of Pseudomonas CrT-8 and Pseudomonas S-3 was greater than Pseudomonas Crm-8. All the strains have great ability to volatilize the mercury except Pseudomonas CrT-8. These strains can uptake zinc. Pseudomonas CrT-8 and Pseudomonas S-3 can uptake cadmium but in low amount. Pseudomonas Crm-8 cannot tolerate $CdCl_2$. Protein analysis of these strains under metallic stresses revealed that they synthesized new polypeptides with different metals. In Pseudomonas CrT-8 polypeptides of 36KD, 30KD, 26KD and 8KD with Zn and polypeptides of 48KD, 44KD and 15KD with Hg were synthesized. Pseudomonas Crm-8 two new protein bands of 45KD and 30KD were appeared.

**Key words:** Metal, binding peptides, Metal uptake ability, PAGE, Pseudomonas

## 1. INTRODUCTION

Pollution can be defined as an undesirable change in physical, chemical or biological characteristics of air, water and land that can be harmfully effect health, survival or activity of humans or other living organisms [2]. Waste water from industries, serve as major source of water pollution. The water bodies are polluted by the refuse of mines, power stations, steel mills, oil refineries and chemical industries. Hazardous wastes from these industries, consists of vast array of items which are discharge into water. These include, mainly heavy metals and materials that are toxic, inflammable, radioactive, infectious and corrosive agents. Different methods were devised for wastewater treatment such as biological as well as chemical reduction of most toxic forms of heavy metals. Biological wastewater treatment particularly relays on microorganisms and plants. Phytoremediation of heavy metals by non-food crops could be a reliable source in controlling the heavy metals content of agriculture soils and industrial wastes [3].

Hence heavy metal resistant bacteria can be exploited for detoxification/removal of metals from the effluents. The ever increasing concern about the toxicity of Cr in industrial waste waters, therefore, constrain the isolation of Cr resistant bacteria for detoxification and uptake of chromium has been reported by many workers [4].

Mercury is the heavy metal with strongest toxicity. The reason for its highest toxicity is that affinity of mercury to thiol group (-SH) (of cell membrane) is even stronger then the affinity of cadmium to sulphide. Bacteria can tolerate heavy metal stresses due to presence of cellular mechanisms of combating the toxic effects. They exhibit metabolism dependent and independent mechanisms for uptake, accumulation and detoxification of heavy metals [5]. Metal resistant bacteria are important as an index of pollution as well as clearing agent for heavy metals from the environment. Bacteria exhibit a number of mechanisms for mercury detoxification [6]. Methylation of mercury by micro-organisms allow the organisms to dispose of heavy metal ions as small organometallic complexes, which depends upon narrow range of pH, redoxpotential, composition of microbial population, availability $Hg^{2+}$ and temperature.

Similarly cadmium may exist in soluble form in soil, water or in insoluble complexes with inorganic or organic soil constituents [7]. The divalent cations of cobalt, zinc and nickel are essential nutrients of bacteria, required as trace element at nano molar concentration. However at micro or millimolar concentrations they are toxic. Cadmium resistance is wide spread in many bacterial strains. In Staphylococcus aureus cadmium resistance is mediated by different genes. Two of these genes, cad A and cad B have been studied extensively. In Pseudomonas aeruginosa prevention is observed in secretin-induced stress rather than response and identifies a novel protein involved in secretin function [8].

Bacillus subtillus Zur regulates the expression of two pulative zinc transport systems, an ABC transporter encoded by the ycd HI-YceA operon and a conserved membrane protein encoded by yci C [9]. In addition, B. subtilis was recently shown to express a third zinc uptake system, Zos A, under conditions of oxidative stress. The zos A gene is not repressed by zinc and is expressed under the regulation of the peroxide sensing repressor per rather than Zur [10]. New fuctions of Ded A membrane protein functions have been studied in this regard [11].

To probe whether the chromium resistant strains Pseudomonas CrT-8, Pseudomonas Crm-8, Pseudomonas S-3 isolated by Faisal and Hasnain (1998)[1] from different polluted environments, could resist Hg, Zn and Cd in rich

medium (L-broth).Present work accomplished, pH and temperature range of these bacterial strains with salts of Hg, Zn, Cd and Cr. Hg, Zn and Cd uptake ability of these strains was checked by determining the Hg, Zn and Cd content in pellet, washing of pellet and supernatant of their cultures. This was analyze by growing these strains at pH, temperature and concentration of salts at which they showed maximum growth.

## 2. METHODS

### 2.1 Bacterial Strains Used

Three chromium resistant strains (Pseudomonas CrT-8, Pseudomonas Crm8, Pseudomonas S-3) were isolated by Faisal and Hasnain (1998)[1] from effluents of tanneries (Din garh Kasur), effluents of ICI and soil samples of G. T. road Muridke.

### 2.2 Metal Resistance

Metal resistance of these strains with different concentrations of $HgCl_2$, $ZnSO_4$ and $CdCl_2$ on L-agar was checked. Three different concentrations (10, 20, 30 μg $ml^{-1}$ of $HgCl_2$, ten different concentrations of (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 μg $ml^{-1}$) of $ZnSO_4$ and six different concentrations (25, 50, 100, 200, 300, 400, 500 μg $ml^{-1}$) of $CdCl_2$ were mixed in 20 ml of L-agar and plates were prepared and bacteria were grown on this medium to check their resistance against these metals.

### 2.3 PH Range of Bacteria

Different pH levels (5-11) of L-broth (with stress of three different metals i.e., Mercury, Zinc and Cadmium) were adjusted, inoculated with Chromium resistant Strains (Pseudomonas CrT-8, Pseudomonas Crm-8, Pseudomonas S-3).Incubated at 37°C, optical density was recorded at 600nm.

### 2.4 Temperature Range of Bacteria

Different pH levels (5-11), of L-broth with the stress of three different metals i.e., Mercury, Zinc and Cadmium was inoculated with chromium resistant strains (Pseudomonas CrT-8, Pseudomonas Crm-8, Pseudomonas S-3). Incubated at different temperatures i.e. 28°C, 30°C, 37°C, 45°C, optical density was recorded at 600nm.

### 2.5 Acid Digestion and Estimation of Mercury, Zinc and Cadmium Uptake

Acid digestion and estimation of mercury, zinc and cadmium in pellet, supernatant and washing of bacterial strains performed by following methods of Rand et al., 1979[12]

### 2.6 Protein Analysis

To compare polypeptides of bacterial strains under different stresses, studied by protein gel electrophoresis according to method of Laemmli., 1970[13]

## 3. RESULTS

Under Mercury stresss all strains resist 10 μg $ml^{-1}$ concentration with pH 8 (Pseudomonas CrT-8, Pseudomonas Crm-8) and pH 7 (Pseudomonas S-3) after incubation at 45°C (Pseudomonas CrT-8), 30°C (Pseudomonas Crm-8) and 37°C (Pseudomonas S-3)

With Zinc stress microorganisms show best growth with concentration of 700 μg $ml^{-1}$ (Pseudomonas CrT-8,) and 200μg $ml^{-1}$ (Pseudomonas CrT-8, Pseudomonas S-3) with pH 8 (Pseudomonas CrT-8, Pseudomonas Crm-8) and pH 7 (Pseudomonas S-3) after incubating at 45°C (Pseudomonas CrT-8), 30°C(Pseudomonas Crm-8) and 37°C(Pseudomonas S-3).

While with cadmium stress maximum growth was observed at 300μg $ml^{-1}$ (Pseudomonas CrT-8) and 500 μg $ml^{-1}$ (Pseudomonas S-3) with pH 5 (Pseudomonas CrT-8)and pH 8 (Pseudomonas S-3) with incubation at 30°C (Pseudomonas CrT-8, Pseudomonas S-3). Pseudomonas Crm-8 showed no growth with cadmium stress.

### 3.1 Estimation of Mercury, Zinc and Cadmium

Heavy metal resistant bacteria, which have developed, some strategies to combat polluted environment by converting toxic forms to less toxic or harmless forms. To probe whether the chromium resistant bacteria (Pseudomonas CrT-8, Pseudomonas Crm-8, Pseudomonas S-3) isolated from polluted water could also detoxify other metals, mercury, zinc and cadmium was estimated. Estimation was done in supernatant, pellet and washing of pellet of bacterial cultures, grown under stress with the pH and temperature where they exhibit maximum growth.

#### 3.1.1 Mercury

In case of strain Pseudomonas CrT-8 mercury content in pellet and supernatant was relatively more, mercury content in washing was low which reflect that content of loosely bound mercury is low. Mercury content detected in pellet, washing and supernatant was accountable and most of the given to Pseudomonas CrT-8 was accountable, only minute quantity was unaccountable which was volatilized (Table-3.1).

In case of strain Pseudomonas Crm-8 mercury content in supernatant and pellet was relatively greater. Mercury content in washing was low. Mercury content detected in washing, supernatant and pellet was less than that

Pseudomonas Crm-8 was not accountable which revealed that this strain might have transformed mercury to volatile form (Table-3.1).

**Table-3.1 Estimation of mercury (Mean of Three Replicates)**

| Strains | Growth conditions | | | Mercury Content in | | |
|---|---|---|---|---|---|---|
| | Conc. µg/ml | pH | Temp°C | Pellet µg/g | Washing µg/ml | Supernatant µg/ml |
| **Pseudomonas CrT-8** | 10 | 8 | 45 | 19.34 | 0.906 | 4.034 |
| **Pseudomonas Crm-8** | 10 | 8 | 30 | 4.42 | 1.729 | 4.012 |
| **Pseudomonas S-3** | 10 | 7 | 37 | 5.77 | 0.527 | 0.788 |

In case of strain Pseudomonas S-3 mercury content was comparatively greater. Mercury content in washing and supernatant was low. Mercury content detected in pellet, supernatant and washing was not equal to that supplied. Most of the mercury given to Pseudomonas S-3 was unaccountable which revealed that this strain might have transformed mercury to volatile form (Table-3.1).

These results showed that mercury uptake ability of Pseudomonas CrT-8 and Pseudomonas S-3 was greater than Pseudomonas Crm-8. Loosely bound mercury was greater in Pseudomonas CrT-8 and very low in Pseudomonas Crm-8 and Pseudomonas S-3. Relatively more mercury was unavailable in case of Pseudomonas S-3. Very low quantity of mercury was detected in unavailable form.

### 3.1.2 Zinc

In case of bacterial strain Pseudomonas CrT-8 zinc was only present in pellet. Zinc was absent in washing and supernatant. Relatively high amount of zinc was detected in pellet but all the zinc given to Pseudomonas CrT-8 was not taken up by the bacteria (Table-3.2).

In case of bacterial strain Pseudomonas Crm-8 zinc was only detected in pellet. Zinc was absent in supernatant and washing. High amount of zinc was detected in pellet but all the zinc given to Pseudomonas Crm-8 was not taken up by bacteria (Table-3.2).

**Table-3.2 Estimation of zinc content (Mean of three replicates)**

| Strains | Growth conditions | | | Zinc Content in | | |
|---|---|---|---|---|---|---|
| | Conc. µg/ml | pH | Temp°C | Pellet µg/g | Washing µg/ml | Supernatant µg/ml |
| **Pseudomonas CrT-8** | 700 | 8 | 45 | 1786.76 | -- | -- |
| **Pseudomonas Crm-8** | 200 | 8 | 30 | 634.8 | -- | -- |
| **Pseudomonas S-3** | 200 | 7 | 37 | 814.90 | -- | -- |

-- Zinc was not detected.

In case of strain Pseudomonas S-3 zinc was only present in pellet and absent in supernatant and washing (Table-3.2).

These results showed that all the strains had zinc uptake ability. Zinc uptake ability of Pseudomonas Crm-8 and Pseudomonas S-3 was greater than Pseudomonas CrT-8. In case of Pseudomonas S-3 it could tolerate 200 µg $ml^{-1}$ but could take up relatively high level of zinc.

### 3.1.3 Cadmium

In case of bacterial strain Pseudomonas CrT-8 cadmium content in pellet and supernatant was greater. Cadmium content in washing was low (Table-3.3).

**Table-3.3 Estimation of cadmium (Mean of Three Replicates)**

| Strains | Growth conditions | | | Cadmium content in | | |
|---|---|---|---|---|---|---|
| | Conc. µg/ml | pH | Temp.°C | Pellet µg/g | Washing µg/ml | Supernatant µg/ml |
| **Pseudomonas CrT-8** | 300 | 5 | 30 | 25.41 | 2.68 | 25.56 |
| **Pseudomonas S-3** | 500 | 8 | 30 | 6.70 | 17.22 | 5.5 |

In case of bacterial strain Pseudomonas S-3 cadmium content in supernatant and pellet was low (Table-3.3).
These results showed that Pseudomonas CrT-8 relatively more was in the supernatant. Relatively more Cd was loosely bound with Pseudomonas S-3.

### 3.2 Polypeptides of Chromium Resistant Strains (Pseudomonas Crt-8, Pseudomonas Crm-8, Pseudomonas S-3) Under Different Metal Stresses

The protein profiles of all the three strains revealed that polypeptides of different weights were present under the stress of mercury, zinc, cadmium and chromium.
Polypeptides of 35KD, 32KD, 31KD, 28KD, 23KD, 18KD, 14KD and 9KD were present in Pseudomonas CrT-8 with chromium stress. In Pseudomonas CrT-8, a protein band of 14KD was same under the three metals stresses i-e Cr, Hg and Zn. Under Zn stress 35KD, 32KD and 18KD polypeptide were also present. Polypeptide of 36KD, 30KD, 26KD and 8KD appeared denovo under Zn-stress and that of 48KD, 44KD and 15KD under Hg stress(Table-3.4).
In Pseudomonas Crm-8 polypeptides of 46KD, 43KD, 40KD and 20KD were present under chromium stress. 43KD, 40KD and 20KD polypeptides were commonly under Cr and Zn stresses, while 45KD and 30KD appeared specifically under Zn stress(Table-3.4).
In Pseudomonas S-3 polypeptides of 42KD, 38KD, 35KD, 34KD, 30KD and 27KD were formed in the presence of 1000 µg ml-1 of chromium. The band of (38KD, 35KD, 34KD, 30KD and 27 KD) were also visible with cadmium stress. 38KD, 35KD, 34KD, 30KD and 27KD polypeptides specially synthesized under Zn stress.

**Table-3.4 Polypeptides of Chromium Resistant Strains Under Different Metal Stresses**

| Peptides (KDa) | Pseudomonas CrT-8 | | | | Pseudomonas Crm-8 | | | | Pseudomonas S-3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $K_2CrO_4$ | $HgCl_2$ | $ZnSO_4$ | $CdCl_2$ | $K_2CrO_4$ | $HgCl_2$ | $ZnSO_4$ | $CdCl_2$ | $K_2CrO_4$ | $HgCl_2$ | $ZnSO_4$ | $CdCl_2$ |
| **48** | - | + | - | - | - | - | - | - | - | - | - | - |
| **46** | - | - | - | - | + | - | + | - | - | - | - | - |
| **45** | - | - | - | - | - | - | + | - | - | - | - | - |
| **44** | - | + | - | - | - | - | - | - | - | - | - | - |
| **43** | - | - | - | - | + | - | + | - | - | - | + | - |
| **42** | - | - | - | - | - | - | - | - | + | - | - | + |
| **40** | - | - | - | - | + | - | + | - | - | - | - | - |
| **38** | - | - | - | - | - | - | - | - | + | - | + | + |
| **36** | - | - | + | - | - | - | - | - | - | - | - | - |
| **35** | + | - | + | - | - | - | - | - | + | - | + | + |
| **34** | - | - | - | - | - | - | - | - | + | - | - | + |
| **32** | + | - | + | - | - | - | - | - | - | - | - | - |
| **31** | + | - | - | - | - | - | - | - | - | - | + | - |
| **30** | - | - | + | - | - | - | + | - | + | - | + | + |
| **28** | + | - | - | - | - | - | - | - | - | - | + | - |
| **27** | - | - | - | - | - | - | - | - | + | - | + | + |
| **26** | - | - | + | - | - | - | - | - | - | - | - | - |
| **23** | + | - | - | - | - | - | - | - | - | - | - | - |
| **20** | - | - | - | - | + | - | + | - | - | - | - | - |
| **18** | + | - | + | - | - | - | - | - | - | - | - | - |
| **15** | - | + | - | - | - | - | - | - | - | - | - | - |
| **14** | + | + | + | - | - | - | - | - | - | - | - | - |
| **9** | + | - | - | - | - | - | - | - | - | - | - | - |
| **8** | - | - | + | - | - | - | - | - | - | - | - | - |

*KDa Kilo Daltons

## DISCUSSION

Heavy metals are probably the oldest toxins known to humans and represent a significant source of pollution for the aquatic environment. Frequent industrial usage of chromium is alarmingly elevating its concentration in the environment. Its potential hazards on biostratum and diwindling resources, demands the removal and reuse of chromium from the industrial effluents. Isolation of chromium resistant bacteria and their characterization is the first step in this direction.
Faisal and Hasnain (1998)[1] isolated chromium resistant bacterial strains from polluted environment i.e. effluents of tanneries (Kasur), effluents of ICI and soil samples of G.T road Muridke. They resist chromium ($K_2CrO_4$) upto 40 mg/ml. Bacterial resistance to chromate has been found in many genera such as Pseudomonas, Alcaligenes, Streptococcus, Enterobacter, Bacillus [14]. Presently, mercury, zinc and cadmium resistance of three chromium resistant strains Pseudomonas CrT-8, Pseudomonas Crm-8 and Pseudomonas S-3 was checked. The characteristic of

these strains align them to family Pseudomonodaceae. Many Pseudomonas have been reported to be chromium resistant [15,16].

Many of the chemical forms of mercury are toxic to living organisms. Nevertheless bacteria have developed different mechanisms of cellular and molecular level to encounter many of these chemical forms [17], and play a major role in the global cycling of mercury in the natural environment. Mercury uptake ability of Cr-resistant strains was checked. Mercury content in supernatant, pellet and washing was evaluated. The mercuric ion detoxify in the environment and involves the uptake of $Hg^{2+}$ into the bacterial cell which is followed by reduction by cytoplasmic reductase enzyme. Finally without disturbing cellular activities of bacterial cell extracellular $Hg^{2+}$ has to transport across to the bacterial surface. Some other mechanisms involved in microbial detoxification of Hg salts include membrane permeability uptake, intracellular and extracellular precipitation of mercury [18].The availability/uptake of Hg from the medium changed with change in pH and temperature [19]. Important aspect of detoxification of Hg in the medium is the metal uptake ability of bacteria. The first step in the uptake of Hg is binding of Hg to bacterial cells. The second important step corresponds to accumulation/entry of metal into the cytoplasm/periplasm. Accumulation of metal ion inside cell/cytoplasm determines whether phenomenon is active or not. The important thing towards accumulation of metal ion inside cell is the chemical nature of the Hg transported into the cell. Some metallic ions modified chemically outside the cytoplasm, which other not do so or some create change within membrane to be transported inside [20]. The accumulation of Hg inside cell was determined in thoroughly washed cells. Thoroughly washed cells do not show the phenomenon of loosely bound Hg [21]. The Hg content in the pellet of Pseudomonas CrT-8, Pseudomonas Crm-8 and Pseudomonas S-3 was greater. This shows that all the strains have great ability to uptake Hg. Generally different strains behaved differently in accumulation of Hg ions inside cytoplasm and they preferred alkaline pHs in most of cases [19].

Zinc is an essential element for living organisms. It plays a vital role as a cofactor for numerous enzymes and DAA binding proteins and serves as a structural scaffold for several proteins [22]. Pseudomonas CrT-8 could grow in the presence of 700 μg $ml^{-1}$ $ZnSO_4$, while Pseudomonas Crm-8 and Pseudomonas S-3 could tolerate 200 μg $ml^{-1}$ $ZnSO_4$. Zinc resistant negative strains AnZn-1 and AnZn-2 could tolerate 12.5 mg $ml^{-1}$ $ZnSO_4$ in the medium. Zinc uptake ability of all the strains was evaluated. Zinc content was determined in pellet, supernatant and washing of pellet. Consequently, living cells have developed systems for high-affinity zinc uptake. Zinc was absent in washing of strains. Zn-content in the pellet of Pseudomonas CrT-8, Pseudomonas Crm-8 and Pseudomonas S-3 was greater. It means all the strains had zinc uptake ability. These strains differ in their ability to accumulate Zn. Pseudomonas CrT-8 showed low amount of zinc uptake whereas Pseudomonas Crm-8 and Pseudomonas S-3 showed high amount of zinc uptake. Two Zn-resistant strains AnZn-1 and AnZn-2 differ in their ability to accumulate Zn. AnZn-1 showed very low amount of Zn-uptake whereas in AnZn-2 it was almost 30 folds more [23]. Zinc was absent in the supernatant of Pseudomonas CrT-8, Pseudomonas Crm-8 and Pseudomonas S-3.

Cadmium is a toxic metal. Pseudomonas CrT-8 could tolerate upto 300 μg $ml^{-1}$ $CdCl_2$ and Pseudomonas S-3 could grow in the presence of 50 μg $ml^{-1}$ $CdCl_2$. Pseudomonas Crm-8 was sensitive to $CdCl_2$ and could not grow in the presence of $CdCl_2$. Cadmium resistance is widespread in many bacterial species. Pseudomonads from environmental sources vary widely in their sensitivity to cadmium, but the basis for their resistance is largely uncharacterized. A chromosomal fragment encoding cadmium resistance was cloned from Pseudomonas putida, a rhizosphere bacterium, and sequence analysis revealed two divergently transcribed genes, cad A and cad R. cad A was similar to cadmium-transporting ATPases known mostly from Gram-positive bacteria, and to znt A, a lead-, zinc-, and cadmium transporting ATPase from Escherichia coli. cad R was related the mcr R family of response regulators that normally control mercury detoxification in other bacterial systems [24]. Cadmium content in pellet, supernatant and washing of pellet was evaluated. Cadmium content in the washing of Pseudomonas CrT-8 was very low, but in washing of Pseudomonas S-3 cadmium content was greater. Pseudomonas S-3 had the ability to loosely bind cadmium with its membranes. Cadmium content in the pellet of Pseudomonas CrT-8 was relatively greater than cadmium content in pellet of Pseudomonas S-3and Pseudomonas CrT-8 had relatively greater ability to uptake cadmium. Cadmium content in supernatant of Pseudomonas S-3 was very low but cadmium content in supernatant of Pseudomonas CrT-8 was relatively greater. A gene coding for a de novo peptide sequence containing metal binding motif was chemically synthesized and expressed in Escherichia coli as a fusion with the maltose binding protein. Bacterial cells expressing the maltose binding protein, bacterial cells expressing the metal binding peptide fusion demonstrated enhanced binding of $Cd^{2+}$ and $Hg^{2+}$ compared to bacterial cells lacking the metal binding peptide [25].

All bacterial strains showed maximum growth in neutral and alkaline media under all metal stresses except Pseudomonas CrT-8, preferred acid medium in the presence of $CdCl_2$. Temperature preference vary with metals and strains. Most of strains under metal stresses preferred 37°C and 45°C for growth. In the presence of $CdCl_2$, strains preferred low temperature for growth. Pseudomonas CrT-8 can uptake more mercury and zinc relative to cadmium. Pseudomonas Crm-8 can uptake more zinc than mercury and it was sensitive to cadmium. Pseudomonas S-3 can uptake better both mercury and zinc. It can also uptake cadmium better than other strains. Pseudomonas CrT-8 had maximum ability to uptake mercury, Pseudomonas CrT-8, Pseudomonas Crm-8 and Pseudomonas S-3 had maximum ability to uptake zinc and Pseudomonas S-3 can uptake cadmium better than other. These are chromium resistant strains and have very good ability to uptake chromium than all other metals [1]. According to Ledin et al., (1997)[26] different sites on bacterial surface varying affinity and capacity for binding metal ion. The adsorbtion of

metal determined by many interacting processes implying the properties of metals, the bacterial surface as well as composition of the solution phase (pH and ionic strength) are important.

Protein analysis was accomplished by using SDS polyacrylamide gel electrophoresis. In Pseudomonas CrT-8 48KD, 44KD and 15KD polypeptide with mercury and 36 KD, 30KD and 26KD polypeptides with zinc were nearly formed. Pseudomonas Crm-8 formed a 30KD polypeptide under zinc stress. An increase in amount of soluble protein and expression of four new polypeptides under salt stress was reported [27]. A cluster of four homologous small RNAs modulates $C_1$metabolism and the pyruvate dehydrogenase complex in Rhodobacter sphaeroides under various stress conditions [28].

Oveall synthesis or inhibition of some proteins or even change in protein processing affect the metal resistance or metal binding ability. The protein profiles of strains described here exhibited a variety, which is indicating the involvement of different proteins in metal resistance or removal mechanisms in these strains.

## REFERENCES

[1] Faisal M, Hasnain S. Gram-negative rod shaped bacteria exhibiting high level resistance to chromium. Proc Pak Congr Zool, 19:121-132, 1998.

[2] Prabharkar VK. Prefix in environment and agricultural pollution. In Kumar JL (Eds) Encyclopedia of Environmental pollution and awareness in 21st century series. Anmol publication Pvt Ltd. Environ Sci Techno, 33: 1717-1725, 2001.

[3] Chakravarty P, Bauddh K, Kumar M. Phytoremediation Potential of Bioenergy Plants In Kuldeep B, Bhaskar S, John K (Eds.), Phytoremediation of Heavy Metal-Contaminated Soil Using Bioenergy Crops (pp63-96). Singapore, Springer Nature, 2017.

[4] Gadd GM. Microbial influence on metal mobility and application for bioremediation. Geoderma, 122(2-4):109-119, 2004.

[5] Vrancken K, Mellaert LV, Anné J. Characterization of the Streptomyces lividans PspA Response. J Bacteriol, 190:3475-3481, 2008.

[6] Naik MM, Dubey SK. Lead and Mercury resistant marine bacteria and their application in lead and mercury bioremediation. In Milind MN, Santosh KD (Eds.), Marine Pollution and Microbial Remediation (pp 29-40). Singapore, Springer Nature. 2017.

[7] USPHS. Toxicological profile for toxic substances and disease registry. 1997.

[8] Seo J, Brencic A, Darwin AJ. Analysis of Secretin-Induced Stress in Pseudomonas aeruginosa Suggests Prevention Rather than Response and Identifies a Novel Protein Involved in Secretin Function. J Bacteriol, 191:898-908, 2009.

[9] Gabballa A, Helmann GD. Identification of zinc-specific metalloregulatory protein, Zur, controlling zinc transport operons in Bacillus subtilis. J Bacteriol, 180: 5815-5821, 1998.

[10] Gabballa A, Helmann JD. A peroxide-induced zinc uptake systems plays an important role in protection against oxidative stress in Bacillus subtillis. Mol Microbiol, 45: 997-1005, 2002.

[11] Doerrler WT, Sikdar R, Kumar S, Boughner LA. 5-New Functions for the Ancient DedA Membrane Protein Family. J Bacteriol, 195:3-11, 2013.

[12] Rand MC, Arnod E, Michel J. In standard methods for the examination of water and waste water. American Public Health Association. 1979.

[13] Laemmli UK. Cleavage of structural proteins during the assembly of head of bacteriophage $T_4$. Nature, 227: 680-685, 1970.

[14] Pcitzsch N, Eberz G, Nies HD. Alcaligenes eutrophus as a bacterial chromate sensor. Appl Environ Microbiol, 64: 453-458, 1998.

[15] Nair S, Krishnamurthi VS. Effect of chromium on growth of Pseudomonas aeruginosa. Indian J exp Biol, 29: 140-144, 1991.

[16] Raja CR, Anbazhagan K, Selvam GS. Isolation and characterization of a metal-resistant Pseudomonas aeruginosa strain. World J Microbiol Biotech, 22(6): 577-585, 2006.

[17] Messaoudi N, Gautier V, Kthiri F, Lelandais G et al. Global Stress Response in a Prokaryotic Model of DJ-1-Associated Parkinsonism. J Bacteriol, 195:1167-1178, 2013.

[18] Hou HPS, Imura N. Involvement of mercury methylation in microbial mercury detoxification. Arch Microbiol, 133: 131-176, 1982.

[19] Hassan Z, Sabri AN, Hasnain S. Plasmid associated, temperature and pH dependent mercury detoxification ability of mercury resistant bacteria. Pro Pak Congr Zool, 18: 57-70,1998.

[20] Moscoso J, Schramke H, Zhang Y, Tosi T et al. Binding of Cyclic Di-AMP to the Staphylococcus aureus Sensor Kinase KdpD Occurs via the Universal Stress Protein Domain and Downregulates the Expression of the Kdp Potassium Transporter.J Bacteriol, 198:98-110, 2016.

[21] Corcuera LG, Basitidas M, Dubourdiev M. Molybdenum uptake in Escherichia coli K12. J Gen Microbiol, 139: 1869-1875, 1993.

[22] Vallee BL, Falchuck KH. The biochemical basis of zinc physiology. Physiol Rev, 73: 79-118,1993.

[23] Altaf T, Sabri AN, Hasnian S. Curing of zinc resistant plasmids from Gram-negative bacteria and their effects on growth of Triticum aestivum under Zn stress. Proc Pak Congr Zool, 15: 189-200, 1995.

[24] Seon WL, Glickmann E, Cooksey DA. Chromosomal locus for cadmium resistance in Pseudomonas putida consisting of a cadmium-transporting ATPase and a Mer R family response regulator. Appl Environ Microbiol, 67: 1437-1444, 2001.

[25] Chung H, Lee S, Park SJ. Oxidation Protection in Metal-Binding Peptide Motif and Its Application to Antibody for Site-Selective Conjugation. https://doi.org/10.1371/journal.pone.0159451, 2016.

[26] Ledin M, Pedersen K, Allard B. The effects of pH and ionic strength on adsorbtion of Cs, Sr, Eu, Zn, Cd and Hg by Pseudomonas putida. Wat Air Soil Pollut, 93: 367-381. 1997.

[27] Jain S, Nainwatee HS, Jain RK, Chowdhury JB. Salt tolerance in Brassica juncea L:II. Salt stress induced changes in polypeptide pattern of in vitro selected sodium chloride tolerant plants. Euphytica, 65: 107-112, 1993.

[28] Billenkamp F, Peng T, Berghoff BA, Klug G. A cluster of four homologous small RNAs modulates $C_1$Metabolism and the pyruvate dehydrogenase complex in Rhodobacter sphaeroides under various stress conditions. J Bacteriol, 197:1839-1852, 2015.