SUMMARY OF THE THESIS

CHARACTERIZATION OF ARSENITE DETOXIFYING BACTERIA AND STUDY THE DETOXIFICATION MECHANISM

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1. INTRODUCTION

Arsenic is a toxic metalloid and a well known poison that is ubiquitous in the environment. The acute minimal lethal dose of arsenic in adults is estimated to be 70 to 200 mg or 1 mg/kg/day (Dart, 2004). The permissible limit in drinking water for arsenic is 10 µg/L according to World Health Organization (WHO) and 50 µg/L as per Bureau of Indian Standards (BIS), India. The concentration of arsenic in drinking water has been found to be much higher in several parts of the world including India. In India, some portions of the seven states namely, West Bengal, Jharkhand, Bihar, Uttar Pradesh, Assam, Manipur and Chhattisgarh have so far been found to be contaminated with arsenic at concentrations much above the permissible limit.

Arsenic exists in environment in four oxidation states (+5, +3, 0 and -3) with pentavalent arsenate [+5, As (V)] and trivalent arsenite [+3, As (III)] being most common soluble forms (Joshi *et al.*, 2009). The inorganic forms of arsenic are more toxic than organic forms (Hopenhayn, 2006). In the inorganic form, trivalent arsenite [+3, As (III)] is considered to be more toxic than less mobile pentavalent arsenate [+5, As (V)] (Valenzuela *et al.*, 2009).

The trivalent arsenite [+3, As (III)] can be converted to 100 times less toxic pentavalent arsenate [+5, As (V)] by the process of oxidation. Thus the oxidation of arsenite to arsenate is considered as primary method of detoxification. This oxidation process can be achieved by using chemical as well as biological methods. The chemical methods are costly and result in secondary pollution. Hence, alternatively biological or bioremediation methods of arsenite remediation are preferred.

Arsenic oxidation is being mediated by both heterotrophic and chemoautotrophic microorganisms. These arsenite oxidizing bacteria possess *aox* gene that codes for arsenite oxidase enzyme responsible for arsenite oxidation (Cai *et al.*, 2009; Branco *et al.*, 2009).

Thus, realizing the importance of bioremediation of arsenite by biological oxidation we undertook the exploration and characterization of arsenite oxidizing bacteria from arsenic contaminated soil.

2. REVIEW OF LITERATURE

After the introduction of arsenic into the environment through natural or anthropogenic sources it enters to the biosphere. This creates local arsenic stress environment to the microbiota. In order to survive the stress microorganisms have shown to develop resistance mechanisms.

The arsenic resistant bacteria can survive in presence of arsenic but usually their growth declines with the increase in arsenic concentration *in vitro* and at a point the growth ceases. This dose is considered as Minimum Inhibitory Concentration (MIC). Various workers have computed MIC of arsenic by their isolates which range from 2-120 mM (Joshi *et al.*, 2009; Dave *et al.,* 2010).

The resistance in microorganisms is because of various detoxification mechanisms in these bacteria. Arsenite is a toxic metalloid and its detoxification frequently involves its oxidation to less toxic forms. This reaction is achievable through chemical methods using strong oxidants but this is very expensive moreover it generates secondary pollution (Kim and Nriagu, 1999). Hence, bioremediation overcomes the limitations posed by chemical technique by bringing out the detoxification of arsenite to less toxic form that is arsenate at a lower cost.

The first arsenite oxidizing bacterium *Bacillus arsenoxydans* was isolated by H. H. Green in 1918. Since then numerous arsenite oxidizing bacteria have been isolated and studied including *Alcaligenes faecalis* (Santini *et al.,* 2000), *Pseudomonas arsenitoxidans* (Ilyaletdinov and Abdrashitova, 1981), *Microbacterium lacticum* (Mokashi and Paknikar, 2002), *Agrobacterium tumefaciens* 5A (Kashyap *et al.,* 2006), *Microbacterium oxydans* (Aksornchu *et al.*, 2008), *Pseudomonas strutzeri*, *Aeromonas* sp., *Agrobacterium* sp., *Comamonas* sp., *Enterobacter* sp., *Pantoea* sp. and *Pseudomonas* sp. (Chang *et al.*, 2010) and *Pseudomonas lubricans* (Rehman *et al.*, 2010).

At national level few arsenite oxidizing strains were identified by various researchers. In 2002, Mokashi and Paknikar isolated *Microbacterium lacticum* from a municipal sewage sample by the enrichment culture technique which exhibitted 50 mM of minimum inhibitory concentration for arsenite. A chemolithoautotroph namely *Arthrobacter* sp. 15b was identified from a sewage treatment plant site by Prasad *et al.*, 2009. Bachate *et al.*, 2012 isolated two heterotrophic arsenite oxidizing bacteria from garden soil that were closely related to genus *Bordetella* (MIC- 15 mM) and *Achromobacter* (MIC- 40 mM) based on 16S rRNA sequencing analysis.

Arsenic oxidation is mediated by both heterotrophic and chemoautotrophic microorganisms. Some microbes gain energy from oxidizing arsenite (Inskeep *et al.*, 2007), although this activity could be an exception limited to chemolithotrophic bacteria. Under standard conditions, arsenite oxidation is a thermodynamically exergonic reaction and can provide sufficient energy to support chemoautotrophic microbial cell growth (Ehrlich, 1996). Heterotrophic bacteria have not been shown to derive major energy from arsenite in growth experiments. In heterotrophic bacteria, this is generally considered to be a detoxification mechanism instead of supporting the growth.

Arsenite oxidation is generally presumed to be a detoxification mechanism without experimental observations of any toxic effects in some cases. Salmassi *et al.*, 2002 isolated a bacterium namely, *Agrobacterium albertmagni* strain AOL15 from the surface of aquatic macrophytes collected in Hot Creek, California. The result suggested that the oxidation of arsenite by this strain is a detoxification mechanism (Salmassi *et al.*, 2002).

The arsenic detoxification potential of the bacterial strains is carried out with the arsenite oxidation gene, *aox* genotype which showed the activity of arsenite oxidation to arsenate (Chang *et al.*, 2009).

A number of bacteria with arsenite oxidase enzyme activity have been isolated and genes apparently encoding arsenite oxidase are found widely in various groups of bacteria and archea (Muller *et al.*, 2003). Various arsenite oxidizing bacteria that possess *aox* genes responsible for arsenite oxidation have been reported (Anderson *et al.,* 1992; Vanden-Hoven and Santini, 2004; Cai *et al.*, 2009; Branco *et al.*, 2009). The *aox*B gene acts as a functional marker for aerobic arsenite oxidizers and is responsible for the oxidation reaction which results in the formation of an enzyme, arsenite oxidase facilitating oxidation of As(III) to As(V) species (Quemeneur *et al*., 2008). The bacteria possessing *aox*B gene encoding the large subunit of arsenite oxidase has been found in different soil and water systems containing arsenic (Inskeep *et al.*, 2007).

3. JUSTIFICATION

Arsenic contamination in ecosystem promotes the development of its detoxification by microbiota. Although arsenic concentration in soils of Rajasthan has not been reported earlier, but understanding the introduction of arsenic in soil through textile dyes and mordants seem necessary. We planned the study of analyzing textile dyes contaminated soil for arsenic and other heavy metals and development of arsenic detoxification system in microorganisms present in soil.

4. OBJECTIVES AND SCOPE

The major objectives of the study included:

- Characterization of arsenite detoxifying bacteria.
- Determining the mechanism of arsenite detoxification.

Soil with long-term arsenic contamination may result in the evolution of highly diverse arsenite-resistant bacteria. Bacteria capable of both arsenite oxidation and arsenite efflux mechanisms had an elevated arsenite resistance level and hence can be used as potential candidate for bioremediation. Further, suitable *in situ* methodologies may be developed for the isolated strain and there is potential of improvement in strain by genetic engineering.

5. DESCRIPTION OF THE RESEARCH WORK

5.1 THE RESEARCH PROBLEM

The research focused upon the isolation of arsenite tolerant bacteria from contaminated soil sample followed by the characterization of the bacteria. Further, the arsenite detoxifying mechanism was explored in them.

5.2 THE METHODOLOGIES EMPLOYED

1. Soil Sampling and its Physico-chemical Characterization:

Soil samples were collected from different places and at various depths from a textile dyeing industry in a previously ethanol cleaned polypropylene zip locked bags. Soil samples were physico-chemically characterized for pH, electrical conductivity, organic carbon, organic matter, exchangeable calcium, water holding capacity and metal content (APHA, 2005; Maiti, 2003).

2. Isolation of Arsenite Resistant Bacteria:

For isolation of arsenite tolerant bacteria from the contaminated soil samples serial dilution method was applied. Soil samples were serially diluted and then inoculated in nutrient broth supplemented with increasing concentration of sodium arsenite. Pure colonies were obtained with repeated spreading, streaking and Gram's staining.

3. Determination of Minimum Inhibitory Concentration:

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration that completely inhibits bacterial growth (Courvalin *et al.*, 1985; Muller *et al.*, 2003). MIC for purified bacterial strains against arsenite was determined in nutrient broth amended with different concentrations of sodium arsenite. Cell density was measured by measuring the culture turbidity using a spectrophotometer (Systronics UV-Vis. Spectrophotometer-106) at 600nm.

4. Preliminary Determination of Arsenite Oxidizing Ability:

Isolated strain showing the maximum MIC was subjected to the following preliminary tests to study its ability of oxidizing arsenite to arsenate-

a. Silver Nitrate Test: The oxidizing ability of the isolate was checked by using silver nitrate method. Arsenite, after reacting with silver nitrate, give a yellow precipitate of silverorthoarsenite, while arsenate generates brown coloured precipitate of silver-orthoarsenate exhibiting arsenite oxidation. Twenty four hour culture growth agar plates with sodium-meta-arsenite (1 g/L concentration) were flooded with a solution of 0.1M $AgNO_3$ and were incubated in dark for 24 hours along with a control plate. A brownish precipitate on flooding the plate with silver nitrate solution revealed the presence of arsenate in the medium (arsenite oxidizing bacteria), while the presence of arsenite was detected by a bright yellow precipitate (Lett, *et al*, 2001; Krumova *et al*, 2008).

b. **Microplate screening assay:** Qualitative estimation of arsenic species transformation in the culture was carried out by Microplate Screening Assay (MSA) using silver nitrate. The presence of arsenate in the culture, its pellet as well as supernatant along with control was detected in microplate by incubating at 37°C for 4 days. Development of brown color precipitate in the well showed the presence of arsenate (Mokashi and Paknikar, 2002; Simeonova *et al.*, 2004; Krumova *et al*, 2008).

c. **Paper chromatography:** The two arsenic species were separated by running a chromatograph in an isopropanol:water (7:3) solvent system. The two forms of arsenic, arsenite and arsenate were distinguished by spraying 0.1 M silver nitrate reagent. Violet spot revealed for arsenate and yellow spot for arsenite (Mokashi and Paknikar, 2002). The retardation factor (R_f) values are calculated.

5. 16S rDNA Sequencing and Biochemical Characterization of the Isolated Strain:

The strain capable of arsenite oxidation was further identified by 16S rDNA sequencing. DNA was isolated from the culture using QIAamp DNA Purification Kit **(**Qiagen). The 16S rDNA gene fragment was amplified by PCR from genomic DNA using 16S rDNA gene universal primers: 8F and 1492R (Sacchi *et al.*, 2002; Maniatis *et al.*, 1989) 8F: (5’ AGA GTT TGA TCC TGG CTC AG 3'), 1492R: (5' ACG GCT ACC TTG TTA CGA CTT 3') and a single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel. The concentration of the purified DNA was determined and was subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). The 16S rDNA gene sequence was used to carry out BLAST with the non redundant database of NCBI genbank database. Phylogenetic analyses were conducted in MEGA4 (Tamura *et al.*, 2007).

The sequencing results were supported by biochemical characterization from Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994).

6. Study of Metal and Antibiotic Sensitivity:

The disc diffusion method of Huysmans and Frankenberger (1990) was used to screen the isolated strain for resistance to various metals like Cadmium chloride, Cobalt (III) nitrate, Lead nitrate, Nickel chloride, Zinc sulphate, Mercuric chloride, Chromium (III) chloride, Sodium selenate, Stannous chloride and Antimony (III) chloride at the concentration of 100 µg/ml. further MIC was determined for bacterial strain against various metals was determined in nutrient broth amended with different concentrations of metal salts. Cell growth was measured by measuring the culture turbidity using a spectrophotometer (600 nm) for the metals like Cadmium chloride, Chromium (III) chloride, Lead nitrate, Mercuric chloride and Nickel chloride.

Isolated strain was screened for resistance to various antibiotics such as: Erythromycin (15 mcg); Penicillin G (10 µg); Oxacillin (1 µg); Cephalothin (30 µg); Clindamycin (2 µg); Amoxyclav (30 µg); Tobramycin (10 µg); Co-Trimoxazole (25 µg); Cephotaxime (30 µg); Ampicillin (10 µg) and Gentamicin (10 µg). Antibiotic sensitivity was established on the basis of zone of inhibition around the disc.

7. Standardization of Growth Conditions:

For standardization of optimum growth of the isolate, following parameters were studied:

a. Temperature: The standardization of optimum temperature for growth of the isolate was done using variable temperature as 4°C, 30°C, 37°C, 45°C and 60°C. It was grown in nutrient broth at different sets of temperatures.

b. **pH:** The standardization of optimum pH for growth of the isolate was done using variable pH as 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The isolated strain was grown in nutrient broth at different pH.

c. **Media:** The standardization of optimum media for the growth of the isolated strain was done using Minimal Media and Chemically Defined Media. Culture was grown in minimal media with and without sodium-meta-arsenite at the concentration of 1 g/L as described by Santini *et al.*, 2000 and Lugtu *et al.*, 2009. Carbon source that is lactose monohydrate at different concentrations (10 g/L, 15 g/L and 20 g/L) was also added to the minimal media. Culture was grown in chemically defined media with and without sodium-meta-arsenite at the concentration of 1 g/L as described by Weeger *et al.*, 1999 and Liao *et al.*, 2011.

8. Confirmatory Determination of Arsenite Detoxifying Ability (Oxidation):

For detecting the arsenite oxidizing ability of the strain following methods were employed:

a. Molybdene Blue Method: The pellet of the strain was suspended in 0.1 g/L arsenite solution for a period of 72 hours (0, 24, 48 and 72 hours) along with appropriate controls was estimated for the quantitative oxidation of arsenite to arsenate by molybdene blue method. The basic principle involved in this method is that initially arsenate can react with molybdate to form a complex and then gets reduced by ascorbic acid to produce blue color under conditions of certain acidity and temperature, while arsenite does not react under the same conditions. The blue complex has an absorbance peak at 838 nm and can be measured by spectrophotometric method (Cai *et al.*, 2009).

b. Anion Exchange Chromatography followed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS): The analytical technique for measuring inorganic arsenic species in the culture involves separation of different chemical forms of arsenic by solid phase extraction followed by detection of arsenic species by ICP-MS. The cultures used for Sodium Molybdene Method were further used for separation of arsenic species by anion exchange chromatography. The tubes containing eluted arsenate by anion exchange chromatography were further quantified by ICP-MS.

c. **Genetic basis of oxidation of arsenite:** The isolated strain was checked for the presence of *aox* gene which is responsible for arsenite oxidation at molecular level. This was performed by PCR amplification of *aox* genes using specific primers (Chang *et al*. 2010, Quemeneur *et al.*, 2008).

Statistical Analysis: The results of each experiment performed in triplicate are represented as mean±standard error. The results were compared using Student's t-test and significance level were considered at $p<0.001$ as highly significant, $p<0.05$ as statistically significant and $p<0.01$ as significant.

5.3 OBSERVATIONS AND RESULTS

1. **Soil Sampling and its Physico-chemical Characterization:**

   Four soil samples were collected at different distances (100-200 m) and various depths (0-40 cm) from a textile dyeing industry and agricultural land adjoining the textile effluent channel in Sanganer area, Jaipur. The physical and chemical properties of the soil samples were determined. The pH of the samples ranged from 7.27-8.61 and Electrical Conductivity ranged from 0.356-2.58 mS. Range of organic carbon and organic matter observed was 0.396 ± 0.019% to 1.924 ± 0.086% and 0.683 ± 0.181% to 3.317 ± 0.087% respectively. The minimum Ca^{2+} present in the soil samples determined was 42.08 ± 3.532 mg/L while maximum was 543.08 ± 15.57 mg/L. The minimum Mg^{2+} present in the soil samples was 28.1 ± 2.144 mg/L while maximum was 254.11 ± 8.33 mg/L. The fluoride content of the samples ranged from 3.405-4.43 mg/g of soil. The water holding capacity ranged between 43.8-71.0%. The arsenic content in samples ranged from 68-464 mg/kg of the soil sample. The iron, magnesium, lead and zinc content were also determined in the soil samples and were in variable ranges.

2. **Isolation of Arsenite Tolerant Bacteria:**

Four arsenite resistant bacterial strains were isolated from soil sample number 3 and 4 which were collected at the depth 10-40 cm and distance of 200 meters from textile effluent channel. These soil samples showed neutral pH and medium range of electrical conductivity, exchangeable calcium and water holding capacity.

These soil samples were also found contaminated with arsenic ranging between 68-84 mg/kg of soil. All the isolated strains were studied for their colony morphology and Gram’s reaction. Morphologically bacterial colonies were yellow and cream colored having circular shape with smooth or lobulated margins. All the bacterial strains were found to be Gram’s positive bacilli.

3. **Determination of Minimum Inhibitory Concentration:**

The isolated four resistant strains exhibited MIC in range of 3-9 g/L of sodium arsenite (23.09-69.2 mM). The strains namely IB-1 and IB-2 isolated from sample number 3 showed the MIC of 8 g/L and 5.5 g/L respectively. Strains IR-1 and IR-2 isolated from sample number 4 exhibited MIC of 9 g/L and 3 g/L respectively.

4. **Determination of Arsenite Oxidizing Ability:**

Oxidizing ability of the strain exhibiting highest MIC was determined qualitatively by silver nitrate test followed by microplate screening assay and it was further confirmed by performing paper chromatography.

The strain showed arsenite oxidizing activity by developing brown color precipitate in the culture plate after supplementation with 0.1 M silver nitrate in silver nitrate test. In microplate screening assay, presence of brown color precipitate when treated with silver nitrate indicated the presence of arsenate in the culture pellet as well as in the supernatant exhibiting the oxidation of arsenite to arsenate. The paper chromatographic separation of 24 hour nutrient broth supplemented only with arsenite indicated the presence of arsenite as well as arsenate (oxidized product) in the media which was confirmed by comparing their R_f value for arsenite and arsenate with standard arsenite and arsenate solution. No significant difference was observed when R_f values of standards of arsenate and arsenite were compared with arsenic forms present in the culture.

5. 16S rDNA Sequencing and Biochemical Characterization of the Isolated Strain:

Based on nucleotide homology and phylogenetic analysis of the 16S rDNA gene sequence, the bacterial strain, IR-1 with highest MIC of 9 g/L was identified as *Microbacterium paraoxydans* strain CF36 (GenBank Accession Number: NR_025548.1).

The bacterial strain was tested for its morphological and biochemical properties using *Bergeys manual of Derterminative Bacteriology*, 1994. The strain appeared as rods in cultures arranged singly or in pairs. It was found to be Gram's positive, non acid fast and negative for spore staining (non-spore forming). It was observed that the strain was aerobic and catalase positive. Acid was not produced from carbohydrates like sucrose, dextrose, xylose, glucose and arabinose in carbohydrate fermentation test. The strain exhibited negative result for oxidase and nitrate reductase tests.

6. Study of Metal and Antibiotic Sensitivity:

Microbacterium paraoxydans isolated from soil contaminated with arsenic, lead, zinc and iron exhibited resistance not only to arsenite but also to heavy metals like cobalt, cadmium, mercury, lead, nickel, zinc, chromium, selenium, stannous at the concentration of 100µg/ml of each. The MIC was determined for the selected heavy metals for the isolate. It was found to be highest for chromium (1.6 g/L) than the other metals employed under study namely cadmium (0.7 g/L), lead (1.2 g/L), mercury (0.7 g/L) and nickel (1.4 g/L). It was found to be resistant to antibiotic cephalothin (30 µg). It was found to be resistant to antibiotic cephalothin (30 µg) and sensitive to all others.

7. **Standardization of Growth Conditions:**

For standardization of optimum growth conditions of the isolate, the parameters studied were temperature, pH and media:

a. **Temperature:** The maximum growth in term of optical density was observed at 37°C as significant difference in growth was observed at 37°C as compared to growth at 30°C and 45°C at significant level of $p<0.01$ and $p<0.001$ respectively. Hence it can be concluded that isolate is mesophilic in nature.

b. **pH:** The optimum pH condition of the medium for growth was found to be neutral to alkaline. Although non-significant difference in growth pattern was observed at pH 7, 8 and 9.

c. **Media:** The strain grew well in nutrient broth, minimal media with carbon source and chemically defined media when supplemented with sodium arsenite. *Microbacterium paraoxydans* grows best in nutrient broth as compared to minimal media and chemically defined media as highly significant difference ($p<0.001$) in growth was obtained when culture was grown in nutrient broth as compared to minimal media and chemically defined media (with arsenite concentration in all the three types of media) whereas no significant difference was obtained when culture was grown in minimal media and chemically defined media when both were supplemented with arsenite concentration. No significant difference was observed in the growth when culture was grown in minimal media supplemented with varying concentration (10, 15 and 20 g/L) of carbon source. Although statistically significant difference ($p<0.05$) in growth was observed when the strain was grown in minimal media with 1g/L of sodium arsenite amended with different concentration of carbon source. On the basis of the nutritional requirement, this bacterium was considered as chemoheterotrophic because it utilizes carbon source when supplemented in media.

8. Confirmatory Determination of Arsenite Detoxifying Ability (Oxidation):

Quantitative determination of the arsenite oxidized to arsenate was done by molybdene blue method and it confirmed the oxidizing ability of the strain. Highly significant ($p<0.001$) increase was observed in the quantity of oxidized product (arsenate) during the first 24 hours of incubation. Statistically significant ($p<0.05$) rise in oxidation was observed from 24 to 48 hours. The results of molybdene blue method were further supported by arsenate estimation by ICP-MS after arsenate extraction by anion exchange chromatography.

The arsenite detoxification mechanism that is its ability of oxidizing arsenite to less toxic arsenate in *Microbacterium paraoxydans* strain CF36 was studied and confirmed. Presence of oxidizing genes *aoxB* and *aoxC* as observed by PCR amplification of the genes showed that the bacterium is able to tolerate arsenite and these *aox* genes may code for the arsenite oxidase enzyme responsible for the oxidation. *aoxB* codes for the large molybdopterin subunit of enzyme, arsenite oxidase and *aoxC* encodes for cytochrome c required in oxidation.

6. CONCLUSIONS

The soil samples from which the arsenite hypertolerant bacteria were isolated were contaminated with arsenic and other heavy metals like lead, iron, zinc and magnesium. The pH was neutral or alkaline and with high Electrical Conductivity. Out of four isolates *Microbacterium paraoxydans* strain CF36 exhibited high MIC of 9 g/L. This strain posses multi-metal resistance as well as resistant to one antibiotic namely cephalothin. The strain grows best in nutrient broth supplemented with sodium arsenite at neutral pH and 37°C at 120 rpm in shaker incubator. On the basis of the nutritional requirement, this bacterium was considered chemoheterotrophic because it utilizes carbon source when supplemented in media. *Microbacterium paraoxydans* strain CF36 also possess detoxifying ability of arsenite by the process of oxidation of arsenite to arsenate which is on an average 100 times less toxic form of arsenic. The arsenic oxidizing ability of the strain as determined by presence of *aoxB* which codes for enzyme, arsenite oxidase and *aoxC* which encodes for cytochrome c which gives it advantage to survive in environment with arsenite as it possess the property of arsenite detoxification. This gram positive mesophilic bacteria growing best in nutrient broth at pH 7 can be used to develop method of arsenic detoxification.

7. LIST OF PUBLICATIONS BASED ON THE RESEARCH WORK

Published Research Papers

- **Title:** Arsenic Hyper-tolerance in Four *Microbacterium* Species Isolated from Soil Contaminated with Textile Effluent

  Name of the Journal: *Toxicology International*

  Year: 2012 **Volume:** 19 **Issue:** 2 **Page:** 188-194 **ISSN:** 09716580 **H Index:** 3

  Authors: Pallavi Kaushik, **Neha Rawat,** Megha Mathur, Priyanka Raghuvanshi, Pradeep Bhatnagar, Harimohan Swarnkar and Swarn Flora

- **Title:** Arsenic Resistant *Microbacterium* Species and Evolution of Arsenic Resistance in Bacteria Isolated from Soil Receiving Textile Discharge

  Book: Microbial, Plant and Animal Research

  Series: Biotechnology in Agriculture, Industry and Medicine, Microbiology Research Advances

Published by: Nova Science Publishers, USA. [Proceedings of conference (ICMPAR, 2012) by Thomson Publishing House]

Year: 2013 **Section:** I (Microbial Research) **Chapter:** 2 **Page:** 9-16

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International Conference Articles

- **Rawat, N.,** Mathur, M., Kaushik, P. and Bhatnagar, P. (2010). Characterization of Fluoride Hyper-tolerant *Microbacterium* sp. CQ0110Y. In Abstract book- XXIX International Conference of International Society for Fluoride Research (ISFR-2010): 147.

- Kaushik, P., **Rawat, N.,** Mathur, M. and Bhatnagar, P. (2010). Antagonistic effect of Fluoride to Arsenite [As(III)] toxicity in arsenite oxidizing *Microbacterium paraoxydans* strain CF36. In Abstract book- XXIX

- International Conference of International Society for Fluoride Research (ISFR-2010): 155.

- **Rawat, N.,** Mathur, M., Kaushik, P. and Bhatnagar, P. (2011). Characterization of Arsenite oxidizing *Microbacterium paraoxydans* strain CF36. In Abstract book– Indo-Swiss Collaboration in Biotechnology (ISCB) International Conference: 100-101.

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- **Rawat, N.,** Mathur, M., Kaushik, P., Krishna Mohan, M. and Bhatnagar, P. (2011). Multi-metal resistance in *Microbacterium paraoxydans* strain CF36 isolated from metal contaminated soil. In Abstract book-52nd Annual Conference of Association of Microbiologists of India (AMI), International Conference on Microbial Biotechnology for Sustainable Development: 37.

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- Kaushik, P., Mathur, M., **Rawat, N.,** Krishna Mohan, M. and Bhatnagar, P. (2011). Antagonistic effect of Fluoride on arsenite hyper-tolerant *Microbacterium paraoxydans* strain CF36. In Abstract book-52nd Annual Conference of Association of Microbiologists of India (AMI), International Conference on Microbial Biotechnology for Sustainable Development: 35.
- **Rawat, N.,** Mathur, M., Kaushik, P., Krishna Mohan, M., Flora, S.J.S. and Bhatnagar, P. (2011). Heavy metals tolerance by *Microbacterium paraoxydans* strain CF36 and its bioremediation approach. In Abstract book-XXXI Annual Conference of Society of Toxicology (STOX), India & International Symposium on Current Trends in Environmental Toxicology: 148.
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- Mathur, M., **Rawat, N.,** Kaushik, P., Krishna Mohan, M., Flora, S.J.S. and Bhatnagar, P. (2011). Reduced Arsenite toxicity by coexposure of Fluoride in Arsenite hyper-

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- Mathur, M., **Rawat, N.,** Saxena, T., Kaushik, P., Krishna Mohan, M., Flora, S.J.S. and Bhatnagar, P. (2012). Antagonism of Fluoride and Arsenite in *Microbacterium paraoxydans* strain CF-36. In Abstract book- International Conference on Microbial, Plant and Animal Research (ICMPAR-2012): 131-132.
- Kaushik, P., **Rawat, N.,** Mathur, M., Saxena, T., Flora, S.J.S. and Bhatnagar, P. (2012). Arsenic resistant *Microbacterium* species and Evolution of arsenic resistance in bacteria isolated from Soil receiving textile discharge. In Abstract book- International Conference on Microbial, Plant and Animal Research (ICMPAR-2012): 78-79.

National Conference Articles

- Kaushik, P., **Rawat, N.,** Mathur, M., Krishna Mohan, M. and Bhatnagar, P. (2011). A bioremediation approach for arsenic contaminated site by arsenite oxidizing *Microbacterium paraoxydans* strain CF36. In Abstract book- National Conference on Contemporary trends in Biological and Pharmaceutical Research (CTBPR-2011): Oral Presentations CT-B020 1-2.
- **Rawat, N.,** Mathur, M., Kaushik, P., Krishna Mohan, M. and Bhatnagar, P. (2011). Arsenite oxidizing *Microbacterium* sp. CQ0110Y exhibiting Arsenite and Fluoride antagonism. In Abstract book- National Conference on Contemporary trends in Biological and Pharmaceutical Research (CTBPR-2011): Poster Presentations CT-B021 29-30.

- Speciation Chemistry of Arsenic- National Conference on "Symposium for Celebrating International Year of Chemistry and Centenary Year of Marie Curie Nobel Prize" (2011)
- **Rawat, N.,** Mathur, M., Saxena, T., Mobar, S., Kaushik, P., Krishna Mohan, M., Flora, S.J.S. and Bhatnagar, P. (2012). Environmental Sustainability- Heavy Metal tolerance by Bacterium. In Abstract book- Interdisciplinary National Conference on sustainable Rural Development in India: Efforts and Challenges. 79.
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