World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

REMOVAL OF ARSENIC FROM ARSENIC CONTAMINATED WATER THROUGH *LACTOBACILLUS SPOROGENES*

Ranjit Kumar[1]*, Pratibha Kumari [2], Shalini Kumari [3], Arun Kumar[1], Mohammad Ali[1] and Ashok Ghosh[1]

[1]Resaearch Centre, Mahavir Cancer Sansthan and Research Centre, Patna.
[2]Banasthali University, Rajasthan.
[3]B.B.R.A. Bihar University, Muzaffarpur, Bihar.

***Corresponding Author: Dr. Ranjit Kumar**
Resaearch Centre, Mahavir Cancer Sansthan and Research Centre, Patna.

Article Received on 16/04/2017 **Article Revised on 05/05/2017** **Article Accepted on 26/05/2017**

ABSTRACT

Arsenic contamination in the groundwater was reported worldwide. The major cause of human arsenic toxicity is from contamination of drinking water from natural geological sources. Microbiota plays an important role in human health, not only due to its participation in the digestion process, but also it plays role in immune system. Lactobacillus was introduced into different concentration of arsenic. 15 million spores and 30 million spores were added in 200 ppb, 100 ppb and 50 ppb respectively in six falcon tubes for 2 hours incubation period at 37℃ in incubator. Incubation with 15 million and 30 million spores of lactobacillus caused marked reduction in arsenic level it was decreased to 26.51 ± 1.139 ppb and 21.49 ± 1.605 ppb from 50 ppb, 43.16 ± 1.06 ppb and 29.54 ± 1.30 from 100 ppb and 80.89 ± 1.48 ppb and 67.09 ± 1.07 form 200 ppb. Arsenic levels were decreased to almost sixty percent in almost all concentration of arsenic after two hours of incubation with lactobacillus. It is concluded from study that lactobacillus causes effective removal of arsenic from water samples. When number of spores were increased removal of arsenic from water sample are more effective.

KEYWORD: Microbiota, Spores, Lactobacillus, Immune.

1. INTRODUCTION

Arsenic contamination in the groundwater was reported worldwide and it causes many diseases in people residing in these countries.[1] Arsenic contamination has been found in the States of Bihar, Uttar Pradesh, Jharkhand, Assam, Chhattisgarh and Andhra a Pradesh.[2] The major cause of human arsenic toxicity is from contamination of drinking water from natural geological sources.[3] Chronic arsenic exposure is a risk factor for ischemicheart disease.[4] Blackfoot disease is a form of severe peripheral vascular disease associated with systemic atherosclerosis due to arsenic toxicity.[5] Different types of neurologic deficits in adults and in children were reported due to long use of arsenic contaminated water.[6] Clinical signs of gastrointestinal irritation, including nausea, vomiting, diarrhoea and abdominal pain, are observed in all cases of short term high dose and longer term lower dose exposures to inorganic arsenic.[7,8] Long term arsenic exposure has been reported to cause a malignant transformation of human keratinocytes in vitro.[9]

Occupational exposure of arsenic among workers in a glass plant in India whose levels of blood arsenic were five times higher than in the control group were reported with increased DNA damage in leukocytes.[10] Many mechanistic studies of arsenic toxicity have suggested that reactive oxygen species and reactive nitrogen species were generated during arsenic metabolism in living cells.[11] Microbiota plays an important role in human health, not only due to its participation in the digestion process, but it also plays role in the development of the gut immune system.[12] Probiotics also have antimutagenic, anticarcinogenic, hypo-cholesterolemic, antihypertensive, anti-osteoporosis and immuno- modulatory effects.[13] Probiotics have been susceptible to wide range of pH conditions and moisture.[14, 15]

L. sporogenes are unique among probiotics in that it possesses a protecting, spore-like protein covering, which allows it to survive stomach acid, arrive at the small intestine, germinate, and grown. This protein is also helpful in anchorage and binding.[16]

Thus the present study is designed to know *In Vitro* effect of *Lactobacillus sporogenes* on removal of arsenic from ground water samples which have high arsenic contamination.

2. MATERIALS AND METHODS

2.1 Chemical used

Arsenic stock were prepared using standard of arsenic (1000µg/ml) provided by Perkin Elmer [(CAS No. :- As 7440-38-2)].

Arsenic stock of 200 ppb is prepared by As standard and it was diluted gradually to get 100ppb and 50ppb solution.

2.2 Probiotics Used

Lactobacillus sporogenes from Sanzyme (P) Ltd. were used as probiotics (Mfg Lic No.:-41/UA/LL/SC/P-2011) containing 150 million spore per gram.

2.3 Experimental Design

Lactobacillus was introduced into different concentration of arsenic. 15 million spores and 30 million spores were added in 200 ppb, 100 ppb and 50 ppb respectively in six falcon tubes for 2 hours incubation period at 37°C in incubator.

After completion of incubation period the mixture were filtered using whatman filter paper No:40 and CAT No: 1440-125.

The absorbance of filtrate and arsenic stock solution is taken on Atomic Absorption Spectrophotometer (Perking Elmer "PINAcle-900") at 193.7°A.

3. RESULTS

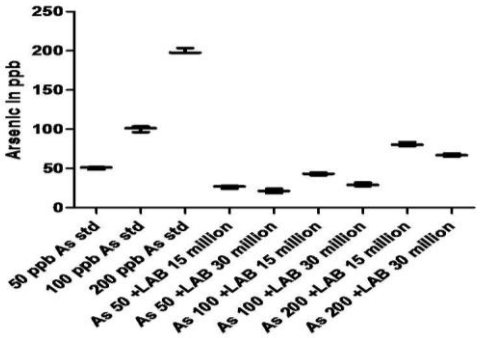
Stock solution of arsenic were prepared in different known concentration of 50 ppb, 100 ppb and 200ppb and analyzed on Atomic Absorption Spectrophotometer (AAS). It was 50.73 ± 0.944, 100.4 ± 2.15 and 199.5 ± 1.93 gradually (Graph – 1,).

Samples of 50 ppb arsenic water were incubated at 37 °C for two hours with 15 million and 30 million spores of *Lactobacillus sporogenes*. Then arsenic level in water samples was gradually decreased to 26.51 ± 1.139 ppb and 21.49 ± 1.605 ppb respectively (Graph - 2).

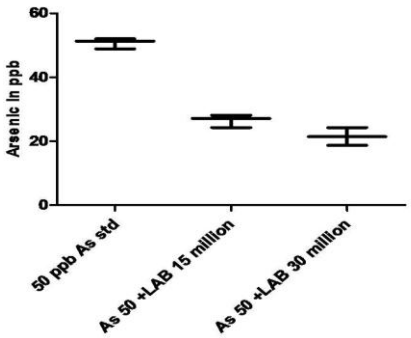
Samples of 100 ppb arsenic water were incubated at 37 °C for two hours with 15 million and 30 million spores of *Lactobacillus sporogenes*. Then arsenic level in water samples was gradually decreased to 43.16 ± 1.06 ppb and 29.54 ± 1.30 ppb respectively (Graph - 3).

Samples of 200 ppb arsenic water were incubated at 37 °C for two hours with 15 million and 30 million spores of *Lactobacillus sporogenes*. Then arsenic level in water samples was gradually decreased to 80.89 ± 1.48 ppb and 67.09 ± 1.07 ppb respectively (Graph - 4).

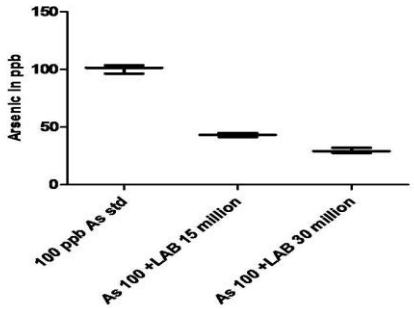
Graph -1: Arsenic level in different samples after 2 hours of Incubation



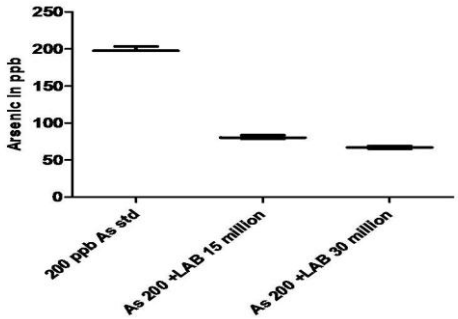
Graph -2: 50 ppb Arsenic level in different samples after 2 hours of Incubation



Graph- 3: 100 ppb Arsenic level in different samples after 2 hours of Incubation



Grap- 4: 200 ppb Arsenic level in different samples after 2 hours of Incubation



4. DISCUSSION

Natural contamination of groundwater with arsenic is a global health problem especially in India and Bangladesh.[17,18] There may be some degree of skin absorption of trivalent arsenic oxide since it is more lipid-soluble than the pentavalent form and causes dermal pigmentation and keratosis.[19] Arsenic is a protoplasmic poison due to its effect on sulphydryl group proteins of cells interfering with many cellular enzymes and mitosis.[20]

Resistances of poisonous metals in bacteria perhaps reproduce the degree of environmental contamination with these substances and may be straight connected to exposure of bacteria to them.[21]

The metals have an effect on microorganisms by reducing their number, diversity, biochemical activity and changing the community structure.[22] In spite of this many bacteria develop resistance to many metals and even involved in degradation of these metals.[23]

The therapeutic benefit of *L. Sporogenes* is due to secretion of bacteriocin and coagulin, which is active against a broad spectrum of enteric microbes.[24] As probiotic microorganisms should support the balance of enteric micro flora, which can be altered by antibiotics.[25] Probiotic lactic acid have potential to remove mixed concentrations of heavy metals lead, cadmium and copper from water with different degrees and also observed that from binary metal solutions containing lead, cadmium, zinc, nickel, cobalt, potassium, sodium, calcium and magnesium[26]. In our study we observed effective removal of arsenic through *Lactobacillus sporogenes*. In only two hours of incubation almost fifty percent arsenic were removed. *Lactobacillus sporogenes* contains surface-associated proteins, which make up around 80% of predicted secreted proteins.[27] Secreted proteins can be covalently attached to the cell surface by sortase-mediated reactions or non-covalently attached with trans-membrane anchors, lipid anchors and different cell wall binding domains. In addition to an N-terminal signal peptide, it also contains a conserved LPXTG motif at their C-terminal, that is followed by a stretch of hydrophobic residues and a positively charged tail. Bacteria are surrounded by a complex cell envelope that performs a variety of functions.[28] Cell envelopes are varied in structure, but all contain layers of peptidoglycan (PG), a cross-linked matrix of linear carbohydrate chains connected to one another via covalent bonds between attached peptides.[29] These may be associated with heavy metal binding and its removal. In our study when number of spores were increased removal of arsenic from water sample are more effective. Which supports the hypothesis that cell wall of lactobacillus has specific charged protein which was involved in removal of arsenic from water samples.

A number of microorganisms containing the *ars* genetic system and are capable of using the reduced inorganic form arsenite and the oxidized form arsenate in their metabolism, hence capable of resisting arsenic toxicity by the *ars* genetic system.[30,31] Our study also supports that lactobacillus not only resisting arsenic toxicity but it also removes fifty percent of arsenic in only two hours.

5. CONCLUSIONS

It is concluded from study that lactobacillus causes effective removal of arsenic from water samples. When number of spores were increased removal of arsenic from water sample are more effective. Which supports the hypothesis that cell wall of lactobacillus has specific charged protein which was involved in removal of arsenic from water samples. *Lactobacillus sporogenes* removes fifty percent of arsenic from water in only two hours.

6. ACKNOWLEDGEMENT

The authors are thankful to Mahavir Cancer Institute and Research Centre for providing infrastructural facility during this work. We are also thankful to our entire research team who provided us every support during this study.

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