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IN VITRO ELIMINATION OF ARSENIC FROM WATER THROUGH *LACTOBACILLUS SPOROGENES*

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ABSTRACT

The problem of the occurrence of arsenic in groundwater has gained worldwide attention and studies in high-exposure regions of the world have established health issues including skin lesions, melanosis and arsenicosis. Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health. *Lactobacillus sporogenes* is a gram-positive, spore-forming, lactic-acid producing bacillus. It is used in prevention and cure of viral, bacterial and antibiotic or radiotherapy induced diarrheas, immunomodulation and even blood cholesterol reduction. The present study is designed to understand effect of *Lactobacillus sporogenes* on In-Vitro elimination of arsenic from water. Arsenic samples of 100 ppb, 200 ppb and 500 ppb is freshly prepared and 50,000 spores were introduced into each culture in one arm study in second arm 50,000 spores were introduced into these samples with nutrient agar as culture medium. It is observed that lactobacillus without culture media eliminate arsenic effectively only in 24 hours while lactobacillus with nutrient agar media causes more effective elimination of arsenic from water sample in 48 hours. It is concluded from study that *Lactobacillus sporogenes* causes effective In Vitro elimination of arsenic from water. When Lactobacillus is introduced with Nutrient agar culture is eliminate arsenic more effectively indicates that whenever *Lactobacillus sporogenes* grows properly arsenic adhere with its growing cell wall and eliminated with the bacterial colony. *Lactobacillus sporogenes* removes more than 90% of arsenic from water samples effectively.

KEYWORDS: *In Vitro*, *Lactobacillus sporogenes*, bacteria, Diarrhea, Spores.

1. INTRODUCTION

The problem of the occurrence of arsenic in groundwater has gained worldwide attention, and studies in high-exposure regions of the world have established its human carcinogenicity (Smedley and Kinniburgh, 2002). Additional health effects have been increasingly linked with arsenic exposure, including associations with diabetes, heart disease and H1N1 viral susceptibility (Kozul et al., 2009).

Major regions affected with arsenic are in the river basin of the Ganga, Brahmaputra and Meghna in India and Bangladesh. It was estimated that 25 million people in Bangladesh and 6 million people in West Bengal, India were exposed to arsenic contaminated groundwater (Chakraborti et al, 2002). In India, though cases of arsenic toxicity including liver fibrosis due to drinking of arsenic contaminated water were reported from Chandigarh in early 1978 (Data et al, 1979), occurrence of large number of cases of arsenic induced skin lesions were reported from Kolkata, West Bengal in 1984 (Garai et al, 1984). Arsenicosis, melenosis and cancer cases were reported from Tilak rai ka hatta of Buxar district, Bihar (Kumar et al, 2016).

Human exposure to naturally occurring arsenic in some world regions is significant because of the use of arsenic-contaminated groundwater as a primary source of drinking water (Chiou et al., 2001; Guo et al., 2004)

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (Salminen *et al*,1998). *Lactobacillus sporogenes*is a gram-positive, spore-forming, lactic-acid producing bacillus. *L sporogenes* is considered a semi-resident, indicating it takes up only a temporary residence in the human intestines. *Lactobacillus* have been suggested to be associated with alleviation of lactose intolerance (Ouwehand 1999); prevention and cure of viral, bacterial and antibiotic or radiotherapy induced diarrheas (Parvez *et al.*, 2006); immunomodulation (Forsythe and Bienenstock, 2010); antimutagenic (Chalova *et al.*, 2008) and anti-carcinogenic effects (Liong, 2008); and even blood cholesterol reduction (Ooi and Liong, 2010).

Outermost layer of lactobacilli contains the S-layer which remains in direct contact with bacterial environment and involved in many of their surface properties. Indeed, different studies found lactobacilli S-layer proteins to mediate bacterial aggregation as well as adhesion to epithelial cells and to intestinal components like mucus or extracellular matrix proteins. It also inhibit the growth and the activity of pathogens (Golowczyc et al, 2007, Jakava and Palva 2007). This facilitates binding of heavy metals with these surfaces. This study is performed to understand effect of *Lactobacillus sporogenes* on in Vitro elimination of arsenic from water.

2. MATERIALS AND METHODS

2.1: Chemical Used: Sodium arsenate, Sigma
2.2: Probiotics used: *Lactobacillus sporogenes*,
2.3: Culture Medium Used: Nutrient Agar, Merk

Standard sample of arsenic is prepared in 100ppb, 200ppb and 500ppb concentrations. Lactobacillus sporogenes spores were introduced in these in 50,000 spores, 1,00,000 spores and 2,50, 000 spores for 24 and 48 hours in nine falcon tubes. Three for standard, three for 24 hours degradation assay and rest three for 48 hours study. In second arm of experiment Lactobacillus sporogenes were introduced in arsenic 100ppb, 200ppb and 500ppb sample with Nutrient agar as culture medium in nine falcon tube. The falcon tube is kept at 37^{o}C for 24 hours and 48 hours.

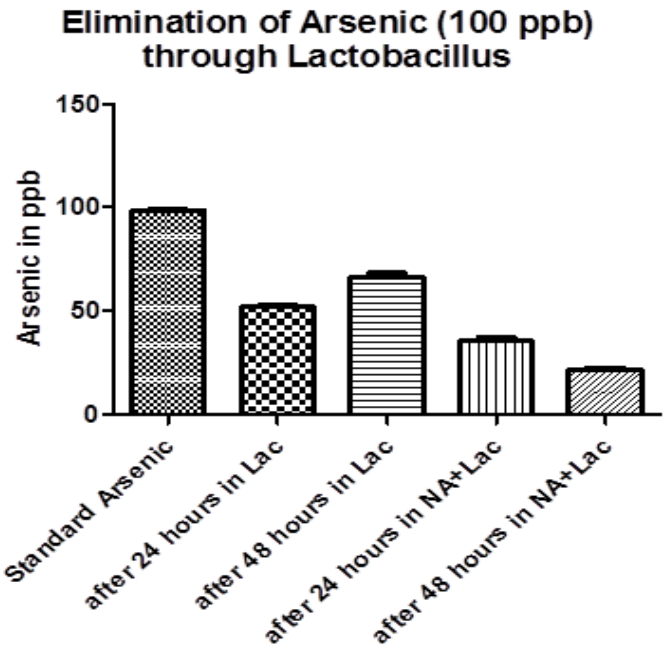
On completion of schedule of 24 and 48 hours mixture of probiotics and arsenic is filtered through filter paper. Probiotics is filtered out from falcon tube which only contains *Lactobacillus sporogenes*. Nutrient agar is also filtered out with Lactobacillus in second group of experiment. The samples were analyzed on Atomic Absorption Spectrophotometer of Perkin Elmer of our laboratory using standard protocol.

3. RESULTS

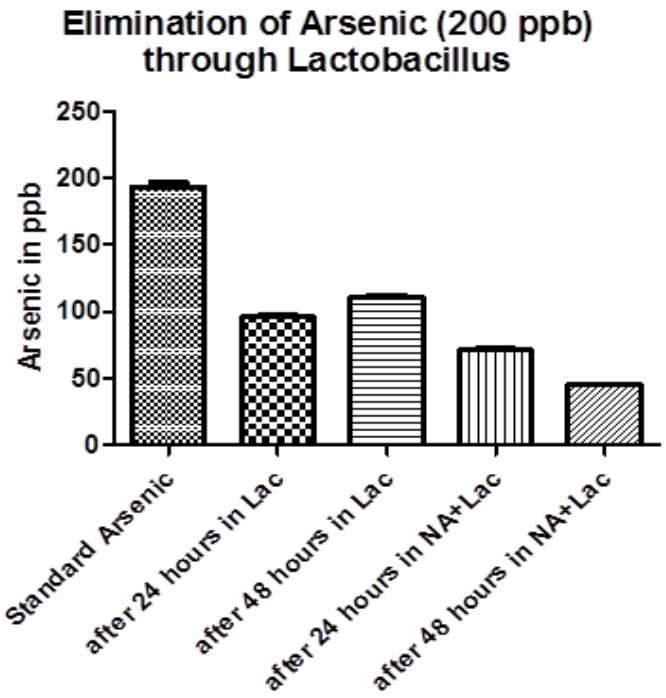
In arsenic standard solution slight changes were observed. 100 ppb were observed 98.1 ppb after 24 hours and 96.8 after 48 hours of study. While it was 48 ppb after 24 hours and 64 after 48 hours when 50,000 spores were applied on 100 ppb sample. When culture of Lactobacillus sporogenes were applied with culture media in Nutrient agar marked elimination in arsenic level were observed with 28 ppb after 24 hours and 16 ppb after 48 hours (Text Figure: 1).

200 ppb were observed 194.7 ppb after 24 hours and 192.6 ppb after 48 hours of study. While it was 91 ppb after 24 hours and 108 after 48 hours when 50,000 spores were applied on 100 ppb sample. When culture of Lactobacillus sporogenes were applied with culture media in Nutrient agar marked elimination in arsenic level were observed with 74 ppb after 24 hours and 36 ppb after 48 hours (Text Figure: 2).

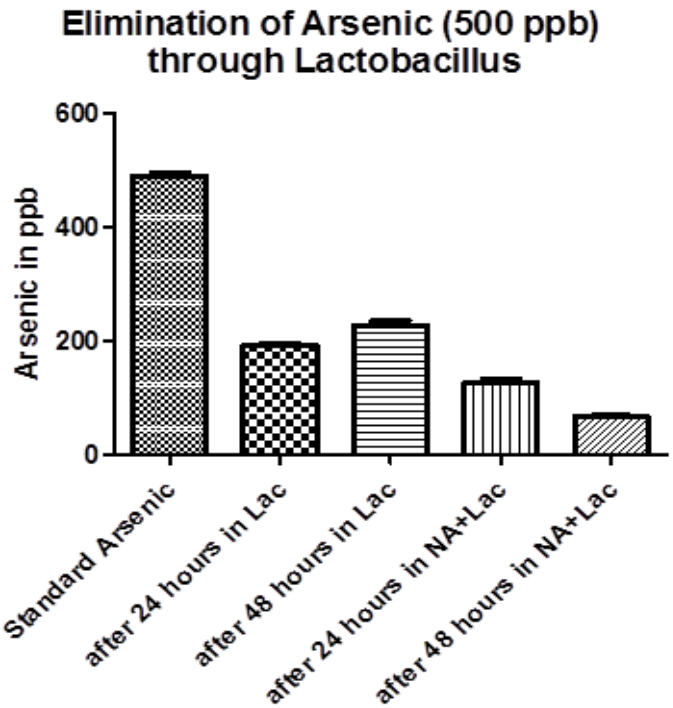
500 ppb were observed 489.3 after 24 hours and 485.1 after 48 hours of study. While it was 185 ppb after 24 hours and 213 after 48 hours when 50,000 spores were applied on 100 ppb sample. When culture of Lactobacillus sporogenes were applied with culture media in Nutrient agar marked elimination in arsenic level were observed with 89 ppb after 24 hours and 68 ppb after 48 hours (Text Figure: 3).



Text Figure: 1



Text Figure: 2



Text Figure: 3

4. DISCUSSION

Bacteria are known to develop heavy metal resistance mostly for their survivals, most of resistant bacteria were found in environmental strains. Bacterial plasmids contain specific genes for resistances to various toxic heavy metal ions (Silver and Ji, 1994).

Uses of probiotic bacteria such as Lactobacilli represent an exciting prophylactic treatment in the prevention of various gastrointestinal disorders including antibiotic-associated diarrhea, infectious bacterial diarrhea, inflammatory and irritable bowel diseases. Probiotics provide protection to intestinal health and are known to convert toxic forms of heavy metal ions into their less

toxic forms followed by detoxification (Rolfe, 2000; Shrivastava et al., 2003). Blocking bio absorption of metal is critical to preventing toxicity. Researchers have shown that the gut micro biota does play a role in metal bioavailability (Reichardt et al, 2011).

Geomicrobiology deals with microbial processes interact with geological and geochemical processes. Studies in the early 1980s (Beveridge and Koval, 1981) explained how Bacillus subtilis was able to interact with a range of toxic metals including copper, iron, magnesium, gold and lead. This ability was attributed to differences between the net negative charge of bacteria and the cationic charge of many metals. The theory stated that nucleation sites on the cell surface had the ability to bind metals of opposite charge. Once bound to the cell wall this resulted in a nucleation site where a large concentration of metals could bind and precipitate on the cell wall (Beveridge, 1989).

In the human intestinal tract the presence of commensal bacteria including species of *Lactobacillus* may be playing an important role in binding these metals and preventing their absorption. It also protects intestinal epithelial layer from increased toxin and pathogen translocation (Farmer et al, 2011 and Madi et al 2010).

Lactobacilli are known to be able to enhance intestinal barrier function (Moorthy et al, 2009). There are more than hundred strains of lactobacillus and many of them effectively remove heavy metals from their surrounding water and soil. The non-pathogenic nature of lactobacilli thereby represents a potentially safe and practical way to sequester metals ingested in food (Johnston et al, 2011). Gram-positive bacteria have high adsorptive capacity, particularly the Bacillus, due to high peptidoglycan and teichoic acid content in their cell walls. Gram-negative bacterial cell membranes are lower in these components and are poorer metal absorbers (Gavrilescu, 2004). In our study we also used Gram positive bacteria *Lactobacillus sporogenes* which effectively absorbs more than 90% of arsenic from water samples and eliminate arsenic from the samples.

Lactobacillus causes microbial detoxification of mercury (Robinson et al, 1984). Lactobacillus causes biosorption of copper by wine (Schut, 2011). *Lactobacillus acidophilus* causes removal of arsenic trioxide from waste water (Singh and Sharma, 2010). Amplification of arsH gene in *Lactobacillus acidophilus* makes it resistant to arsenic toxicity (Sinha et al 2011). Cadmium and lead is reversibly bind and removed through surface of bifidobacterium and removes these heavy metals (Teemu et al, 2008). In our present study we observed that cultures of *Lactobacillus sporogenes* in Nutrient agar causes more effective elimination in arsenic from water samples in 48 hours in different concentration of arsenic.

It is not inconceivable that home or community-based yogurt containing lactobacilli able to remove arsenic could be of practical use in countries like India and Bangladesh (Monachese et al, 2011, Reid, 2010).

5. CONCLUSIONS

It is concluded from study that *Lactobacillus sporogenes* causes effective In Vitro elimination of arsenic from water. When Lactobacillus is introduced with Nutrient agar, it eliminates arsenic more effectively indicates that whenever *Lactobacillus sporogenes* grows in optimum condition, arsenic adheres with its growing cell wall and eliminated with the bacterial colony. *Lactobacillus sporogenes* removes more than 90% of arsenic from water samples effectively.

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