Arsenic bioremediation by *Lactobacillus acidophilus* from water in weightlessness condition

Maryam Salavatifar[1*], Zahra Afsharian[2], Kianoush Khosravi_Darani[3]

[1*]Aerospace Research Institute, Ministry of Science, Research and Technology, Tehran, Iran
[2] Department of Food Science and Technology, National Nutrition and Food Technology Research Institute, Faculty of Nutrition Science and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Research Department of Food Technology Research, National Nutrition and Food Technology Research Institute, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Abstract

The pollution of water with heavy metal ions has generated great concern for public health due to the high toxicity, persistence, and non-degradability. Arsenic has been added to the list of heavy metals due to its carcinogenicity and toxicity. There is an emerging trend of employing microorganism's bioremediation of heavy metals, due to several benefits including their ability to heavy metal binding and arresting. Earth gravity is an influential physical force under which life appeared and developed. There are several reports about the effect of gravity removal on some characteristics of living organisms, including microorganisms. In this study bioremediation ability of *Lactobacillus acidophilus* for arsenic ions in water was evaluated. In addition, the effect of thermal and alkaline pretreatment of *L. acidophilus* on the amount of metal bioremediation was also investigated. Furthermore, simulated gastrointestinal effects were evaluated on stability of *L. acidophilus*-heavy metals complex. The results showed that *L. acidophilus* can bind to arsenic and reduce its concentration in water. In addition, it's ability for arsenic adsorption enhanced following heat-pretreatment in weightlessness as well as normal gravity. Furthermore, *L. acidophilus* ability for arsenic bioremediation was lower in weightlessness at first, but, the stability of the complex in simulated gastrointestinal conditions was higher in weightlessness compared to normal gravity. Thus, the results of our study support the idea of using bacteria to solve arsenic pollution.

Keywords: *"Arsenic", "Heavy metal", "weightlessness", "Lactobacillus acidophilus".*

1. Introduction

Heavy metal ions contain a large portion of industrial and feedlot water wastes. Releasing these heavy metal ions into rivers, and the ocean severely damages the environment, eventually leading to the pollution of food sources. Consuming polluted food can cause illness, even leading to death. Beyond legal standards, these heavy metal ions can lead to irreparable damage to public health [1].

Among these heavy metals, arsenic has been introduced as a threat to human health. Arsenic is also called as "king of poisons" because of its potency and the discreetness [2]. It is naturally present at high levels in the groundwater of several countries. Contaminated water used for drinking, food preparation and irrigation of food crops poses the greatest threat to public health from arsenic [3]. WHO recommended limit of arsenic in drinking water is 10µg/L [2]. Long-term exposure to arsenic from drinking-water and food can cause cancer and skin lesions. It has also been associated with cardiovascular disease and diabetes. In utero and early childhood exposure has been linked to negative impacts on cognitive development and increased deaths in young adults. The most important action in affected communities is the prevention of further exposure to arsenic by provision of a safe water supply [3-5].

Gut microorganism and its metabolites in addition to modifying absorption, metabolism of heavy metals, oxidative stress, and modulating the pH act as a physical barrier and regulate detoxification enzymes or protein expression. The detoxification mechanism of heavy metals by gut microorganism is carried out via the binding of metallic ions to the cell wall of bacteria. Further, they transformed from a more toxic form to less toxic [6]. The Lactobacillus species have a high adsorption capacity for heavy metals because of the large amounts of exopolysaccharides (peptidoglycan and teichoic acids) in their cell walls (Fig. 1) [7]. Many studies have shown that Lactobacillus has a high tolerance and tendency to absorb heavy metals in water and food [8].

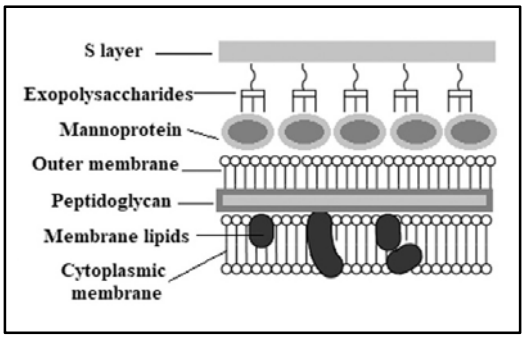

Fig. 1: Cell wall structure of lactic acid bacteria

1. Assistant professor, P.O. Box: 1465774111, Fax and Tel: 02188366030, *Salavati@ari.ac.ir*
2. Master of Science
3. Professor

Gravity is one of the most important physical factors in the environment of the earth, which has a great impact on them. Microorganisms such as other organisms have emerged and developed under the influence of gravity. Gut microorganism, like other organisms, have been affected by this force and have adapted to that. Any alterations in gravity have led to changes in gut microorganism composition, as well as, growth rate, secondary metabolites production, biofilm formation, pathogenicity, and gene expression of them [9]. Recent studies have shown that gravity reduction or removal can affect the physiology and morphology of microorganisms. One of the consequences of gravity changes on microorganisms can be related to human health [10].

There are not enough reports about the gravity removal (weightlessness) effect on the bioremediation of heavy metals by *Lactobacillus* bacteria. In this study, the effect of weightlessness condition as well as heat and alkaline pretreatments have been investigated on bioremediation of arsenic from water by *L. acidophilus*. Also, the stability of *L. acidophilus*-heavy metal complexes under simulated gastrointestinal tract (GIT) conditions was evaluated.

2. Materials and Method

L. acidophilus ATCC 4356 was taken from Tak Gene Zist, Tehran, Iran. It was routinely aerobic cultured in MRS broth at 37°C for 24 h seed culture was prepared with 5 cc of master culture to 50 cc MRS broth and incubated at 37°C for 48h [11]. *L. acidophilus* cell autoclaved 20 min at 121°C for Heat pretreatment. The NaOH pretreatment *L. acidophilus* cell at the first mixed 0.1 N NaOH at 37°C for 1h, then samples were centrifuged to remove supernatants. Bacterial cells were washed triple time with sterile distilled water and centrifuged them. Then bacterial cells were ready to removal of arsenic solution [12].

At the first, 700 µg of arsenic (10 mgL^{-1} in HCl 2%) was mixed with 9.3 ml of sterile deionized water and adjusting pH to 4 by HCl, then added *L. acidophilus* to a solution (2.6 $\times 10^{12}$ $CFU.ml^{-1}$) [13]. Finally, this solution was incubated for 24h at 37°C under weightlessness on clionostat. One-axis clinostat was used (UN00SA, USA) for weightlessness, which rotates samples perpendicular to the direction of the gravity vector (Fig.2). Clinostat was placed in an incubator at 37°C. The falcons were filled with samples without any bubbles that disrupt weightlessness. Then samples were well-fixed around the center, and rotational speed was adjusted to 15 rpm [14, 15].

Fig. 2: Clinorotation of samples

In order to investigation the strength of *the L. acidophilus*-arsenic in the body, a simulation of the digestive system was prepared. Simulated gastric juice was prepared by pepsin (3 $g.L^{-1}$)(Sigma-Aldrich, Darmstadt, Germany) in a sterile NaCl (0.5 % w/v) and adjusting the pH to 2 using HCl. Simulated small intestinal juice was prepared by pancreatin (1 $g.L^{-1}$) (Sigma-Aldrich, Darmstadt, Germany) and bile salt (1.5 $g.L^{-1}$) (Sigma-Aldrich, Darmstadt, Germany) in sterile NaCl (0.5 % w/v) and adjusting the pH to 8 with 0.1 mol/L NaOH. Both gastric and small intestinal juices were sterilized using 0.45-µm membranes filter (Nalge Co., Rochester, USA). After 24 h of biosorption under weightlessness, 10 ml of each metal-bacteria solution was inserted to 40 ml of simulated gastric juice then vortexed (Vortex Genie 2, Scientific Industries, Bohemia, USA) for 10 s, and incubated at 37°C for 2h under weightlessness conditions. After sampling for arsenic analysis, 10 ml of gastric solution, was added to 50 ml of simulated small intestinal juice and incubated at 37°C for 2h under weightlessness conditions, and repeated sampling for arsenic analysis [16].

In this study, the amount of arsenic concentration was measured by ICP-MS (Perkin Elmer ELAN 6100 DRC-e). Device conditions for testing were included, Nebulizer Gas Flow: 0.69 L/min, ICP Radio Frequency (RF) generator power: 1100W, Lenz Voltage: 6V, Analog Stage Voltage: -2300V, Pulse Stage Voltage: 1600V.

In this study all experiments were carried out in triplicate. Statistical analysis was performed by SPSS software, version 22 (IBM SPSS, Armonk, NY, USA) and graphs were drawn using GraphPad Prism, version 9, (GraphPad Software, USA). Results were used in one-way analysis of variance (ANOVA) to estimate p-values and confidence levels. For all data $p\text{-values} < 0.05$ were considered.

3. Results and Discussion

Pretreating Lactobacilli cells affects the bioremediation process. Treatment method leads to alteration in bacterial surface arrangement, and therefore alter adsorption manner [17]. Lactobacillus strains heat treatment enhances heavy metal bioremoval because of cell surface change. Indeed, heat expands heavy metal binding sites and facilitate absorption [18]. Due to existence of polysaccharides, peptidoglycans, teichoic

acids, and proteins in *L. acidophilus* cell wall, heat pretreatment may reduce the thickness and structure. Furthermore, denaturation of proteins occurred with heat. As shown in figures 3, 4, and 5, the bioremediation of arsenic by *L. acidophilus* in liquid phases after 24 h of exposure to untreated, heat and NaOH pretreated, had significant differences between natural gravity and weightlessness conditions. There were significant differences in bioremediation of all conditions *(p-values < 0.05)*.

As shown in Figs.3, in untreated *L. acidophilus* of weightlessness conditions, no significant differences between initial concentration of arsenic and after 24hr exposure. Also, it was reported that in both conditions (weightlessness and normal gravity), the lower liquid phases of arsenic concentration was observed in Heat-treated *L. acidophilus* cells. Heat-treated *L. acidophilus* for removing arsenic in weightlessness and normal gravity conditions 31.4% and 91.28%, respectively. The reason for this was exposed more hydrophobic regions to bind to the toxins which are due to denaturation of the cell wall proteins in *L. acidophilus* strains and establishment of Maillard reaction products. Several studies have shown similar results [19, 20]. It has been reported that an increments in surface activity and kinetic energy of the solute with an increase in temperature causes the removal of heavy metals [21].

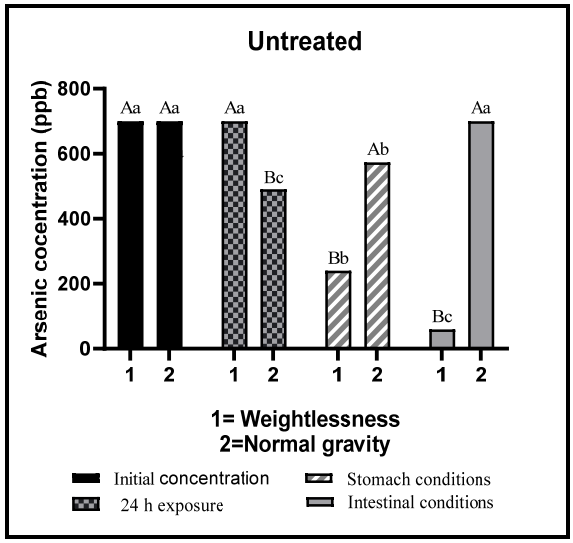

Fig. 3: Concentration of arsenic after 24h exposure of Liquid phases with *L. acidophilus* in weightlessness and normal gravity as well as simulated gastrointestinal conditions (untreated). Error amount 5% different small letters among the same samples, differ significantly (*P < 0.05*). Capital letters between weightlessness and normal gravity conditions, differ significantly (*P < 0.05*).

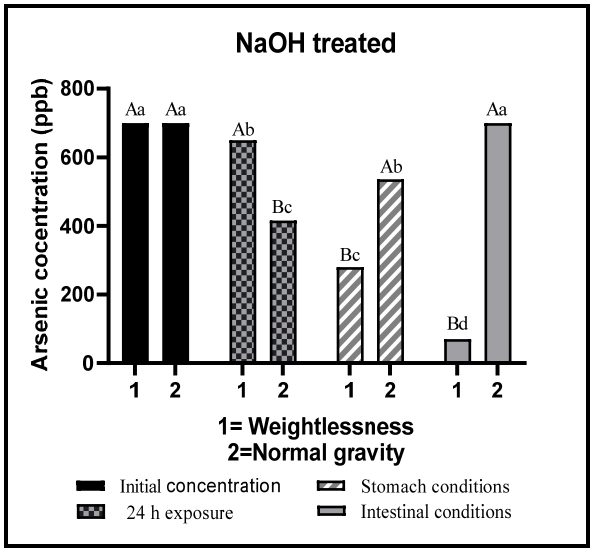

Fig. 4: Concentration of arsenic after 24h exposure of Liquid phases with *L. acidophilus* in weightlessness and normal gravity as well as simulated gastrointestinal conditions (NaOH- treated). Error amount 5% different small letters among the same samples, differ significantly (*P< 0.05*). Capital letters between weightlessness and normal gravity conditions, differ significantly (*P < 0.05*).

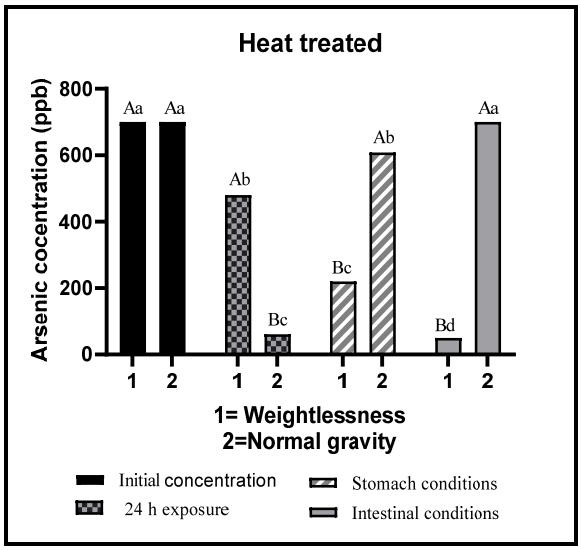

Fig. 5: Concentration of arsenic after 24h exposure of Liquid phases with *L. acidophilus* in weightlessness and normal gravity as well as simulated gastrointestinal conditions (Heat-treated). Error amount 5% different small letters among the same samples, differ significantly (*P < 0.05*). Capital letters between weightlessness and normal gravity conditions, differ significantly (*P < 0.05*).

Alterations in arsenic concentration after 2 h exposure to simulated gastric juice and 2 h exposure to the simulated small intestinal condition were shown in Figs.3, 4 and 5 too. As shown in Fig. 5, considering that Heat-treated *L. acidophilus* was powerful to reduce the concentration of arsenic in normal gravity conditions, but after simulated stomach conditions more bonds were released and it could not maintain any arsenic

binding. Also, no significant differences between initial concentration and intestinal conditions in all treated *L. acidophilus* (untreated, heat, and NaOH treated) in normal gravity conditions. These results were similar to the Petruzzi et al results [22]. However, in weightlessness conditions, the best ability of arsenic bioremediation belongs to heat- treated *L. acidophilus* were after simulated stomach conditions (68.57%) and after simulated intestinal conditions (92.85%).

In general, simulated intestinal conditions were enhanced bioremediation of arsenic in weightlessness conditions by treated and untreated *L. acidophilus*. According to previous studies, environmental conditions, pH, temperature, microbial strains, cell wall structure and surface charge are the factors affecting the metal removal by microorganisms [13, 23, 24].

According to the results, bioremediation of heavy metals using *L. acidophilus* was somewhat reversible under simulated GIT conditions in normal gravity conditions. It might be due to the simultaneous chemical and physical adsorption in the heavy metal adsorption process. Zoghi et al., reported reversibility of complex indicating the importance of non-covalent electrostatic bonds (hydrogen and Van der Waals bonds) [17].

The GIT treatment results of weightlessness conditions showed considerable performance of *L. acidophilus* to arsenic bioremediation. So that in the simulated intestinal conditions had the highest absorption of heavy metals compared to the simulated stomach conditions. This differences may be due to the effect of pH on the arsenic removal. According to studies, the lowest heavy metal removal occurs at pHs below 2-3 and the highest Heavy metal removal occurs at pHs above 3 (especially at pH 4-6) [21].

Alteration in gravity can lead to changes in the cells. Thus, gravity removal is an especially environmental condition. Some of the considerable effects of this environment for cells include reduction or elimination of shear stress and low-turbulence environment. Such conditions lead to changes in microorganisms, such as changes in growth rate, changes in the production of secondary metabolism, changes in pathogenicity, changes in resistance to environmental stresses, including antibiotics, changes in genetic, and changes in morphology and physiology are some of these [25, 26]. In the human body, environments with low shear stress and low turbulence due to weightlessness were found [26].

4. Concluding Remarks

Food and water heavy metal contamination is the major problems for public health. Removal of these types of contaminations by safe strategy is very important. Bacterial application can be a solution in removing these pollutions. Exopolymeric substances in bacterial outer membrane are capable to efficiently bound to positively charged heavy metals and arresting them [27]. Any factor that increases the thickness of the polymeric outer material of the bacteria will improve the heavy metal removal capacity. Due to the increase of foreign polymeric materials of some bacteria in microgravity conditions compared to natural gravity [28], in this research arsenic bioremediation by *L. acidophilus* was investigated. The results showed that bioremediation of arsenic was lower in weightlessness compare to normal gravity, but stability of complex was highest in weightlessness on GIT condition. The results of this research can be used in the food industry.

5. References

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