Confidence Interval Analysis of Lead Bioremoval from Water by Lactobacillus Acidophilus in Simulated Microgravity

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Abstract

Lead as a heavy metal, is a harmful environmental pollutant which have high toxic effects to many body organs. Even though lead can be absorbed from the skin, it is mostly absorbed from respiratory and digestive systems. Lead is a highly toxic metal whose widespread consumption has caused extensive environmental contamination and health problems. There is an emerging tendency of using microorganism's bioremediation of heavy metals, due to several benefits including their ability to heavy metal binding and arresting as well as efficiency, relatively low cost and minimal negative effects for the surrounding environment. Earth gravity is an influential physical force under which life appeared and developed. There are several reports about the effect of simulated microgravity on some specifications of living organisms. In this study bioremediation ability of Lactobacillus acidophilus for lead in water was measured. In addition, the effect of alkaline and thermal pretreatment of L. acidophilus on the rate of lead bioremediation was also studied. Furthermore, simulated gastrointestinal effects were measured on stability of L. acidophilus-heavy metals complex. The results showed that L. acidophilus can bind to lead and decrease its concentration. In addition, highest amount of absorption was done under natural gravity conditions without any treatment. The results standard deviation was calculated with 95% confidence interval. Therefore, simulated microgravity has no possitive effect on lead absorption by L. acidophilus. Although, L. acidophilus ability for lead bioremediation was lower in simulated microgravity, but, the stability of the complex in simulated gastrointestinal conditions was higher in simulated microgravity.

Keywords: Heavy metal; *Lactobacillus acidophilus*; Lead; Microorganism; Simulated microgravity.

Introduction

Heavy metals are commonlly a specific density of more than 5 g/cm3 and have negative effect on environment and organisms. Heavy metals are basic to maintain various biochemical and physiological functions in living organisms when in very low concentrations, however they become noxious when they exceed certain threshold concentrations. Heavy metals are significant environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons [1]. The most commonly found heavy metals in waste water include arsenic, cadmium, chromium, copper, lead, nickel, and zinc, all of which cause risks for human health and the environment [2]. Heavy metals enter the surroundings by natural means and through human activities. Various sources of heavy metals include soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insect or disease control agents applied to crops, and many others [3].

Lead (Pb) as a heavy metal is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Lead is a bright silvery metal, slightly bluish in a dry atmosphere. It begins to tarnish on contact with air, thereby forming a complex mixture of compounds, depending on the given conditions. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water and domestic sources. The sources of lead were gasoline and house paint, which has been extended to lead bullets, plumbing pipes, pewter pitchers, storage batteries, toys and faucets [4]. Part of this heavy metal is taken up by plants, fixation to soil and flow into water bodies, hence human exposure of lead in the general population is either due to food or drinking water. Lead is an extremely toxic heavy metal that disturbs various plant physiological processes and unlike other metals, such as zinc, copper and manganese, it does not play any biological functions. A plant with high lead concentration fastens the production of reactive oxygen species (ROS), causing lipid membrane damage that ultimately leads to damage of chlorophyll and photosynthetic processes and suppresses the overall growth of the plant [3]. Some research revealed that lead is capable of inhibiting the growth of tea plant by reducing biomass and debases the tea quality by changing the quality of its components [5]. Even at low concentrations, lead treatment was found to cause huge instability in ion uptake by plants, which in turn leads to significant metabolic changes in photosynthetic capacity and ultimately in a strong inhibition of plant growth [6].

Intestinal symbiotic bacteria 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 intestinal symbiotic bacteria is carried out via the binding of metallic ions to the cell wall of bacteria. Further, they transformed from a more toxic to less toxic [7]. The Lactobacillus species have a high adsorption capacity for heavy metals because of the large amounts of exopolysaccharides in their cell walls [8]. Many studies have proved that Lactobacillus has a great tendency and endurance to attract heavy metals in water and food [9].Earth gravity as the most important physical factors, has a great impact on its inhabitants. Microorganisms such as other organisms have emerged and developed under the influence of this force. Intestinal symbiotic bacteria, like other organisms, have been affected by this force and have adapted to that. Any alterations in gravity have led to changes on their composition, as well as, growth rate, secondary metabolites production, biofilm formation, pathogenicity, and gene expression of them [10]. 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 [11]. In this study, the effect of gravity removal as well as heat and alkaline pretreatments have been investigated on bioremediation of lead from water by *L. acidophilus*. Also, lead- *L. acidophilus* complexes durability under simulated gastrointestinal tract (GIT) conditions was measured.

Materials and Methods

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 [12]. *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 lead solution [13].

At the first, 700 μg of lead (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 ×1012 CFU.ml-1) [14]. Finally, this solution was incubated for 24h at 37°C under simulated microgravity on clionostat. One-axis clinostat was used (UN00SA, USA) for simulated microgravity, which rotates samples perpendicular to the direction of the gravity vector (Fig.1). Clinostat was placed in an incubator at 37°C. The falcons were filled with samples without any bubbles that disrupt simulated microgravity. Then samples were well-fixed around the center, and rotational speed was adjusted to 15 rpm [15, 16].

Fig. 1: Clinorotation of samples

In order to investigation the strength of *the L. acidophilus*-lead 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 simulated microgravity, 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 simulated microgravity conditions. After sampling for lead analysis, 10 ml of gastric solution, was added to 50 ml of simulated small intestinal juice and incubated at 37°C for 2h under simulated microgravity conditions, and repeated sampling for lead analysis [17].

In this study, the amount of lead 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-values < 0.05* were considered. The results standard deviation were calculated with 95% confidence interval.

Results and Discussion

Pretreating Lactobacilli cells impressed bioremediation process. Treatment method ultimate to alteration in bacterial surface arrangement, and therefore alter adsorption manner [18]. Due to existence of polysaccharides, peptidoglycans, teichoic acids, and proteins in bacterial cell wall, heat pretreatment may reduce the thickness and structure. Furthermore, denaturation of proteins occurred with heat. As shown in figures 2, 3, and 4, the bioremediation of lead have taken place by *L. acidophilus* in liquid phases after 24 h of exposure to untreated, heat and, NaOH pretreated, and significant differences were found between natural gravity and simulated microgravity conditions. There were significant differences in bioremediation of all conditions (*p-values < 0.05*).

As shown in Fig.2, in untreated *L. acidophilus* of normal gravity conditions, significant differences was found between initial concentration of lead and after 24hr exposure. Also, it was reported that in normal gravity, the lower liquid phases of lead concentration was observed in untreated *L. acidophilus* cells. This reduction was 92% of the initial lead concentration, while in untreated condition, the reduction amount was only 25% in simulated microgravity conditions. As shown in fig 3, the highest lead decrement in simulated microgravity conditions was for NaOH treatment (84.5%). 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].

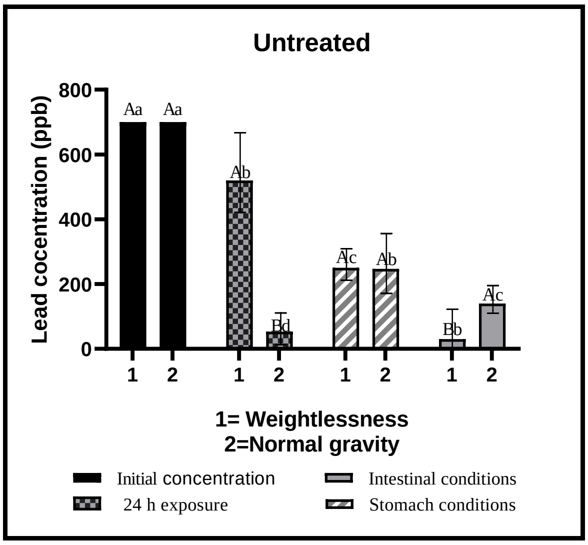

Fig. 2: Concentration of lead after 24h exposure of Liquid phases with *L. acidophilus* in simulated microgravity and normal gravity as well as simulated gastrointestinal conditions (untreated). Error amount 5% different small letters among the same samples, differ significantly (*p-values < 0.05*). Capital letters between simulated microgravity and normal gravity conditions, differ significantly (*p-values < 0.05*).

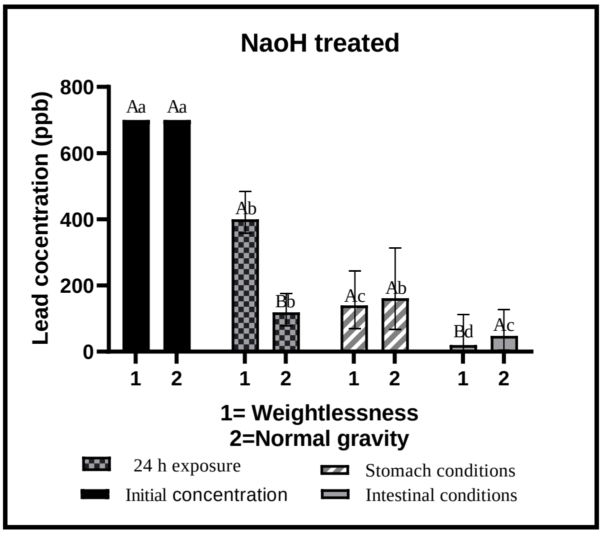

Fig. 3: Concentration of lead after 24h exposure of Liquid phases with *L. acidophilus* in simulated microgravity and normal gravity as well as simulated gastrointestinal conditions (NaOH- treated). Error amount 5% different small letters among the same samples, differ significantly (*p-values < 0.05*).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

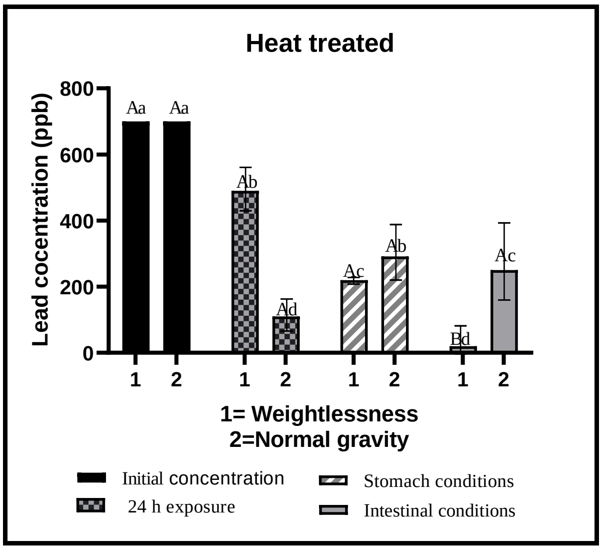

Fig. 4: Concentration of lead after 24h exposure of Liquid phases with L. acidophilus in simulated microgravity and normal gravity as well as simulated gastrointestinal conditions (Heat- treated). Error amount 5% different small letters among the same samples, differ significantly (p-values < 0.05).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

Alterations in lead concentration after 2 h exposure to simulated gastric juice and 2 h exposure to the simulated small intestinal condition were shown in Figs.2, 3 and 4 too. As shown in Figs, despite the negative effect of simulated microgravity on initial metal absorption, bond strength in simulated microgravity was much higher than natural gravity.

In general, simulated intestinal conditions were enhanced bioremediation of lead in simulated microgravity 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 [14, 22, 23].

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) [18].

Gravity removal is an especially environmental condition that maybe have considerable effects on 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 [24, 25]. In the human body, environments with low shear stress and low turbulence due to simulated microgravity were found [25].

Concluding Remarks

Bioremediation of heavy metals by microorganisms have introduced as a solution in water and food decontamination. Bacterial outer membrane are capable to efficiently bound to positively charged heavy metals and arresting them [26]. The results of this project showed that bioremediation of lead was lower in simulated microgravity compare to normal gravity, but stability of complex was highest in simulated microgravity on GIT condition. The results of this research can be used in the food industry.

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