

The Effects of Taheri Consciousness Fields on the Growth of *Escherichia Coli BL21* and Absorption of Lead Heavy Metal by *Saccharomyces Cerevisiae* in Altered Gravity Conditions

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DOI: doi.org/10.61450/joci.v2i12.165

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Abstract

In previous experiments, the growth of different microorganisms under the influence of T-Consciousness Fields (TCFs) was investigated. These fields with non-material and non-energetic properties can have traceable effects on the system under study, and it has been determined that they are different from magnetic, electric, and gravity fields. This study aims to investigate the effects of T-Consciousness Fields on the growth of *Escherichia coli* (*E. coli*) strain *BL21* as a prokaryotic model organism under microgravity (MG) conditions and also to investigate the bioabsorption behavior of the heavy metal lead by *Saccharomyces Cerevisiae*, a eukaryotic model, in MG and Earth's gravity (1G). In these experiments, which were conducted separately, samples without the influence of T-Consciousness Fields were considered as controls, and the duration of the experiment was 24 hours. First, bacterial growth was evaluated by measuring optical density at 600 nm (OD600). The results showed that the microgravity environment increased the growth of bacteria by about 1.4% compared to the earth's gravity (1G). While under the influence of T-Consciousness Fields, no significant difference was observed between MG and 1G samples. In the second study, the inductively coupled plasma mass spectrometry (ICP-MS) technique was used to evaluate heavy metal absorption. The results showed that yeast was able to absorb heavy metal in both gravities and MG environment increasing the absorption significantly and about 13.5% compared to Earth's gravity. Although the effect of TCFs on the MG sample was not statistically significant, it significantly reduced the absorption of lead metal by about 9% in the conditions of the earth's gravity. In conclusion, the microgravity environment increased the growth of this strain of *E. coli* bacteria, and the application of T-Consciousness Fields inhibited this growth induction. Also, these fields affected the eukaryote model differently in 1G and MG gravity. It is suggested to investigate the effect of TCFs on the behavior of prokaryotic and eukaryotic organisms under microgravity stress in further studies.

Keywords: Taheri Consciousness Fields, Microgravity, *Escherichia coli*, Growth, Yeast, Lead

Introduction

Microorganisms can withstand harsh environmental conditions and grow and reproduce even at very high temperatures (1). Therefore, it is important to know if they can live in different stresses of space. In other words, it is necessary to conduct studies on microbes in the space environment to understand their possible risks and maintain the health of the crew in long missions (2). The growth of *Escherichia coli* (*E. coli*) has been repeatedly investigated in microgravity (MG) conditions, and there are various reports about their physiological responses and altered gene expression in this stress (3). The yeast *Saccharomyces cerevisiae* is one of the best-known eukaryotic models. This model has also attracted attention due to its ability to absorb heavy metals (4). Heavy metals can be a threat to living organisms. Today, due to the Industrial Revolution, human life has been affected by the high production of heavy metals. For this reason, several studies have been conducted to investigate their effects on the food chain and its long-term harm (5). Various techniques are used to remove these pollutants, including non-biological methods such as electrochemical technologies and bioremediation methods that rely on the use of microorganisms (6).

In this study, firstly, the effect of the combination of three types of T-Consciousness Fields (TCFs) on the growth of *E. coli* strain *BL21* in MG conditions were investigated, and after observing the results, another experiment was designed on the bioabsorption behavior of lead metal by *Saccharomyces cerevisiae* in two environments of earth's gravity (1G) and MG under the influence of these fields.

Materials and Methods

Using T-Consciousness Fields: according to the explanations given in the considerations section of this issue, the samples were affected by these Fields.

Bacteria culture medium

First, *Escherichia coli* strain *BL21* was cultured using Luria-Bertani (LB) liquid culture medium, which contains (per 100 ml): one g of peptone (QUELAB), 0.5 g of yeast extract (QUELAB), one g of NaCl (Merck, Germany) was used. Then five μL of bacterial stock were cultured in LB liquid culture medium with five μL of ampicillin and incubated in a shaker incubator at 37 °C for 16-18 hours.

Applying microgravity

In this study, microgravity conditions were performed using a clinostat (donated by the United Nations Office for Space Affairs in Vienna to Iran Aerospace Research Institute). Although it is not possible to eliminate the gravity vector on the Earth's surface, this device can provide a plausible example of real microgravity space experiments. For this purpose, the clinostat was sterilized with UV light and ethanol (70%) and then placed in an incubator at 37 °C.

To prevent the formation of air bubbles, the samples were fixed in 50 ml syringes in the circular holder in the clinostat. There were six syringes in the clinostat, three of which were exposed to TCFs for 24 hours, and the rotation speed of the machine was 30 rpm (7).

In the yeast test, the rotation speed of the device was set to 15 rpm (8) and it was performed at a temperature of 27 °C inside a standard incubator. The samples were placed in the incubator next to the clinostat under 1G conditions.

Three types of T-Consciousness Fields were applied to half of the samples simultaneously with the rotation of the clinostat and continued for 24 hours. Samples without TCFs influence in MG medium and earth's gravity conditions were considered as controls. The groups and samples tested on yeast are as follows: a standard sample with specific and initial amounts of heavy metals, a sample under the influence of T-Consciousness Fields in microgravity

conditions (MG-TCFs), a control in microgravity conditions (MG-Control), a sample under the Fields in Earth's gravity conditions (1G-TCFs) and control located in Earth's gravity conditions (1G-Control).

Measuring optical density (OD)

Bacterial cell growth was measured by measuring OD₆₀₀ for samples under the influence of T-Consciousness Fields in Earth's gravity and microgravity. Each measurement was repeated three times. To estimate growth, one milliliter of samples was used.

Yeast culture medium and biomass

S. cerevisiae ATCC 9763 was obtained from the Department of Research and Technology of the Ministry of Science in a lyophilized form at a growth time of 48-72 hours and stored at a temperature of 25-30 °C under aerobic conditions. The culture medium contained one gr of glucose, 0.25 gr of yeast extract, 1 gr of ammonium chloride, 0.75 gr of Na₂HPO₄, and 1.25 gr of KH₂PO₄. All culture components and chemical reagents were purchased in pure form from Merck (Darmstadt, Germany).

These materials were autoclaved separately in three 250 ml Erlenmeyer flasks containing 50 ml of deionized water at a temperature of 121 °C and a pressure of 1.4 atm for 20 minutes. It should be noted that all the empty glass containers were autoclaved for three hours at 180 °C under dry heat. After cooling in sterile conditions, the ingredients were mixed and reached a final volume of 150 ml. Then 100 ml of the prepared culture was poured into a 250 ml sterile Erlenmeyer flask and 10 colonies of yeast were inoculated in the plate.

Then it was placed in a shaker incubator at a temperature of 25-30 °C for 16-20 hours at a speed of 80 rpm so that the yeast grows and reaches the end of the exponential growth stage (main culture). After this period, the culture medium becomes cloudy, which indicates the proliferation of yeast cells. The purpose of this

work is to increase the amount of biomass. The original culture solution was placed in the refrigerator under sterile conditions until the time of biosorption studies.

Biomass colony count

To perform the bioabsorption test, it is necessary to prepare an active seed culture on a daily basis. For this purpose, 10 ml of the original culture (master) was added to 100 ml of prepared culture under sterile conditions and placed in a shaking incubator like the original culture.

The first seed culture was counted, and since the same method and primary culture solution were used in all the yeast biosorption experiments, this first cell count was considered a criterion for the next seed culture. Activated seed culture was counted using the serial dilution method. One ml of seed culture was transferred to nine ml of physiological serum in an innertube and diluted at a ratio of 1:10 and repeated 10 times consecutively. After that, from each dilution, two volumes of one ml were taken and poured into two separate plates. Then Saburo dextrose agar (SDA) was added to the plates at a lukewarm temperature. The plates were rotated clockwise and counterclockwise in the shape of number 8 and then kept in an incubator at 30 °C for 72 hours.

Counting of yeast microcolonies was done with two repetitions and showed an average of 2.5×10^8 CFU/ml. This method is based on the observation of real colonies and accurate counting of *S. cerevisiae* yeast (8).

Standards and solutions

In this study, deionized water was used in all stages to prepare aqueous solutions. To prepare a solution of 10 mg/L from the standard solution of lead (Pb²⁺) with an initial concentration of 1000 mg/L (Merck), first one ml of the standard solution of 1000 mg/L lead was mixed in nine ml of 1% nitric acid and after mixing the solution 100 mg/liter was obtained.

We repeated this process to prepare a solution of 10 mg/liter. In addition, 0.83 μ l of 36% HCl and 0.4 g of NaOH were diluted in 100 ml of deionized water to prepare 0.1 N from these solutions, and 0.5% (w/v) NaCl by adding 0.5 gr of it was prepared in 100 ml of deionized water.

Exposure of yeast to heavy metal

Before conducting the biosorption test, first, all the glass containers were soaked in 15% nitric acid for 24 hours to remove possible metal contamination, then they were washed with deionized water and dried properly.

In addition, the cleaned dishes and tools were sterilized in an oven at 180 °C for three hours. Then the required volume of the spiked solution containing 28 μ g per liter of lead was prepared, and the pH of the solution was adjusted to five by 0.1 N NaOH and 0.1 N HCl, after that 10 ml of the solution containing yeast (seed) was transferred to the Falcon and kept for 10 minutes. It was centrifuged at 4000 g.

The supernatant was carefully decanted to leave one ml of it in the Falcon. Finally, nine ml of spiked solution was added to the solution, and the yeast concentration was 2.5×10^7 CFU/ml. Using syringes, the solution was degassed and then placed in the clinostat device under either microgravity or normal Earth gravity conditions (six syringes in each condition).

In this study, the concentration of heavy metals was measured using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer ELAN 6100 DRC-e, USA) (8).

Statistical analysis

Each experiment was repeated three times. Data were presented as mean \pm standard error, then a two-way analysis of variance followed by multiple comparisons with a 95% confidence interval was performed using GraphPad Prism software (version 9), and significant values, less than 0.05 ($p < 0.05$) were presented.

Results and discussion

Measurement of optical density (OD) showed that in the MG environment, the growth of bacteria without the influence of TCFs (control) increased by 1.4% compared to Earth's gravity (Figure 1). However, no significant change was observed in the samples that were affected by the Fields compared to the earth's gravity.

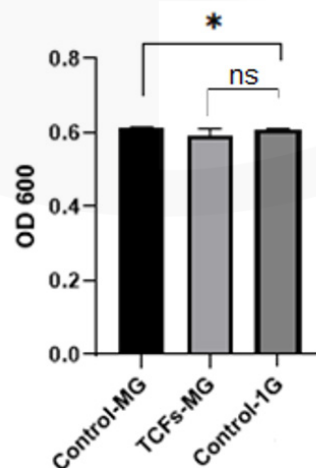


Figure 1. Optical density at 600 nm (OD600) of *Escherichia coli* strain *BL21* under the influence of TCFs in microgravity (MG) conditions and compared to Earth's gravity (1G) at 24 hours. The bar error represents the mean \pm standard error of three replicates. *: p -value < 0.05 in the Brown-Forsythe statistical test. ns: non-significant.

In a previous study, the effect of T-Consciousness Field 1 or Faradarmani on the growth of bacterial population has been confirmed (6). In the present experiment, bacteria were simultaneously affected by three types of T-Consciousness Fields, and it was found that under the influence of these Fields, they grew less in microgravity conditions. *E. coli* bacteria is a part of normal human flora and is known as the best-living organism.

Therefore, studies on this model can expand our horizons regarding its possible responses under microgravity conditions and its potential risks to the health of the space crew. It has been reported that microgravity increases *E. coli* biofilm formation (9). In addition to *E. coli*, a similar experiment was conducted on the eukaryotic model *Saccharomyces cerevisiae*, and the bioabsorption behavior of lead heavy metal by this yeast was investigated in MG and 1G conditions with and without the effect of three types of TCFs. The residual amounts of lead in each experimental group after removing

the yeasts from the medium and comparing it with the standard solution are shown in Figure 2. As can be seen, the absorption of this heavy metal by yeast is confirmed.

Regarding the microgravity environment, these stress conditions have had a positive effect on the absorption of lead metal, so that its absorption has increased by about 13.5% compared to the earth's gravity. It has been found that this eukaryotic single cell can sense MG stress conditions and give cellular responses to this mechanical stimulation, such as changes in morphology, growth rate, and gene expression (10). The application of TCFs had different effects on the biosorption behavior of yeast in two gravities. At 1G gravity, the amount of remaining metal is significantly more than the control sample (about 9%). While, in the microgravity environment, the application of fields does not change the absorption behavior.

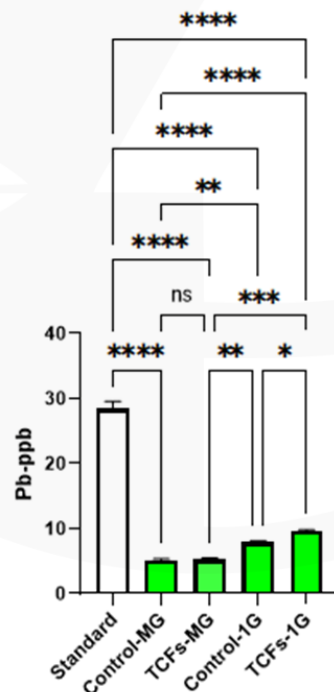


Figure 2. The effect of T-Consciousness Fields (TCFs) on changes in the concentration of residual lead (Pb) compared to the standard value. *, **, *** and **** indicate p. value is <0.05, <0.01, <0.0002 and <0.0001

In addition to the fact that these studies are important to prevent contamination in space missions, they can provide a deeper understanding of life outside the Earth (11). Also, the observation that the application of the T-Consciousness Field inhibited the induction of the growth of *E. coli* bacteria by the microgravity environment, shows that the effect of these Fields is independent of gravity. More studies are needed to understand their mechanism of action.

For example, in this experiment, how to reduce the absorption of the heavy metal lead by yeast in the earth's gravity is unclear and needs more experiments. Therefore, it is suggested to investigate the effect of T-Consciousness Fields on the behavior of prokaryotic and eukaryotic models in microgravity and hypergravity conditions.

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