Adaptive Responses of Escherichia Coli to Space Conditions and Analog Environments: An Updated Review

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Abstract - *Research on E. coli conducted on Earth have identified it as a bacterium capable of rapidly adapting to various environments, such as a free-living organism, in drainage strains, water, and as a commensal in the digestive tract, blood, urogenital tract, and secondary environments. It has been determined that the most populated areas and those at lower altitudes present higher levels of E. coli. However, E. coli samples have been identified from 2610m to more than 5000m above sea level, even surpassing the Kármán line, within the International Space Station. Therefore, it is suggested that bacteria will accompany humans in our exploration of the cosmos. In this review, we summarize the results of recent research on the relationship between humans and microbes, their alteration and adaptation in a microgravity environment, their presence in strategic areas of Mexico, and examine prospects.*

***Key Words*: Escherichia coli, Space, Analog environments.**

1.INTRODUCTION

Microbial surveillance and health monitoring in upcoming space exploration missions will be fundamental for the safety of astronaut crews, as bacteria will accompany us to space [1]. Both human physiology and microbial communities will adapt to the various conditions of spaceflight [2], particularly those that are part of our microbiota. Escherichia coli is of special interest because, even under the strictly controlled conditions of an isolation and confinement environment like space, the human gut microbiota is intrinsically dynamic [3].

E. Coli is a facultative anaerobic bacterium, primarily implicated in gastrointestinal infections and highly adaptable to environmental changes. It has been identified in water from 2,610m to over 5,000m above sea level on Earth. Studies have shown its optimal growth temperature range to be between 35-43ºC, with the capability to survive temperatures exceeding 50ºC [4]. Additionally, there is evidence suggesting an exponential increase in its growth rate in space [5]."

Furthermore, recent research on E. coli in a controlled microgravity environment showed alterations in its metabolism secondary to ionizing radiation, increased growth during specific phases [6–8], changes in its morphology, and enhanced resistance to attacking factors [9]. A thirteenfold increase was observed in the final cell count in space compared to ground controls, and the spaceflight cells were able to grow in the presence of normally inhibitory levels of gentamicin sulfate [1].

However, after the flights, various mutations in E. coli were identified upon their return to Earth. These mutations exhibited variations based on the specific conditions and the timeframe studied. In different mission scenarios, multiple space factors influenced the bacteria in terms of morphology, function, and resistance, leading to a diverse range of mutation types and frequencies [10]. This suggests a need to investigate the short- and long-term effects of E. coli in space and the potential health implications it may have for future space missions.

The International Space Station (ISS) provides an invaluable testing ground for measuring microbial resilience [11]. Indeed, fungi biofilms have been detected on various surfaces of spacecraft, such as windows, pipes, and cables [12] Furthermore, it has been identified that the microgravity condition can modify microbial characteristics, gene expressions, and biofilm formation [13].

However, logistics and operations on the ISS limit the collection of samples both in quantity and frequency. For this reason, it's essential to study Earth's analog environments, such as high-altitude locations and spaces involved in space mission preparations, like spacecraft assembly clean rooms. It's crucial to start characterizing the presence of microbes and their impact on human health.

In this preliminary review, we delve into the latest research on E. coli's adaptation and changes in space, and its interactions with humans. We leverage insights from its presence in key analog research zones in Mexico and highlight prospective avenues for future study.

2. AN IN-DEPTH EXAMINATION OF E. COLI BEHAVIOR AND ITS RESPONSES TO MICROGRAVITY IN SPACE

Microgravity response

After research conducted on the ISS, Zea and Prasad [1] suggest a general increase in the metabolic rate of E.coli. They observed a 69% activation of genes linked to glucose catabolism, including an operon that encodes for thiamine biosynthesis. This activation leads to an overexpression of these genes, resulting in an increase in thiamine synthesis.

Furthermore, the proteomic analysis of E. coli aboard the Shenzhou VIII spacecraft conducted by Zhang [14] identified alterations at the genetic and protein levels, mainly related to biochemical metabolism after spaceflight.

This study reported the downregulation of genes associated with the catabolism of arginine and proline through transcriptomic and proteomic analyses. Since arginine regulates the cellular response to oxidative stress, the decrease in these genes might induce oxidative stress, which could lead to bacterial death [6].

Long-term space missions affects the intestinal microbiome of astronauts, especially the viability of some pathogens [8]. Voorhies [15] identified during the analysis of the astronaut's microbiota on the ISS that pathogen cell adhesion increases under microgravity conditions.

However, for the case of E. coli Nissle 1917, changes in its expression might interfere with its adherence to epithelial cells and prevent intestinal colonization [8]. This makes it less competitive in microgravity, promoting an imbalance in the intestinal microbiota during flights [16].

In response to stress from simulated microgravity, E. coli Nissle 1917 exhibited resistance to heat, osmotic pressure, and acidic environments. During its exponential growth phase, this strain demonstrated elevated expression levels of both heat shock and cold shock proteins compared to its counterparts grown under normal gravity [8].

However, its diminished resistance to acidic stress implies that it may not effectively serve as a probiotic during space flights when taken orally.

Table -1: E. coli responses to microgravity.

Article	**E. coli response to Microgravity**	**Reference**
The impacts of microgravity on bacterial metabolism	1. Alteration in amino acid metabolism. 2. Biofilm formation. 3. Shortening of the lag phase and increase in cell density.	Gayatri Sharma, 2022 [6]
E. coli antimicrobial satellite	1. Size reduction. 2. Thickening and development of vesicles in the cell membrane. 3. Clustering into aggregations.	*NASA's Ames Research Center, 2017 [9]*
Transcriptional profiling of the probiotic escherichia coli nissle 1917 strain under simulated microgravity	1. Low cell adherence. 2. Reduced competitiveness in intestinal microbiota. 3. Resistance to acidic environments.	Jaewoo Yim, 2020 [8]
Microbial growth at hyperaccelerations up to 403,627 x g	1. Shortening of the latency phase. 2. Increase in the duration of exponential growth. 3. Doubling of the final cell density. 4. No induction of the repair system is generated.	Shigeru Deguchi, 2011 [7]

Radiation changes

Rainey [17] suggests that external stress factors might induce the activation of specific genes that promote the organism's adaptation to fluctuating conditions. These changes involve an alteration in the organism's surface characteristics, intensifying the production of EPS (extracellular polymeric substances), and thereby, influencing the auto-aggregation process [18].

An experiment was conducted with water samples from the Rudeau River in Ottawa, where E. coli was exposed to four different doses of low-pressure radiation (20, 40, 60, and 80 mj/cm2). As a result, an increase in the size of the bacteria to up to 10 micrometers was observed (given that its usual size is 2 micrometers), along with auto-aggregation induced by UV rays. It's important to note that at higher radiation doses, the growth of the bacteria was more pronounced [19].

Peltek and Meshcheryakova [20] found that after exposing E. coli to waves of 1 and 2 terahertz (THz), the expression measured by RNA-seq in the irradiated cells increased by more than fourfold. Furthermore, they observed an increase in the number of pili, enhancing their adherence capability, even among the bacteria themselves, through their apical regions. This caused a rupture in the outer membrane and multiple V-shaped invaginations.

Temperature alterations

To understand how the growth curves of E. coli respond to dynamic temperature variations, Dey and Bokka [21] in India exposed E. coli to different temperatures and monitored the results using mathematical models such as the classic logistic.

The tests were conducted in a range of 29-37 °C using the E. coli MG1655 strain. They observed that the increase in the maximum growth rate as the temperature rises within the set range is concurrent with a reduction in the period during which this maximum rate is maintained.

Responses associated with the culture methods

The environment of a spacecraft imposes atypical conditions for terrestrial bacteria, inducing physiological adaptations due to high cellular stress [6]. Therefore, E. coli has been grown in various media, including in suspension, on solid or semi-solid mediums, and in liquid cultures, each of which has yielded different results.

In suspension cultures, there was a higher growth rate and a greater final cell density [22]. With the aim of comprehensively analyzing the impact of spaceflight on various phases of microbial growth (lag, exponential, and stationary), experiments were conducted with in vitro suspension cultures of Escherichia coli during seven missions of the United States space shuttle.

The results indicated that, because of spaceflight, the lag phase shortened, the duration of exponential growth increased, and the final cell population density approximately doubled [23]. In liquid cultures, faster growth and greater biomass production were observed, due to fluid dynamics and the distribution of the liquid medium [22].

Resistance

In 2017, NASA [9] sent E. coli to the International Space Station using a system called EcAMSat to cultivate it and analyze the impact of gentamicin on it. It was observed that the cell count in space was 13 times higher than on Earth, and that the bacteria continued to grow despite the presence of this drug, demonstrating its resistance to the antibiotic.

In the space environment, E. coli reduced its size, and its cell envelope became thicker, enhancing its resistance. Additionally, it developed vesicles on its outer membrane that facilitate cell communication, increasing its virulence. It also demonstrated its ability to cluster into conglomerates, forming protective biofilms.

3. COMPREHENSIVE INVESTIGATION OF E. COLI BEHAVIOR ON EARTH COMPARED TO ENVIRONMENTS THAT SIMULATE SPACE CONDITIONS

This section presents the research conducted to identify the presence of E. coli in high mountain regions, Latin America, Mexico, and in analogous space missions. The aim is to understand space health research as a tool that benefits both space activities and humans on Earth.

High-altitude zones

In 2016, Nicholson and Haye [4] conducted research in the Sagarmatha National Park, Nepal, examining the quality of drinking water and the levels of bacteria present to identify water contaminated with fecal matter. They found that drinking water samples from lower altitudes had higher levels of E. coli and coliform bacteria than samples from higher altitudes, and that the bacterial content of the samples was greater in summer than in winter.

In Kathmandu Valley, Nepal, Malla and Shrestha [24] found that the E. coli levels exceeded the World Health Organization's guideline value for drinking (<1 MPN/100 mL) in 66% of the tested groundwater samples. This suggests that the groundwater is unsuitable for drinking purposes. Additionally, their results showed a seasonal trend in E. coli concentration, with levels increasing in the wet season compared to the dry season. These findings align with studies conducted in Latin America, where E. coli has been identified in waters contaminated with fecal matter, posing a risk for disease outbreaks.

At 2850 m above sea level in Quito, Ecuador, Vinueza [25]identified the presence of E. coli and found that the main rivers in Ecuador display microbial, physicochemical, and metal levels that are unacceptable for preserving aquatic life and freshwater wildlife, as well as for human consumption, bathing waters, and agricultural activities. All samples were collected from urban sites, where the population lived close to the rivers.

Mexico

In Mexico, the presence of E. coli was analyzed in two of the main cities, Mexico City (CDMX) at an altitude of 2240 m above sea level and Guadalajara at 1566 m. In CDMX, Rosas and Salinas [26] assessed E. coli, viewing it as a significant indicator of fecal matter presence, in both indoor and outdoor environments. The frequency of indoor isolation was up to 33%, and 21% of the identified serotypes displayed multi-drug resistance. Furthermore, they suggest that E. coli could be damaged during the aerosolization process.

In Guadalajara, in 2018, Rubino and Corona [27] studied a small sample for the presence of E. coli in water. They found that drinking water in Guadalajara households was contaminated with coliform bacteria and/or had chlorine residues outside regulatory standards.

The presence of E. coli in various regions of the planet suggests that they will be one of the bacteria that will continue with us in space exploration. Therefore, their immediate analysis under isolation and confinement conditions is necessary.

Space analog missions

Analog space missions are multidisciplinary activities that test multiple features of future space missions in an integrated manner to gain a deeper understanding of interactions and system-level [28]. These missions allow for the development and testing of countermeasures that can prevent potentially dangerous situations and, ultimately, help enhance the efficiency and safety of human space missions [29].

The MARS500 project is the longest terrestrial space simulation (520 days), focused on analyzing changes in the microbiota of six analog astronauts under the conditions of a regulated environment. Twenty-seven fecal samples were taken per astronaut. These data suggests that even under the strictly controlled conditions of a closed environment, human gut microbiota is inherently dynamic [3].

Therefore, given the presence of E. coli in human microbiota [30], its behavior and adaptation might be intrinsically dynamic in space. While we cannot reproduce genuine microgravity conditions terrestrially, certain facets of the microgravity environment can be approximated using sophisticated simulators.

However, microgravity experienced in space doesn't equate to the microgravity analogs utilized in earthbound simulators [22]. The rotating wall vessel (RWV) bioreactor has been increasingly utilized to enhance our understanding of microbial responses that might occur during spaceflight l [31], [32].

The RWV (Fig. 1) is an optimized form of suspension culture in which cells are grown under conditions of low shear fluid that are physiologically relevant [2].

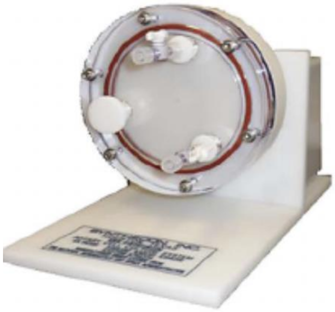

Fig -1: The Rotating-Wall Vessel (RWV) Bioreactor. Modified from [2].

Through the study of various microorganisms grown in the RWV bioreactor, similarities have been observed in responses to spaceflight as well as between simulations [32].

Under the simulated microgravity conditions of the RWV bioreactor, E. coli has demonstrated notable adaptations to environmental stressors. Specifically, the bacteria showcased an augmented capacity to withstand thermal conditions post-cultivation in the RWV [33]. Moreover, when faced with osmotic stress challenges, E. coli exhibited heightened resilience [34].

Additionally, E. coli is more capable of resisting oxidative stress [2]. A summary of E. coli's responses to simulated microgravity conditions within the RWV bioreactors can be found in Table 2, based on various experiments conducted by different authors over several years.

Table -2: E. coli responses to modeled microgravity. Modified from [2]

Microorganism	**Response to Modeled Microgravity within the RWV bioreactor**	**Reference**
E. coli AMS6	Increased biofilm formation and resistance to osmotic, ethanol, and antibiotic stresses	Lynch, 2006 [33]
E. coli O83:H1	Increased resistance to thermal and oxidative stresses and adhesion to epithelial cells	Allen, 2008 [34]
E. coli E2348/69	Increased intimin production	Carvalho, 2005 [35]
E. coli MG1655	Decreased growth and differential gene expression	Tucker, 2007 [36]

In Japan, Deguchi [7] examined the behavior of E. coli under hypergravity using centrifugal acceleration with hyper-accelerations of 3 and 5xg, followed by centrifugation at 1000xg. It was observed that under these conditions, the lag phase shortened (although no cellular growth was observed, size changes were noticeable).

The duration of its exponential growth phase increased, and the final cell density doubled. Meanwhile, in microgravity conditions, microcin B17 was suppressed. This molecule inhibits DNA synthesis, indicating that the repair system wasn't triggered despite its apparent need.

4. DISCUSSION

This section presents the statistics of articles published in international journals between 1997 and 2023. To analyze the number of articles and identify the research trend in this field, an integrative document matrix was developed to provide a data summary.

To initiate the assessment of the studies, relevant keywords were selected. The initial keywords were "E. coli" and "microgravity". Subsequently, the research was narrowed down based on the publication years. After analyzing the studies, additional keywords such as "high mountain", "astronauts", and "bacteria" were identified. The number of articles retrieved is 34, highlighting a consistent increase in research interest in E. coli in space starting from the year 2000.

This suggests a growing interest in the field and underscores the importance of research in understanding changes in the microbiota in space as well as the various responses to the hostile and diverse factors of space. Research articles make up the largest proportion among the different types of studies. This suggests that a significant amount of research in this field is dedicated to presenting original findings and contributing to the existing body of knowledge.

Based on the analysis, several countries stand out as major contributors to the research on E. Coli in space. The United States, Russia, and Japan have the highest number of publications, showcasing their involvement and research output in this field.

4. CONCLUSIONS

After conducting a thorough analysis of the results, considering their specific contexts, it is evident that Escherichia coli demonstrates a notable adaptive capacity in various scenarios, thus optimizing its growth and resistance. These scenarios, which vary from one study to another, include factors such as simulated microgravity, different altitudes, temperature variations, radiation levels, the cultivation methods used, the inherent resistance of the strain, and the nutrient concentrations in the medium.

While this observation is revealing, it underscores the urgent need for more detailed and systematic research. Such studies should be aimed at precisely discerning the genetic, physiological, and morphological adaptations of this bacterium in analog environments. It is especially relevant to mention the importance of its research in Mexico, as it offers a unique geographical and environmental context, which could yield distinctive results with significant implications for the field. In this way, we can gain a deeper understanding of its adaptability in conditions that closely emulate the challenges of space. This is essential not only from a microbiological perspective but also to anticipate and mitigate potential risks associated with space travel and its impact on human health.

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