The Title of the Thesis and the proposed Plan of Work for the Degree of Ph.D. in Science (Food and Nutrition), University of Calcutta

(1000 words synopsis)

Title of the thesis

Effect of Microgravity on Growth, Antibiosis, and Pathogenicity of Probiotic and Pathogenic Bacteria

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Introduction:

For the health of an astronaut and the prolonged survival of man in a space habitat, microorganisms are essential for maintaining the balance between healthy homeostasis and disorder. Understanding the microbial processes behind these consequences is crucial as people, plants, and animals spend increasing amounts of time in space. When in space, microorganisms and their connections with their hosts are transformed. In the influence of space analogs and space travel, some research suggested that pathogenic bacteria and fungi become more virulent due to changes in microbial gene expression, the structure of cells, physiology, and metabolism which are not observed during conventional terrestrial culture conditions (Senatore et al., 2018). An altered environment boosts the microbial secretion of protein known as secretome. Through effective colonization of their host, secreted proteins are essential for many bacteria's pathogenicity (Tsolis et al., 2019). In addition to other factors, from the bottom to the higher part of the body, fluid shifts, which may affect gastrointestinal function (Zwart et al., 2014). This is caused by several effects on crew members, from an impairment in cognitive function to more extensive physiological reactions brought on by microgravity, like hampered cardiovascular function, accelerated muscular atrophy, and weakened immune system (Turroni et al., 2020).

Most studies use the term "microgravity" to describe the "weightlessness" or "zero-g" circumstances that can only be experienced in space. It depends on where it is in a spaceship and its vibration frequency, "microgravity" levels typically range from about 10^{-3} to 10^{-6} g (Huang et al., 2018a). The best way to do biological research in microgravity is with actual spaceflight studies, but due to the high cost and lack of spaceflight possibilities, simulation in Ground-Based Facilities (GBFs) is frequently required. Because of this, several kinds of GBFs with different gravitational theories have been designed to mimic microgravity on Earth. The concept of " simulated microgravity " originated from GBF-based microgravity analogs like Clinostats and Rotating Wall Vessels " (Herranz et al., 2013).

For research on Earth-based microgravity, GBFs are initially developed by the NASA Johnson Space Center (Houston, TX). This device is named a Rotating Wall vessel (RWV). RWVs have been used as an optimized suspension culture technology that can produce Low-Shear Modeled Microgravity (LSMMG). The effects of microgravity on biological samples thorough investigation using RWV analogs, such as Rotating Wall Bioreactors (RWBs), Rotating Cell Culture Systems (RCCSs), and High-Aspect Rotating Vessels (HARVs), which were created using different configurations but similar physical principles (Acres et al., 2021).

Another device is likely used to mimic microgravity on Earth with a small variation in a style called clinostats which is Two dimensional (2D) and three-dimensional (3D). It has been demonstrated that 3D clinostats work significantly at mimicking microgravity than 2D clinostats, specifically for larger quantities samples, and that 3D clinostats induce responses approximating those experienced in the International Space Stations (ISS) microgravity. The 3D clinostat is made up of an inner chamber where the samples are rotated horizontally, vertically, clockwise, and anticlockwise. An outside chamber provides vertical and horizontal motions. Small motors are used to drive each chamber. The dynamic simulation of gravity in any direction was canceled using microcontroller rotation cycles at an outer frame rotation speed of 20 rpm and an inner frame speed of 13 rpm.

The formula for microgravity (g′) is $g' = \omega^2 R / g$ where $g = 9.8$ m/s ^{2}, R= radius from the center of rotation, and the clinostat rotates with a constant angular velocity (ω) where angular velocity is equal to angular displacement in radians/time taken (θ/τ). The angular velocity obtained using the clinostat was 2 rads/s. At this angular velocity, the simulated microgravity is 10^{-3}. Each sample was held accurately in the middle of the clinostat (Siamwala et al., 2010).

In the present study, the pathogenicity and growth of the food pathogens in simulated microgravity conditions will be assessed. The effect of simulated microgravity conditions on the growth, antibacterial activities of the probiotic bacterial species will also be evaluated.

Review of Literature:

According to some findings, cells smaller than 10 micrometers in diameter cannot directly feel gravity, and the reaction shown in a microgravity experiment was explained by indirect impacts on the cell's immediate fluid microenvironment (Zea et al., 2016, 2017). On the other hand, a ground-based study in low-shear modeled microgravity found that *Escherichia coli* biofilms were significantly denser compared to those in normal gravity and showed better tolerance to stressors including salt, ethanol, and antibiotics (Lynch et al., 2006). When compared to the ground, *Escherichia coli ATCC 4157* cells displayed a higher ultimate cell density in microgravity, which was explained by the activation of genes related to hunger and taking up carbon-based sources (Zea et al., 2016). However, additional research utilizing different E. coli strains was unable to support this discovery following space travel (Gasset et al., 1994), although a simulation of a microgravity experiment revealed medium-dependent alterations (Baker et al., 2004; Santomartino et al., 2020). Other experiments revealed that *S. maltophilia* in simulated microgravity showed a greater growth rate, higher biofilm-forming capacity, enhanced swimming motility, and altered metabolic activity in contrast to *S. maltophilia* in real gravity and the original strain. Clusters of Orthologous Groups (COG) annotation analysis suggests that the increased growth rate may be related to the upregulation of genes that are differentially expressed (DEGs), involved with energy metabolic activity and utilization, secondary metabolite biosynthesis, transportation and degradation, release, and vesicular movement. (Su et al., 2021).

Other studies explore that when in contrast to *P. aeruginosa* grown under normal gravity (NG) conditions, multiple drug-resistant *P. aeruginosa* cultivated under the low earth gravity environment showed less ability to form biofilms and swim. The additional investigation demonstrated that the downregulated expression of the genes in charge of type IV pili biosynthesis (pilDEXY1Y2VW) and flagellar synthesis (flhB, fliQ, and fliR) could be the cause of the lower swimming motility and biofilm formation capacity (Zhang et al., 2021). Some research experts' experiments on the transcriptome properties and development characteristics of *Proteus mirabilis* cells were subjected to continuous modeling techniques of microgravity and short-term modeling techniques of microgravity. Their findings demonstrated that, in contrast to short-term modeling techniques of microgravity, in continuous modeling techniques of microgravity the ability to develop biofilms was hindered, growth declined, and various transcriptome profiles were impacted, including those associated with stress response, transportation through membranes, metallic ion transportation, biological attachment, metabolism of carbohydrates, and metabolism of lipids. (Zhang et al., 2022).

Significance of the study:

Humans have had to create isolated self-sustaining habitats like submarines, Arctic or desert stations, and spacecraft to enable the crew's long-term survival in hostile settings like deep water, the Arctic or desert, and space. The spaceship finds an unusual ecological niche where factors like gravity and radiation differ from those on Earth (Senatore et al., 2018). Astronauts completing space missions are known to experience a variety of stresses due to the microgravity environment. These stressors may negatively impact astronauts' health by affecting the composition of their gut microbiomes. It has been proven that the host's health and behaviour are significantly influenced by the gut microbiota (Siddiqui et al., 2021).

Despite the immense interest in bacterial biological responses to the surroundings in space, microgravity's influence on the proliferation of bacteria has been argued and investigated inconclusively. Most of the knowledge on bacterial growth in weightless is derived from numerous independent research projects, each of which seems to use a distinct set of experimental techniques and a smaller number of replicate samples overall, however, they were all focused on different outcomes for different scientific goals. (Huang et al., 2018b; W. Kim et al., 2013; Milojevic and Weckwerth, 2020; Nicholson and Ricco, 2020; Zea et al., 2016, 2017). As a result, it is challenging to derive findings that can be applied generally about how gravity affects bacterial cell growth. So, to generalize the physiological changes of bacterial strains under ground-based facilities like three-dimensional clinostat with randomization and without randomization following experiments can be done.

On the other hand, the virulence activities of pathogenic bacteria will be assessed through biofilm analysis and isolation of the secretome. The antibacterial efficiency of probiotics against food pathogens under simulated microgravity will be evaluated.

Randomized and non-randomized Clinostat will be used for the generation of simulated microgravity.

Objectives:

- To study the physiological characteristics of some food-borne pathogenic bacteria and probiotics bacteria under simulated microgravity (SMG).
- To isolate the bacterial secretome under SMG conditions.
- To find out the antibacterial activities of probiotics on pathogenic strains under SMG conditions.
- To analyze the adhesive abilities of bacteria to Caco-2 cells under SMG conditions.
- To find out antibiotic resistance of bacteria under SMG conditions.
- To interpret randomization and the non-randomization effect of clinostat on bacterial populations.

Plan of work:

- ✓ All the bacterial strains are cultured with their respective nutrient and temperature overnight.
- ✓ Collect the individual strains and treated them under simulated microgravity and observe their physiological changes through the different methods.
- ✓ Secreted proteins are isolated under an altered environment such as SMG condition.
- ✓ Collection of Caco -2 cells from the cell laboratories and adhesive abilities of probiotics are noted.
- ✓ Under stressed condition drug resistance of bacterial strains is evaluated.

Methodology:

A. Study of Physiological Characteristics

i) **Analysis of the growth rate:** The growth rate will be measured as per the method of Zhao et al., 2019a.

ii) **Growth kinetics analysis with modified media:** This analysis will be determined by the method of H. W. Kim et al., 2014.

iii) **pH measurement:** pH measure will be performed by the method of H. W. Kim et al., 2014.

iv) **Analysis of cellular morphology:** Cellular morphology will be done by the method of Senatore et al., 2020.

v) **Qualitative and quantitative analysis of biofilm formation:** This will be measured by the method of Wang et al., 2017; Zhao et al., 2019b.

vi) **Analysis of biofilm formation abilities of bacteria:** Biofilm formation abilities via Confocal Laser-Scanning Microscopy will be observed by the method of Su et al., 2021.

vii) **Swarming Motility Assay:** Swarming Motility will be determined as per Zhang et al., 2022.

viii) **Survival of bacteria during heat treatment:** Survival of bacteria will be determined by the method of H. W. Kim & Rhee, 2016.

ix) **Cold Survival assay:** Cold Survival assay will be performed by the method of Sheet et al., 2020.

x) **Acid Tolerance Assay:** This assay will be measured according to the method performed by Hingston et al., 2017**.**

B. Isolation of Secretome:

Bacterial secretory protein isolation will be performed by the method of Bach and Bramkamp, 2013.

C. Analysis of the antibacterial activity of probiotics on pathogenic strains:

This will be measured by the method of Shao et al., 2017.

D. Analysis of adhesive abilities of bacteria to the Caco-2 cell:

The adhesive abilities of bacteria will be evaluated by the method of Chopra et al., 2006.

E. Analysis of antibiotic resistance:

Antibiotic resistance will be done by the method of Wang et al., 2017.

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