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Evaluation of Microalgae Prebiotic Activity in Long-term Humans Isolation in Confined Habitat

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Abstract: It is known that long-term isolation in sealed compartment the first 10-14 days is called a period of "acute adaptation". It is manifested by an increase of number of opportunistic microflora species together with quantitative decrease of protective microflora species. This is fraught with the development of auto- and cross-infections. In this regard, it is necessary to develop countermeasures against maladjustment processes in these conditions. IBMP conducted research on possibility of using an autotrophic element of biological life support systems, including as an additive in diets. A comparative assessment of the microflora of 6 people, participants of a long-term isolation experiment, was carried out. As countermeasures pills based on spirulina were used. Two pills was taken by all participants times per day. For comparison, archival data from experiments in which subjects did not use any countermeasures were used. The results indicate the stabilization of the microbiocenosis of the intestines and upper respiratory tract when using the spirulina pills. The concentration of Enterococci was maintained at a high level, most of the participants did not have Proteus, Staphylococci or yests. However there were minor fluctuations in the content of protective groups in the intestinal microflora (Bifidobacteria and Lactobacilli), which determines the need to include probiotics as countemesures. Thus, studies have demonstrated the positive results of the use of spirulina as a countermesure. Therefore, it is expedient to consider the issue of equipping the sealed compartments planned for use for the implementation of deep space exploration programs with water-mixture bioreactors.

Keywords: microbial fuel cell, isolation, microflora, spirulina.

1. INTRODUCTION

The human microflora is an integral and plastic system, considered by some researchers as a separate “organ” that functions as part of a particular biotope and is influenced by various factors [2,9].

Maintaining the qualitative and quantitative balance of the microbiota seems to be especially important taking into account planned long-term space missions beyond the Earth's orbit. Previous studies of the microflora in sealed compartments revealed that due to the constant microbial exchange between crew members, combined with stress and intense psycho-emotional state associated with the performance of a space mission, there is a deterioration in the microflora of the upper respiratory tract and intestines. Under such conditions, there is an activation and increase in the number of opportunistic microorganisms, and at the same time decrease in protective species for a given biotope, which leads to decrease in colonization resistance and increase in the risk of developing dysbiotic conditions, and in case of too strong an imbalance in the microflora – to inflammatory processes of one or more other localization [3,8].

The most dangerous from the point of view of the development of such situations is a so-called period of acute adaptation, which is observed from 1 to 10–14 days of the isolation experiment [1]. The experience of the volunteers' previous participation in isolation

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experiments slightly "smoothes" the severity of changes in this period, but still, it seems appropriate to develop effective countermeasures to reduce risks for crew members. One of such couontermeasures is probiotic and autoprobiotic pills [10], however, other means can also be used as a food additive, the effectiveness of which can also be high, especially when combined with the above mentioned drugs. One such option is spirulina. Spirulina contains a large amount of protein, some vitamins and minerals, as well as phenolic acids. The toxicological safety of spirulina has already been proven, in addition, this algae is easy to cultivate. A number of studies have shown that spirulina has a good anti-inflammatory effect by inhibiting the release of histamine from mast cells [6,11]. Also, spirulina is able to stimulate an increase in the production of IgA immunoglobulin, an increase in which was noted in saliva in a study by Ishii et al. [5]. This indicates the influence of algae on the formation of immunity of the oral mucosa.

The purpose of this study was to analyze the data of the intestinal microflora and the microflora of the upper respiratory tract of the subjects who participated in a 3-month isolation experiment and used a spirulina-based preparation as a dietary supplement.

Research objectives:

1. Assess the state of the microflora of the upper respiratory tract and intestines from 1st to 60th days of isolation;
2. Determine the timing of the most pronounced changes;
3. Evaluate the effectiveness of taking a biological food supplement based on spirulina to maintain the stability of the intestinal microflora and upper respiratory tract.

2. METHODS AND MATERIALS

This study is included in the scientific program of the isolation experiment, which simulates the classical adverse factors of space flight and the sealed compartment experiment, such as sensory deprivation, monotony, limited living volume and controlled habitat. The experiment also simulates the factors of autonomous interplanetary flight, including limiting the resources of the expedition and modeling the professional activities of the space crew. According to the scenario of the experiment, there is a delay in communication with the Mission Control Center up to 5 minutes one way, as well as working out emergency situations.

This study analyzed data on the microflora of the upper respiratory tract and intestines in 5 volunteer crew members. The study was reviewed and approved by the bioethical commission of the State Scientific Center of the Russian Federation - IBMP RAS.

The subjects used a preparation with spirulina, which included microalgae spirulina (Spirulina platensis) and an auxiliary component: aerosil (anti-caking agent).

The drug was taken two pills three times a day with meals for one month (30 days). Spirulina was taken from the 1st to the 30th day of the experiment. Daily dose - 6 pills.

Samples of microflora from the upper respiratory tract were taken 2–3 days before the start of the experiment (background) with a sterile swab, followed by inoculation on selective nutrient media, then by the crew doctor from each tester on the 30th and 60th day of isolation.

Fecal samples were also taken in the background period 2–3 days before the start of the isolation experiment, and then on the 14th, 30th, and 60th days of the experiment. A number of tenfold dilutions in sterile saline from 10^1 to 10^9 were prepared from fecal samples, and 100 μl of the inoculum were sown in Petri dishes with agar nutrient media: blood agar, Endo agar or MacConkey agar, mannitol-salt agar, Sabouraud medium, MRS, Bactofoc medium, citrate agar, enterococcal agar, bifidoagar.

Samples from the upper respiratory tract were diluted with saline and plated on the following nutrient media: blood agar, chocolate agar, MacConkey agar, mannitol-salt agar, Sabouraud's medium. Manufacturer of all media - Himedia, India.

Growing colonies were counted and identified.

Statistical processing of the results was carried out using the STATISTICA 7.0 program; discriminant and variance analysis for small groups was used. [7]

To assess changes in the microflora of the gastrointestinal tract, the eubiotic index was used, which shows the ratio of the number of positive changes in the microflora to the number of negative changes. [4]

3. RESULTS AND DISCUSSION

When analyzing the composition of the microflora of the upper respiratory tract, a significant difference was found between the state of the microbiota on the 30th day after the monthly use of the drug with spirulina and in the background period, as well as on the 60th day (30 days after stopping the use of the supplement). Discriminant analysis showed a significant difference between groups in Staphylococcus spp. Taking into account the value of the contribution of various groups of microorganisms to canonical variables (Root 1 and Root 2), we can conclude that when moving along the abscissa axis (from left to right), the number Staphylococcus and fungi (together in the "nose" and "mouth" biotopes) decreases, and when moving along the y-axis (from bottom to top), the number of Neisseria spp increases (Figure 1). Thus, analyzing the position of the groups on the graph, we can conclude that after 30 days of using the drug with spirulina, there is a decrease in the opportunistic component of the microbiota of the upper respiratory tract.

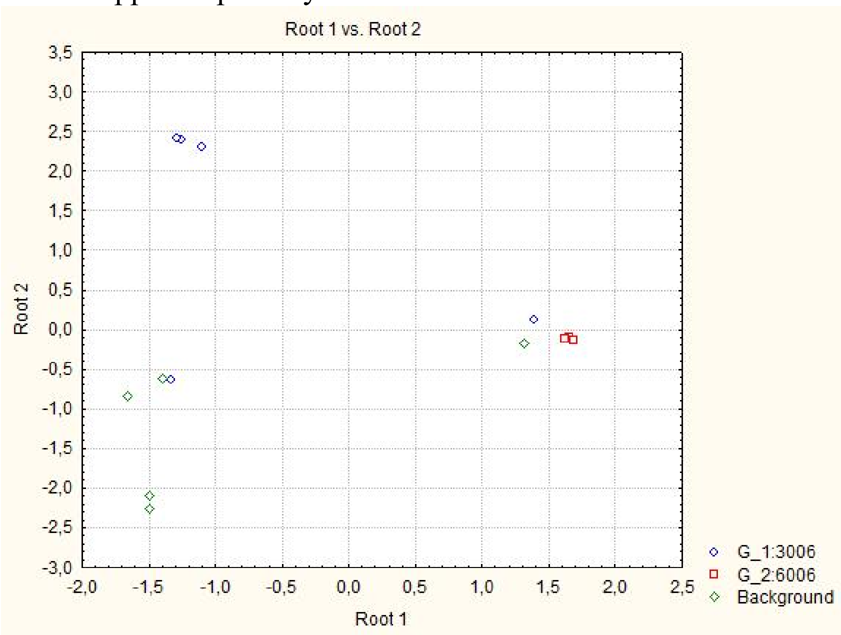

Figure 1. Projections of cenoses on two canonical variables (Root 1 and Root 2). Each point on the graph corresponds to one participant. G_1:3006 - 30th day of isolation, G_2:6006 - 60th day of isolation.

When analyzing the intestinal microflora, discriminant analysis revealed significant differences in 4 groups of microorganisms: Enterobacteriaceae spp, Enterococcus spp, Lactobacillus spp and Clostridium spp.

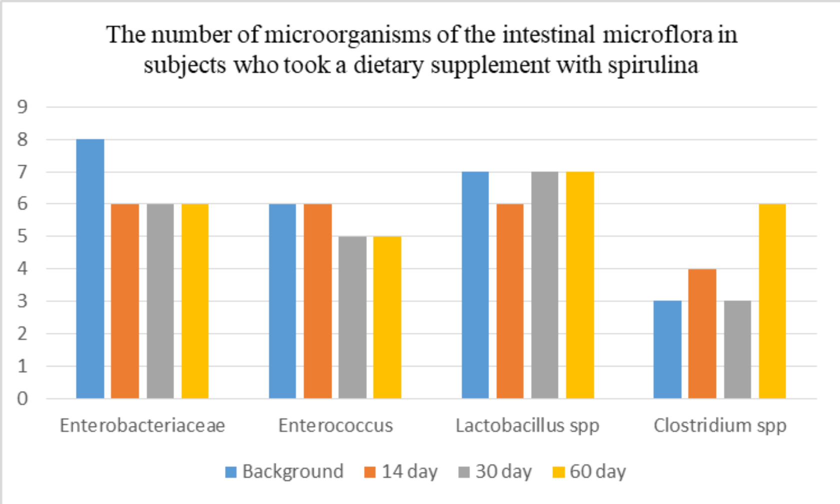

Figure 2. The number of microorganisms of the intestinal microflora of the participants who took pills with spirulina from days 1 to 30 of the isolation experiment (lg (CFU)).

Attention is drawn to the decrease and stabilization at a relatively low level of opportunistic microorganisms, such as Enterobacteriaceae spp and Clostriduim spp, while the number of Clostridiim spp. by 14 days (by the end of the acute period of adaptation) increases, and then decreases by 30 days, and after discontinuation of the drug sharply increases again. The number of Lactobacilli (a protective component of the intestinal microbiota) slightly decreases by the end of the acute period of adaptation, and then increases to the level of normocenosis (Figure 2).

In addition to assessing the number of different groups of microorganisms, the eubiotic index was also calculated using data on genera, for which statistically significant differences were found (Figure 3). The figure shows that the index increases by day 30 (that is, the number of protective microorganisms increases and stabilizes, while the number of conditionally pathogenic microorganisms decreases), and then decreases slightly, while remaining slightly above one, which indicates the predominance of positive changes in the microflora over negative ones.

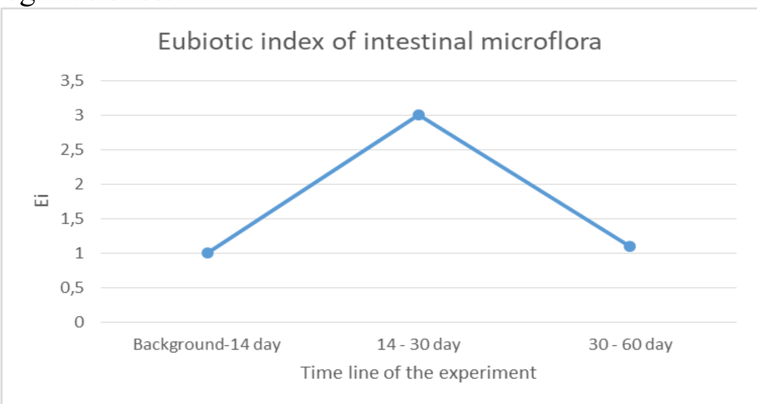

Figure 3. Dynamics of the eubiotic index of intestinal microflora in subjects who took a supplement with spirulina from days 1 to 60 of the isolation experiment. E_i – eubiotic index.

Separately, it is noteworthy that some subjects do not have any Staphylococci, or Proteus, or yeast, which indicates the stability of the colonization resistance of the intestinal microflora. Nevertheless, a slight decrease in Lactobacilli on the 14th day of the experiment as well as the absence of a significant difference in Bifidobacteria, which are also a protective species, determines the need to include additional probiotic or autoprobiotic preparations based on these groups of microorganisms in the diet.

Thus, it seems appropriate to consider the issue of completing long-term isolation experiments with algal bioreactors for cultivating spirulina under conditions of long-term autonomous stay of isolated groups in a hermetic facility. It also appears that this bioreactor may be compatible with other biotechnological processes for regenerative life support systems, such as microbial fuel cells, in which the cathode chamber would be used for oxygen production purposes, where this latter would be optimized by the operation of the algae reactor.

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