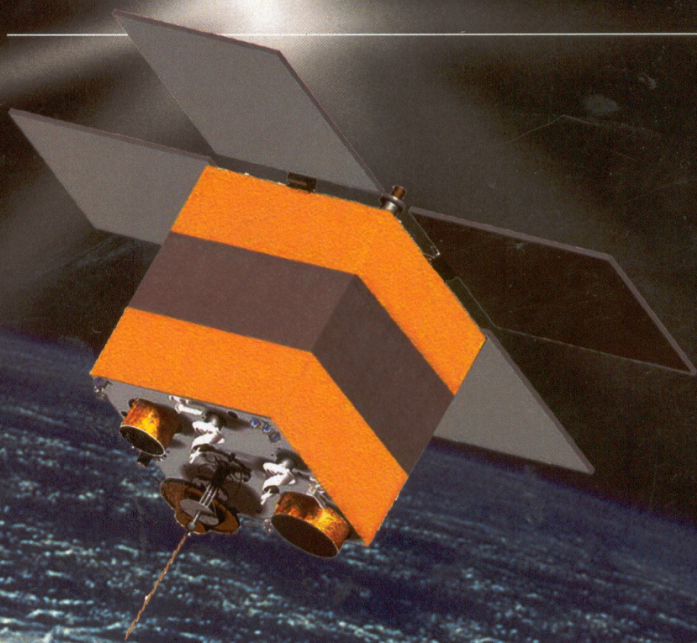


# SPACE RESEARCH IN UKRAINE

2010-2012



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## ROBUST SYMBIOTIC MICROBIAL COMMUNITIES IN SPACE RESEARCH

O. Kukhareno<sup>1</sup>, O. Podolich<sup>1</sup>, A. Rybitska<sup>1</sup>, G. Reshetnyak<sup>1</sup>, L. Burlak<sup>1</sup>,  
L. Ovcharenko<sup>1</sup>, T. Voznyuk<sup>1</sup>, O. Moshynets<sup>1</sup>, I. Rogutskyi<sup>2</sup>, I. Zaets<sup>1</sup>,  
O. Reva<sup>3,4</sup>, E. Rabbow<sup>5</sup>, J.-P. de Vera<sup>6</sup>, V. Yatsenko<sup>7</sup>, N. Kozyrovska<sup>1</sup>

<sup>1</sup>Institute of Molecular Biology & Genetics of NAS of Ukraine (IMBG NASU)

<sup>2</sup>Institute of Physics of NAS of Ukraine (IPh NASU)

<sup>3</sup>Institute of Microbiology and Virology of NAS of Ukraine (IMV NASU)

<sup>4</sup>University of Pretoria, South Africa

<sup>5</sup>German Aerospace Center (DLR) Cologne, Institute of Aerospace Medicine Radiation Biology

<sup>6</sup>German Aerospace Center (DLR), Berlin, Institute of Planetary Research

<sup>7</sup>Space Research Institute of NAS and SSA of Ukraine (SRI NASU and SSAU)

Naturally occurring symbiotic microbial communities (SMC) are the most robust assemblages for a multipurpose use in keeping humans healthy and soil fertile. Especially, safe and reliable SMC are needed for producing probiotics and ferments valuable for health problems prophylaxis. This is true for long-term expeditions, outposts, extraterrestrial permanently-manned bases where humans are exposed to adverse environmental factors, weakening the immune system. The kombucha beverage has been used in human society within millennia as a probiotic drink which is produced by naturally occurring mixed populations of living microorganisms. Here, we discuss the potential of the kombucha culture for outposts in far future missions.

### Stable symbiotic microbial communities as promising ecosystems to be in use in space research

The kombucha culture (*Medusomyces gisevii* Lindau) represents a Symbiotic Culture of Bacteria and Yeast (SCOBY) known as tea «fungus» or a Kombucha Tea (KT). The KT microorganisms co-exist in interdependent symbiotic relationships: during fermentation in sweet tea sugar is converted into organic acids and ethanol by yeast, and bacteria use the latter to produce cellulose fibres, forming a jellyfish-like zoogloeal mat (Fig. 1a, see colour plate). Bacterial cellulose (see Fig. 1b), synthesized by *Gluconacetobacter hansenii* and other acetic acid bacteria in KT, is chemically pure, free of lignin and hemicellulose. Microcellulose fibres may have several practical implications in nanobiotechnology and biomedicine. Finally, SCOBY gives a pleasant sweetish sour sparkling beverage under aerobic conditions. Organic acids, ethanol and antibiotics protect SCO-

BY from the colonization by other microorganisms. The species of yeast found in the kombucha culture can vary and may include *Brettanomyces/Dekkera*, *Schizosaccharomyces*, *Torulasporea*, *Zygosaccharomyces*, *Pichia*, etc. [1]. Few acetic acid bacterial species such as cellulose-forming bacteria *Gluconacetobacter hansenii* (earlier *xylinus*), *G. kombuchae* sp. nov. are the most known species isolated from different ecotypes of KT [2]. Kombucha-derived microorganisms produce a cocktail of diverse metabolites: fiber, organic and amino acids, vitamins, antioxidants, enzymes, essential elements [1]. Potential health effects of the SCOBY's metabolites have created an increased interest in kombucha drink. Kombucha's greatest health benefits include its ability to detox and energize the body, improve digestion, boost the immune system. A kombucha tea is known for ages; however, scientific interest to SCOBY was increased at the beginning of the last century. Due to efforts of many scientific teams, positive effects of the kombucha culture were substantiated on immunological, endocrinological, cardiovascular, gastro-intestinal, urogenital and other levels [3]. The wide expansion of synthetic pharmaceuticals has kombucha drink effects hidden. At the beginning of this millennium we observed kombucha's re-birth due to irreversible return to natural healing products, as well as research activity on the background of functional nutrition study. Antimicrobial and antiproliferative activity; health improvement after oxidative stress and cytotoxicity induced by environmental pollutants; hypcholesterolaemic and antioxidant effects; ulcer-healing activity etc attract attention to the Kombucha Microbial Community (KMC) as a promising product for use in unfavourable conditions.

## Symbiotic kombucha microbial community could be helpful in outposts

### *A valuable synbiotic*

Work in extreme conditions such as space stations, mines, submarines, manned bases etc needs the robust immune system and balanced indigenous intestinal microbiota in crews. Combined with sharp changes in diet and the stringent sanitary conditions in which crews live, their state of gut colonization may be changed by normal microbiota. Protective gut commensals and mutualists may disappear, leading to secondary infection by opportunistic microbes always present in the gut, and may have negative health consequences such as altering predisposition to allergies and autoimmune disease. Correction with probiotics and prebiotics may be reasonable. Probiotics (live microorganisms which when administered in adequate amounts confer a health benefit on the host, — the World Health Organization and the Food and Agriculture Organization of the United Nations) are essential to establishing and maintaining optimal immune health. However it is important to note that probiotics can be efficient only when nondigestible (foods) food-stuffs are available in the diet. This is why *prebiotics* (food ingredients that selectively stimulate the growth and/or activity of beneficial microorganisms already in people's colons) are highly recommended, and along with probiotics these supplements form *synbiotics* (a combination of probiotics and prebiotics).

It is a time to find out how to use probiotics in foods for astronauts. A kombucha tea/mat may be a promising formulation of a synbiotic for extreme expeditions for several reasons [4, 5, 6]. First of all, the kombucha culture provides a source of both probiotic bacteria and yeast, as well as microcellulose fibres — prebiotics, which help to fuel the growth of helpful microorganisms in the digestive tract, i.e. the kombucha culture is a synbiotic. Secondly, KMC provides short-chain fatty acids (acetate, butyrate, propionate and other metabolites) which boost immunity. Finally, SCOBY is a rich source of vitamins B, C, as well as other biologicals and essential minerals. In addition, *in situ* processed a jellyfish-like zooglycal SCOBY's mat, rich in crude microfibrils, polysaccharides and protein will serve as a permanent source of foods for both crews and animals.

### *A component of autonomous regenerative life-support system*

In addition to probiotics for crews, the kombucha culture is a promising probiotic for growing plants [3]. The ability to grow plants in extraterrestrial greenhouses is a practical necessity for providing an advanced life support system for humans. Plants will provide fresh food, oxygen, and clean water for explorers living in manned extraterrestrial bases. The concep-

tion of the first generation plants growing in a lunar base anticipates them to play a main role in forming a protosoil of acceptable fertility needed for purposely growing second generation-plants (wheat, rice, etc.) at a low cost [7]. The residues of the first generation-plants could be composted and transformed by microorganisms along with local regolith into a soil-like substrate within a loop of regenerative life support system. The use of a permanent source of organics which is a SCOBY mat will be helpful for a protosoil formation. The enzyme system of *Dekkera* sp. yeast, an active player in the KMC, is enough powerful to degrade plant waste polymers. In the coming future, metabolic engineering of the kombucha microbiota to improve their direct interaction with plant waste material will be reasonable. Novel cooperative robust synthetic ecosystems composed of yeast and bacteria strains will be constructed for these purposes. Both natural and genetically engineered kombucha microbiome will be the indispensable components of the regenerative life-support system.

### **Advantages**

The diversity of naturally occurring symbiotic microorganisms and appropriate wide range of their activity are advantageous over single strain-probiotics or artificially assembled cocktails of beneficial microbial strains. Due to biofilm-formation, the starter kombucha culture is practically immortal and can be activated when needed. This means that the longevity of the KMC shelf-life has no sense, and this is probably the major advantage of SCOBY. Slow-growing biofilms produce substantial numbers of persisters (a sub-population of dormant cells) which tolerate adverse factors. The KMC has metabolic plasticity and can be adapted to various economically reasonable feeding resources. SCOBY can be easily and safely reproduced *in situ* (at home, missions, outposts etc) at low cost and low laboriousness using low cost and labor. Importantly, the final KT product is a slightly carbonated acidic-sweetish beverage that creates positive emotions in addition to health effects.

### **Perspectives**

In spite of tremendous interest to the health-promoting effects of the KT, there is no systemic scientific approach to characterizing KMC collective genome and its expression in changed environment, using new research opportunities. Metagenomics approach in study of microbial complex ecosystems allows reconstructing the community structure on the base of a high-throughput DNA sequencing and analysis of annotated sequences with bioinformatics programs [8]. The use of such a approach for study of the KMC structure will unmask not-yet-cultivated microorganisms which are expected in such biofilms as SCOBY. This approach may allow the compre-

hensive documentation of changes in the gut microbiome of a given individual as a function of probiotic administration, including consumption of KT. It is important for the evaluation of dietary products, being consumed as probiotics. Recent advances in Human Microbial Project (NIH, USA) have furthered our understanding of probiotic functionality and the specific interactions between probiotics and human hosts [9]. A parallel study of the effects of KMC on the immune system markers allows elaborating the personal kombucha-based corrections of functional disorders in a patient-friendly manner. The ability to examine fully sequenced and annotated SCOBY's genomes will promote the application of genetic approaches to elucidate many important functional roles of probiotic kombucha microbes.

The kombucha culture is a biochemical unit, producing valuable health-promoting substances [3]. Up to date, there is inconsistency in the results of chemical analysis of kombucha metabolome and a comprehensive study of kombucha metabolites that requires understanding of their role in healing diseases and the immune system modulation. The annotated KMC metagenome and deciphered KMC metabolic pathways and their networks will create a basement for separation and study of unknown compounds. This will be helpful for the metabolic engineering approach and construction of more efficient pathways of sugars utilization, including waste plant material by KMC during fermentation.

The study of SCOBY's effect on environmental factors, influencing its functional properties, will be addressed to functional genomics and transcriptomics. The knowledge about the KMC transcriptomes at standard and changed conditions will invest data in the evaluation of putative hidden effects on human health, as well as in the study of bacteria-yeast interactions under influences of adverse factors.

#### The first future astrobiological study of the KMC

The interdisciplinary and international space research project BIOMEX (Biology and Mars Experiment) will take place on the space exposure facility EXPOSE-R2 on the International Space Station (ISS) in 2013/2014 under aegis of European Space Agency and DLR. One of the scientific objectives is to analyze the effect of space environment on survival and interaction between biological samples and selected minerals (including terrestrial, Moon- and Mars analogue varieties) under space and Mars-like conditions [10]. The samples will be exposed outside the module Zvezda at the ISS. Currently, space simulation experiments, the Experiment Verification Tests (EVTs) and Scientific Verification Tests (SVTs), were performed, using the simulation facilities at the German Aerospace Center (Cologne).

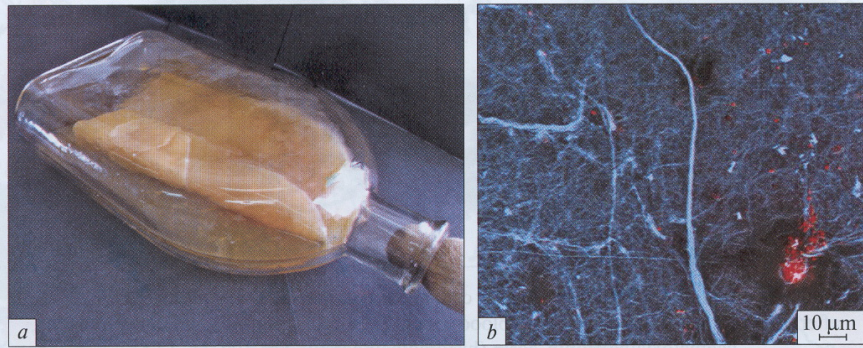
The kombucha culture from the collection of Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine is selected for the BIOMEX preparation. The kombucha biofilm was unified with a sterile rock material, and the biomineral specimens have been formed under aseptic conditions (Fig. 2a, b, see colour plate). The samples were placed in the holders for the experiment verification tests, imitating some space factors. Preliminary results show a high survival rate of the both bacteria and yeasts, as well as a biofilm production capacity. However, a delay in a term of the biofilm formation and a change of its quality were observed after EVTs in the selected SCOBY compared to the untreated laboratory variant (Fig. 2c).

#### Conclusion

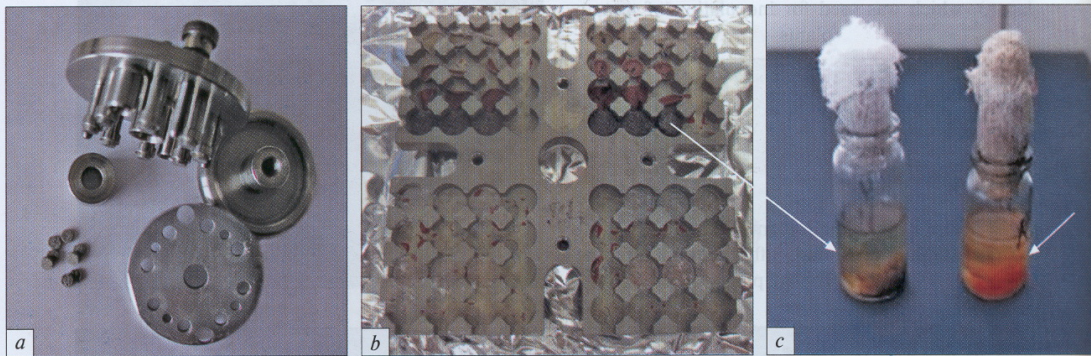
Post-genomic tools will make a progress in the research of naturally occurring symbiotic microbial communities, and the output of post-genomics studies will promote the investigation of KMC for astrobiology and astromedicine. Studies on functional metagenomics and transcriptomics will provide additional evidences on healing effects of KT and kombucha mat and create a basement for safe use of these products in adverse conditions of the future space missions or in autonomous regenerative life-support systems at extraterrestrial manned bases.

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**Fig. 1.** The kombucha culture produces a floating jellyfish-like zoogeleal mat (a). Microphotograph (confocal laser scanning microscopy) of the mat; staining with calcofluor white to reveal a cellulose matrix (a blue signal) and ethidium bromide to detect microbial cells (a red signal) (b)



**Fig. 2.** A sample preparation for the Experiment Verification Tests and Scientific Verification Tests: a device for tablets fabrication (a); samples in the holders after tests (b); a biofilm formation by the kombucha culture after tests (left) and without treatment (right) (c)