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PRE-FLIGHT KOMBUCHA SAMPLES TESTING BEFORE EXPOSITION OUTBOARD THE INTERNATIONAL SPACE STATION

Kombucha is a multispecies microbial community which produce bacterial cellulose — a polymer molecule to be a candidate for a biomarker of life. For the pre-flight ground-based phase of Biology and Mars Experiment (BIOMEX), the multi-microbial cellulose-based biofilm was embedded in mineral material to test the structural integrity of the bacterial cellulose and a survival of community-members under Mars-like CO_2-rich atmosphere, pressure and solar irradiation spectrum similar to that on the surface of Mars. During the preparatory testing stage it was found that after the synergistic action of a set of stressful space- and Mars-associated factors the mineralized cellulose preserved the characteristic molecular fingerprints, which might be detected instrumentally. The flight stage of the BIOMEX begun on July, 2014 and will last for 12—18 months on the EXPOSE-R2 platform mounted by the astronauts outside the ISS.

Key words: *astrobiology, Biology and Mars Experiment (BIOMEX), biosignature, bacterial cellulose.*

THE INTERNATIONAL AND INTERDISCIPLINARY "BIOLOGICAL AND MARS EXPERIMENT (BIOMEX)" ON LOW EARTH ORBIT (LEO)

Interest to the Red Planet existed since ancient times. In modern times since the sixties of the last century, in the former SU, Sergey Korolyev elaborated a program of manned flights to Mars. Simultaneously, NASA's Mars Exploration Program was initiated and implemented. At present time, two American landers are exploring the Martian surface. The European ExoMars program will deliver a European rover in the next coming years. All missions in the Mars exploration programs aim to find signs of primitive life on Mars. However, questions remain, are there or were there Earth-like life forms on this planet; could terrestrial organisms survive under harsh Martian conditions. This means that future Mars missions have to be designed much more for a systemic investigation of its habitability and to search for life.

The international and interdisciplinary project BIOMEX headed by the DLR (Berlin) and realized by the European Space Agency (ESA) proposed another, relatively cheaper approach compared to several other projects to investigate the probability of life existence on Mars [1]. The idea is to simulate Martian conditions in LEO where, by using filters, solar irradiation similar to the Martian spectrum can be

simulated, which might have significant impact on a variety of extremophiles (bacteria, archaea, algae, fungi, lichens and mosses), and to test their vitality, as well as the structural integrity of biomolecules as possible biosignatures. The samples were integrated on the EXPOSE-R2 platform at the outer side of the ISS.

The best chance to find signs of the present or past life on Mars is to investigate and model possible changes of biomolecules and the potential of organisms or bio-communities to form fossil remnants [3, 5]. In the framework of BIOMEX, we use a mineralized cellulose-based biofilm, where multi-component pro- and eukaryotic microbial assemblage may reside protected from the harsh conditions. In this work, we suggest to use as a model assemblage the complex kombucha microbial culture (KMC), where diagenetically changed bacterial cellulose may serve as a biosignature. A scheme of the Ukrainian segment of BIOMEX is shown on Figure.

Kombucha beverage has been used for millennia as a healthy and restorative drink. Now it may serve us beyond Earth as a promising model to study synbiotics (combined probiotic and prebiotic products) for space crew [2] and also nanocellulose as a multipurpose nanomaterial, which could be used also for construction of space-related devices, *e.g.,* supercapacitors [4]. An additional objective of our miniproject within BIOMEX was to study the cellulose stability to be used as a nanomaterial under extreme conditions.

The project BIOMEX began in the late 2010, and within a 3.5 year period it passed the way from an idea into a real experiment in space. Collection of samples and a field /laboratory study of pro- and eukaryotic objects for BIOMEX have been done by 25 institutions. A series of pre-flight experiments, using the Planetary and Space Simulation facilities (PSI) at the Institute of Aerospace Medicine (DLR, Cologne) (http://www.dlr.de/spacesim), showed the potential of model organisms to survive in a 1.0-1.5-year space experiment on the EXPOSE-R2 platform. In the final stage of BIOMEX, the KMC cellulose-based pellicle was prepared as a bio-mineral sample for the exposure experiment at the ISS, and it was used in pre-flight assessments along with other model objects aforementioned.

BIO-MINERAL SAMPLES, TESTS FACILITIES AND EXPOSURE CONDITIONS

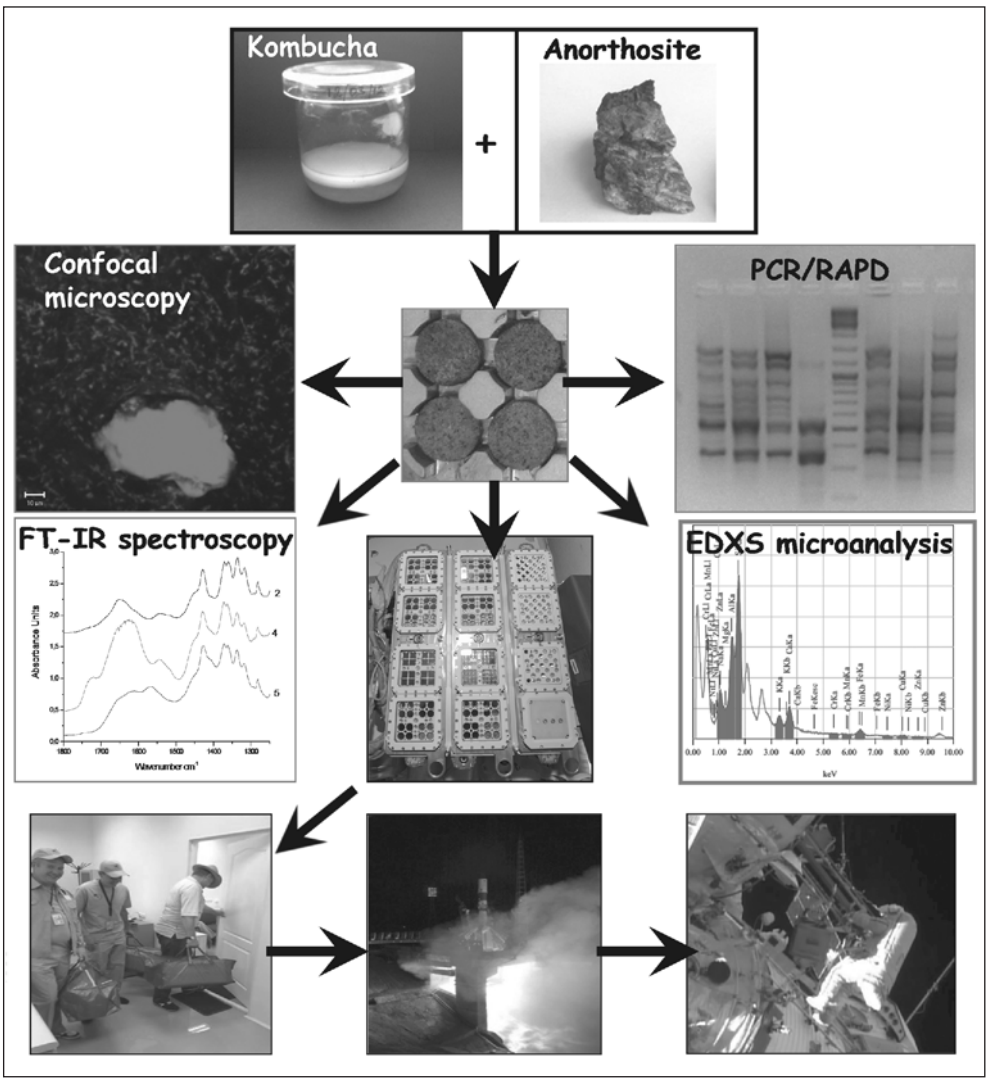
Bio-mineral samples comprised tiny 'pills' made from the KMC biofilm fragments ($d = 7$ mm) enclosed inside of the mineral-organic capsule, one of components of which was a grinded anorthosite rock from Ukrainian deposit (Zhytomyr oblast, Turchynka).

Two ground-based pre-flight Experiment Verification Tests (EVT) and two Science Verification Tests (SVT) were performed and realized at the PSI (DLR, Cologne), which aimed at modelling the space-flight conditions on EXPOSE-R2 platform outside the ISS and partially the conditions on Mars. Exposure of bio-mineral samples was performed to achieve two main objectives: to investigate the effects of space-flight factors like vacuum, temperature fluctuations, short wavelength UV irradiations >200 nm, Martian atmosphere factors like gas composition and low pressure of 10^3 Pa (EVTs), and to integrate fluencies of the 1-year experiment at the ISS (SVTs).

For the SVT run 2, a fully functional and a flight-identical ground hardware was provided. Samples were glued such as it is foreseen to be realized for the flight and integrated under sterile conditions into the appropriate 3-ground sample carriers at their positions. Four kombucha mineralized biofilm specimens in either carrier in the top, medium and bottom positions were located in the tray 2 along with other bio-mineral samples prepared by partners from the institutions affiliated to BIOMEX and objects from other projects.

A META-ANALYSIS OF BIO-MINERAL SAMPLES AFTER CUMULATIVE EFFECTS OF SIMULATED STRESSFUL FACTORS

In the final SVT, KMC specimens were studied after a period of 5 months since their preparation and the beginning of the experiment to determine the rates of survival of the KMC members. It was found that survival of the organisms depended on sample location in the experimental tray. Specimens from the middle and bottom carriers, which were shielded from UV-irradiation by the top layer, survived under Mars atmosphere and pressure simulated conditions and extreme temperature fluctuations, except for two KMC-members (one bacteria and one yeast species), which did not withstand the conditions.



A scheme of the path of the kombucha microbial community from laboratory to space. Photo credit: ESA/Roskosmos.

The specimens from the top carrier were completely sterilized by the UV-radiation applied in addition to the other harsh conditions. Alteration in species diversity of KMC samples from the middle and bottom layers in comparison to the laboratory (untreated) control was confirmed by a DNA technique that allows to analyze microbial polymorphic DNA segments (RAPD/PCR).

Remarkably, the bacteria which were located during the experiment in bottom and medium carriers kept the ability to create biofilms, however, with a little delay after recovering of the community, if compared to the laboratory and transport control samples. The FT-IR absorption spectra of the BC-based matrices produced by KMC after its recovery in the post-treatment period have not changed. BC from bio-mineral samples preserved spectral characteristics (*e. g.,* 960 — 730 cm^{-1} fingerprint region of anomeric carbons, wherein a band at 897 cm^{-1} confirmed the presence of specific β-1,4-linkages). After the treatments, KMC was able to mobilize and accumulate more actively inorganic ions from the

anorthosite mineral carrier, if compared to the control. This was confirmed by confocal scanning laser microscopy and EDX-ray microanalysis data.

CONCLUSION AND FUTURE PERSPECTIVES

For the pre-flight ground-based experiments, the multi-microbial cellulose-based biofilm was embedded in mineral material to test the survival of community-members and integrity of the bacterial cellulose under Mars-like CO_2-rich atmosphere, pressure and solar irradiation spectrum similar to that of the surface of Mars. During the preparatory testing stage it was found that after the synergistic action of a set of stressful space- and Mars-associated factors the mineralized cellulose preserved the characteristic molecular fingerprints, which might be detected instrumentally. These results demonstrated the stability of the cellulose and its applicability as a biomarker.

The final stage of the BIOMEX begun at the ISS in July 25, six hours after the launch. It will continue for 12-18 months on the EXPOSE-R2 platform mounted by the astronauts outside the ISS on August 18, 2014. The post-flight studies will include several previously worked out microbiological, molecular genetic and physical methods, which were severely tested in advance to achieve the basic scientific information on the impact of space-flight and simulated Martian factors on the KMC members and cellulose structural integrity.

The further space-related research on KMC will focus on the following aspects.

1. *Kombucha-related live products as functional food or supplements for crew and animals.* Being a synbiotic, KMC combines valuable metabolites, immune-stimulatory nucleic acids and other compounds produced by micro-organisms, with crude cellulose fibres, fuelling human gut microbiota and providing biological calcium and other valuable minerals important for the crew.

2. *KMC as a component of the regenerative life-support systems,* performing *in situ* biomobilization of essential elements from local resources; functioning as a biosorbent; providing biological additives for animals and fertilizers for plants, a promising low cost and easy handling terraforming agent. Also it may contribute to crew positive relaxation and socialization as a joyful microbial 'pet'.

3. *Bacterial cellulose is a biotechnologically attractive environmentally friendly multipurpose nanomaterial for space research* applicable for construction of space-related electronic devices such as supercapacitors, power suppler facilities, etc.

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ПЕРЕДПОЛІТНІ ТЕСТУВАННЯ ЗРАЗКІВ КОМБУЧІ ПЕРЕД ЕКСПОНУВАННЯМ ЗА БОРТОМ МІЖНАРОДНОЇ КОСМІЧНОЇ СТАНЦІЇ

Чайний гриб є багатовидовим угрупованням мікроорганізмів, яке виробляє бактеріальну целюлозу — полімер, що є кандидатом у біомаркери живого. Для наземної фази експерименту BIOMEX (біологічний марсіанський експеримент) мультимікробну целюлозну плівку було закладено у мінеральний матеріал, щоб перевірити структурну цілісність бактеріальної целюлози та виживання членів угрупування в імітованих умовах Марса (атмосфера, збагачена CO_2, тиск і спектр сонячного випромінювання, подібні до марсіанських). У ході підготовчого етапу встановлено, що після синергічної дії комплексу космічних і марсіанських факторів мінералізована целюлоза зберегла характерні молекулярні фінгерпринти, які може бути виявлено інструментально. Політний етап BIOMEX розпочався у липні 2014 р. і триватиме протягом 12—18 місяців на платформі EXPOSE-R2, встановленій астронавтами зовні МКС.

Ключові слова: астробіологія, Biology and Mars Experiment (BIOMEX), біомаркер, бактерійна целюлоза.

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ДОПОЛЕТНЫЕ ИСПЫТАНИЯ ОБРАЗЦОВ КОМБУЧИ НАКАНУНЕ ЭКСПОЗИЦИИ ЗА БОРТОМ МЕЖДУНАРОДНОЙ КОСМИЧЕСКОЙ СТАНЦИИ

Чайный гриб является многовидовым сообществом микроорганизмов, которое производит бактериальную целлюлозу — полимер, являющийся кандидатом в биомаркеры живого. Для наземной фазы эксперимента BIOMEX (биологический марсианский эксперимент) мультимикробная целлюлозная пленка была заложена в минеральный материал, чтобы проверить структурную целостность бактериальной целлюлозы и выживание членов сообщества в имитированных условиях Марса (атмосфера, обогащенная CO_2, давление и спектр солнечного излучения, подобные марсианским). В ходе подготовительного этапа установлено, что после синергичного действия комплекса космических и марсианских факторов минерализованная целлюлоза сохранила характерные молекулярные фингерпринты, которые могут быть обнаружены инструментально. Полетный этап BIOMEX начался в июле 2014 г. и продлится в течение 12—18 месяцев на платформе EXPOSE-R2, установленной астронавтами снаружи МКС.

Ключевые слова: астробиология, Biology and Mars Experiment (BIOMEX), биомаркер, бактериальная целлюлоза.