

Bacterial cellulose may provide the microbial-life biosignature in the rock records

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Abstract

Bacterial cellulose (BC) is a matrix for a biofilm formation, which is critical for survival and persistence of microbes in harsh environments. BC could play a significant role in the formation of microbial mats in pristine ecosystems on Earth. The prime objective of this study was to measure to what extent spectral and other characteristics of BC were changed under the performance of BC interaction with the earthly rock – anorthosite – via microorganisms. The spectral analyses (Fourier Transform Infrared FT-IR, spectroscopy, and atomic absorption spectroscopy) showed unprecedented accumulation of chemical elements in the BC-based biofilm. The absorption capacity of IR by BC was shielded a little by mineral crust formed by microorganisms on the BC-based biofilm surface, especially clearly seen in the range of 1200–900 cm^{−1} in FT-IR spectra. Confocal scanning laser microscopy analysis revealed that elements bioleached from anorthosite created surface coats on the BC nanofibril web. At the same time, the vibrational spectra bands showed the presence of the characteristic region of anomeric carbons (960–730 cm^{−1}), wherein a band at 897 cm^{−1} confirmed the presence of β-1, 4-linkages, which may serve as the cellulose fingerprint region. Results show that BC may be a biosignature for search signs of living organisms in rock records. © 2014 COSPAR. Published by Elsevier Ltd. All rights reserved.

Keywords: Astrobiology; Search for extraterrestrial life; Biosignature; Bacterial cellulose; Anorthosite rock; Spectroscopy

1. Introduction

The Lithopanspermia hypothesis is to explain that life exists throughout the Universe distributed by rocky bodies like meteorites, asteroids or planetoids (Arrhenius, 1903; Crick, 1981). Recently, a new argument was received in a support of this hypothesis: the low-velocity process called weak transfer wherein solid materials have been opened (Belbruno et al., 2012). This mechanism could have allowed the exchange of life-bearing rocks amongst the planetary systems in the star cluster, when Earth and its

planetary neighbors would have been close enough to each other. Organic compounds, which played a key role in life's origin, were already present in the early solar system, as well as microorganisms embedded in the fragments of distant planets that might have been the seeds of life. A high degree of probability of organic matter preservation on meteorites (Parnell et al., 2012) or its formation in the interstellar medium (Gudipati and Yang, 2012) were shown.

Some solid arguments in a support of the hypothesis can be gained in simulation experiments. The probability of Lithopanspermia has been tested in laboratory and some space experiments like FOTON/BIOPAN, STONE 6, LITHOPANSPERMIA (de la Torre Noetzel et al., 2007;

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de la Torre et al., 2010; Horneck et al., 2008; Meyer et al., 2011; Parnell et al., 2012), and recently in the EXPOSE experiments located on the ISS (Cockell et al., 2011; Onofri et al., 2012; Bertrand et al., 2012). The data gained imply that biomarker compounds (molecular organic compounds or inorganic material as the evidence of their presence or previous biological activity) survived in some extent to prove the biological origin of the organic matter, however, some cellular microbial bioactive molecules were destroyed after exposure to outer space factors (Cockell et al., 2011; Bertrand et al., 2012). Search of new robust biomarkers preserved on extraterrestrial bodies remains one of primary tasks for astrobiologists at present time.

A list of possible biomarkers that would be signatures of life has been proposed by Parnell et al. (2007) and reviewed recently by Davé et al. (2013). The nitrogen-centric life detection approach anticipates organic primary amines (amino acids, the nucleobases of nucleic acids) to be priority molecular compounds for astrobiological investigation as biosignatures. These compounds offer ability to discriminate between their biogenic or abiotic origin – a critical requirement for a definitive and unequivocal life detection. Biogenic organic molecules and biogenic atmospheric gases also considered as definitive and most readily detectable by sensitive instruments (Summons et al., 2011). In extracellular matrix produced by pro- and eukaryotic microbial organisms, various biogenic organic molecules have been identified, e.g. proteins, nucleic acids, lipids and polysaccharides (alginate, cellulose, etc.) (Wingender et al., 1999). Bacterial cellulose (BC) is a widespread biopolymer used as a three-dimensional matrix for a biofilm formation in multi-microbial communities (Ross et al., 1991). Biofilms have been found to collect together different microbial populations in a close proximity for a metabolic cooperation and information processing, as well as to protect the microbial community from environmental stresses (Elias and Banin, 2012; Lee et al., 2013). Cellulose is an insoluble polysaccharide, consisting of a linear chain of β -1, 4-linked D-glucose units with the different degree of inter- and intramolecular hydrogen bonding (O'Sullivan, 1997). Cellulose biosynthesis has been reported in plants, algae, metazoans (tunicates), fungi, cellular slime molds, and bacteria (Ross et al., 1991; Okuda et al., 2004; Grenville-Briggs et al., 2008; Kawano et al., 2011). Microbial cellulose as the extracellular polymeric substance is produced by phylogenetically diverse bacteria of the genera *Gluconacetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Salmonella*, *Alcaligenes*, *Sarcina* (Hestrin et al., 1947; Zogaj et al., 2001; Matthysse et al., 2005), the sulfur-oxidizing bacteria (Ogawa and Maki, 2003), cyanobacteria (Nobles et al., 2001; Nobles and Brown, 2004). Cellulose-producing microorganisms inhabit a seawater, hot springs, dry lands, and other extreme niches. Microbial cellulose has remarkable physical properties, which cause its stability under high temperature and pressure, irradiation, etc. and provide a protection for inhabiting microbes against these stressors (Williams and Cannon, 1989; Kato et al.,

2007). This allows to conclude that cellulose is a widespread biopolymer on Earth, and it might play a role in the survival of microbial organisms in the harsh conditions of primordial Earth around 3.5 billion years ago (Nobles et al., 2001). Fossilized microbial polymeric substances and biofilms are better preserved than fossil bacteria themselves (Westall et al., 2000). Consequently, microbial cellulose might serve as a biosignature for the search of life records. Presumably, a life is being disseminated by microbial communities on rocks, and we may predict that, in natural settings, e.g. on rocks colonized by cellulose-producing microbes, this molecule is influenced with numerous physico-chemical factors, which may change this biomolecule diagenetically. Could spectral characteristics of mineralized cellulose be recognized in such a case? In this study, a cellulose-producing polymicrobial community (kombucha, known as a tea “fungus”) was used for model experiments on a BC-based biofilm formation in the presence of anorthosite – the earthly rock found also on the Moon. Nearly all biofilms in nature composed by a variety of microorganisms. It was clearly shown that multispecies biofilms provide a higher community level protection, e.g. to chemical agents than the single-species biofilms (Lee et al., 2013). Kombucha culture based on a metabolic cooperation between pro- and eukaryotic microbes (bacteria and yeast) is a promising object for astrobiology (Kukhareenko et al., 2012; Kozyrovska et al., 2012). Selection of a multispecies community as model is more reasonable than a producing cellulose mono-culture from the point of interspecies communications in the mixed community. Due to the presence of a variety of species in the selected biofilm system, the bioleaching of elements from anorthosite could be expected, as well as a filling the cellulose-based matrix with the leached out inorganic ions and reduced elements.

This study have been performed within the frames of the preparation of the international interdisciplinary space low Earth orbit *Biology and Mars Experiment* (BIOMEX) (de Vera et al., 2012), which aims to assess the stability of biomolecules (pigments, cellular components, biopolymers, etc.) in a contact with geological samples in a support of Lithopanspermia hypothesis. The strategic approach in the search of life signs focused mainly on the examination of surface and the subsurface rock/dust material of meteorites or the nearest Earth neighbors (Davila et al., 2010; Summons et al., 2011; Davé et al., 2013). Non-invasive *in situ*-probing of biomass of microbial communities or deposits of bioorganic and inorganic compounds may be sufficient for detection with the sensitive instruments like Infrared (IR) spectroscopy (Igisu et al., 2006, 2009), Raman spectroscopy (Frosch et al., 2007; Böttger et al., 2012, 2013; de Vera et al., 2012), mass-spectroscopic method (Gudipati and Yang, 2012). Among a variety of modern techniques IR- and Raman-spectroscopies are the most relevant for the detection of characteristic bonds in biogenic molecules – fingerprints – by which the molecule can be identified in non-destructive manner, since it is a scattering technique, and specimens do not need to be fixed

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