# Bacterial Cellulose Retains Robustness but Its Synthesis Declines after Exposure to a Mars-like Environment Simulated Outside the International Space Station

Iryna Orlovska,[1] Olga Podolich, [1*] Olga Kukharenko, [1] Iryna Zaets, [1] Oleg Reva,[2] Ludmila Khirunenko,[3]Danica Zmejkoski,[4] Sergiy Rogalsky,[5] Debmalya Barh,[6] Sandeep Tiwari,[7] Ranjith Kumavath, [8]Aristóteles Goés-Neto,[9] Vasco Azevedo,[7] Bertram Brenig, [10] Preetam Ghosh,[11] Jean-Pierre de Vera,[12] and Natalia Kozyrovska[1]

[1]Institute of Molecular Biology and Genetics of NASU, Kyiv, Ukraine.
[2]Centre for Bioinformatics and Computational Biology, University of Pretoria, Pretoria, South Africa.
[3]Institute of Physics of NASU, Kyiv, Ukraine.
[4]Vinca Institute of Nuclear Sciences—National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia.
[5]V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of National Academy of Sciences of Ukraine, Kyiv, Ukraine.
[6]Centre for Genomics and Applied Gene Technology, Institute of Integrative Omics and Applied Biotechnology, West Bengal, India.
[7]Laboratório de Genética Celular e Molecular, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[8]Department of Genomic Science, Central University of Kerala Tejaswini Hills, Kerala, India.
[9]Laboratório de Biologia Molecular e Computacional de Fungos, Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[10]Institute of Veterinary Medicine, University Göttingen, Göttingen, Germany.
[11]Department of Computer Science, Virginia Commonwealth University, Richmond, Virginia, USA.
[12]German Aerospace Center (DLR) Berlin, Institute of Planetary Research, Planetary Laboratories, Astrobiological Laboratories, Berlin, Germany.

*Address correspondence to: Olga Podolich
Institute of Molecular Biology and Genetics of NASU Acad. Zabolotnoho str., 150 Kyiv 03680
Ukraine. E-mail: podololga@ukr.net

**Abstract**

Cellulose is a widespread macromolecule in terrestrial environments and a major architectural component of microbial biofilm. Therefore, cellulose might be considered a biosignature that indicates the presence of microbial life. We present, for the first time, characteristics of bacterial cellulose after long-term spaceflight and exposure to simuled Mars-like stressors. The pristine cellulose-based pellicle membranes from a kombucha microbial community (KMC) were exposed outside the International Space Station, and after their return to Earth, the samples were reactivated and cultured for 2.5 years to discern whether the KMC could be restored. Analyses of cellulose polymer integrity and mechanical properties of cellulose-based pellicle films, as well as the cellulose biosynthesis-related genes' structure and expression, were performed. We observed that (i) the cellulose polymer integrity was not significantly changed under Mars-like conditions; (ii) *de novo* cellulose production was 1.5 times decreased in exposed KMC samples; (iii) the dry cellulose yield from the reisolated *Komagataeibacter oboediens* was 1.7 times lower than by wild type; (iv) there was no significant change in mechanical properties of the *de novo* synthesized cellulose-based pellicles produced by the exposed KMCs and *K. oboediens*; and (v) the gene, encoding biosynthesis of cellulose (*bcsA*) of the *K. oboediens*, was downregulated, and no topological change or mutation was observed in any of the *bcs*

operon genes, indicating that the decreased cellulose production by the space-exposed samples was probably due to epigenetic regulation. Our results suggest that the cellulose-based pellicle could be a good material with which to protect microbial communities during space journeys, and the cellulose produced by KMC members could be suitable in the fabrication of consumer goods for extraterrestrial locations.

**Key Words**: Bacterial cellulose—Extraterrestrial stressors—Microbial biosignature—Kombucha multimicrobial community—*Komagataeibacter oboediens*—The *bcs* operon.

## 1. Introduction

Cellulose is one of the oldest biopolymers on Earth. Studies have shown remnants of microbial cellulose to be 250 Ma years old (Griffith *et al.*, 2008). Cellulose synthesized by various bacteria could be used as a biosignature in investigations of ancient microbial communities (Westall *et al.*, 2000; Zaets *et al.*, 2014). Bacterial cellulose (BC) is composed of a three-dimensional (3D) nanofibrous network of linear polysaccharide polymer, where d-glucose units are linked by β-(1,4) glycosidic linkages (Nishiyama *et al.*, 2003). In cellulose-based biofilm, microbial microcolonies are arranged close together in layers that facilitate communication and protection from harsh conditions within a 3D hub. The capability to synthesize cellulose has been documented in a wide variety of bacteria, including cyanobacteria, which occupy practically all terrestrial eco-niches in nature (Romling and Galperin, 2015). Biosynthesis of BCcontaining biofilms occurs in a variety of Proteobacteria that inhabit diverse ecological niches, such as fruits, flowers, and particularly fermented beverages. *Komagataeibacter* spp. (acetic acid cellulose-based biofilm-forming bacteria) are the most practically valuable among them (Ross *et al.*, 1991; Vigentini *et al.*, 2019).

BC is quite similar to that found in plants; however, contrarily to vegetative cellulose, BC does not contain traces of lignin, hemicellulose, or pectin (Azeredo *et al.*, 2019). The robust cellulose properties such as high crystallinity, water holding capacity, thermostability, mechanical properties, biocompatibility, and biodegradability offer a wide range of applications in different fields, including optics, electronics, and medical and food industries (Villarreal-Soto *et al.*, 2018; Sales *et al.*, 2019). BC has a high sustainable value and a great potential to be used in extraterrestrial bioeconomy for providing biodegragable and reusable goods and materials in power engineering, water cleaning facilities, health care, and so on (Blanco Parte *et al.*, 2020). Despite its wide applications, stability of cellulose after exposure to an extraterrestrial environment has never been obtained in the context of cellulose as a putative nanomaterial. To the best of our knowledge, there are no records on the cellulose-synthetic activity in bacteria after extraterrestrial journeys.

In our preflight experiments, the BC produced by acetic acid bacteria within the kombucha microbial community (KMC) was classified as a possible biosignature, indicating the presence of life, and was also mentioned as an indicator of bacterial activity in the extraterrestrial environment (Kukharenko *et al.*, 2012; Zaets *et al.*, 2014). We proposed live cellulose-based pellicle samples for an astrobiological flight experiment to investigate further the previous findings and to determine the stability of this biomolecule after exposure to Mars-like conditions realized directly in space. The BIOlogy and Mars Experiment (BIOMEX) was performed in low Earth orbit, where various biological samples, including KMC pellicles, were exposed on the outer side of the International Space Station (ISS) for 18 months. Samples were placed in a three-level compartment, where the top

samples were exposed to ultraviolet (UV)-irradiation, and the bottom samples were protected by the two upper sample holders. The entire compartment was mounted on the EXPOSE-R2 platform fixed outside of the ISS (Supplementary Fig. S1A, B). The aim of the exposure experiment was to investigate the survivability of selected pro- and eukaryotes, as well as to analyze the stability of biomolecules as potential biosignatures under simulated Mars-like environmental conditions (de Vera *et al.*, 2019).

In our project within the BIOMEX, the purpose was to assess the endurance of BC after exposure to the harsh conditions of the space/Mars-like environment and evaluate a perspective of BC usage beyond the Earth. We present the first data on the structural integrity of bacterially produced cellulose film exposed beyond the Earth to the space/ Marslike conditions for an 18-month period of time and show changes in BC-based pellicles after a course of postflight culturings of the recovered BC producers.

## 2. Materials and Methods

### *2.1. Biomineral samples*

Desiccated mineralized pellicle specimens from KMC (ecotype IMBG-1) (*d* = 7 mm) were prepared for the astrobiological project BIOMEX as described in the work of Podolich *et al.* (2017). In brief, the KMC was grown in a filter-sterilized sugared black tea infusion (0.5% brew, 7.0% sugar) in stationary conditions at room temperature (average 21°C), and the 21-day-old KMC pellicle fragments (log7 CFU/mg dry weight) were covered with the northosite-egg white mixture to form sample ‘‘tablets.’’

At the Institute of Aerospace Medicine (German Aerospace Center, DLR, Cologne, Germany), the desiccated samples were aseptically allocated to a three-level compartment on top, middle, and bottom settings, designated as tKMC, mKMC, and bKMC, respectively, four at each level (Supplementary Fig. S1). Samples exposed at the top level underwent solar irradiation, including UV-radiation similar to that on Mars, in addition to a Mars-like atmosphere (95.55% $CO_2$, 2.70% $N_2$, 1.60% Ar, 0.15% $O_2$, ~370 ppm $H_2O$) and pressure of 980 Pa (de Vera *et al.*, 2019). Other samples were placed below them and therefore were protected from solar UV radiation, although they remained under the same Mars-like conditions. KMC samples were exposed on the EXPOSE-R2 platform along with other biomineral samples of the BIOMEX consortium for 18 months outboard and within 7 months onboard of the ISS in Mars-like conditions. The ground control samples were simultaneously stored at the laboratory at 20°C in darkness during the spaceflight as follows: (i) lKMC, organo-mineral samples, analogous to spaceflight-exposed samples, and (ii) a wild type (wt), designated as initial KMC, iKMC, used for sample preparation. In parallel, a Mission Ground Reference (MGR) experiment was performed with analogous samples at the German Aerospace Center (DLR), in simulated analogous conditions for the same duration period.

After the flight experiment, samples were taken with sterile forceps from the tubes. The samples’ surfaces were treated with sterilant, and the samples were transferred to sterile glass vials under aseptic conditions, which were prefilled with 5mL of the BTS medium (a filter-sterilized black tea [*Camellia sinensis*] [Lipton,1.2%, w/v] with white sugar [3%, w/v] with pH 2.9). The vials were then placed in an incubator at 24°C for 1 h (Podolich *et al.*, 2017). After rehydration, the embedded KMC pellicles (Fig. 1A, left) were gently released by using a sterile forceps to avoid destruction of the cellulose film.

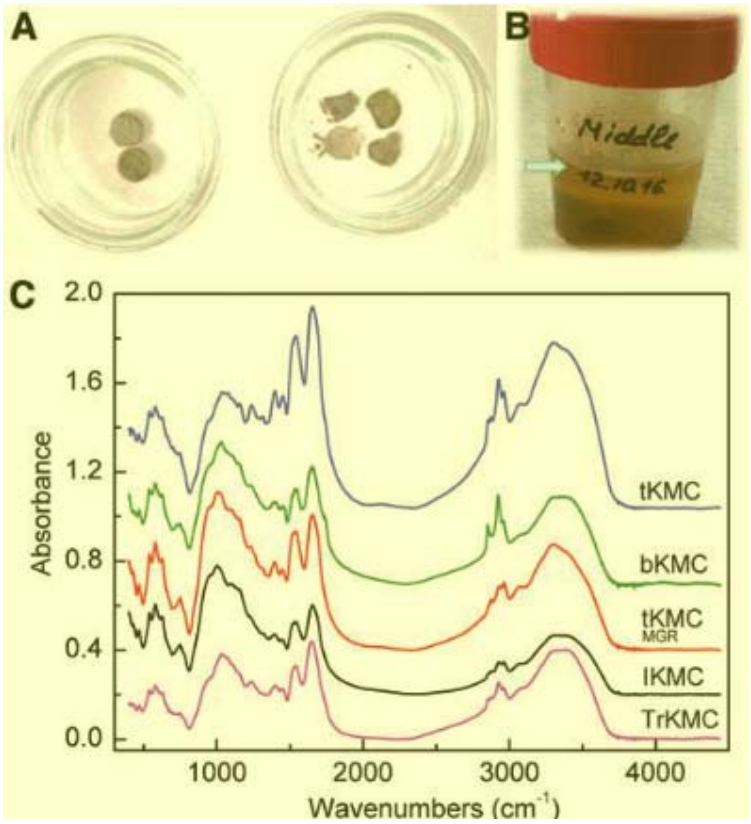


**FIG. 1**. The cellulose-based pellicle characteristics of the postflight KMC. (A) KMC cellulose-based dry pellicle samples prepared for spaceflight experiment (on the left—organomineral samples, containing dry pellicle film inside; on the right—the mined films from the dry returned samples). (B) After the spaceflight experiment, a newly formed pellicle was released from the returned KMC sample, which was exposed in the middle irradiation-protected position of the sample holder on compartment 1 of tray 2 on the EXPOSE-R2 platform mounted outside the International Space Station. (C) The absorption spectra of the exposed KMC cellulose-based pellicle samples obtained by ATRFTIR (500–4000 $cm^{-1}$): tKMC—returned samples from the unprotected to irradiation top level of the sample holder; bKMC—returned samples from the irradiation protected bottom level of the tray; tKMC/MGR—samples exposed on the top level of the compartment in Mission Ground Reference experiment (DLR, Cologne, Germany), lKMC—laboratory control samples, and TrKMC—transportation control samples. The spectra are shifted along the vertical axis for clarity (lKMC by 0.2, tKMC—0.4, bKMC—0.7, tKMC/MGR—1.04, TrKMC—1.04). ATR-FTIR, attenuated total reflection Fourier-transform infrared spectroscopy; KMC, kombucha microbial community; MGR, Mission Ground Reference.

Half of the released films (Fig. 1A, right) were used for the spectrometry analysis, and the remainder were involved in the miniproject on survivability (Podolich *et al*., 2019; 2020; Goés-Neto *et al.*,2021). KMC samples from all three levels of exposure (top, middle, and bottom), as well as a laboratory-kept reference, were reactivated with BTS at room temperature (average 21°C). Figure 1B shows the reactivated sample exposed at the middle-level position on the ISS. After the consortium restoration, aliquots of the culture from each sample (10%) were transferred to a new flask with 50mL of BTS for serial monthly culturing over the course of 2.5 years in parallel with the initial KMC ecotype.

### *2.2. Attenuated total reflection Fourier-transform infrared spectroscopy*

The absorption spectra of biofilm samples were obtained by attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) to evaluate the macromolecular composition. The ATR-method makes it possible to increase the thickness of the

investigated material due to the multiple reflectance of a beam from a prism surface. Dry samples of returned pellicles were mounted on the KRS-5 prism. The prism with the sample was clamped in a special holder for ATR studies. The ATR-FTIR analyses were carried out with a Bruker-113v Fourier Transform spectrometer. Measurements were performed at room temperature in the range of 500–4000 $cm^{-1}$ with a spectral resolution of 1.0 $cm^{-1}$. The accuracy of determination of the line position was –1 $cm^{-1}$.

### *2.3. The whole-genome bcs cluster analysis*

Given that the *Komagataeibacter* spp. (the key member in the model KMC) have multiple *bcs* operons (Gullo *et al.*, 2019; La China *et al.*, 2020), we used draft genomes of the ground sample (iKMC) and extraterrestrial stress-exposed and recovered samples (tKMC) of the *Komagataeibacter oboediens* strain to compare the topographical changes and mutations in the cellulose encoding *bcs* genes. The draft genomes were retrieved from the NCBI with accession numbers SAMN14942824 and SAMN14942470, respectively, for Ikmc and tKMC. The metabolic pathway was generated with the ‘‘Pathologic’’ software. This specific software can auto-assign pathways and compare with in-build Kyoto Encyclopedia of Genes and Genomes pathways (Santos *et al.*, 2020). By that the cellulose encoding key *bcs* operons were constructed, following the *Komagataeibacter xylinus* K2G30 *bcs* genes (Gullo *et al.*, 2019). The comparison of the extraterrestrial stress-exposed sample (tKMC) with the ground sample (iKMC)was realized by following basic methods of comparative genomics using BLAST against Uniprot database (McGinnis and Madden, 2004), and the visual analysis of the genomes was carried out by using Pathway tools (Karp *et al.*, 2010).

### *2.4. Cellulose synthesis gene expression assay*

The complete RNA of the 2-day *K. oboediens* cultures ($10^7$ CFU/mL) was isolated by using innuSOLV RNA reagent, according to the manufacturer’s instructions (Analytik Jena AG, Germany). The RNA quality was determined by agarose gel electrophoresis very close to the time of cDNA synthesis from RNA. The RNA concentration and purity were determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), by measuring the $A_{260}$ and $A_{260}/A_{280}$ values, respectively. The cDNA synthesis was performed with the RevertAid™H Minus First Strand cDNA Synthesis Kit (Fermentas, Lithuania) using random hexamer primers according to the manufacturer’s instructions. Four oligonucleotide primer pairs designed by Augimeri and Strap (Augimeri *et al.*, 2015) for the genes encoding the cellulose biosynthesis (*bcsA, bcsB, bcsC,* and *bcsD*) were used in the RT-qPCR along with the housekeeping gene *gyrB*. The syntheis was performed with the iCycler iQ5 Real-time PCR System and Bio-Rad iQ5 software (Bio-Rad Laboratories, Germany), using cycling conditions with annealing temperature gradient (10 min at 95°C; 45 cycles of 95.0°C for 20 s, the $_{Ta,opt}$. for each primer set for 30 s and 72.0°C for 30 s). The PCR mixture contained EvaGreen®qPCR Mix Plus (NO ROX; Solis BioDyne, Estonia), 0.6 µ*M* of gene-specific forward and reverse primers, and cDNA in a 20-µL reaction. ‘‘No-template’’ control contained RNase-free water instead of cDNA. The specificity of PCR was analyzed with melting curves and by agarose gel electrophoresis. The amplification efficiencies for individual reactions were calculated with the LinRegPCR software version 11.0 (http://LinRegPCR.nl). Relative expression analysis correction for PCR efficiency and normalized with respect to the reference gene *gyr*B was performed with Gene Expression Macro™ version 1.1 (Bio-Rad Laboratories).

### *2.5. Production of BC*

Rates of bacterial growth within a sample were tracked before estimation of pellicle biomass. Monocultures of *K. oboediens* strain reisolated from tKMC (*K. oboediens*/tKMC), as well as wt strain, were grown in HS medium (Hestrin and Schramm, 1954) in 250mL flasks at 30°C in stationary conditions for 14 days and plated daily on the HS agar medium, using serial dilutions.

For a cellulose-based pellicle biomass yield determination, revived postflight KMC samples from all exposure locations (top, middle, and bottom) were grown in 24-well sterile plates in BTS (final volume 300 µL), respectively, at 30°C in 10-fold repetition. Cultures of *K. oboediens*/tKMC and wt *K. oboediens* were grown in 250mL flasks with 50mL of HS medium, and the pellicle films were harvested on the 10th and 7th days, respectively. Pellicles were dried at 50°C until a constant weight was obtained. The measurement of dry weight of cellulose-based pellicles was carried out by using an analytical balance KERN 770 (Kern & Sohn Gmbh, Germany).

### *2.6. Mechanical properties of BC in postflight bacteria*

BC-based pellicle specimens were cut into samples of 50 X 50 X 0.05mm dimensions. Mechanical testing of the BC samples was performed by using the P-50 universal tensile testing machine (Milaform, Russian Federation) at a deformation rate of 10 mm/min. An average value (with standard deviation [SD]) for the tensile strength was obtained from three samples of each film.

### *2.7. Statistical analyses*

The statistical analyses of the results were analysed with SPSS version 11.0 software for Windows (SPSS, Inc., Chicago, IL). The presented data are expressed as mean values ± SDs, where each value was the average of triplicate reaction. The *t*-test was performed to estimate the differences between space-exposed and reference samples. *p*-Values lower than 0.05 were considered to be significantly different. Statistical significance is indicated by asterisks (*$p \leq 0.05$, ** $p \leq 0.01$, ***$p \leq 0.001$).

## 3. Results

### 3.1. No significant change in cellulose polymer integrity was recorded after exposure to space/Mars-like conditions

ATR-FTIR analysis is a suitable analytic method to uncover cellulose degradation through changes or lack of changes in the obtained vibration spectra (Talari *et al.*, 2017; Fuller *et al.*, 2018). Earlier, we showed through spectral characterization that mineralized BC samples were changed diagenetically, but the feature characteristics could be recognized by FTIR spectroscopy (Zaets *et al.*, 2014). A series of preflight experiments, simulating the influence of space/Mars-like factors, clearly showed small differences in the FTIR spectra between treated and laboratory specimens (Fuller *et al.*, 2018). It has been observed that if anorthosite was present in the samples (it was used to mineralize a pellicle film), a shielding effect can be determined in the vibration spectra range of 800–950 $cm^{-1}$, which can be assigned to anomeric carbons. In the postflight cellulose film samples, we observed the same findings. Moreover, resulting vibration spectra for specific $\beta C_1$-O-$C_4$ glycosidic linkage (1090, 992 $cm^{-1}$), as well as skeletal stretching vibration spectra, involving -CH- stretching at $C_3$ (1074–1078 $cm^{-1}$) and C-O at $C_6$ (1028–1035 $cm^{-1}$) in pyranose rings, were detected in the

samples tKMC, mKMC, and bKMC (Supplementary Table S1). Furthermore, there were shifts of peaks to higher frequencies within a range of 1000–1238 $cm^{-1}$ (Fig. 1C).

A shift of the skeletal vibration spectra, involving C-O stretching at $C_6$, was detected at lower frequencies. Specific $\beta C_1$-O-$C_4$ glycosidic linkage vibration spectra were also lower both in the exposed samples and the reference laboratory or transportation controls in comparison with native (initial) cellulose-based pellicle samples. The intensity of C-O stretching of COH/C-O-C in pyranose ring skeletal vibration at 1105 $cm^{-1}$ decreased in the KMC specimens located at the unprotected top-level sample (tKMC) carrier exposed to solar UV. The -CH- stretching vibrations on $C_3$ (1001 $cm^{-1}$) were not observed here. In contrast, in films removed from KMC samples exposed at the protected bottom level (bKMC), the identical -CH- stretching vibrations at $C_3$ were detected, as well as in films that originated from the top-level KMC samples from the parallel ground-based simulation experiment (tKMC/MGR).

The ATR-FTIR analysis did not reveal the presence of C =O stretch at 1740 $cm^{-1}$ (free –COOH; –COOR of normal and/or cyclic ester group: δ-valerolactone) as a probable sign of hydroxyl group oxidation or cellulose degradation in the returned samples. Preservation of OH-stretching vibration at 748 $cm^{-1}$, which is characteristic of hydroxyl bonding in cellulose structure due to the Iα crystalline phase, may serve as the evidence of structural integrity of cellulose polymer in the space-exposed samples. The FTIR spectra on the samples exposed to the ground MGR experiment, in parallel with a spaceflight exposure, showed quenching of C-O-C bond vibrations. The reason for this could be the stronger stress conditions of the ground simulation experiments compared with the conditions in space.

BC is characterized by a higher purity compared with plant-derived cellulose; however, BC-based pellicle, as a host of resident microbes, includes the cells, free nucleic acids, extracellular membrane vesicles, and so on in its 3D network matrix. In this astrobiological experiment, egg white and anorthosite rock powder were used for sample preparation to create their final structure, and therefore, there were additional ‘‘impurities’’ (Podolich *et al.*, 2017). The FTIR analysis clearly demonstrated expected groups of BC ‘‘impurities’’ (Supplementary Table S1). Bands around 3300–3400 $cm^{-1}$ were attributed to the stretching vibrations of the OH and NH bonds of proteins and amino acids (amide A) present in the pellicle and generated from microbial cells, as well as from protein present in the egg white. The protein spectra exhibited vibrations at 1633 $cm^{-1}$, which is the dominant feature of the amide I region, as well as at 1540 $cm^{-1}$—amide II and 1311 $cm^{-1}$—amide III. Lipids were represented by the $CH_2$- and $CH_3$-groups of fatty acids at 2960 $cm^{-1}$ (asymmetric C-H stretching of $CH_3$), 2929 $cm^{-1}$ (asymmetric C-H stretching of $CH_2$), 1450 $cm^{-1}$ (scissoring C-H bending vibration of $CH_2$), and 1398 $cm^{-1}$ (scissoring C-H bending vibration of CH3). The phosphate groups were represented by -P =O asymmetric vibration at 1224 $cm^{-1}$.

Asymmetric $CH_3$ and $CH_2$ stretching vibrations at ~2959 and ~2931 $cm^{-1}$, respectively, and symmetric $CH_3$ and $CH_2$ stretching vibrations at 1398 and 1450 $cm^{-1}$, respectively, can be used to represent changes in lipids by ratio $CH_3/CH_2$. In this study, the mean value of 2959/2931 was 1.09 for reference samples, while it was 0.72 ( $p < 0.05$) (bKMC) or 0.92 ( $p < 0.05$) (tKMC) for space samples, which suggests that the lipids in exposed bKMC samples had more methylated and/or probably more branched chains than the lipids in reference samples.

Taken together, our analysis shows that the integrity of the cellulose polymer was not changed significantly under space/Mars-like conditions compared with the ground-based reference samples.

### *3.2. The structure of the bcs operons was not disturbed, but its expression was found to be deregulated in space-exposed K. oboediens*

BC producers have a potential to be used for biofabrication of cellulose in extraterrestrial bases after long-term space travel. The aim of this study was to investigate the impact of the environmental space conditions on the expression of cellulose synthesis genes. In KMCs, cellulose is synthesized by acetic acid bacteria, mainly representatives of the *Komagataeibacter* genus (Ross *et al.*, 1991). Komagataeibacters produce cellulose by a BC synthase (*Bcs*) complex, containing subunits *BcsA, BcsB, BcsC*, and *BcsD*, which are encoded by three (*bcsAB, bcsC*, and *bcsD*) or four (*bcsA, bcsB, bcsC*, and *bcsD*) genes (Augimeri *et al.*, 2015).

In the KMC ecotype used for BIOMEX, *Komagataeibacter* spp. are the dominant cellulose-synthesizing bacteria (Podolich *et al.*, 2019), and *K. oboediens* represents the most abundant population in t KMC and is one of the best candidates to analyze. Our aim was to assess the topological and expression difference of the *bcs* genes in the *K. oboediens* between the exposed and ground-based reference samples. Since it is reported that K. xylinus probably belongs to *K. oboediens* and the *bcs* operon of *K. oboediens* 174Bp2 is ''unusual'' (Ryngajłło *et al.*, 2019), we constructed the established cellulose synthesis key *bcs* operon following the K. xylinus K2G30 (a relatively close member of *K. oboediens*, belonging to the same clade) cellulose synthesis *bcs* operon as described in the works of Liu *et al.* (2018) and Gullo *et al.* (2019). The full set of the cellulose-synthesizing *bcs* genes in K. xylinus consists mainly of the *bcs* operon I that contains four genes: *bcsA, bcsB, bcsC*, and *bcsD*, and three additional genes: *bcsZ (cmcAx*) and *bcsH* (*ccpAx*) in upstream position, and *bglX (bglAx*) in downstream position (Liu *et al.*, 2018; Gullo *et al.*, 2019; La China *et al.*, 2020).

Our draft genome-based *bcs* operon analysis shows the presence of four *bcs* operons of the *K. oboediens* (Fig. 2), and this is in line with results described in the work of Ryngajłło *et al.* (2019). To achieve this, we first identified the *bcs* genes in our *K. oboediens* sample based on the *bcs* genes of K. xylinus K2G30 to confirm that these genes are also present in the available complete genome of *K. oboediens*. Next, we performed BLASTn against *K. oboediens* strain BPZTR01 (the GenBank Accession No. CP043481.1). The BLASTn results show the presence of four operons of the *bcs* genes both in our *K. oboediens* and the *K. oboediens* BPZTR01 genomes (Supplementary Table S2). The K. xylinus-based construction shows that the *bcsI* operon of *wt K. oboediens* in the iKMC sample consists of all four key genes*: bcsA, bscB, bcsC*, and *bcsD* along with three regulatory genes *cmcax, ccpAx*, and *bglAx* in proper orientation (Fig. 2). The *K. oboediens bcsII* operon from iKMC also shows the topology to be similar to that of *K. oboediens* 174Bp2 consisting of four genes: *bcsAB-II, bcsX, bcsY*, and *bcsC-II* (Fig. 2). The *bcsIII* and *bcsIV* operons also had the same organization as described in the work of Ryngajłło *et al.* (2019) (Fig. 2). While we compared the *bcs* operons of the ground sample (iKMC) with that of the space-exposed sample (tKMC), we observed no topological change in any of the *bcs* operon genes (Fig. 2). We also observed no mutations in any of the *bcs* operon genes in the exposed *K. oboediens* genome (Supplementary Tables S3 and S4).

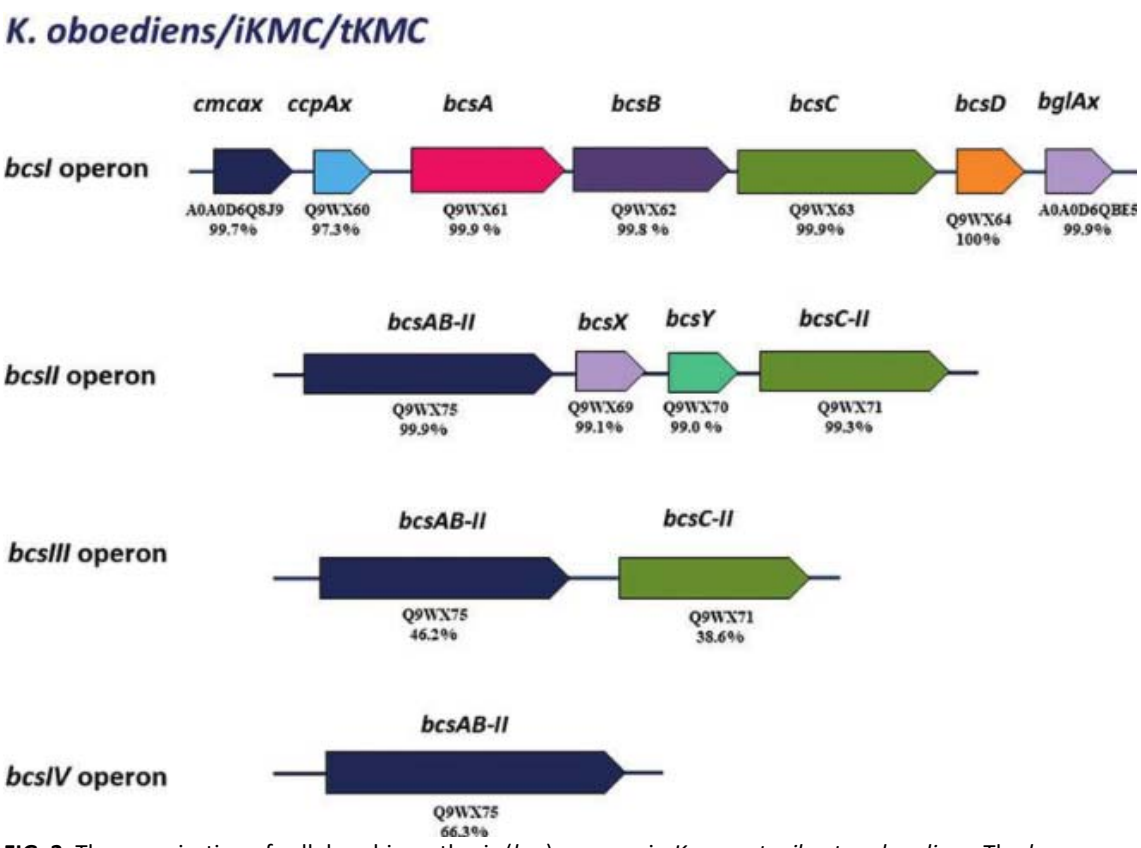


**FIG. 2.** The organization of cellulose biosynthesis (*bcs*) operons in *Komagataeibacter oboediens*. The *bcs* operons of *K. oboediens* strain isolated from KMC (ecotype IMBG) (*K. oboediens*/iKMC) were designed based on the *bcs* operons of *Komagataeibacter xylinus* K2G30 (Gullo *et al.*, 2019) and compared with the *bcs* operons of K. oboediens 174Bp2 (Ryngajłło *et al.*, 2019). The sequence identity percentage among the retrieved coding sequence of *K. oboediens*/iKMC and *K. oboediens*/tKMC (isolated from KMC exposed to Mars-like stressors [UV, atmosphere, pressure] simulated at the International Space Station) and the sequence in Uniprot databases of K. xylinus K2G30 were shown under each represented gene. UV, ultraviolet.

Interestingly, however, while we performed the expression analysis of the key four genes (*bcsA, bcsB, bcsC,* and *bcsD*) in *K. oboediens* originated from exposed tKMC, as well as in control variant of bacteria from iKMC, we observed that in postflight *K. oboediens*, the *bcsA*, encoding the BC-synthase, was downregulated, while the *bcsD* was upregulated significantly compared with a control. There is no significant change in expression observed for *bcsB* and *bcsC* (Fig. 3).

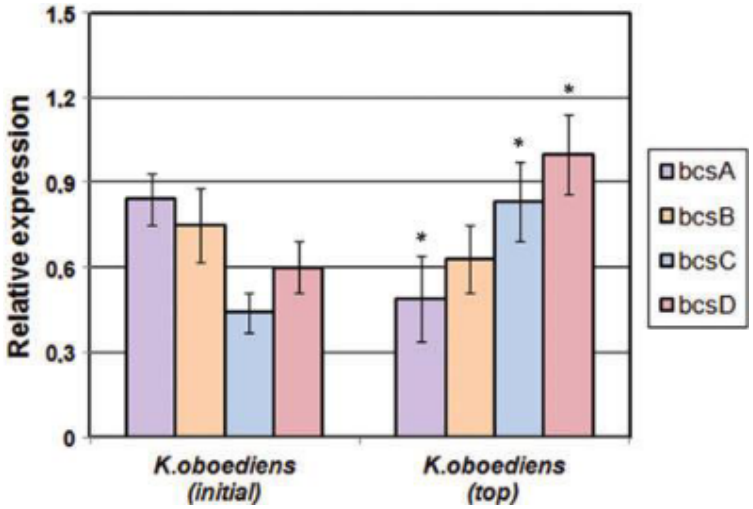


**FIG. 3.** Differentially expressed genes of cellulose synthesis (*bcsA, bcsB, bcsC,* and *bcsD*) in *Komagataeibacter oboediens* originated from KMC exposed to Mars-like conditions at the top level (tKMC) of the compartment 1 in tray 2 mounted on the EXPOSE-R2 platform outside the International Space Station, as well as in control variant of bacteria from initial KMC ({KMC). Data are shown as mean – SD (n = 3). Statistical significance was assessed between the experimental samples and initial KMC (*p £ 0.05). SD, standard deviation.

To summarize, our data reveal that the expression of the *bcs* operon is deregulated without the topology changes under Mars-like stress, indicating a probable epigenetic regulation of cellulose synthesis under such extraterrestrial conditions.

### *3.3. Poststress de novo synthesis of BC is decreased*

After investigating the potential changes in the *bcs* operon genes, we aimed to determine the *de novo* cellulose production by the experimental KMC samples. KMC-related acetic acid bacteria produced *de novo* cellulose-based pellicle film, which appeared to be morphologically similar to native kombucha biofilm (Fig. 4). The BC-based membrane is a branched 3D network organized by adjoining fibrils into 20–50-nm-wide flat and twisted ribbons (Reddy and Yang, 2015) and possesses pores of 100–300 nm (Hutchensa *et al.*, 2006). In a comparison between BC membranes produced by exposure to the space/Mars-like KMCs and BC membranes of both reference KMCs, the laboratory-stored and initial KMC ecotype showed a similar average in pore size and fibril thickness (Fig. 4F, G). However, there were some differences in the yield of cellulose production (in gram of dry cellulose per milliliter) between space-exposed and initial KMCs (Fig. 5A). The yield of dry cellulose decreased with a factor of about 1.5 times ( $p < 0.05$) in the unprotected tKMC samples, but in the samples from bottom-level KMCs, it was not significantly decreased compared with the initial iKMC samples. The mKMC samples produced a thicker pellicle, comparing with initial KMCs, however, a difference in dry biomass between these samples was not statistically significant (Fig. 5A).

Our goal was also to study the cellulose-synthesizing efficiency of the *Komagataeibacter* genus representatives after space exposure. The cellulose yield of *K. oboediens* was tied to the culture growth rate peculiarity. Comparative dynamics of culture growth between the exposed and reference ground-based strains of *K. oboediens* showed that the growth rate in postflight strain was lower compared with initial *K. oboediens*: if the wt reaches a stationary phase of growth on the 7th day, the strain isolated from the

exposed tKMC sample—only on the 10th day (Fig. 5D). In the stationary phase of growth, cellulose pellicles of both starins were harvested and dried. As expected, similar to the observation from the gene expression analysis, we found that the cellulose-based pellicle biomass of *K. oboediens* reisolate recovered from tKMC decreased, comparing with the yield of dry cellulose by the homological wt *K. oboediens* produced, and the difference was a 1.7-times ( $p < 0.05$) (Fig. 5E).

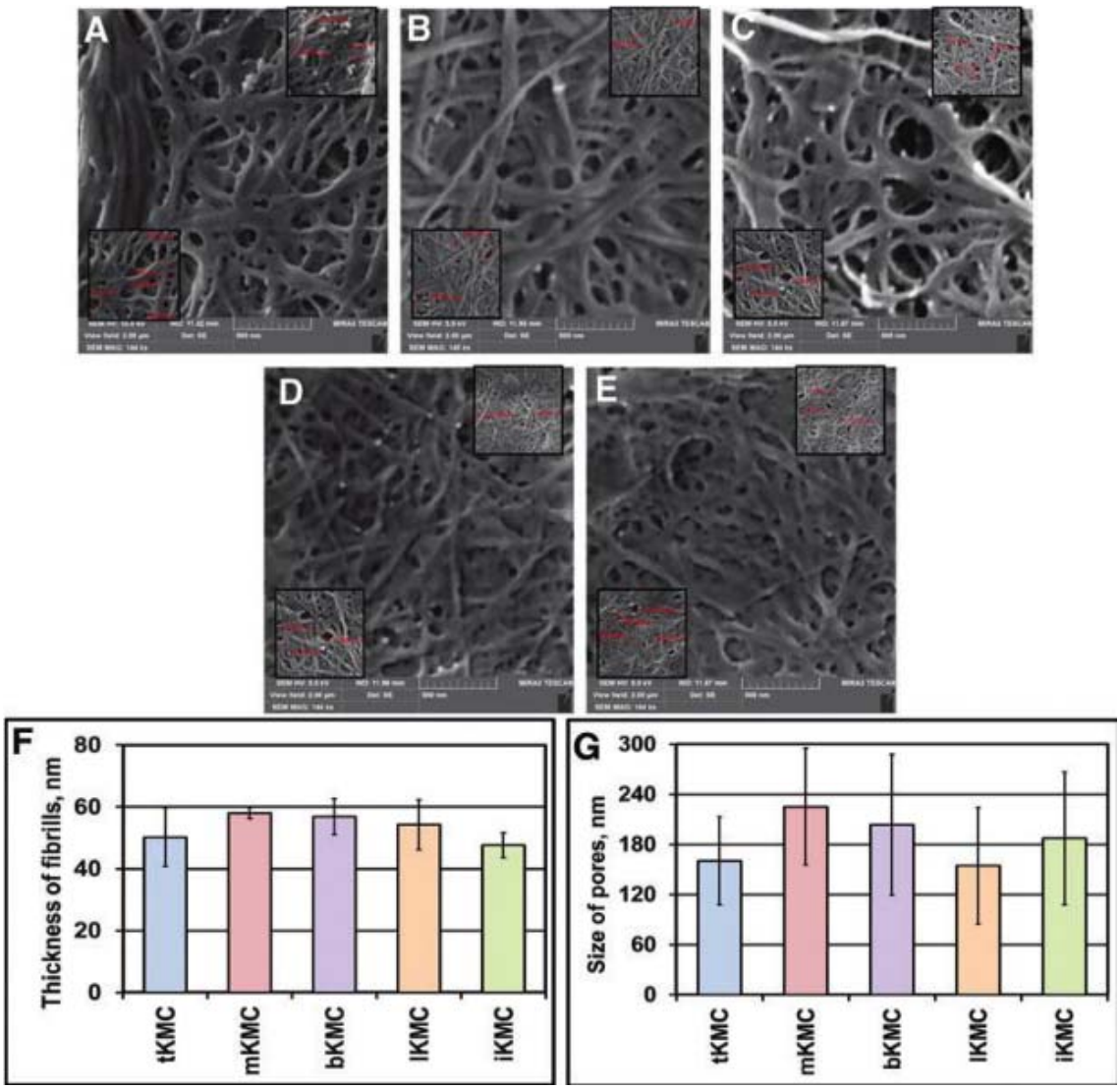


**FIG. 4.** The scanning electron microscopy images of *de novo* synthesized cellulose embranes originated from pellicles produced by bacterial members of KMC samples. The KMCs were exposed at the different levels of a tray mounted on EXPOSE-R2 platform installed outside the International Space Station: top (A), middle (B), bottom (C) levels; the ground-based eference samples stored on the ground during the spaceflight experiment: KMC samples kept in the laboratory (D); and initial KMC used for sample preparation (E). Scale bars 500 nm. A thickness of fibrils (F) and the size of pores (G) selected randomly on the scanning electron microscopy images of cellulose membranes originated from pellicles produced by the KMCs. Data are shown as mean – SD (n = 10). Statistical significance was assessed between the experimental samples and wild-type reference (iKMC) ( p £ 0.05).

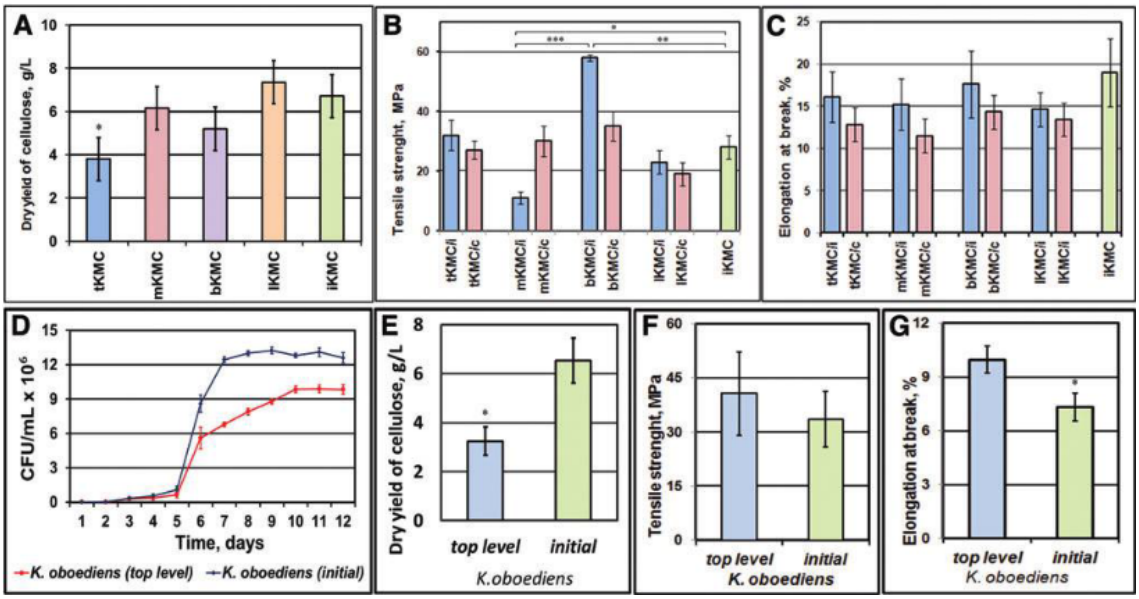


**FIG. 5**. Poststress de novo synthesized cellulose-based pellicle films by KMCs and *Komagataeibacter oboediens* reisolated from the appropriate postspaceflight KMCs. Bacterial cellulose-based pellicle yield in the revived KMCs (A) and K. oboediens (E). Mechanical properties of the cellulose-based pellicle films (B, tensile strength; C, elongation at break) of the returned KMC samples (designated as KMC/i) from the top, middle, and bottom levels in compartment 1 of tray 2 exposed on the EXPOSE-R2 platform outside the the International Space Station and KMCs after a series of culturing (KMC/c). Mechanical properties of cellulose-based K. oboediens pellicle films (F, tensile strength; G, elongation at break). Data are shown as mean – SD (n = 10). Statistical significance was assessed between the experimental samples and wild-type reference (iKMC) (*p £ 0.05, **p £ 0.01, ***p £ 0.001). Comparative dynamics of culture growth between the space-exposed and reference ground-based strains of *Komagataeibacter oboediens* (D). Data are shown as mean – SD (n = 3). Statistical significance was assessed between the experimental sample and wild-type reference ( p £ 0.05).

### 3.4. Mechanical properties of BC were not altered significantly under Mars-like stress

Another objective of our research was to investigate the tensile properties of cellulose-based pellicles produced by space-exposed KMCs that were revived after spaceflight and by reisolated *K. oboediens* monocultures. We also wanted to compare the mechanical strength between cellulose derived from returned KMC samples and KMC samples cultured within 2.5 years after a spaceflight. The tensile strength of cellulose-based pellicle specimens produced by the postflight KMCs varied between the exposure locations (top, middle, and bottom). Nevertheless, they showed similar strength in comparison with initial KMC pellicles, that is, except for the bKMC, which showed higher tensile strength than the ground references (Fig. 5B). Notably, after a series of culturings, the difference in values of tensile strength between postflight and control samples was reduced.

The pellicle film elasticity tends to decrease in space experiment-related samples, in comparison with the initial KMC cellulose-based pellicles. However, the differences were insignificant (Fig. 5C). The scattering of tensile strength and elasticity demonstrates the nonuniform structure of preserved BC after exposure to the Mars-like stressors. With respect to the *K. oboediens* monocultures, the elongation at break of the BC film produced by *K. oboediens*/tKMC showed a higher elasticity compared with the *K. oboediens* pellicle films of the original KMC ecotype (Fig. 5G).

## 4. Discussion

### *4.1. Changes of cellulose polymer FTIR spectra and morphology were minimal after impact of space/Mars-like stressors*

In this spaceflight experiment, cellulose as a KMC pellicle constituent underwent different stringent environmental stressors such as desiccation, solar irradiation, temperature fluctuations, changed gravity, and atmosphere. Besides the environmental exposure conditions during the exposure period outside the ISS, some stressors also occurred during the sample preparation, transportation to the launch place, the launch *per se*, and on the return transport to Earth and back to the laboratory. Remarkably, after the impact of the aforementioned events and impact of appropriate stressors, specific $\beta C_1$-O-$C_4$ glycosidic linkage vibration spectra were recorded by using ATR-FTIR in cellulose polymer. Moreover, a characteristic hydroxyl bonding in cellulose structure due to the Iα crystalline phase was preserved in the cellulose polymer, which may serve as evidence of cellulose structural integrity in the postspace-exposed samples. However, there were some alterations in the cellulose polymer structure that caused shifts of vibration spectra to higher frequencies or to lower wave numbers. Such changes in the cellulose-based pellicles could have been due to the effect of ionizing radiation (IR) (gamma rays, ion beams, or electron beams) that crosslinks or degrades polymers, breaking polymer chains and causing reactive intermediates (Kabanov *et al.*, 2009).

Earlier experiments on the impact of IR on vegetative cellulose have shown (i) depolymerization by chain scission, (ii) free-radical formation followed by other degradation processes, and (iii) a decrease in the amount of Iα cellulose (Ershov, 1998; Iller *et al.*, 2002; Morin *et al.*, 2004). It has also been reported that IR has an impact on dissociated cellulose that leads to oxidation in the crystalline areas (Henniges *et al.*, 2013). The *in vitro* degradation of the irradiated cellulose polymer resulted in weight loss and decreased tensile strength with increasing irradiation dose (Darwis *et al.*, 2013).

During the BIOMEX mission, the cosmic radiation dose was low (0.5 Gy) because of the location of the ISS, which is within the shielding Earth's magnetosphere. At the EXPOSER2 platform on the outer side of the ISS, the radiation risk of galactic cosmic ray particles and their secondary products—protons, relativistic electrons, and/or solar energetic particle events—was estimated to be much smaller than safe doses that are permissible for crews (Henniges *et al.*, 2013). Therefore, we expect a significant bombardment of the exposed samples with such rays or particles only to be a rare event. Notably, exposed kombucha cellulose-based biofilm samples were not wet, but desiccated; an irradiation of wet samples is known to facilitate oxidation compared with chain degradation, as water provides a medium for radicals to encounter each other and recombine (Darwis *et al.*, 2013).

Light-induced polymer degradation, or photodegradation, is another known factor for polymer decomposition (Dachev *et al.*, 2017). Photodegradation of vegetative cellulose under UV light catalyzed by TiO2 is well known (Yousif and Haddad, 2013). Solar UV irradiation at the top level of the sample carrier was recognized to be the most damaging factor to both the cellulose pellicle and microbial cells.

The KMC pellicle samples were exposed on the top-level location within the exposure compartment without any neutral-density filters, which normally reduce the irradiation on the sample site. Total UV (>200 nm) fluencies of about $4.92 \cdot 10^2$ kJ/m$^2$ were received by the exposed samples (de Vera *et al.*, 2019). Likely, the absorption of UV quanta

by pentose rings at C3 resulted in the loss of the -CH-stretching vibrations, also the intensity of C-O stretching of COH/C-OC of pyranose ring skeletal vibration decreased in the exposed KMC samples. In another BIOMEX project, pure vegetative cellulose in a mixture with Mars analog minerals was detectable only in UV-protected samples (pers. comm.). Such different levels of degradation can likely be explained by the presence of life-relevant organic matter, that is, microbial organisms associated with cellulose polymer and their metabolites that could quench harmfull action of the UV quanta.

Desiccation stress for cellulose polymer within the organomineral mixture of prepared samples was also observed to be minimal. The dry KMC control samples, which were kept desiccated in the laboratory, did not show essential changes in the cellulose FTIR absorption spectra.

### *4.2. Changes in the cellulose producion capability by postflight reactivated KMC and K. oboediens monoculture*

Our experiment showed that, after long-term spaceflight, the cellulose biosynthetic capability, as well as mechanical properties of cellulose-based pellicle, changed in KMCs according to the exposure locations in the compartment. For example, samples exposed at the protected middle and bottom levels had a dry cellulose output practically equal to the controls. In contrast, the cellulose pellicle weight decreased in the samples from an unprotected exposure level, tKMC, compared with the initial KMC. In a submitted article, we show that in returned and reactivated tKMC, the cellulose-synthesizing bacterial community structure was dramatically changed after exposure to stressors as indicated by metagenomic analysis, and this could have resulted in the decreased capacity to produce cellulose. After serial culturings, the community structure was more changed, but for 2.5 years, it did not return to the initial composition. Similarly, after a long period of permanent culturings, mechanical properties of cellulose produced by KMCs improved but did not come to normality.

The same tendency of consequences of stress factor influence was observed on cellulose produced by monoculture *K. oboediens*, which maintained ‘‘memory’’ about the past exposure to stressors, and this is in agreement with known facts of alterations in bacterial physiology during spaceflight experiments (Nickerson *et al.*, 2004; Huang *et al.*, 2018; Yu *et al.*, 2019). In *K. oboediens*/tKMC, whose growth rate is lower than in the reference strain, cellulose synthesis could depend on the culture cell number, as well as on regulation of gene expression. Earlier, we detected alterations in cell outer membranes in exposed bacteria, and this could result in onset of the cellulose synthesis process, as the cellulose thread moves through the membrane outside, and so on (Podolich *et al.*, 2020). Cellulose synthase operon proteins BcsCI and BcsCII are beta-barrel proteins, likely forming channels in the outer membrane (Hayat *et al.*, 2016). Since proteins may be involved in the formation of a membrane complex for extrusion of the cellulose product, a disturbance noticed in *K. oboediens*/tKMC outer membrane may result in an incorrect integration of *BcsCI* and *BcsCII* barrels with the membrane, an influence on secretion of the polymer, and finally, a decrease in cellulose production.

Current genomic data analyses of cellulose-producing bacteria show a significant diversity in the *bcs* operons and their link to cellulose synthesizing capacity (Gullo *et al.*, 2019; Hernandez-Arriaga *et al.*, 2019; Jacek *et al.*, 2019; Ryngajłło *et al.*, 2019). Among several such operons, the *bcsI* operon is the only one that contains all the genes required for cellulose biosynthesis (Hernandez-Arriaga *et al.*, 2019; Lu *et al.*, 2020).

*K. oboediens* has four *bcs* operons, and among the four, the *bcsI* is the key operon for cellulose synthesis (Ryngajłło *et al.*, 2019). In genome-based *bcs* operon analysis, we observed the same organization of four *bcs* operons similar to those of K. xylinus (Liu *et al.*, 2018; Gullo *et al.*, 2019) and *K. oboediens* 174Bp2 (Ryngajłło *et al.*, 2019). Furthermore, we observed that the Mars-like stressors did not alter the topology or induce any mutation in any of the *bcs* operon genes of *K. oboediens*, reisolated from tKMC. However, in the revived *K. oboediens* from the tKMC sample, the cellulose synthase gene bscA was downregulated, despite the full homology/topology of the *bcsABCD* to wt gene cluster, supporting the observation of the lower cellulose output by revived bacteria compared with the ground reference. We may assume that changes in *komagataeibacter*'s cellulose synthetic apparatus or in the regulation of the *bsc*-genes occurred at the epigenetic level or due to other mechanisms to be defined further.

### *4.3. Future perspectives to the biofabrication of cellulose composites for extraterrestrial habitable bases*

BC is a potentially interesting astrobiological material for *in situ* good fabrication (Bacakova *et al.*, 2019; Dutta *et al.*, 2019) and offers several advantages as a starting material for different biotechnology applications due its properties and easy modification of its crystalline structure, including functionalization with other materials to form advanced fiber composites. In early-stage development of environmentally sensitive materials at permanently manned extraterrestrial bases, biofabrication will play a key role because this practice will correspond to the principles of self-organization and existence of extra-terrestrial settlements aimed at the rational use of resources and energy, environmental protection, reutilization, and contribution to a cleaner cost-effective production (Camere and Karana, 2018). In this study, we have shown high resistance of impure cellulose polymer to a set of stressors under exposure experiments in Earth orbit at the ISS. Interest in BC will likely increase, given its multiple applications, and thus, BC is a key subject in interdisciplinary research toward generation of smart cellulose materials for both terrestrial and extraterrestrial purposes.

Currently, applied controlled biosynthesis of patterned cellulose and functionalization of cellulose could be used in extraterrestrial settings. Similarly, increasing attention is paid to cellulose-producing organisms. For example, the reprogrammed acetic acid bacterial strains that are capable of producing high yields of BC in low-cost media with a precise control of heterologous cellulose biosynthesis gene expression have been designed (Florea *et al.*, 2016; Buldum *et al.*, 2018; Teh *et al.*, 2019). Our results show that cellulose-producing bacteria of the *Komagataeibacter* genus are tolerant to spaceflight and Mars-like stressors. However, the rate of tolerance may depend on the genotype of the species. As komagataeibacters show a significant potential of survival in extraterrestrial conditions, these bacteria have a chance to be safely delivered there within a cellulose-based pellicle after long-distance travels, and they can achieve efficient cellulose production at extrarettestrial, permanently manned bases (Cichan *et al.*, 2017).

## 5. Conclusion

For the first time, the results of the BIOMEX experiment on BC stability in harsh conditions beyond the Earth have shown significant cellulose polymer stability within the pellicle film inhabited by microbial organisms. Furthermore, ATR-FTIR absorption spectra analysis of BC exposed to space and Mars-like environmental conditions showed preservation of the

characteristic cellulose vibration spectra. Such a spectral pattern could also serve as a biosignature for future life detection during space missions. The location of samples on the exposure platform outside the ISS correlated with a rate of alterations in cellulose polymer. The study showed that cellulose-based pellicle could serve as a reasonable carrier matrix of KMC members in extraterrestrial journeys. The cellulose produced biotechnologically by KMC/komagataeibacters would be suitable for the fabrication of diverse consumer products in extraterrestrial environments.

**Acknowledgment**
J.-P.d.V. is very thankful to DLR programatic support through the BIOMEX project.

**Author Disclosure Statement**
No competing financial interests exist.

**Funding Information**
This study was supported by the National Academy of Sciences of Ukraine (Grant 49/2018-2019) and the DLR programatic management (Grant BioSigN PF/FE).

**References**
Augimeri RV, Varley AJ, and Strap JL (2015) Establishing a role for bacterial cellulose in environmental interactions: lessons learned from diverse biofilm-producing *Proteobacteria. Front Microbiol* 6:1282.

Azeredo HMC, Barud H, Farinas CS, *et al.* (2019) Bacterial cellulose as a raw material for food and food packaging applications*. Front Sustain Food Syst* 3:7.

Bacakova L, Pajorova J, Bacakova M, *et al.* (2019) Versatile application of nanocellulose: from industry to skin tissue engineering and wound healing. *Nanomaterials* 9:164.

Blanco Parte FG, Santoso SP, Chou C-C, *et al.* (2020) Current progress on the production, modification, and applications of bacterial cellulose. *Crit Rev Biotechnol* 40:397–414.

Buldum G, Bismarck A, and Mantalaris A (2018) Recombinant biosynthesis of bacterial cellulose in genetically modified *Escherichia coli. Bioprocess Biosyst Eng* 41:265.

Camere S and Karana E (2018) Fabricating materials from living organisms: an emerging design practice. *J Clean Prod* 186:570–584.

Cichan T, Bailey SA, Antonelli T, *et al.* (2017) Mars base camp: an architecture for sending humans to Mars. *New Space* 5:203–218.

Dachev TP, Bankov NG, Tomov BT, *et al.* (2017) Overview of the ISS radiation environment observed during the ESA EXPOSE-R2 mission in 2014–2016. *Space Weather* 15: 1475–1489.

Darwis D, Khusniya T, Hardiningsih L, *et al.* (2013) *In vitro* degradation behaviour of irradiated bacterial cellulose membrane. *Atom Indones* 38:78–82.

de Vera J-P, Alawi M, Backhaus T, *et al.* (2019) Limits of life and the habitability of Mars: the ESA space experiment BIOMEX on the ISS. *Astrobiology* 19:145–157.

Dutta SD, Patel DK, and Lim K-T (2019) Functional cellulose-based hydrogels as extracellular matrices for tissue engineering. *J Biol Eng* 13:1–19.

Ershov BG (1998) Radiation-chemical degradation of cellulose and other polysaccharides. *Russ Chem Rev* 67:154–196.

Florea M, Hagemann H, Santosa S, *et al.* (2016) Engineering control of bacterial cellulose production using a genetic toolkit and a new cellulose-producing strain. *Proc Natl Acad Sci U S A* 113:3431–3440.

Fuller ME, Andaya C, and McClay K (2018) Evaluation of ATR-FTIR for analysis of bacterial cellulose impurities. *J Microbiol Methods* 144:145–151.

Goes-Neto A, Kukharenko O, Orlovska I, *et al.* (2021) Shotgun metagenomic analysis of kombucha mutualistic community exposed to Mars-like environment outside the International Space Station. *Environ Microbiol* (in press).

Griffith JD, Willcox S, Powers DW, *et al.* (2008) Discovery of abundant cellulose microfibers encased in 250 Ma Permian halite: a macromolecular target in the search for life on other planets. *Astrobiology* 8:215–228.

Gullo M, La China S, Petroni G, *et al.* (2019) Exploring K2G30 genome: a high bacterial cellulose producing strain in glucose and mannitol based media. *Front Microbiol* 10:58.

Hayat S, Peters C, Shu N, *et al.* (2016) Inclusion of dyad-repeat pattern improves topology prediction of transmembrane B-barrel proteins. *Bioinformatics* 32:1571–1573.

Henniges U, Hasani M, Potthast A, *et al.* (2013) Electron beam irradiation of cellulosic materials—opportunities and limitations. *Materials* 6:1584–1598.

Hernandez-Arriaga AM, del Cerro C, Urbina L, *et al.* (2019) Genome sequence and characterization of the *bcs* clusters for the production of nanocellulose from the low pH resistant strain *Komagataeibacter medellinensis* ID13488. *Microb Biotechnol* 12:620–632.

Hestrin S and Schramm M (1954) Synthesis of cellulose by *Acetobacter xylinum*. 2. Preparation of freeze-dried cells capable of polymerizing glucose to cellulose. *Biochem* J 58: 345–352.

Huang B, Li DG, Huang Y, *et al.* (2018) Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. *Mil Med Res* 5:18.

Hutchensa A, Roberto S, Benson RS, *et al.* (2006) Biomimetic synthesis of calcium-deficient hydroxyapatite in a natural hydrogel. *Biomaterials* 27:4661–4670.

Iller E, Kukielka A, Stupinska H, *et al.* (2002) Electron-beam stimulation of the reactivity of cellulose pulps for production of derivatives*. Radiat Phys Chem* 63:253–257.

Jacek P, Dourado F, Gama S, *et al.* (2019) Molecular aspects of bacterial nanocellulose biosynthesis. *Microbial Biotechnol* 12:633–649.

Kabanov VY, Feldman VI, Ershov BG, *et al.* (2009) Radiation chemistry of polymers. *High Energ Chem* 43:1–18.

Karp PD, Paley SM, Krummenacker M, *et al.* (2010) Pathway tools version 13.0: integrated software for pathway/genome informatics and systems biology. *Brief Bioinform* 11:40–79.

Kukharenko O, Podolich O, Rybitska A, *et al.* (2012) Robust symbiotic microbial communities in space research. In *Space Research in Ukraine (2010–2011). The Report to the COSPAR, edited by OP Fedorov. Academ Periodyka*, Kyiv, pp 102–105.

La China S, Bezzecchi A, Moya F, *et al.* (2020) Genome sequencing and phylogenetic analysis of K1G4: a new *Komagataeibacter* strain producing bacterial cellulose from different carbon sources. *Biotechnol Lett* 42:807–818.

Liu M, Liu L, Jia S, *et al.* (2018) Complete genome analysis of Gluconacetobacter xylinus CGMCC 2955 for elucidating bacterial cellulose biosynthesis and metabolic regulation. *Sci Rep* 8:6266.

Lu T, Gao H, Liao B, *et al.* (2020) Characterization and optimization of production of bacterial cellulose from strain CGMCC 17276 based on whole-genome analysis. *Carbohydr Polym* 232:115788.

McGinnis S and Madden TL (2004) BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids* Res 32:20–25.

Morin FG, Jordan BD, and Marchessault RH (2004) High-energy radiation-induced changes in the crystal morphology of cellulose. *Macromolecule*s 37:2668–2670.

Nickerson CA, Ott CM, Wilson JW, *et al.* (2004) Microbial responses to microgravity and other low-shear environments. *Microbiol Mol Biol Rev* 68:345–361.

Nishiyama Y, Sugiyama J, Chanzy H, *et al.* (2003) Crystal structure and hydrogen bonding system in cellulose iα from synchrotron x-ray and neutron fiber diffraction*. Am Chem Soc J* 125:14300–14306.

Podolich O, Kukharenko O, Haidak A, *et al.* (2019) Multimicrobial kombucha culture tolerates Mars-like conditions simulated on low-earth orbit. *Astrobiology* 19:183–196.

Podolich O, Kukharenko O, Zaets I, *et al.* (2020) Fitness of outer membrane vesicles from *Komagataeibacter intermedius* is altered under the impact of simulated Mars-like stressors outside the International Space Station. *Front Microbiol* 11: 1–14.

Podolich O, Zaets I, Kukharenko O, *et al.* (2017) The first space-related study of a kombucha multimicrobial cellulose-forming community: preparatory paboratory experiments. *Orig Life Evol Biosph* 47:169–185.

Reddy N and Yang Y (2015) Bacterial cellulose fibers. In: *Innovative Biofibers from Renewable Resources*. Springer-Verlag, Berlin, Heidelberg, pp 307–328.

Romling U and Galperin MY (2015) Bacterial cellulose biosynthesis: diversity of operons, subunits, products, and functions. *Trends Microbiol* 23:545–557.

Ross P, Mayer R, and Benziman M (1991) Cellulose biosynthesis and function in bacteria. *Microbiol Rev* 55:35–58.

Ryngajłło M, Kubiak K, Jędrzejczak-Krzepkowska M, *et al.* (2019) Comparative genomics of the *Komagataeibacter* strains—efficient bionanocellulose producers. *Microbiology open* 8:e00731.

Sales AA, Beekmann U, Laromaine A, *et al.* (2019) Opportunities of bacterial cellulose to treat epithelial tissues. *Curr Drug Targets* 20:808–822.

Santos RG, Hurtado R, Gomes LGR, *et al.* (2020) Complete genome analysis of *Glutamicibacter creatinolyticus* from mare abscess and comparative genomics provide insight of diversity and adaptation for *Glutamicibacte*r. *Gene* 741: 144566.

Talari ACS, Martinez MA, Movasaghi Z, *et al.* (2017) Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues. *Appl Spectrosc Rev* 52:456–506.

Teh MY, Ooi KH, Teo SXD, *et al.* (2019) An expanded synthetic biology toolkit for gene expression control in *Acetobacteraceae. ACS Synth Biol* 8:708–723.

Vigentini I, Fabrizio V, Dellaca` F, *et al.* (2019) Set-up of bacterial cellulose production from the genus *Komagataeibacter* and its use in a gluten-free bakery product as a case study. *Front Microbiol* 10:1953.

Villarreal-Soto SA, Beaufort S, Bouajila J, *et al.* (2018) Understanding kombucha tea fermentation: a review. *J Food Sci* 83:580–588.

Westall F, Steele A, Toporski J, *et al.* (2000) Polymeric substances and biofilms as biomarkers in terrestrial materials: implications for extraterrestrial samples. *J Geophys Res Planets* 105:511–527.

Yousif E and Haddad R (2013) Photodegradation and photo-stabilization of polymers, especially polystyrene: review. Springerplus 2:1–32.

Yu Y, Zhao X, Guo Y, *et al.* (2019) Identification of potential tobramycin-resistant mutagenesis of *Escherichia coli* strains after spaceflight. *Future Microbiol* 14:315–330.

Zaets I, Podolich O, Kukharenko O, *et al.* (2014) Bacterial cellulose may provide the microbial-life biosignature in the rock records. *Adv Space Res* 53:828–835.