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Original Article

Furnishing spaceship environment: evaluation of bacterial biofilms on different materials used inside International Space Station

Elena Perrin [a, 1], Giovanni Bacci [a, 1], Laurent Garrelly [b], Francesco Canganella [c], Giovanna Bianconi [c], The Biowyse Consortium[3, 2], Renato Fani [a, **], Alessio Mengoni [a, *]

[a] Department of Biology, University of Florence, Via Madonna del Piano 6, Sesto Fiorentino (FI), I-50019, Italy
[b] GLBiocontrol, 9, avenue de l'Europe, Cap Alpha, 34 830 Clapiers, France
[c] Department of Biological, Agricultural and Forestry Sciences, Università della Tuscia, Via San Camillo de Lellis snc, I-01100 Viterbo Italy

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ABSTRACT

Performed inside International Space Station (ISS) from 2011 to 2016, VIABLE (eValuatIon And monitoring of microBiofiLms insidE International Space Station) ISS was a long-lasting experiment aimed at evaluating the bacterial contamination on different surface space materials subjected to different pre-treatment, to provide useful information for future space missions. In this work, surfaces samples of the VIABLE ISS experiment were analyzed to determine both the total bacterial load (ATP-metry, qPCR) and the composition of the microbial communities (16S rRNA genes amplicon sequencing).

Data obtained showed a low bacterial contamination of all the surfaces, with values in agreement with those allowed inside ISS, and with a taxonomic composition similar to those found in previous studies (*Enterobacteriales*, *Bacillales, Lactobacillales* and *Actinomycetales*). No pre-treatment or material effect were observed on both the bacterial load and the composition of the communities, but for both a slight effect of the position (expose/not expose to air) was observed.

In conclusion, under the conditions used for VIABLE ISS, no material or pre-treatment seems to be better than others in terms of quantity and type of bacterial contamination.

1. Introduction

The International Space Station (ISS), circulating in low Earth orbit about 400 km above our planet, represents an extreme-situed indoor environment [1]. It is characterized by i) constant temperature (~22 °C), ii) stable humidity (~ 60%) and iii) microgravity [1]. The various space agencies are strongly interested on the bacterial contamination inside this particular environment, because it could be a model for similar systems and/or for future space missions [2] and the development of life-support facilities, as those envisaged within the BIOWYSE project [http://biowyse.eu/].

First studies on microbial contamination inside ISS were mainly focused on cultivable bacteria and fungi [2]. Recently, Next Generation Sequencing (NGS) of the 16S ribosomal RNA (rRNA) genes and of the fungal internal transcribed spacer (ITS), has been used on different ISS samples, providing information on the composition of the entire microbial and fungal communities [1–7]. All these studies highlighted that the main source of the ISS microbiota are members of the crew, since in most cases the identified bacteria were known as human-associated and in particular as skin-

Abbreviations: BIOWYSE, Biocontamination Integrated cOntrol of Wet sYstems for Space Exploration; FBG, Functional Cargo Block; ISS, International Space Station; ITS, internal transcribed spacer; NGS, Next Generation Sequencing; OTU, Operational taxonomic unit; PVD, Physical vapor deposition; qPCR, Quantitative Real Time PCR; RLU, Relative Light Units; ST solution, 0.15 M NaCl, 0.1% Tween 20; VIABLE ISS, eValuatIon And monitoring of microBiofiLms insidE International Space Station.

* Corresponding author.
** Corresponding author.
E-mail addresses: elena.perrin@unifi.it (E. Perrin), giovanni.bacci@unifi.it (G. Bacci), l.garrelly@gl-biocontrol.com (L. Garrelly), canganella@unitus.it (F. Canganella), giovannabianconi@libero.it (G. Bianconi), renato.fani@unifi.it (R. Fani), alessio.mengoni@unifi.it (A. Mengoni).
[1] These authors contributed equally to the work.
[2] Biowyse: Emmanouil Detsis. edetsis@esf.org
[3] The Biowyse consortium is formed by: 1) European Science Foundation, France 2) Thales Alenia Space Italia SPA, Italy 3) Consiglio Nazionale Delle Ricerche, Italy 4) GL Biocontrol, France 5) Società Metropolitana Acque Torino SPA, Italy 6) Liewenthal Electronics Ltd, Estonia 7) Università Degli Studi Di Firenze, Italy 8) AquiSense Technologies (Europe) Ltd, 9) UKA-ETC s.r.o, Czech Republic.

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associated, and that the microbiota composition is similar to what is found on surfaces of human dwellings [1–7].

VIABLE ISS (eValuatIon And monitoring of microBiofiLms insidE International Space Station) was a long-term study sponsored by the Italian Space Agency, aimed at checking the microbial contamination of model surfaces formed by different materials and pre-treated to reduce microbial adhesion and growth (www.nasa.gov/mission_pages/station/research/experiments/806.html).

The specific objectives of the experiment included i) the investigation of bacterial biofilm growth on space materials inside ISS, ii) the determination of the effects of biosurfactants on biofilm growth, and iii) the establishment of the effect of H_2O_2 and silica/silver surface pre-treatment on biofilm growth.

Here we report the results obtained by the analysis of microbial contamination on such surface samples with both total microbiota analysis (16S rRNA genes amplicon sequencing) and microbial activity measurement (as ATP-metry).

2. Materials and Methods

2.1. Description of sampled materials

The surface samples were placed inside 4 foam-lined Nomex bags as well on their covers (Fig. 1). Metallic and textile space materials either conventional or innovative (Aluminum, Armaflex and Betacloth), were supplied by ThalesAleniaSpace of Turin and tested as follows (Fig. 1):

- pouch 1 - untreated space materials;
- pouch 2 - space materials pre-treated with biosurfactants;
- pouch 3 - space materials pre-treated with hydrogen peroxide;
- pouch 4 - space materials physically pre-treated with silica and silver coating (supplied by Polytechnic of Turin, only Aluminum and Betacloth were subjected to these pre-treatment).

For pouch 2, the anti-biofilm product was represented by the sterile extract supernatant containing biosurfactants (obtained after grown with glucose) of the thermotolerant hydrocarbon-degrading microorganism *Pseudomonas aeruginosa* strain AP02-1, originally isolated from muddy sediments collected in sulphataric hot springs in Viterbo, Italy [8]. The biosurfactants produced were rhamnolipid in nature, and their maximal concentration in the solution used for coating was 0.5%. The amount of solution per sample treated was 5 ml.

For pouch 3, the pre-treatment was 6% H_2O_2, while for pouch 4, the antibacterial thin coating was based on a nano-metal/glass composite deposited on the different materials via PVD techniques, with a thickness ranging from 25 nm to 200 nm tuned upon the applicable substrate. The coating shows reduced density, increased adhesion interface strength and ductility.

The four experimental pouches remained on ISS from March 2011 to August 2016. Inside the ISS they were initially exposed for 12 days inside the Columbus module, the research laboratory module, and were subsequently moved to the FBG (Functional Cargo Block) module inside a storage locker. During this second, prolonged experimental phase, in order to maintain a sufficient level “human contamination”, all pouches were regularly and intentionally exposed to hand touching and blowing performed by astronauts. After returning to Earth, the Viable ISS bags have been sent to Kayser Italia Srl and then have been taken over for the analysis by University of Florence on September 13, 2016. Samples analysis began the next day.

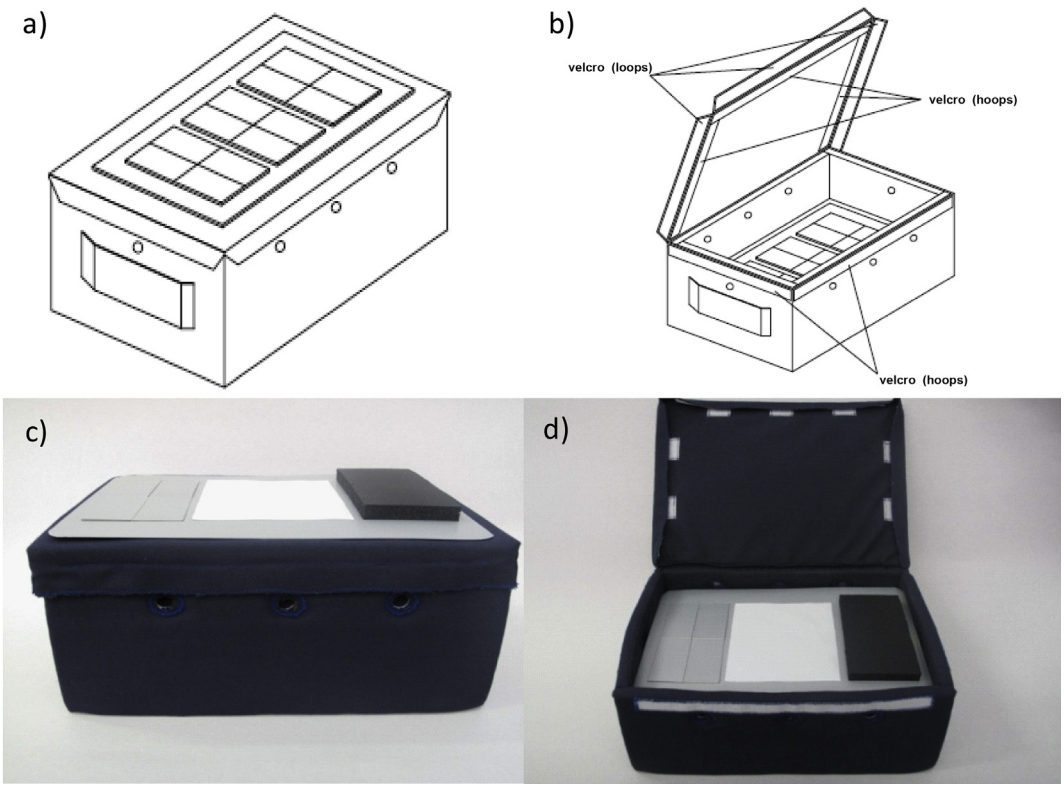

Fig. 1. VIABLE ISS bags. A schematic representation (a,b) and a photo (c,d) of the exterior (a,c) and interior (b,d) of the bags and of the surfaces samples.

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