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Journal Pre-proof



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Journal Pre-proof

Migration of surface-associated microbial communities in spaceflight habitats

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1 Migration of surface-associated microbial 2 communities in spaceflight habitats

3 **Abbreviations:**

- 4 CFU: Colony forming unit
- 5 CLSM: Confocal Laser Scanning Microscopy
- 6 ECLSS: Environmental Control and Life Support System
- 7 e-DNA: extracellular DNA
- 8 EPS: Extracellular polymeric substance
- 9 ESA: European Space Agency
- 10 ESKAPE: acronym describing six highly virulent and antibiotic-resistant bacterial pathogens of major
11 interest in human health including (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella*
12 *pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp.*)
- 13 EVA: extra-vehicular activity
- 14 FGB: Functional Cargo Module
- 15 ISS: International Space Station
- 16 LSMMG: low-sheared modelled microgravity
- 17 NASA: National Aeronautics and Space Administration (America's civil space program)
- 18 OMV: outer membrane vesicles.
- 19 PRW: Persistence Random Walk model
- 20 QS: Quorum-sensing
- 21 RNA-seq: RNA-sequencing for high-throughput transcriptomics analysis
- 22 SUS: super-hydrophilic and underwater superoleophobic material
- 23 T4P: type-IV-pili
- 24 WPA: Water Process Assembly
- 25 WRS: Water Recovery System

26 Abstract

27 Astronauts are spending longer periods locked up in ships or stations for scientific and exploration
28 spatial missions. The International Space Station (ISS) has been inhabited continuously for more than
29 20 years and the duration of space stays by crews could lengthen with the objectives of human
30 presence on the moon and Mars. If the environment of these space habitats is designed for the comfort
31 of astronauts, it is also conducive to other forms of life such as embarked microorganisms. The latter,
32 most often associated with surfaces in the form of biofilm, have been implicated in significant
33 degradation of the functionality of pieces of equipment in space habitats. The most recent research
34 suggests that microgravity could increase the persistence, resistance and virulence of pathogenic
35 microorganisms detected in these communities, endangering the health of astronauts and potentially
36 jeopardizing long-duration manned missions. In this review, we describe the mechanisms and
37 dynamics of installation and propagation of these microbial communities associated with surfaces
38 (*spatial migration*), as well as long-term processes of adaptation and evolution in these extreme
39 environments (*phenotypic and genetic migration*), with special reference to human health. We also
40 discuss the means of control envisaged to allow a lasting cohabitation between these vibrant
41 microscopic passengers and the astronauts.

42 **Keywords:** Biofilm, space flight, microgravity, transcriptomic, adaptation, evolution, control.

43

44 1. Introduction

45 Human space exploration presents many challenges for space agencies, habitability engineers and
46 microbiologists, especially in the upcoming new era of human expansion in the universe, such as
47 future space travel to Mars. Internal spacecraft must provide safe levels of biological, chemical and
48 physical parameters to astronauts. Space spaceships and stations are closed systems inhabited by

49 microorganisms that originate from different sources including the initial contamination of space
50 flight materials during manufacturing and assembly, the delivery of supplies, the automicroflora of the
51 crew and other biological materials present on board [1]. In space habitats, environmental conditions
52 (gas composition, pressure, temperature, and humidity) are set to the comfort of astronauts (e.g. 22°C,
53 60% of relative humidity in the International Space Station (ISS) [2]) and are also favourable to other
54 forms of terrestrial life such as embarked bacteria, yeasts, moulds or viruses. Microorganisms are
55 ubiquitous and will in general accompany human-inhabited spacecraft without imposing dramatic
56 safety concerns. However, if biological contamination were to reach unacceptable levels or if it
57 contains microorganisms at risk (for astronauts and their equipment), long-term human space flights
58 could be jeopardized. In these environments, most microorganisms are associated with surfaces in
59 spatially organised microbial communities termed biofilms which can be defined as surface-
60 associated communities of microorganisms embedded in self-produced extracellular polymeric
61 substances (EPS) [3]. This microbial mode of life significantly differs from free planktonic cultures in
62 homogeneous Newtonian liquid environments. Cells in a multilayered biofilm experience a diversity
63 of local microenvironments within the matrix and intensive cell-to-cell interactions with other
64 community members. Biofilm structures are associated with emerging community functions such as a
65 dramatic tolerance to the action of antimicrobials [4]. Important material degradation associated with
66 microbial biofilm development has been reported in several space stations (**Figure 1**). The affected
67 parts were for example piping and equipment behind panels, headphone of extra-vehicular activity
68 (EVA) suit, thermal control system, rubber of hatch locks, electrical connectors, radiators, air
69 conditioning, water recycling systems and oxygen electrolysis block [5]. The microbially-induced
70 degradation of a navigation window was associated with the presence of *Bacillus polymyxa*,
71 *Penicillium rubens* and *Aspergillus sp.* [5]. On the ISS, the most severely affected units are
72 wastewater collection reservoirs, also known as the Water Process Assembly (WPA) of the Water
73 Recovery System (WRS) which is part of the Environmental Control and Life Support System
74 (ECLSS). For WPA the most common microbial organisms isolated are *Ralstonia pickettii*,
75 *Bulkholderia sp.* and *Cupriavidus metallidurans* [6]. Biofilm formation is critical in any spacecraft

76 system, however, it is of utmost relevance when it affects ECLSS, given the relevance of this system
77 to the health of the crew.

78 Lessons learnt in previous space missions suggest that prevention of microbiological problems is
79 preferred over mitigation, and prevention steps must be taken into consideration from the very early
80 design phase. Requirements to control free water from condensate, hygiene activities, humidity,
81 condensate and other releases must be included in every spacecraft system development. Water is one
82 of the main driving elements for microbial outgrowth and its accumulation must be avoided and
83 controlled either by hygienic design or by water processing techniques, such as thermal inactivation,
84 filtration and biocide treatments. Furthermore, the materials selected must not promote microbial
85 growth and system design must include the onboard capability to achieve recovery of the system from
86 microbial contamination. Robust housekeeping procedures that include periodic cleaning and
87 disinfection are required. In addition, routine and systematic microbial monitoring of surfaces, air, and
88 water using culture-based techniques is conducted by each space agency [7]. Monitoring includes two
89 levels of sample analysis. The first level corresponds to a real-time assessment of the microbial load
90 and dynamics on the basis of total microbial counts. The second level is the ground-based assessment
91 of species composition, properties, and characteristics of archived samples which were collected in-
92 flight, as well as samples that are collected 1-2 days before crew return [8]. However, culture-based
93 analysis limits the understanding of the diversity of microorganisms in space habitats as only a small
94 fraction of organisms can be cultured under standard laboratory conditions [9]. Implementing
95 molecular methods on board the spaceship will enable the identification and quantification of both
96 culturable and unculturable organisms providing a more in-depth assessment of the microbial
97 population and density [10]. This is of utmost importance considering the long-term human space
98 exploration and associated protection of planet contamination [11]. On the International Space
99 Station, air cleanliness is ensured through the implementation of the Potok system [12]. The microbe-
100 killing principle of Potok is through the use of an electrical field of alternating polarity with fine
101 electrostatic filtration of microbe decomposition products. In the framework of ESA's Microbial
102 Detection in Air System for Space (MiDASS) project a miniaturised automated system was developed
103 for the sampling and monitoring of the microbiological quality of air, surfaces, and also potentially

104 water and food [13]. The system comprises two modules: sample preparation with nucleic acids
105 extraction, and module with nucleic acids amplification and detection [14].

106 In regards to biocontamination analysis onboard spacecraft, Nokivoka et al. [15] reported that in the
107 Mir orbital station, bacterial concentration in airborne contamination was below 5×10^2 Colony
108 Forming Unity (CFU)/m³ where bacterial genera *Staphylococcus sp.*, *Corynebacterium sp.*, and
109 *Bacillus* species were dominant. The concentration of airborne fungi fluctuated between 2 and 5×10^4
110 CFU/m³, with *Penicillium* and *Aspergillus* as the dominant genera. Contamination levels of surfaces
111 and equipment on board were also variable, with bacterial and fungal concentrations between 10 and
112 10^5 CFU cm², where the dominant bacterial and fungal genera were closed as for airborne
113 contamination. Dominant opportunistic pathogenic bacteria were also identified, compiling among
114 others *Flavobacterium meningosepticum*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella*
115 *pneumoniae*, *Staphylococcus sp.*, etc. Some of these microorganisms have been associated with
116 infectious diseases in respiratory organs and the digestive tract. Biocontaminants isolated on board the
117 Mir orbital station are to a great extent comparable [16] to the results obtained from the ISS [17] [18]
118 [19] [20] [21] [22] [23].

119 These reports highlight that the microbiota in inhabited space crafts is mainly associated with surfaces
120 (often in contact with the crew) and dominated by human automicrobiota, including pathogenic
121 species. Specific concerns about detected pathogens were pinpointed recently including a high
122 prevalence of antibioresistant isolates, many of them listed in the ESKAPE list (the six most highly
123 virulent and antibiotic-resistant human bacterial pathogens: *Enterococcus faecium*, *Staphylococcus*
124 *aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and
125 *Enterobacter spp*) [26] [27] [28]. 76% of isolates from the Russian segment on the ISS show
126 resistance to one or more antibiotics, questioning the evolution of this microbiota and its interactions
127 with astronauts in long-term missions [27].

128 Recent fundamental studies on bacterial biofilms exposed to microgravity also pinpointed specific
129 traits of serious concerns for the crew safety in long-term spaceflight missions: i) an important global
130 regulator involved in the pathogenesis of *Salmonella typhimurium* was shown to be highly
131 overexpressed aboard the space shuttle mission STS-115 compared to the ground control condition,

132 suggesting hypervirulent physiology of this pathogen under microgravity exposure [29], ii)
133 *Pseudomonas aeruginosa* cultivated in microgravity condition (space shuttle missions STS-132 and
134 STS-135) generate more biomass and adopted a unique canopy-like biofilm structure instead of the
135 flat architecture observed in terrestrial conditions [30]. The impact of this specific structure on
136 pathogens persistency and virulence is not elucidated yet, but the knowledge acquired on the links
137 between biofilm architecture and their functions would suggest specific adaptive processes in these
138 biological systems exposed to microgravity [31] [32] [33]. To illustrate the impact of microgravity
139 exposure on *P. aeruginosa* cell microenvironments, we computed a reaction-diffusion model from
140 real microscopic images from [30] showing sharper gradients of nutrients for biofilm cells exposed to
141 microgravity (**Figure 2**).

142 Altogether, the accumulation of data from spaceflight habitats and microgravity exposition of
143 microorganisms suggest that biofilms' emerging properties make them an essential issue to take into
144 account in long-duration space flights, as they could increase the risk and severity of microbial
145 infection [34]. The objective of this review is to consider not only the mature biofilm traits in long-
146 term spaceflight habitats, but the whole dynamic of the biosystem, including the populations of cells
147 migrating on the surface to initiate new biofilms, the populations migrating inside a biofilm matrix
148 and the populations emigrating from a biofilm to propagate the community (**Figure 3**). We will also
149 discuss the phenotypic and genetic *migration* of these vibrant surface communities that are
150 continuously adapting and evolving to the specific conditions encountered in these biotopes, with
151 special reference to crew health, and discuss envision control strategies.

152

153 2. The mechanisms of microbial migration on surfaces

154 Microorganisms can move across their environment through passive means such as colloidal particles,
155 but also through highly sophisticated and tightly regulated mechanisms involving specific appendages

156 or cellular processes. Each cycle starts with the transport of the organisms from bulk to the host
157 surface.

158 2.1 The effect of gravity on bulk microbial transport

159 In general, the bulk transport of microbes occurs either in gas or liquid phases. In the first case (gas)
160 there are two possibilities for the microenvironment of microbes depending on the cell size [35]: (i)
161 aerosols for cells $< 1\mu\text{m}$ such as bacterial spores or viruses, (ii) suspended droplets for cell units with
162 size $> 1\mu\text{m}$. The transported aerosol units could have sizes quite similar to the cell size whereas
163 several microbes could exist in a liquid droplet of size fairly larger than $1\mu\text{m}$ [36] [37]. From now on,
164 both types of transported units are called droplets (considering their varying amount of liquid). In case
165 of favourable droplet/substrate interactions, droplet deposits through Brownian motion (i.e., diffusion)
166 on a substrate, creating a local droplet concentration deficiency in the gas phase. The combination of
167 the concentration gradient imposed by diffusion and of the gas flow transferring the droplet
168 constitutes the convective-diffusion droplet deposition mechanism. The deposition is affected also by
169 other mechanisms that lead to deviation of the droplet motion from the gas motion. Such mechanisms
170 are the (droplet) inertia plus several external force fields like the gravitational one (leading to
171 sedimentation), the electrical one (leading to electrical precipitation) and the thermal one (leading to
172 thermophoresis) [38]. The relative contribution of gravity and Brownian diffusion to the deposition
173 rate is described by the dimensionless number $N_G = 4\pi r^4(\rho_p - \rho)g / (3k_B T)$ [39] where r is the droplet
174 radius, g is the gravitational acceleration, ρ_p , ρ are the droplet and gas density respectively, k_B is the
175 Boltzmann constant and T is the temperature. Introduction in the equation for N_G of representative
176 sizes yields that for microbes of type (i) gravity plays a negligible to small role in the deposition
177 efficiency (depending on microbe size). On the contrary, under terrestrial conditions, gravity
178 completely dictates the deposition behaviour of microbes of type (ii).

179 The second way of bulk transport is inside the liquid phase. It is noted that motility and several types
180 of taxi motion may affect the bacteria transport in the bulk. In this section, only the passive bulk

181 transport is examined, see section 2.2 for microbial active mechanisms. The deposition rate of
182 microbes depends not only on bulk transport but also on physicochemical interactions between the
183 microbial cell and the substrate. These thermodynamic interactions have been described by the
184 general colloidal model proposed by Derjaguin–Landau–Verwey–Overbeek (DLVO) and reviewed
185 extensively elsewhere [40]. Some specific domains where the liquid phase bulk transport of microbes
186 is of paramount importance are wastewater and potable water pipe networks, groundwater flows, deep
187 bed filtration, and reverse osmosis membrane biofouling. Each particular application determines the
188 geometry of the involved flow field (pipe, structured or unstructured porous media). In general, bulk
189 transport is considered quite important for the generation of a biofilm which refers actually to a
190 biofilm seeding process *i.e.*, transport and deposition of microbes on a clean surface. On the other
191 hand, bulk transport is not so important for the subsequent stage of biofilm growth (where sometimes
192 [41] it is even ignored) which is a very complex process driven by its inherent dynamics. So after the
193 aforementioned clarifications, it is apparent that the passive motion of non-motile microorganisms
194 toward a surface is driven by similar processes to that of inert colloidal particles. There is an
195 enormous amount of studies on the deposition of colloidal particles on surfaces [42]. The main bulk
196 transport mechanism (similar to that discussed before in the context of aerosols) is the combination of
197 diffusion (Brownian motion) and motion within the fluid (*i.e.* convective-diffusion mechanism).
198 Further deposition can occur by causes leading to the deviation between microbe and fluid
199 streamlines. Such causes are microbe inertia and gravity. The inertia effect is associated with the
200 Stokes number which is proportional to the square of particle size. Although no detailed calculation of
201 this number can be performed, as it depends on specific flow velocity and size scales, it can be argued
202 that inertia is negligible for particle size of a few microns. An additional deposition mechanism can be
203 found under the name "interception" [43]. This is simply due to the combination of the flow field and
204 of finite particle size. The relative effect of gravity and diffusion on deposition is described by the
205 number N_G which is discussed before (where ρ_p , ρ are the microbe and the liquid density,
206 respectively). Knowledge of the microbe density is required in order to calculate N_G . Several values
207 between 1.03 g/cm^3 and 1.14 g/cm^3 have been reported in the literature [44]. An average value of
208 1.085 g/cm^3 will be used herein for the calculations. The value of N_G is 0.053 for $r=0.5 \text{ }\mu\text{m}$ and 4.4 for

209 $r=1.5 \mu\text{m}$ which means that gravity has only a small contribution in the deposition for a microbe size
210 of $1 \mu\text{m}$ but dominates it for a microbe size of $3 \mu\text{m}$. Another interesting issue is that most microbes
211 are not perfect spheres (ovocoids, bacilli, filaments...). This makes their interaction with the flow
212 field very complex. Usually, a spheroidal shape is considered [45] for which several hydrodynamic
213 theoretical approaches can be found in the literature [46]. The shape effect typically leads to larger
214 deposition rates compared to the theories based on a spherical shape. The above order of magnitude
215 analysis has been supported by experimental studies on microbe deposition. In [47] it is argued that
216 the measured deposition rate is higher than the theoretical one based on convective diffusion, due to
217 gravitational contribution. More significantly, in [48] it was shown that for microbes with an aspect
218 ratio of 1.91 and an equivalent diameter of $1.7 \mu\text{m}$ the deposition rate strongly depends on the
219 direction of gravity with respect to the surface of deposition. In addition, the deposition rate differs
220 from that taken in the absence of gravitational contribution (achieved by density matching). Finally, it
221 is found that the microbe deposition rate is larger than that of spherical colloidal particles with a
222 diameter close to the equivalent diameter of the microbes (attributed to their non-spherical shape
223 discussed above). It is noted that in [48] the definition of N_G is somewhat different than the original
224 one in [39] having 2 as a constant parameter in the denominator instead of the correct 3. On obstacles,
225 the interplay between fluid shear and microbial motility allows the accumulation of elongated bacteria
226 in unattainable locations for passive particles [49]. The effect of gravity on microbe deposition has
227 also been indirectly confirmed experimentally by observing the spatial distribution of microbes bulk
228 concentration [50]. Furthermore, the use of fluorescence imaging in [50] allowed the measurement of
229 increased deposition rates along with the flow. This behaviour is totally opposite to the one predicted
230 by convective-diffusion, but is in accordance with a sedimentation-based model. In summary, it
231 appears that gravity may be quite important at least at the stage of biofilm seeding and its absence
232 would certainly yield different results in many cases. However, it must be stressed that increased
233 deposition rates due to microbe-specific mechanisms (motility, several taxis) may reduce the
234 contribution of gravity to deposition.

235 In addition to these passive movements, a large proportion of microorganisms can actively propel
236 themselves into an environment governed by viscosity using different appendices. Microorganisms
237 motion can be achieved by different mechanisms: swimming, swarming, gliding, twitching, and
238 sliding [51]. Regardless of the type of motility machinery that is employed, most motile
239 microorganisms use complex sensory systems to control their movements in response to stimuli,
240 which allows them to migrate to optimal environments [51]. Of note, most of these surface motility
241 mechanisms have never been studied in microgravity conditions.

242 2.2 Swimming in the flow

243 Microorganism swimming behaviour is possible through the flagella-driven motion. The structure and
244 functioning of flagella are different between eukaryotic and prokaryotic cells. The prokaryotic
245 flagellum on which we will focus in this review acts as a reversible rotary motor powered by
246 transmembrane proton potential (a different proton concentration is a priority for its function). It is
247 composed of an anchoring basal body, a hook and a long helical filament [52]. The anchoring basal
248 body acts as the rotary motor of the structure, the hook is the junction structure that connects the
249 motor and filament, and the flagellar filament is normally a left-handed helix of a length of 5 to 10 μm
250 and a diameter of 20 nm. When motor rotation is counterclockwise (CCW) the cell body is pushed
251 and starts its motion in a linear trajectory at an average speed that is for example about 40 $\mu\text{m/s}$ in the
252 case of *E. coli*. When motors rotate clockwise (CW), the filaments are placed under right-handed
253 torsional stress, resulting in a filament poorly defined orientation resulting in tumbles and a phase of
254 random reorientation. This type of behaviour can be mathematically characterized in an isotropic
255 environment using the Persistence Random Walk model (PRW) described by Dickinson & Tranquillo
256 [53]. In this model cells trajectories are described by a succession of uncorrelated movements of a
257 characteristic duration (the times between two different tumbles). Motility is quantified by three
258 parameters: root-mean-squared speed, directional persistence time, and random motility coefficient
259 (analogous to a molecular diffusion coefficient) [54]. The random motility of microorganisms is lost
260 in the case of an anisotropic environment where cells sense chemical and physical gradients resulting

261 in directional motility (taxes). These directional motions are categorized based on the stimuli
262 depending on chemical (chemotaxis [55], aerotaxis, [56]), thermal (thermotaxis [57]), electromagnetic
263 (magnetotaxis [58]), and light intensity (phototaxis [59]) special gradients. In an anisotropic
264 environment, random reorientation after a tumble also occurs, but the different duration of motion
265 phases is observed among different directions of motion with respect to the direction gradient. A
266 motion toward the gradient persists for a longer time with respect to the case of motion in the opposite
267 direction [60].

268 Another source of environmental anisotropy can be induced by mechanical stresses, that can be
269 related to flow directionality or force fields, such as gravity. In particular hydrodynamic shear plays a
270 key role in biofilm formation and morphology [61]. In recent work, Rusconi et al [62] demonstrated
271 that shear flow produces spatial heterogeneity in bacterial distribution inside a microfluidic channel.
272 Shear flow seems to affect bacteria accumulation at the channel wall boundary, in a so-called
273 “trapping effect”. This effect is a function of the shear rate in a given range of shear. This flow effect
274 hampers chemotaxis and promotes surface attachment. These results prove that flow influence can
275 overcome taxes and directly affect the first step of biofilm formation (adhesion on surfaces). In other
276 recent works, the effect of flow was evaluated not only for its contribution to cell swimming
277 behaviour but also for its effect on biofilm morphologies [62] [63]. A common observation can be
278 made from these studies: at low shears, biofilms present a lower cohesion resulting in loose top layers.
279 Recent studies show that high shear causes a faster diffusion of nutrients and higher incorporation of
280 bacteria, promoting the formation of more crosslinks in the EPS matrix and, ultimately, a more
281 mechanically stable biofilm [64].

282 In space-relevant applications, specific conditions, such as microgravity, can impact swimming
283 motility, and bacterial growth, but the research available so far seems to be controversial on this
284 aspect. In the review of Benoit and Klaus [65], it is found that spaceflight and devices simulating
285 microgravity enhanced non-motile microbial growth in a liquid medium. A common explanation of
286 this phenomenon is related to two gravity-related effects: the sedimentation of cells, and the potential
287 buoyant convection of less dense fluid in the proximity of the cell. In microgravity conditions, both

288 these phenomena are reduced and as a result, bacterial cells are more uniformly distributed in the
289 liquid medium, in an environment governed by Brownian diffusion. Motile swimming bacteria seem
290 to reduce this phenomenon, by actively agitating the surrounding quiescent fluid with flagella rotation
291 and reducing the difference between 1g gravity and microgravity condition. In contrast with this
292 hypothesis, a recent study [66] observed three different strains (non-motile *Sphingomonas desiccabilis*
293 *CP1D* and motile *Bacillus subtilis* NCIB 3610, *Cupriavidus metallidurans* CH34) exhibiting the same
294 cell final concentration after 21 days in space growth, respect to standard ground controls. This
295 controversy suggests that microgravity effects on bacterial growth and the role of cell motility related
296 to this aspect are still not well understood, and deserve further investigation. The definition of a
297 standard protocol to compare bacterial growth and biofilm formation in different gravity conditions is
298 also still not defined.

299 Bacterial motility deeply affects the colonization of surfaces both in no-flow and flow conditions, due
300 to the forces generated by the flagellar-fluid motion at the microscale and the elongation of the cell
301 body. In no-flow conditions, the surface accumulation of motile bacteria is promoted by the
302 hydrodynamic interaction between the swimming cell with the solid surface [67]. This phenomenon,
303 combined with stop events and transient surface adhesions, allows bacteria to attain optimal surface
304 diffusivity [68]. In flow conditions, hydrodynamic interactions trigger bacterial motion in the
305 direction opposite to the flow, leading to upstream flagellar swimming [69]. Upstream motility can
306 also be achieved by surface motility with type IV pili, as shown in *P. aeruginosa* [70] and
307 *Mycoplasma mobile* [71], with a lower velocity compared to upstream swimming. In both cases, the
308 torque exerted by flow shear rotates the cells around the appendages-free extremity of the body and
309 orients them facing upstream, resulting in a preferential direction of motion. Upstream migration
310 grants an advantage in the colonization of flow networks [72] and promotes the segregation of
311 bacterial species based on their surface motility [73]. Due to its significant implications for bacterial
312 spreading on surfaces, upstream migration should be accounted for while evaluating the origin of
313 bacterial contamination in technological settings.

314

315 2.3 Moving as a free cell or a group on the surface

316 The multiple strategies employed by bacteria to move on surfaces (swarming, twitching, gliding,
317 sliding) are important for survival since they govern the dispersal of progenies, and the way bacteria
318 aggregate into microcolonies under unfavourable conditions, typically starvation or oxygen depletion.
319 Twitching motility is a key mechanism for many pathogenic strains to propagate on surfaces, either as
320 individual cells or collectively. This type of motility is found in many biofilm-forming species, such
321 as *Pseudomonas aeruginosa*, *Neisseria gonorrhoea*, *Myxococcus xanthus* or *Acinetobacter baumannii*
322 [74] [75]. Twitching allows single cells to move on surfaces at typical speeds of the order of a fraction
323 of a micrometre per minute and is powered by type-IV-pili (T4P). T4P are thin (~10 nm) contractile
324 surface appendages, up to several micrometres-long and often polarly localized, with a terminal
325 adhesin that can act like a hook and promiscuously bind surfaces. T4P are formed by the assembly of
326 a protein subunit: polymerization/depolymerization cycles at the base of the appendage power
327 motility, and pili extension-attachment-contraction-detachment cycles propel bacteria through
328 surfaces [76] [77][78]. By allowing bacteria to explore surfaces and efficiently colonize different
329 microenvironments, twitching motility is one of the key strategies allowing bacterial dispersal,
330 pathogenesis and is also an important ingredient of biofilm development [79] [80] [81]. In flow
331 conditions, the polar localization of pili results in the upstream migration of adhered bacteria, a
332 counter-intuitive effect that can provide a dispersal advantage for twitching species [70][73].
333 Recently, it has been shown that mechanical signals sensed and transmitted by T4P regulate virulence
334 factors in *P. aeruginosa* [82] or direct twitching motility of individual bacteria [83], and suggested
335 that substrate rigidity could modulate bacterial twitching, thus impacting colony morphogenesis [84].
336 Together these findings highlight the importance of mechanical interactions between T4P and their
337 environment, which could be modified in space conditions.
338 However, so far it is unclear whether pili-mediated motility is modified under microgravity
339 conditions, and no experimental nor theoretical study has focused on this specific point.
340 One basic question is to determine whether the direction of twitching bacterial displacement steps
341 could be biased by gravity, and thus modified under zero-g conditions. If evaluating the forces at

342 stake, it appears very unlikely that gravity could impact the behaviour of a single twitching bacterial
343 cell: T4P stall forces, which drive bacterial twitching on surfaces, have been measured to range from
344 50 to over 100 pN. In contrast, the gravitational force on a cell (in air) of volume $3 \times 2 \times 2 \mu\text{m}^3$ and
345 density $\rho=1 \text{ g/ml}$ is $\rho Vg=0.1 \text{ pN}$ and could therefore play no role in the twitching process. Following
346 this reasoning, only cohesive colonies of a few dozen bacteria could be exposed to gravitational forces
347 comparable to T4P contractile forces.

348 However, it is entirely possible that microgravity conditions could modify pili expression levels or
349 activity, thus impacting twitching motility. This is supported by several studies that showed that
350 signalling pathways are modified under microgravity [85].

351 A modified twitching behaviour could have important consequences: twitching impacts surface
352 colonization, and more generally the early spatial organization of bacteria into colonies, which can
353 directly impact their tolerance to environmental stresses in general, and in particular to the action of
354 antimicrobials [86]. Second, by translocating across substrates bacteria can actively modify the
355 underlying surface by depositing extracellular polymeric substances [87]. This coupling between
356 environmental conditions (shear flow, substrate mechanical and chemical properties), surface motility
357 and EPS distribution governs microcolony formation and should thus be considered when designing
358 space equipment.

359 Surface motility and dispersal of individual bacteria can also take place through mechanisms that do
360 not require any active surface appendage: gliding or sliding.

361 Gliding is common among Myxobacteria and is also observed in a number of phylogenetically diverse
362 gram-negative, non-flagellated bacteria [88]. It relies on the movement of adhesion complexes along
363 helical tracks on the cell surface, powered by proton-activated molecular motors [89]. Gliding propels
364 the cell body forward at several micrometres per minute. It is known that gliding cells deposit a layer
365 of slime on the substrate, and the role of this thin slime layer as a lubricant for cell displacement is
366 well-established. In addition, the trafficking of adhesion complexes on the helical MreB scaffold
367 results in sinusoidal deformations of the cell surface. Recently, Tchoufag et al. proposed that these
368 periodic deformations, when transmitted to the underlying elastic slime layer, result in local pressure
369 gradients that generate the overall thrust force experienced by bacteria [90]. Interestingly, their

370 elasto-hydrodynamic model accounts for the known substrate-rigidity dependence of gliding motility,
371 which decreases on soft substrates, similar to twitching [84]. Stall forces of gliding molecular motors
372 were measured around 12 pN, resulting in total gliding forces up to 60 pN (with ~ 5 motors/bacteria)
373 [91].

374 In conclusion, the forces exerted by individual gliding bacteria are similar to the ones involved in
375 twitching motility, and thus the physical mechanisms that govern these processes are unlikely to be
376 directly influenced by gravity. However, indirect regulation of these phenomena could exist in space
377 conditions, as a result of changes in bacterial phenotypes (e.g. T4P or EPS expression levels).

378 Once they form a microcolony, bacteria can collectively slide on surfaces thanks to division and
379 growth: dividing bacteria at the centre of a colony generate pressure, pushing their neighbours
380 outwards. The progression of the edge of the colony can be facilitated by the production of
381 biosurfactants that reduce friction [92] [93], EPS that trigger osmotic swelling of the biofilm [94] or
382 capillary forces at the air/liquid interface [95]. This gives rise to very diverse colony morphologies,
383 including fingering instabilities [96]. To our knowledge, this phenomenon has not been specifically
384 studied under microgravity or 0g conditions. Because bacteria adhere to each other and to the
385 underlying substrate, growth gives rise to local stress build-up in the colony, which relaxes through
386 rapid reorganization events. Maximal adhesion forces in these “focal adhesions” under spreading
387 colonies have been measured experimentally of the order of 50 to 100 pN [97] -again in the same
388 range as the forces involved in twitching or gliding motilities.

389 Could the presence of gravity directly impact macroscopic biofilm spreading? The capillary length
390 $\sqrt{\gamma/(\Delta\rho g)}$ of a water droplet under 1g conditions is ~3mm, meaning that gravity would only deform
391 droplets larger than this characteristic size. Considering that biofilms have a density close to water's,
392 and even if the production of biosurfactants decreased surface tension, the capillary length would not
393 go under a few 100 μm . This means that only thick, mature biofilms could potentially be deformed by
394 gravity under their own weight. The structural differences observed for *P. aeruginosa* biofilms in
395 spaceflight conditions [] are most likely due to nutrient or oxygen availability, or changes in the
396 motility of bacteria, rather than the absence of a direct deformation of biofilms by gravitational forces.

397 In specific conditions, billions of bacteria can migrate cooperatively from a colony across distances of
398 centimetres in a matter of a few hours through a phenomenon called swarming [98]. Swarming
399 motility is a process by which bacteria can rapidly advance on moist surfaces in a coordinated manner
400 [99]. It is a multicellular, flagella-mediated surface migration of bacterial groups typically involving
401 surfactant secretion and an increase in flagella numbers [100] [98]. In *Bacillus subtilis*, this
402 developmental process is observed on semi-solid agar (0.6%–1% agar) and has been shown to be
403 completely dependent on flagella and surfactin production [101]. Traditionally, dispersal by microbial
404 swarm propagation has been studied in monoculture, but there is evidence that swarming
405 microorganisms can transport other species by forming multispecies swarms with mutual benefits
406 [102].

407

408 2.4 Active microbial movements in biofilm communities, dispersion 409 and hitchhiking

410 Biofilm structures were initially described as a sessile three-dimensional assemblage of
411 microorganisms immobilized in an EPS organic glue [103]. The combination of new visualisation
412 tools such as confocal laser scanning microscopy (CLSM) along with genetically engineered
413 fluorescent reporter strains allowed the discovery of unexpected mobile subpopulations within
414 biofilms. In the early 2000s, Tim Tolker-Nielson and his collaborators demonstrated in a series of
415 articles on *Pseudomonas aeruginosa* the migration of a subpopulation of cells to the cap of
416 mushroom-like biofilm structures [104]. These movements involved type IV pili and were observed
417 on the interface between the biofilm and the bulk fluid [105]. The biological role of death and lysis in
418 biofilm development and the existence of hollow voids containing cannibal swimming subpopulations
419 of cells involved in active cell dissemination was also pinpointed [106] [107]. Several authors
420 demonstrated that non-flagellated bacteria were also able to actively disperse the biofilm population.
421 This is the case of the coccoid pathogen *S. aureus* for which the induction of the *agr* system in
422 established biofilms detaches cells through a dispersal mechanism requiring extracellular protease

423 activity [108] [109]. More recently, it was shown that flagella-propelled motile bacilli were able to
424 swim and create transient pores within the biofilm matrix, increasing the macromolecular transfer
425 with the bulk phase [110] [111] [112]. While these bacilli swimmers can deliver locally several types
426 of effectors, it was shown that they can actively transport several types of adsorbed organisms taking
427 advantage of a “free ride” inside the biofilm. Described “hitchhikers” on the flagella of motile bacilli
428 comprise several families of non-motile organisms such as the bacterial pathogen *Staphylococcus*
429 *aureus* [113], fungal spores [114] and bacteriophages [115] [116].

430

431 3. Emerging properties of surface-associated 432 migrating communities

433

434 Understanding how microorganisms adapt to stressful space conditions has been the focus of many
435 studies. Most studies involved single bacterial species, often pathogenic, including various
436 *Pseudomonas*, *Enterobacteria* such as *E. coli* and *Salmonella*, *Actinobacteria*, and bacteria of the
437 *Streptococcus* and *Enterococcus* genera [117] [118] [119] [120] [121] [122] [30] [123] [124] [125].
438 Non-pathogenic species such as the soil bacteria *Bacillus subtilis*, the fermentative bacteria
439 *Lactococcus lactis* or the nitrogen-fixating bacteria *Rhodospirillum rubrum* have been also sent to
440 space [126] [127] [128] [129] [130]. Experiments conducted on spaceflight as well as on ground-
441 based simulators established that microgravity triggered various physiological responses by affecting
442 bacterial cell growth, cell morphology, gene expression, gene transfer, virulence, drug resistance,
443 biofilm formation, and secondary metabolism [117] [121] [130] [125] [131].

444

445 3.1 Impact of spaceflight conditions on the adaptation of bacterial 446 populations

447

448 Phenotypic changes with potential implications for astronauts have been reported in various bacterial
449 species exposed to low-sheared modelled microgravity (LSMM). Growth under simulated
450 microgravity conditions increased the cell density of *Stenotrophomonas maltophilia*, *Lactobacillus*
451 *acidophilus* and *Pseudomonas aeruginosa* [30] [130] [132]. Notably, several studies conducted under
452 space flight conditions have linked growth rate to bacteria motility, suggesting that the effect of
453 microgravity could be indirectly caused by a lack of convective flows altering the diffusional access
454 to nutrient and affecting the immediate cellular metabolic environment [30] [131]. A direct
455 consequence of this hypothesis is that this response should be counteracted by motility, as
456 corroborated by a comparative study between a wild type and a $\Delta motABCD$ motility-deficient mutant
457 of *P. aeruginosa* exposed to a space flight environment [30] (Kim et al., 2013b). In *Streptococcus*
458 *mutans*, it was shown that genes involved in carbohydrate metabolism, translation or stress responses
459 were differentially expressed in simulated microgravity conditions, with potential effects on the
460 cariogenic potential of this bacterial species [133]. Phenotypic changes were also observed in *E. coli*
461 when cultured in space, along with an increase in cell size, cell counts, and cell envelope thickness.
462 Compared to earth, *E. coli* cells challenged to microgravity also exhibited higher resistance to
463 gentamicin sulfate coupled with a unique ability to generate numerous outer membrane vesicles
464 (OMG), these two phenotypes being connected to a change in membrane fluidity [134] [125]. Long-
465 term exposure to microgravity indeed affects bacterial virulence as well as susceptibility to diverse
466 antibiotics and drugs in many bacterial species. Increased virulence has been observed in bacteria
467 pathogens grown in simulated microgravity and space conditions [135] [136] [137]. After exposure to
468 simulated microgravity in rotating-wall vessel bioreactors, the pathogen *Salmonella typhimurium*
469 became more virulent in mouse or cellular infection models [138]. In their study, Gilbert and al.
470 revealed that the opportunistic pathogen *Serratia marcescens* was more lethal to *Drosophila*

471 *melanogaster* after exposure to true spaceflight conditions [135]. Importantly, they also established
472 that this characteristic did not persist after the cells resumed normal growth under ground conditions.
473 This observation suggests that microgravity can induce transient physiological changes in
474 microorganisms.

475 Another major concern is that prolonged exposure to microgravity conditions triggers increased
476 antibiotic resistance, as documented for *E. coli*, *S. aureus*, *Streptococcus pyogenes*, *P. aeruginosa*, or
477 *Enterococcus faecalis* [139] [140] [137] [141] [125] [142]. It was proposed that adaptive resistance to
478 antibiotics under low gravity in *S. aureus* and in *E. coli* could be associated with modifications of the
479 cell envelope such as an increase in membrane fluidity and cell wall thickness [143] [144] [125].
480 Short-term microgravity (<50h) also demonstrated the potential to affect *E. coli* resistance to
481 antibiotics from different families including gentamicin, ampicillin, nalidixic acid, penicillin G or
482 chloramphenicol [145] [146] [147].

483 In addition to phenotypic changes, the question of the genetic evolution of bacterial populations under
484 spaceflight and microgravity conditions and its role in the emergence of particular bacterial
485 phenotypes, such as resistance to antimicrobials is particularly of concern in a spacecraft environment
486 during long-term missions. Interestingly, mutation frequency and/or spectrum of mutations in the
487 *rpoB* gene involved in rifampicin resistance was modified in *Staphylococcus epidermidis* and *Bacillus*
488 *subtilis* cultures grown in spaceflight environments (ISS) by comparison to ground control cultures
489 [148] [149]. That supports the idea that space environments can induce unique stresses on bacteria,
490 leading to modulations in their mutagenic potential. Through a pangenomics meta-analysis of 189
491 genomes of *Bacillus cereus* and *Staphylococcus aureus* from different origins, Blaustein et al. (2021)
492 [150] identified genomic signatures specific to International Space Station (ISS) bacteria. Functions
493 related to biosynthesis, materials transport, or stress response were enriched in ISS-derived strains
494 suggesting their involvement in bacterial survival under ISS selective pressures.

495

496 3.2 The importance of biofilm lifestyle in adaptive migration of 497 bacterial populations

498

499 Biofilms are dynamic multicellular edifices and are recognized as a collective strategy for
500 microorganisms to adapt and survive face changing environmental conditions [31]. It is now
501 acknowledged that exposure to the space environment enhances biofilm biomass and thickness in
502 most bacteria [120] [30] [151] [140] [132] [152] [131] [125]. The increased propensity to develop
503 biofilms in space has been first discovered in *P. aeruginosa* [151]. The typical column-and-canopy-
504 like architecture revealed during the space shuttle Atlantis missions illustrated the complex selective
505 forces at play that shaped the 3D structure of biofilms when exposed to microgravity [153] [151]. The
506 formation of such structures requires flagella-driven motility but is not dependent on the carbon
507 source [154]. Alteration of biofilm mass, composition, and architecture, combined with abnormal EPS
508 distribution has been also reported for *Streptococcus mutans* grown under simulated microgravity
509 [155]. Substantial modifications of biofilm architecture and colony morphology, associated with an
510 increase in virulence and resistance to environmental stress and antifungal (amphotericin B), were
511 also observed for *Candida albicans*, an opportunistic fungal pathogen, grown in low-shear modelled
512 microgravity bioreactors [156] [157]. To illustrate the effect of microgravity on biofilm cell
513 metabolism we simulated the growth of microalgae biofilms in both terrestrial and microgravity
514 conditions (**Figure 4**). These simulations suggest that micro-gravity impacts the spatial structure of
515 the biofilm and therefore the resulting substrate consumption and the overall biofilm growth.

516 One key component of the survival strategy of the biofilm community is the ability to withstand
517 externally applied mechanical stresses, thanks to the viscoelastic nature of the EPS matrix. When a
518 force is applied, biofilms instantaneously undergo an elastic deformation as solids and then slowly
519 flow as viscous fluids, further spreading on surfaces while maintaining their structural integrity [155]
520 [156]. The viscoelastic behaviour increases the surface spreading [157] and allows the formation of
521 biofilm filaments suspended in the bulk fluid, known as biofilm streamers [159]. The EPS matrix

522 supports the mechanical stability of the biofilm through physicochemical interactions, and EPS
523 biochemical composition determines its mechanical behaviour [159] [160]. Biofilm mechanical
524 behaviour is key to the impact of biofilms in technological contexts, including spaceflights. However,
525 while the impact of microgravity on biofilm architecture and composition has been elucidated, the
526 microgravity-induced changes in biofilm's mechanical behaviour are still understudied. Measuring the
527 ability of biofilms to withstand stress would provide information and indicates future directions for
528 the design of biofilm-cleaning tools. Additionally, the mechanical protective role of the matrix is
529 largely decoupled from the viability of the cells themselves, so even after successful antimicrobial
530 treatment, the detrimental effects of biofilms due to fouling persist beyond the death of the cells.

531 In multispecies communities, a consequence of microgravity-induced modification of biofilm
532 structure is a modification of competitive interactions, resulting in a modification of ecological
533 balance and an alteration of community functions. This was illustrated by the fitness increase of *S.*
534 *mutants* over *S. sanguinis* when mixed under simulated microgravity compared to ground level [161]
535 which would promote the initiation of dental caries in dental biofilms. Similarly, by performing a
536 shotgun metagenomics analysis of ISS environmental surfaces, Singh et al. [162] demonstrated a
537 specific composition of ISS microbial communities compared to earth analogues. Moreover, the
538 authors reported an increase in antimicrobial resistance and virulence gene factors over the period
539 sampled showing the specific adaptation of functional profiles of ISS microbial surface-associated
540 populations [162]. Overall, these observations emphasize the close interplay between the three-
541 dimensional organization of biofilm, its plasticity and the modulation of functional properties in
542 response to microgravity conditions.

543 Furthermore, biofilm structural changes in spaceflight environments are likely to affect how bacteria
544 evolve toward specific genotypes. Biofilms are considered incubators for microbial genetic diversity
545 as they promote the process of diversity generation and protect genetic diversity [163]. This mainly
546 arises from the multiple micro-environments produced by the chemical gradients and the protective
547 three-dimensional structure. This phenomenon plays a central role in microbial adaptation and in the
548 "migration" toward specific functions expressed at the scale of the whole community such as

549 antimicrobial resistance. This feature, in combination with the fact that spaceflight conditions can
550 independently affect mutagenic potential in bacteria [148] [149] underlines the need to better
551 understand the adaptation of surface-associated migrating communities to spaceflight environments.

552 More generally, biofilms represent a spatial and structural advantage for cell-to-cell communication
553 through both metabolic and genetic exchanges. Indeed, in bacterial populations, the emergence of
554 functional traits is much associated with the horizontal transfer of genetic determinants. Considering
555 antimicrobial resistance, the emergence of resistance at the community scale relies on their propensity
556 to exchange plasmids, transposons and other genetic determinants considered reservoirs for antibiotics
557 genes. In a recent study, Urnaniack et al. established that microgravity stimulated the horizontal
558 transfer of two antibiotic resistance genes, *blaOXA-500* and *isabA1*, from *Acinetobacter pittii*, in four
559 *S. aureus* strains, thus posing the hypothesis that interspecies genetic transfer could also occur
560 onboard of a space station [141]. This study points to the potential role of other modes of genetic
561 transfer such as natural competence and phage transduction in spreading resistance genes and
562 pathogenicity determinants in space. The facilitation of horizontal gene transfer in biofilms is
563 proposed to be part of the mechanisms responsible for the dissemination of virulence and antibiotic-
564 resistance genes in space [164] [137].

565 The microbial stress response to microgravity is thus multifaceted. Understanding how microbes
566 integrate information from a microgravity environment to elicit multiple and interconnected
567 phenotypes requires understanding at a system level. Although the effect of microgravity in biofilm
568 formation is well documented in the literature, knowledge remains to be gained to understand the
569 decision-making genetic circuits underlying this lifestyle switch.

570

571 3.3 The effectors and what we know about their expression in space 572 conditions

573 Deciphering the principles underlying the cellular response to altered gravity is expected to provide
574 important information for the development of countermeasures to control bacteria growth, virulence,
575 antimicrobial resistance and biofilm formation in space. Whole-genome gene expression profiling
576 offers the prospect of gaining insight into gene regulatory pathways and elucidates the effectors
577 involved in adaptation to microgravity. In the last decade, advances in omics approaches have enabled
578 the generation of data to identify potential microgravity-sensitive genes. Several studies made under
579 real or simulated microgravity environments provided the differential-gene expression analysis of
580 bacterial genomes, which partly supported the observed phenotypic changes [120] [165].

581 Transcriptomic analysis in various bacteria exposed to altered gravity highlighted the differential
582 expression of genes involved in motility and transport, including multidrug efflux systems and metal-
583 ion transport and utilization. Confusingly, different *E. coli* strains exhibited either an increase or a
584 decrease in the expression of flagellar and motility-associated genes as well as chemotaxis-associated
585 genes under simulated microgravity [166] [167] [168]. These different responses were reflecting their
586 distinctive motility capabilities, their physiological stages in the experiment, as well as the different
587 nutrient composition of the medium tested [166] [167] [168]. In a different approach, whole-genome
588 sequencing of *E. coli* cells exposed to LSMMG microgravity for up to 1000 generations revealed loss-
589 of-function mutations affecting genes of the flagellar, motility and chemotaxis regulons [169] [170].

590 Genes encoding proteins that compose the flagella apparatus were reproductively down-regulated in
591 the pathogen *Salmonella typhimurium* when exposed to spaceflight or to simulated microgravity
592 conditions [171] [172]. Real and simulated microgravity commonly elicited the differential expression
593 of chemotaxis genes in the gram-negative pathogen *P. aeruginosa* [119] [173]. All these studies
594 underlined the importance of motility and chemotaxis in bacterial adaptation to microgravity
595 conditions. Lately, Su et al., used an integrated multi-omic approach combining transcriptomic and
596 proteomic to investigate the impact of long-term exposure to microgravity on *Stenotrophomonas*

597 *maltoiphilia* physiology and metabolic responses [132]. Gene ontology enrichment analysis revealed
598 that simulated microgravity conditions affect several processes related to cell adhesion, motility and
599 biofilm formation. Most particularly, genes encoding proteins that compose the T4P pilus machinery
600 and two-component systems (TCSs) are up-regulated, in keeping with the physiological changes
601 observed under microgravity such as enhanced biofilm formation ability, and increase growth rates
602 [132].

603 Transcriptomic study in the gram-positive bacteria *Streptococcus pneumoniae* revealed that exposure
604 to microgravity conditions up-regulated many genes involved in the cell envelope biogenesis, DNA
605 replication, recombination and repair as well as ABC-type multidrug transport systems [120].
606 However, a unique comparative transcriptomic analysis from a *B. subtilis* strain grown under identical
607 conditions aboard ISS in two separate spaceflight experiments BRIC-21 and BRIC-23, provided
608 invaluable data on the bacterial response elicited under microgravity [165]. This study revealed higher
609 levels of transcripts related to anaerobic respiration, the production of secondary metabolites (e.g;
610 siderophores), the synthesis of antimicrobials (e.g. bacteriocins), as well as the utilization of various
611 nutrients [165] (**figure 5a**). These observations correlated with the limitation in oxygen and nutrient
612 transport due to the lack of convection in the absence of gravity, as mentioned above. However, one
613 of the most interesting outcomes of this comparative study was the overexpression of genes involved
614 in biofilm and motility pathways [165]. Although the domesticated *B. subtilis* strain 168 used in these
615 experiments was not prone to form strong biofilms, clusters of biofilm-related genes were
616 significantly upregulated in the two experiments, such as parts of the *epsA-O* operon, encoding the
617 exopolysaccharide production machinery, and genes of the *tapA-sipW-tasA* operon encoding
618 important components of the biofilm matrix (**figure 5a**). Another regulatory function related to the
619 biofilm lifestyle switch is also highlighted by the increased expression of the *sivA*, *B* and *C* genes
620 encoding factors that modulate the activation of the sporulation master regulator Spo0A [174].
621 Notably, *sivB* encodes the BslA protein, another component of the extracellular matrix of the *B.*
622 *subtilis* biofilm [175]. Finally, illustrating another form of motility behaviour, the up-regulation of the
623 *srfAA-srfAD* operon, involved in the production of surfactin, suggested an increased ability of *Bacillus*

624 to swarm across solid or semi-solid surfaces under microgravity. An effect of microgravity on
625 swarming motility was also strengthened by the up-regulation of the entire *yrkEFHIJ* operon,
626 encoding genes of unknown function but found to be specifically expressed during swarming
627 conditions [176] [177].

628

629 3.4 The role of global regulators in the adaptive response to space 630 conditions

631 Global RNA-seq based gene expression and proteomic analysis uncovered hundreds of genes
632 differentially expressed under low gravity compared to earth gravity conditions, part of them being
633 involved in cellular pathways governing the observed physiological responses. Their role in bacterial
634 adaptation to microgravity might be direct or indirect. Bacteria adapt to a stressful environment by
635 reprogramming gene expression to produce the necessary effectors to cope with stress. Responses
636 involved key regulators that allow the rapid modulation of the expression of a wide range of genes. In
637 experiments on rotating HARV (high aspect ratio vessel bioreactors) in Salmonella, the LSMMG
638 regulon comprises 163 genes, involved in various cellular processes, part of them known to belong to
639 other specific regulons governed by global regulators [172]. The analysis identified two chromatin-
640 associated proteins, HimA and DPS, able to affect local gene expression by modulating DNA
641 topology [178]. Among other transcriptional factors, the ferric uptake regulator Fur is also involved in
642 the LSMMG response [172]. In *P. aeruginosa*, the alternative sigma factor AlgU controls genes
643 involved in alginate biosynthesis and oxidative stress [179]. AlgU has been found to play a role in the
644 adaptive response to LSMMG, in agreement with the observed enhanced biofilm and virulence
645 phenotypes [119]. Recently, the global regulator Hfq has emerged as a recurring space-responsive
646 gene in *E. coli*, *S. typhimurium*, *Vibrio fischeri* and *P. aeruginosa* [119] [173] [180] [29]. Hfq is a
647 RNA-binding protein acting as a global post-transcriptional regulator of gene expression in bacteria.
648 Hfq acts as a RNA-chaperone, stabilizing RNA-RNA interactions, such as those occurring between

649 small regulatory RNAs (sRNAs) and their messenger RNA (mRNA) targets, thus modulating their
650 function by multiple mechanisms. Owing to its functional flexibility, Hfq participates in regulating
651 various bacterial processes, including motility, biofilm formation and virulence [181] [29] [182]. Hfq
652 expression was found consistently down-regulated in several space transcriptional studies [183] [119]
653 [173] [180] [184] [29] [172]. Considering that most of the flagellar genes are regulated through Hfq-
654 dependent sRNA in many Gram-negative pathogens, Hfq is thus arising as a central player in motility
655 behaviour under microgravity stress across gram-negative bacterial species [183] [173].
656 Contrastingly, Hfq was not identified in the transcriptomic profiling of *B. subtilis* in space conditions
657 [165]. In this Gram-positive bacteria, the Hfq protein is not essential although it plays an important
658 role in survival during the stationary growth phase [185] [186]. Compared to Gram-negative bacteria,
659 Hfq in *B. subtilis* Hfq does not play a central role in post-transcriptional regulation and its absence
660 alters the expression levels of only a limited number of genes. Most particularly, genes involved in the
661 anaerobic respiration and fermentation pathways and belonging to the ResD/Rex regulons, are up-
662 regulated in a Δhfq mutant [185]. Interestingly, the comparative analysis of the *B. subtilis*
663 transcriptomes from the BRIC-21 and BRIC-23 spaceflight missions revealed a down-regulation of
664 expression of many operons regulated by ResD (6 over 17) and Rex (5 over 7) (**figure 5b**). This
665 observation suggests a less direct role of the Hfq-mediated response to space conditions. Further
666 studies are required to decipher the genetic regulatory network at play during the adaptation of a
667 bacillus cell to microgravity stress.

668

669 **4. Controlling microbial migrating communities on spaceflight** 670 **habitats surfaces**

671

672 In an event of a spaceflight biocontamination outbreak, such as the fungal contamination of panel
673 fronts in the “hygiene area” of a functional cargo module or clogged lines in SRV-K line of the

674 condensate recovery system (**Figure 1**), remediation actually relies on cleaning and disinfection with
675 fungistat wipes, air filtration with POTOK 150MK system, or disassembly and replacement of
676 contaminated payload [17]. However, these strategies are not feasible for extended long-term human
677 missions to space and special concern is raised about microbial biofilms because of their difficulty to
678 be eliminated due to their increased resistance to antimicrobials. Indeed, biofilms are the most
679 resilient form on life on Earth and currently available coatings and antibiofilm technologies, are not
680 yet able to permanently avoid biofilm growth. This limitation is relevant both on Space and Earth
681 applications. A possible approach is to adopt combined strategies to delay as long as possible biofilm
682 formation (coatings, biocides, shear stresses...). In a recent review, H.-C. Flemming concluded that to
683 really solve biofouling problems, it is necessary to learn how to live with biofilms and mitigate their
684 detrimental effects instead of trying to eradicate them [188]. Hence, new strategies are being
685 investigated to prevent microbial migrating communities on surfaces in order to reduce microbial risk
686 to crew health, safety, and performance during human exploration in space. In this regard, Zea et al.
687 [3] summarized potential biofilm control strategies for extended human spaceflight missions
688 including, biocides, coatings, ionizing radiation, biofilm detachment, biocontrol as well as chemical
689 removal of nutrients. It was pointed out that solutions developed against biofouling of marine surfaces
690 and medical devices could bring insights useful for biofilm control on spacecraft. The aim is to
691 develop broad-spectrum antibiofilm surface treatments for confined space stations which would be
692 easy to upscale. Representative coatings for biocontamination control are typically based on metal
693 ions (silver(I), copper(II), tributyltin), titanium alloys and mixtures, synthetic polymers (e.g.
694 polyethylene glycol PEG) that can be copolymerised with hydrophobic polydimethylsiloxane (PDMS)
695 or biopolymers such as poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [189] [190] (**Table 1**). The
696 active molecules can be deposited or chemically grafted on the surface. It can also be formulated to be
697 released progressively, locally or on-demand [191]. Special emphasis is put on the lack of toxicity and
698 long-term stability under space station conditions. Wang et al [192] reported the strict standards that
699 antimicrobial coatings must-have for space application, according to the European Cooperation for
700 Space Standardization (ECSS). Forbidden powder (e.g. Cd, Zn, Hg, polyvinyl chloride and
701 radioactive materials (European Cooperation for Space Standardization, <https://ecss.nl>) and key

702 parameters such as toxicity, flammability, stability, effectiveness for the application, maturity of use,
703 program/user acceptability, and material compatibility are taken in the exam. A strong limitation in
704 the validation of these antimicrobial surfaces is the distance between laboratory experiments (short-
705 time scale, monospecies contamination in rich synthetic media...) and environments existing in the
706 real world [188]. Moeller and collaborators designed the ISS experiment “BIOFILMS” [193] [25] to
707 investigate the formation of biofilms on various antimicrobial surfaces in a real space station
708 environment. These materials include inert surfaces such as stainless steel, as well as antimicrobial
709 active surfaces such as copper-formulated materials. Antimicrobial compounds involved in those
710 formulations raised major concerns as their continuous use may lead to the emergence of
711 antimicrobial resistance (AMR) [194]. In this context, coatings free of heavy metals are now under
712 investigation in the framework of ESA’s NBactSpace project implemented by the Luxembourg
713 Institute of Science and Technology [192].

714

715 A promising, biomimetic, method for biofilm prevention is based on the example of several natural
716 super-repellent surfaces [195] which exhibit non-sticking properties by combining hierarchical
717 micro/nano-structures with low surface energy agents. The most prominent example is the surface of
718 the lotus (*Nelumbo nucifera*) leaf which corresponds to a very large water contact angle ($>150^\circ$) and
719 small sliding angle and contact angle hysteresis ($<10^\circ$) [196] thus exhibiting both
720 superhydrophobicity and extreme water repellency [197]. Lotus-like phenomena are typically studied
721 in three-phase systems which include air, a liquid and a solid). However, the same concept can be
722 applied on a three-phase system consisting of microbes, a liquid medium and a solid surface. Several
723 methods have been developed to produce lotus leaf-like materials which have the potential to be used
724 as surface coatings for biofilm prevention [198], according to a two-step process [199]: First, a non-
725 sticking surface can resist or prevent the initial attachment of microbes. Second, even if there are
726 some microbes adhered to the surface, these can be easily removed by slight external forces e.g.
727 wiping, wind, and water impact [199]. According to these concepts, superamphiphobic materials,
728 which show extreme non-sticking properties as they have the ability to repel not only water but

729 virtually any liquid, may offer enhanced protection against biofilm formation [200]. It is stressed,
730 however, that non-wetting conditions do not always promote microbe repulsion. For example, Yuan *et*
731 *al.* [201] showed that polystyrene surfaces with a moderate water contact angle of about 90° produced
732 the highest level of bacterial (*E. coli*) adhesion whereas limited bacterial binding was observed on
733 both superhydrophobic and superhydrophilic structured polystyrene surfaces. Moreover, it is well
734 known that superhydrophilic and underwater superoleophobic (SUS) materials have in general
735 antifouling properties as they repel organic materials [202]. Prevention of biofilm formation by
736 antiadhesive surfaces can be supplemented by a biocidal step. This is of particular interest in the case
737 of brush coatings that have been shown to increase antimicrobial action in addition to reducing
738 bacterial adhesion forces with the material [203]. Some specific material also exhibits strong
739 antibacterial activity without being formulated with biocides. Ivanova *et al.* [204] showed that
740 bacteria entering in contact with the array of pillars of the superhydrophobic surface of cicada
741 (*Psaltoda claripennis*) wings are inactivated within a few minutes. Clearly, this bactericidal ability of
742 the cicada wing surface is a physicochemical effect as it does not involve the action of any biocide
743 [204]. With similar biomimetic nanopatterned surfaces, Michalska *et al.* demonstrated the dependence
744 upon pillar density and tip geometry on the mechanism of bacterial killing [205]. In a recent study, Pal
745 *et al.* [206] produced a highly hydrophobic laser-induced graphene film that can be implemented on
746 reusable surgical protective masks. Several reports described the combination of superhydrophobic
747 coatings with biocidal agents embedded within the structured coatings [199] including quaternary
748 ammonium compounds [207], metal oxides [199], N-halamines [208] and natural antibacterial agents
749 [209].

750 Several papers reported the possibility to interfere with microbial processes involved in adhesion,
751 migration and biofilm maturation. Many bacteria produce extracellular adhesins or appendages to
752 mediate their adhesion to the surface. Several coatings containing pilicide molecules such as disperse
753 red 15 or verstatin were found efficient to interfere with the pili function and reduce pathogen fixation
754 [210] [211]. Drugs specifically interfering with flagellar motor assembly and function (agaric acid,
755 phenamil, amiloride) were also reported and could be integrated into such targeted approaches [212]

756 [213]. A very exciting direction in these applications is to interfere with microorganisms signalling
757 systems and decision-making. Each cell at a specific period integrates hundreds of environmental
758 signals to adopt a specific cell fate. Coatings integrating molecules perturbing the Quorum-sensing
759 (QS) response are already available (Table 1) and [214]. By preventing the biofilm QS-maturation,
760 several important mechanisms of persistence could be bypassed. The cyclic-di-GMP pathway could
761 be similarly targeted to prevent the physiological transition from planktonic to biofilm in many
762 bacterial species. In recent years, several plant metabolites and their formulations (resveramax,
763 cinnamic acid,...) have been identified in motility-swarming-biofilm inhibitors with the advantage of
764 being environmentally friendly and poorly toxic for humans in contact [213].

765 A microbe-based preventive strategy to protect surfaces from being colonized by unwanted
766 microorganisms is based on guided microbial ecology and interspecies competition. The concept here
767 is to consider that if any surface supports microbial life it is worth settling selected beneficial
768 organisms able to exclude unwanted microorganisms. This positive biofilm approach is applied in a
769 One Health context to limit microbial pathogens on the surface of livestock buildings [215]. This
770 biological approach has shifted from labs to farms in recent years with various commercial products
771 now on the market in several countries paving the way for applications in spatial missions [216].

772 When preventive approaches failed and a biofilm is formed, the presence of the extracellular cellular
773 matrix, the spatial organisation of the communities and the associated diversification of cell types will
774 generate emergent properties of the community and recalcitrance to the action of most conventional
775 disinfection treatments [4] [32]. It is frequently stated in scientific publications that microorganisms in
776 a biofilm are typically 1000 times more resistant to biocide action than their planktonic counterparts.
777 While the mode of action of disinfectants depends on the type of biocide employed [224], the low
778 efficiency on surface-associated biofilm communities is still not fully understood. It is now evident
779 that biofilm tolerance to disinfectant is intimately related to the three-dimensional structure of the
780 biofilm, heterogeneous within the biostructure and multifactorial, resulting from an accumulation of
781 different mechanisms [4]. To overcome these disinfection limitations, an attractive strategy is to re-
782 sensitive the biofilm population by targeting the matrix. EPS-degrading enzymes can help disrupt the

783 matrix for more effective removal and disperse bacteria in biofilms for more effective killing when
784 combined with antimicrobial agents [225]. Enzyme treatments are mainly used in the medical context
785 to target recalcitrant biofilm infections by undermining the protective role of the matrix and thus
786 increasing the effectiveness of traditional antimicrobial therapies [226]. Exopolysaccharide-degrading
787 enzymes, such as glycoside hydrolases and glucanohydrolases, have been used to degrade a mixed-
788 species *S. aureus* and *P. aeruginosa* biofilm grown in a mouse model of chronic wounds [227] and to
789 prevent the formation of pathogenic oral biofilms [228]. Since eDNA is a broadly conserved EPS
790 component [228], DNases have also proven to be effective in disrupting biofilms [229], both in vivo
791 and in vitro [230]. In particular, human-derived DNase I is exploited to treat pulmonary infections in
792 cystic fibrosis patients [231] [232]. Moreover, enzyme-based biofilm impairment treatments are
793 finding increasing applications in the food [233] [234] and paper [235] industries, potentially opening
794 the doors for their increased use in the technological sector. In particular, since enzymes require
795 specific physicochemical conditions to maximize their efficacy, their use in the spaceflight context
796 would require studies to assess longevity and effectiveness in the application conditions. It was also
797 demonstrated that tunneling the biofilm matrix by selected bacilli swimmers could resensitize bacteria
798 to the action of biocides by creating a transient vascularization network [110]. Matrix destabilisation
799 is also possible by magnetic disturbance. In a recent paper, magnetic iron oxide nanoparticles were
800 successfully used to disrupt a recalcitrant biofilm upon exposure to a controlled magnetic field [236].

801 Among promising strategies to cope with biofilm development, the use of bacteriophages regained
802 consideration in the last decade. By exploiting their ability to kill their bacterial host, phages have
803 been successfully applied to eradicate biofilm from within [237]. Phage treatments can be based on
804 the use of the whole phage particles, but also on phage-derived antibacterial activities. The main
805 phage-encoded bactericidal enzymes are the depolymerases, lyases and hydrolases, externally
806 associated with the virion tail and able to degrade EPS [238]. Phages also encode lysins acting from
807 inside the bacterial cell and are responsible for cell wall degradation [239]. Similarly to DNase
808 treatments targeting extracellular DNA, endolysins and depolymerases-based treatments have been
809 used to overcome the biofilm matrix barrier. The biocidal potential of these phage-encoded enzymes

810 has been demonstrated to prevent the biofilm formation of pathogens in vitro as well as in vivo [240].
811 The use of integral lytic bacteriophages for bacterial biofilm control has been proven a safe alternative
812 approach to antibiotics and chemical biocides [241]. Although most studies assessing the ability of
813 bacteriophages to reduce biofilm biomass are performed in laboratory conditions, the successful
814 application of phages and phage cocktails has been reported in several medical cases as a last
815 alternative to combat drug-resistant bacterial infections [242] [243]. The narrow-host range specificity
816 of phages combined with the ability of bacteria to rapidly develop defense mechanisms to survive
817 infection could be considered as a limitation of this approach. However, the use of phage cocktails
818 with a broader spectrum of infection has already proved to be very efficient in complement to
819 antibacterial treatments in combating pathogen biofilms within medical devices [244]. We can
820 anticipate that similar strategies could be also used successfully in a spacecraft environment.
821 Furthermore, phages are not motile and the structure as well as the composition of the biofilm matrix
822 acting as a diffusion barrier interfere with their penetration and dispersal within biofilm [245]. The
823 recent discovery that phage could be passively transported by motile carrier bacteria sheds new light
824 on the importance of the role of non-host bacteria–phage interactions on biofilm dynamics [115][116].
825 This behavior, called “hitchhiking”, opens new avenues to improve phage delivery within biofilms. In
826 addition, a growing number of studies highlight the synergistic action of phages combined with other
827 antimicrobials for the effective eradication of biofilms [246] [247] [248]. Emergent approaches are
828 now combining phages and/or phage-derived products with other nano weapons or bactericidal agents
829 to combat biofilms on earth. Phage-based biocontrol could be used in support of other biofilm
830 eradication strategies to delay corrosion and biofouling in space and more generally mitigate biofilm
831 formation on future missions (ICES-2019-271). How the lack of gravity could influence their
832 diffusion and their interaction with their host is currently under investigation
833 (www.issnationallab.org/iss360/phageevolution-rhodium-scientific-studying-viruses-microgravity/).
834 The efficiency of similar treatment applied to biofilm control in the context of spaceflight remains to
835 be studied.

836 The chemical, physical and biological toolbox to control biofilms migrating communities is constantly
837 increasing with innovative prevention and curative strategies. However, their efficacy is not universal
838 and synergetic combined approaches will be needed to prevent biofilm deleterious effects during
839 long-term spatial missions [249].

840

841 5. Conclusions & perspectives

842 The vibrant microbiota migrating and settling on surfaces of spaceflight habitats could jeopardize
843 long-term spatial missions by altering surface and equipment functions and threaten astronauts'
844 health. The most conventional hygienic procedure to control surface-associated microbiota on earth is
845 cleaning and disinfection with highly reactive chemicals [4]. This approach is hardly compatible at
846 large scale with long-term space missions in terms of the quantity of water needed, the absence of
847 drainage in microgravity conditions, the cost of transport of the biocides as well as their potential
848 corrosive, toxic and explosive properties. Space agencies intensify their effort in preventive strategies
849 mostly relying on hygienic design and maintenance and the use of anti-biofilm material in a sensitive
850 part of the spaceflight (e.g. WRS). Most of the activity of those materials and coatings relies on
851 antiadhesive or antimicrobial properties [192]. With our better understanding of the specific
852 physiology of microorganisms living in a biofilm in microgravity conditions, we could envision
853 activating those materials with effectors targeting molecular determinants of biofilm
854 initiation/stability/dispersion (pili, flagella, EPS...) or the regulations pathways involved in the shift
855 between planktonic and biofilm cell fate (cyclic di-GMP pathway, Quorum-sensing signaling...).

856 Physical decontamination procedures based on the intensive exposition of surface to antimicrobials
857 beam (UV, blue light, pulsed light, plasma) are also interesting alternatives to chemical disinfection
858 [250]. Another family of control strategies are based on biological organisms (biological warfare). In
859 several areas, microorganisms are used to kill specific unwanted organisms or to guide the ecology of
860 the surface (microbiota editing). This is the case of phages which are viruses killing specifically a

861 group of (pathogenic) bacteria or of positive biofilm that is composed of bacteria recognized as safe
862 that are settled on purpose on a surface to prevent unwanted colonisation. Both these microbe-based
863 strategies are already in use in the biomedical, agricultural and food industries. A point of interest is
864 that they can be propagated indefinitely very easily in the space habitat.

865 The first demonstration of DNA sequencing in space was performed recently by NASA with the
866 portable MinION device (Oxford Nanopore Technologies) on the ISS. Successful sequencing of
867 mouse microbiota (bacterial and viral DNA) was demonstrated, showing potential for monitoring of
868 microbes in food, water and environment [14] [251]. This opens doors to on-site analysis and
869 monitoring of the biofilm species composition and ecological diversity evolution, but also in terms of
870 functional potential through shotgun metagenomics analysis [252]. Thereby, astronauts will be able in
871 a near future to detect unwanted species in spaceflight habitats in real-time, but also catalogues of
872 genes associated with unwanted microbial functions independently of the hosting species (genes
873 involved in material degradation, biofilm persistence, virulence...). These metagenomic approaches
874 have been developed with success in other biotopes such as the human gastrointestinal tract allowing
875 for stratification of the population (e.g. enterotypes) in different responding groups and identifying
876 biomarkers associated with specific functions [253] [254].

877 Microbial biofilms can also be used for some of their positive effects on crew health [255]. They are
878 envisioned as a source of safe, fresh and valuable food to improve astronauts' health for long-term
879 spatial missions. Several solid fermentation processes involving biofilms are/could be explored in this
880 context e.g. miso and natto resulting from the biofilm formation of *Aspergillus oryzae* or *Bacillus*
881 *subtilis* on cooked beans [256]. Kefir granules and relatives products are centimetre natural symbiotic
882 communities composed of lactic acid bacteria and yeasts embedded in a dense and complex
883 extracellular matrix [257]. Described as functional “super-organisms”, these spatially organised
884 consortia are highly tolerant to environmental stress and could be multiplied for years during long-
885 term missions with low resource requirements. Astronauts do not receive the same replenishment of
886 microbes on a space flight as they do on earth [34]; fermented food along with probiotics can be used
887 to prevent or restore gut microbiota dysbiosis. Another possible source of fresh food is the cultivation

888 of microalgae with nutritive interest (lipids, vitamins...). In order to limit the use of water and
889 energy, biofilm-based microalgae cultivation systems on surfaces are developed [158]. Alternatively,
890 bioprinting of microalgae cells with controlled patterns in hydrogels could allow the formation of a
891 synthetic biofilm with optimum exposure to light and nutrients [258] [259]. An important advantage
892 of these microorganism based-processes involving biofilms is that they could be adapted to recycle
893 organic matter from waste. More generally, biofilms are envisioned in various *in-situ* resource
894 utilization (ISRU) procedures in space travel such as biomining or bioregenerative life-support
895 systems [260].

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903

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- 1654

1655 **Legends to Figures**

1656

1657 **Figure 1.** Biofilms in spaceflight habitats. A) Mold-stained panels in the ISS hygiene area
1658 resulting from mould growth following contact with wet towels. Image: Mold species in
1659 dust from the International Space Station identified and quantified by mould-specific
1660 quantitative PCR [24]; B) Biofilm formation inside the condensate plumbing at the inlet
1661 to the Russian SRV-K condensate processor [25].

1662

1663 **Figure 2.** Different 3D structures obtained in terrestrial or microgravity induce different
1664 molecular diffusion. To illustrate this difference, we computed a reaction-diffusion
1665 process based on confocal microscopy images of *Pseudomonas aeruginosa* grown under 1
1666 g or microgravity taken in [30]. A chemical component diffuses from a bulk source
1667 located in the upper boundary of the image with a heterogeneous diffusion coefficient
1668 that depends on the biofilm density: the higher the local bacterial density, the lower the
1669 local diffusion coefficient. The reactive process corresponds to the chemical consumption
1670 by the biofilm bacteria, the rate of which also varies according to the local bacterial
1671 density with Monod dependency. The steady state of the chemical density map is
1672 displayed, with isolines every 0.05 to better represent the distribution gradients. These
1673 different concentration maps induce different nutrient availability which in turn may
1674 impact the 3D biofilm structure and physiology.

1675

1676 **Figure 3:** Schematic view of surface colonisation by microorganisms. Microorganisms from
1677 the bulk are transported toward a surface through passive and active processes. After
1678 contact with the surface, cells can migrate individually or collectively and initiate the
1679 formation of a 3D structure called biofilm associated with emergent properties such as

1680 extreme environmental stress tolerance. Subpopulations of cells can disseminate from this
1681 primary structure to initiate secondary biofilms with potential phenotypic and genetic
1682 evolution. Those microbial processes were formally described on earth and there is rising
1683 scientific evidence that many of them could be strongly affected under hyper- or micro-
1684 gravity conditions encountered in spaceflight missions.

1685

1686 **Figure 4.** We simulated the growth of a micro-alga biofilm subject to terrestrial or micro-
1687 gravity. The model is a mixture model [158] coupling a fluid dynamics model to a
1688 reaction-diffusion-convection model of biofilm dynamics including biomass growth and
1689 consumption of diffusive nutrients and CO₂. Compared to [158], an additional force is
1690 added to the movement conservation equation modelling the gravity-dependent
1691 sedimentation as a net force between a gravitational force and a buoyant force [154]. We
1692 can observe that micro-gravity impacts the spatial structure of the biofilm and therefore
1693 the resulting substrate consumption and the overall biofilm growth.

1694

1695 **Figure 5:** (A) Cluego representation of common biological process grouping genes involved
1696 in similar pathways from BRIC-21 and BRIC-23 datasets. Each node corresponds to a
1697 GO (Gene ontology) term. Blue and red colours illustrated the contribution of genes
1698 upregulated in BRIC21 and BRIC23, respectively. The size of the node represents the
1699 term enrichment significance. The visualization was obtained with the Cluego Plugin for
1700 Cytoscape 3.9.1. (10.1093/bioinformatics/btp101, 10.1101/gr.1239303). (B) Venn
1701 diagram showing genes of the ResD and Rex regulons significantly downregulated in the
1702 BRIC-21 and BRIC-23 missions (data from [187]).

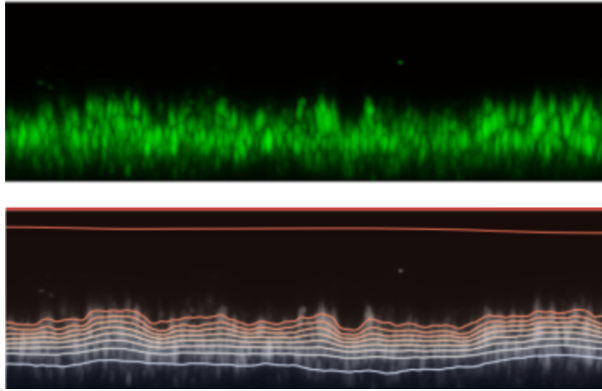
Table 1: Surface modifications to prevent biofilm formation and their mode of action.

Coating type	Coating agent	Description	Action mechanism	References
Polymeric films (organic, synthetic, mixed)	Poly(2-alkylacrylic acids), layer by layer (LdL)	Agents released in response to environmental acidification due to bacterial metabolism	Release-killing	[217]
Metal ions (copper, argent, titanium)	Octadecylamine capped Cu/reduced graphene oxide	Enhancing surface hydrophobicity	Anti-adhesion	[218]
Metal ions (copper, argent, titanium)	Silver oxide film	Photocatalytic antimicrobial surfaces	Contact-killing	[219]
Liquid films	Liquid-infused structured surfaces	Imbibition of porous surfaces with surfactants	Anti-adhesion	[220]
Antimicrobial peptides (AMPs) grafting	Polydopamine peptide coating	Polydopamine coating to immobilize AMPs on surfaces	Contact-killing	[221]
Quorum Quenching enzymes grafting	Acyase and α -amylase coating	Degradation of Quorum Sensing signals	Anti-adhesion	[222]
Nanoparticles grafting	Zinc oxide nanoparticle	Immobilization of antibacterial nanoparticles on surfaces	Contact-killing	[223]

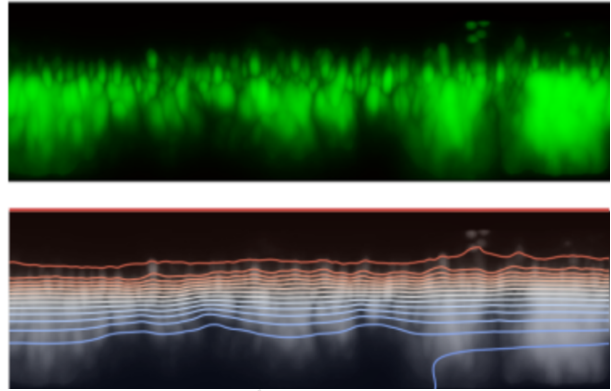


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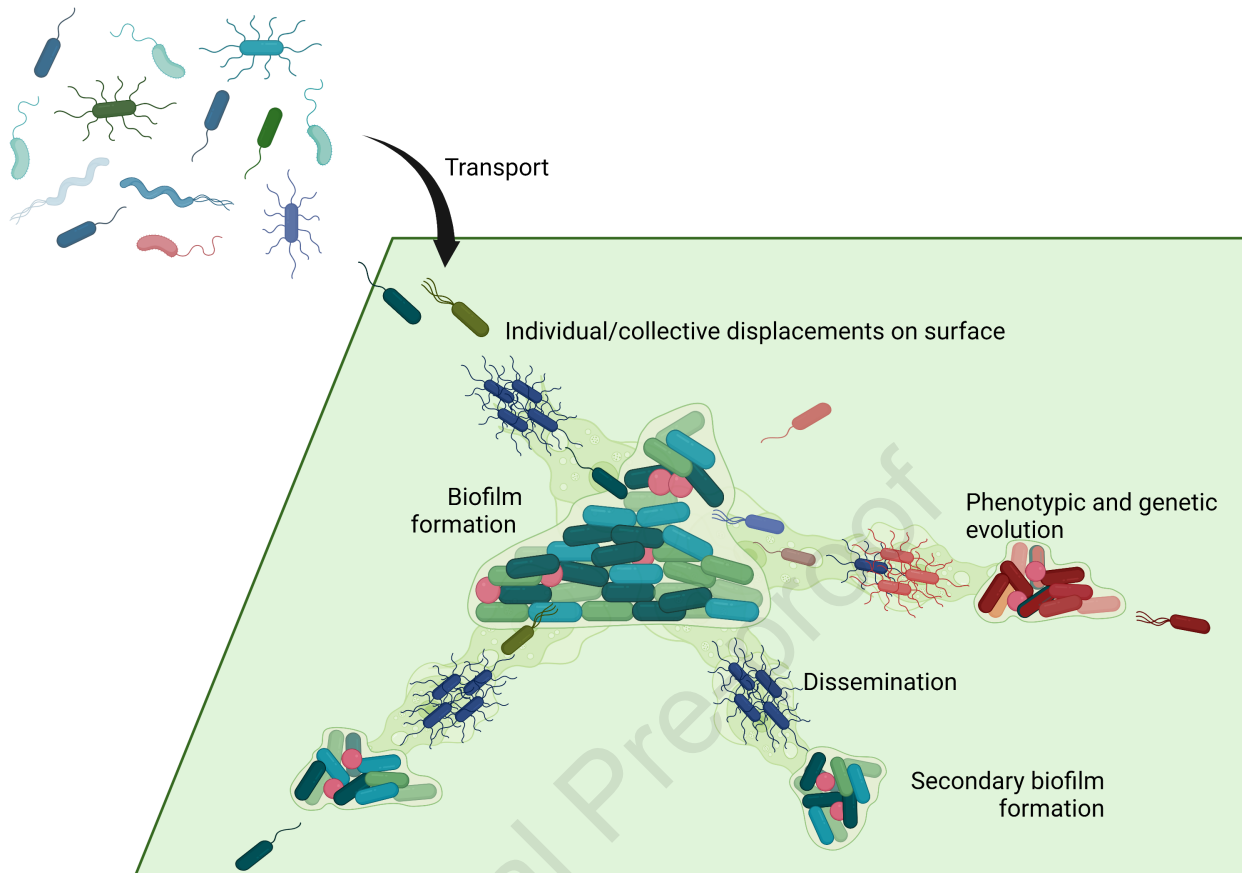
Normal gravity

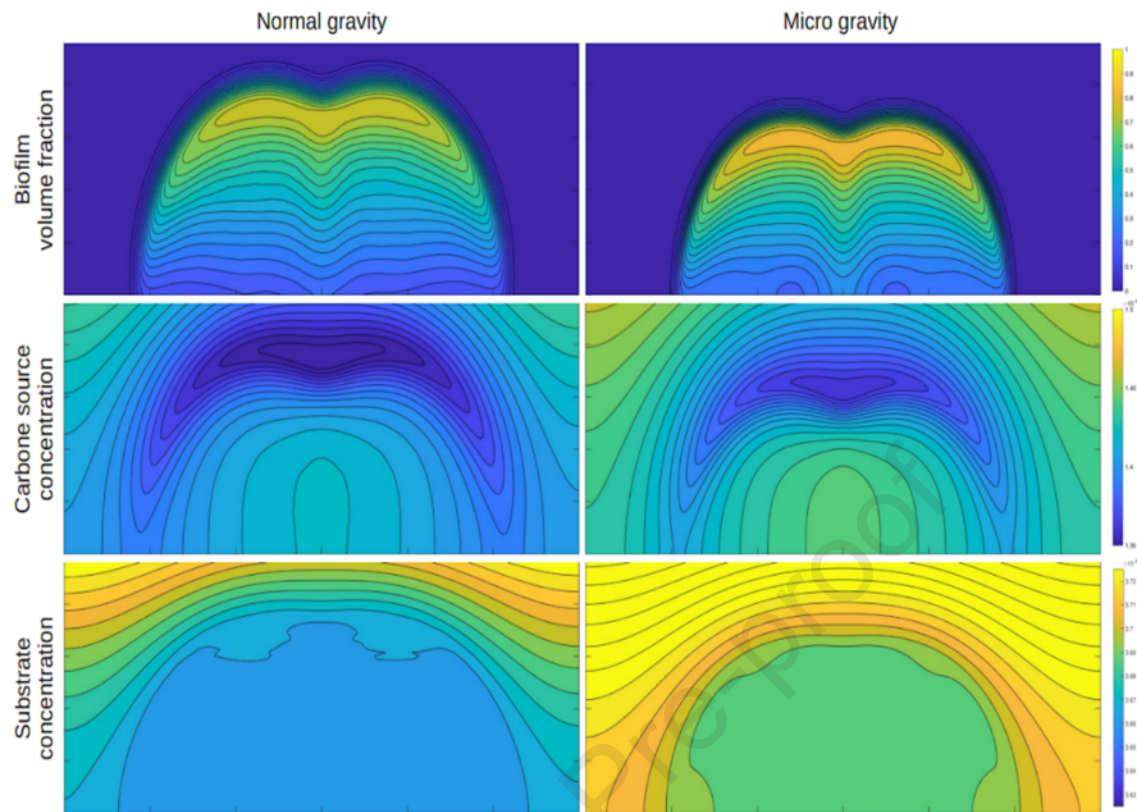


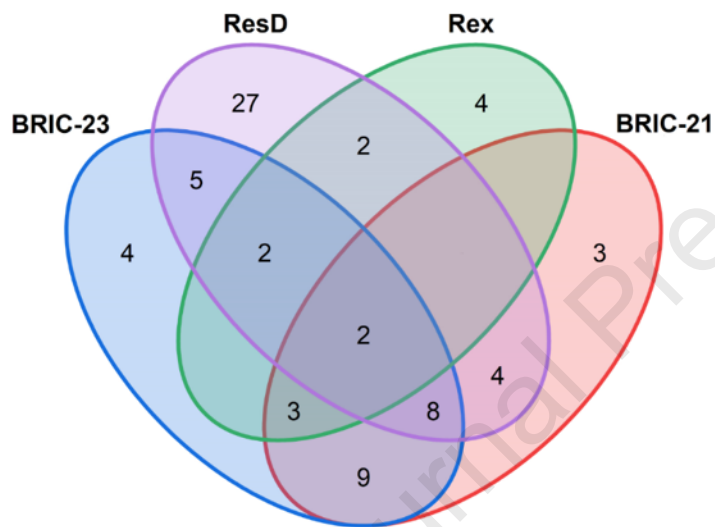
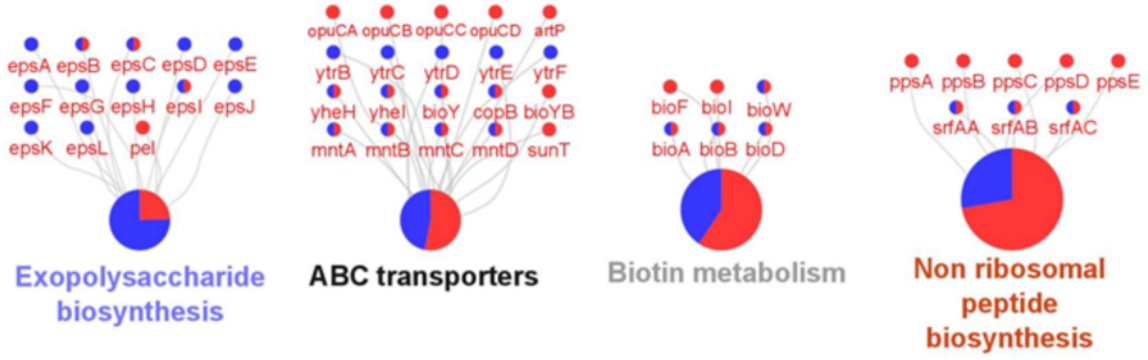
Microgravity



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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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