

1 **Survival of the magnetotactic bacterium *Magnetospirillum***  
2 ***gryphiswaldense* exposed to Earth's lower near space**

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28 Earth's near space (20-100 km above sea level) is one of the most extreme environments on  
29 Earth due to a combination of high radiation, low atmospheric pressure, extreme cold and hyper  
30 aridity [1]. Investigations of the survival strategies of microorganisms in near space are of great  
31 interest in respect of understanding the limits of life on Earth, identifying the upper boundary  
32 of Earth's biosphere, testing the panspermia hypothesis, and assessing long-distance microbial  
33 transfer [2]. High-altitude balloon based research on microorganisms in near space dates back  
34 to the 1930's [3]. Ultraviolet (UV) radiation has been shown to be one of the main causes of  
35 biological mortality in near space. Thus to date many extremophilic microorganisms including  
36 bacteria, archaea and fungi have been tested for their tolerance to extreme conditions in the  
37 stratosphere region of lower near space [4].

38 Magnetotactic bacteria (MTB) are a group of microorganisms able to passively align along  
39 geomagnetic field lines and are characterized by their ability to synthesize intracellularly  
40 magnetic organelles (known as magnetosomes) consisting of lipid bilayer-bounded membranes,  
41 in which nanosized, ferrimagnetic magnetite ( $\text{Fe}_3\text{O}_4$ ) and/or greigite ( $\text{Fe}_3\text{S}_4$ ) crystals are  
42 biomineralized [5]. Magnetoreception is the major function of magnetosomes in modern MTB,  
43 while  $\text{Fe}_3\text{O}_4$ -type magnetosomes have been revealed to play an enzyme-like role to eliminate  
44 intracellular levels of toxic reactive oxygen species (ROS) in *Magnetospirillum*  
45 *gryphiswaldense* strain MSR-1 (MSR-1) [6], indicating the potential of MTB to tolerate high  
46 levels of UV radiation. Moreover, MTB are considered to be one of the earliest magnetic-  
47 sensing organisms that emerged in the Archean Eon when Earth lacked a stable thick ozone  
48 layer and was exposed to extremely high fluxes of UV radiation [7]. Therefore, studying the  
49 tolerance of MTB under high UV doses will lead to a better understanding of the origin and  
50 early evolution of MTB. Previously, UVB radiation (peaking at 306 nm) has been revealed to  
51 affect the cell growth and magnetosome formation in *M. magneticum* strain AMB-1 (AMB-1)  
52 under laboratory conditions [8]. However, our understanding of the effects of natural solar UV  
53 radiation on MTB remains very limited.

54 Here, we performed experimental exposure of wildtype and magnetosome-deficient MSR-1  
55 strains (Fig. S1 and the Supplementary materials for strain description and culture condition)  
56 to Earth's lower near space at ~23 km above sea level (ASL) through a high-altitude scientific  
57 balloon using the Chinese Academy of Sciences Balloon-Borne Astrobiology Platform (CAS-  
58 BAP) during the HH-20-7 flight mission [9]. Samples of MSR-1 were freeze-dried beforehand  
59 to reduce damage from freeze-thawing processes during the flight (Fig. 1a and the  
60 Supplementary materials for sample preparation and experimental design). The BIOlogical  
61 Samples Exposure Payload (BIOSEP) is a box-shaped vessel that accommodates  
62 biological/chemical samples in a 30-cell (61 mm in diameter, Fig. S2) sample chamber and four  
63 96-well plates (Fig. 1b). The BIOSEP was mounted at an elevation angle of  $30^\circ$  relative to the

64 horizon, and hence the normal of the windows of the sample chambers is 60° above the horizon.  
65 Chamber cells were kept sealed from the outside environment to maintain a constant internal  
66 pressure and humidity. The BIOSEP was launched at 01:02 China Standard Time (CST) on  
67 September 3, 2020 into the lower near space at Dachaidan, Qinghai, China (37°44' N, 95°21'  
68 E) and flew for a total of 18 hours and 44 minutes using a 50,000 m<sup>3</sup> high-altitude helium  
69 balloon. The payload stayed at a floating altitude of 23 km for 14 hours (Fig. 1c). Samples were  
70 remained shielded inside the BIOSEP until 07:59 CST, at which point the flight computer  
71 remotely lifted the box lid of BIOSEP to start the exposure experiments. The box lid was closed  
72 at 15:15 CST after 7 hours and 16 minutes of exposure, with a period of direct exposure for 5  
73 hours 18 minutes as monitored by illumination measurements (Fig. 1d and the Supplementary  
74 materials for data analysis). The level of diffuse UV radiation (191-399 nm) was measured by  
75 the UltraViolet Spectrometer (UVS) next to the BIOSEP. The diffuse UV radiation dose  
76 received by samples during the direct exposure was  $\sim 2.42 \times 10^4$  J/m<sup>2</sup> (Fig. 1e and the  
77 Supplementary materials for UV radiation measurement). According to the MODerate  
78 resolution atmospheric TRANsmission (MODTRAN) model [10], the direct solar UV spectrum  
79 at an altitude of 23 km near Dachaidan is approximately two orders of magnitude higher than  
80 the diffuse radiation spectrum (Fig. 1f). Moreover, in situ temperature in the sample chamber  
81 cell was monitored, which gradually increased from -43.9 to 33.8°C with the duration of  
82 continuous exposure (Fig. 1d). All samples were successfully recovered and stored in an  
83 automotive refrigerator at 4°C within 5 hours of landing. The laboratory control group (LCG)  
84 of freeze-dried MSR-1 was remained in a light-free refrigerator at 4°C in the laboratory (Fig.  
85 1a).

86 After the flight, the number of surviving MSR-1 in each sample was estimated by colony-  
87 forming unit (CFU) enumeration. For the near space non-exposed group (NNG, launched to the  
88 near space but shielded by an opaque lid), CFU estimates of MSR-1 capable of magnetosome  
89 synthesis (referred to as MSR-1(+)) did not change significantly as compared to those of the  
90 LCG control group: the CFU estimates of NNG were  $(2.21 \pm 0.15) \times 10^6$  CFU per chamber cell,  
91 and those of LCG were  $(2.03 \pm 0.12) \times 10^6$  CFU per chamber cell (Fig. 2a and Table S1). In  
92 contrast, the survival rates of the near-space exposed group (NEG) decreased significantly ( $F =$   
93  $51.5$ ,  $P < 0.001$ ), and culturable MSR-1(+) of NEG dropped by an order of magnitude to  $(2.50$   
94  $\pm 0.22) \times 10^5$  CFU per chamber cell. For samples that did not form intracellular magnetosomes  
95 (referred to as MSR-1(-)), exposed bacteria suffered more significant inactivation after flight  
96 (Fig. 2a and Table S1). In contrast to MSR-1(+) that retained  $\sim 12.32\%$  of viable cells after  
97 exposure, no culturable colonies of MSR-1(-) were observed in all NEG samples. Moreover, a  
98 considerable degree of inactivation of the NNG MSR-1(-) samples was also noted; that is, only  
99  $\sim 27.97\%$  of bacteria survived even without UV radiation exposure. The different viability

100 between exposed MSR-1(+) and MSR-1(-) indicates that magnetosomes may play an important  
101 role in protecting MTB from UV irradiation.

102 To investigate the effects of near-space exposure on magnetosome formation, we examined  
103 the variations in the number and crystal size of magnetosome of surviving MSR-1(+) using  
104 transmission electron microscopy (TEM). MSR-1 with intracellular magnetosomes accounted  
105 for 95.6% of the total surviving cells in NEG, while only 84.5% of cells in LCG were capable  
106 of synthesizing magnetosomes, suggesting that cells with magnetosomes are more likely to  
107 survive after near-space exposure. Magnetite crystals within each single MSR-1 cell formed  
108 one magnetosome chain (Fig. 2b), with average crystal numbers of  $16.0 \pm 5.4$  in NEG and  $14.8$   
109  $\pm 6.0$  in LCG (Fig. 2c). For NEG, cells that synthesize more than 10 magnetosomal crystals  
110 accounted for 90.3% of the total cells ( $n = 134$ ); however, this proportion in LCG dropped to  
111 75.7% ( $n = 112$ ) (Fig. 2c). There were 86.6% of magnetosomes with grain sizes of 25-55 nm in  
112 NEG ( $n = 822$ ), while this proportion decreased to 81.5% in LCG ( $n = 800$ ) (Fig. 2d).  
113 Magnetosomal crystals less than 25 nm accounted for 8.3% and 11.5% in the NEG and LCG  
114 samples, respectively; and those larger than 55 nm accounted for 5.1% and 7.0% in the NEG  
115 and LCG samples, respectively.

116 Rock magnetic measurements were performed to characterize the bulk magnetic properties  
117 of MSR-1(+) samples. The room-temperature hysteresis loops of both NEG and LCG yield pot-  
118 bellied shapes. The reproducibility of the parallel samples in LCG was better than those in NEG  
119 (Fig. 2e). The values of coercivity of remanence ( $B_{cr}$ ) for NEG and LCG ranged from 26.0 to  
120 31.0 mT and 27.9 to 29.6 mT, respectively. The first-order reversal curve (FORC) diagrams of  
121 NEG and LCG have the typical characteristics of noninteracting uniaxial single domain  
122 particles, indicating that the bulk samples retain intact chains as shown in Fig. 2f. Low-  
123 temperature magnetization curves revealed that the  $\delta_{FC}/\delta_{ZFC}$  ratios of NEG and LCG were 3.4  
124 and 2.8, respectively. Carter-Stiglitz et al. (2002) showed that the value of  $\delta_{FC}/\delta_{ZFC}$  ratios  
125 increased from  $1.97 \pm 0.32$  to  $2.28 \pm 0.52$  when the magnetosome number increased from 5 to  
126 10 in one chain [11]. For our magnetic results,  $\delta_{FC}/\delta_{ZFC}$  ratio of NEG is larger than that of LCG,  
127 indicating that NEG has more magnetosomes per chain than the LCG. Together with TEM  
128 observations, these results suggest that MSR-1 with more magnetosomes may have a better  
129 chance to survive after near space flight exposure. Alternatively, extreme environmental  
130 conditions during flight (e.g., high UV radiation and temperature variation) may affect the  
131 synthesis of magnetosomes in MTB.

132 In a previous study, Wang et al. showed that living cells of AMB-1 exposed to UVB radiation  
133 (peaking at 306 nm) with a maximum dose of  $1800 \text{ J/m}^2$  could affect the cell growth and  
134 magnetosome formation, resulting in more and larger magnetosomes per cell [8]. Our study

135 reveals that a fraction of freeze-dried MSR-1 with magnetosomes are able to survive in near  
136 space conditions with direct UV radiation of  $\sim 10^6$  J/m<sup>2</sup> dose (wavelength recorded from 191 to  
137 399 nm). The surviving cells after exposure synthesize larger numbers of and more uniformly  
138 sized magnetosomes. Previously, organisms used for near-space exposure experiments were  
139 normally extremophiles and spores [4, 12], and most of them are found to suffer from a  
140 significant loss of viability under such high levels of UV radiation (with survivability of 0-50%  
141 under  $\geq 2$  hours of exposure at 20-40 km ASL) [4, 12, 13, 14 and references herein]. The results  
142 of our work are noteworthy because MSR-1 is not an extremophile – it was isolated from a  
143 normal aquatic environment and does not produce spores. The finding that after near space  
144 exposure MSR-1(+) remain viable while MSR-1(-) are completely inactivated in this study,  
145 together with previous studies [6], indicates that magnetosomes very likely play an important  
146 role in protecting MTB from UV radiation. Although the underlying mechanisms of the  
147 magnetosome-mediated UV defense are not fully understood yet, the peroxidase-like activity  
148 of magnetite crystals is thought to be one possibility. UV-induced ROS could damage important  
149 cellular components such as peptide chain fragmentation, enzyme inactivation, lipid  
150 peroxidation and DNA oxidative damage [15]. Magnetosomes in MSR-1 have been shown to  
151 exhibit peroxidase-like activity that could eliminate intracellular levels of ROS [6]. Therefore,  
152 magnetosomes may act as antioxidative compounds to protect MSR-1 from UV damage.  
153 Another possible mechanism is the optical screening of UV wavelengths by magnetosomes.  
154 Some bacteria and yeasts accumulate pigments to absorb a range of light spectrum. MTB invest  
155 a great deal of energy to synthesize magnetosomes which enable the accumulation of  
156 intracellular magnetite, resulting in colonies dark brown in color. Visual inspection of the  
157 exposed samples revealed slightly alterations in color that indicates a potential photochemical  
158 process mediated by magnetosomes that may serve to reduce the UV dose or deplete irradiation  
159 energy. The third mechanism is that the lower layers of MSR-1 may be physically protected  
160 from UV by upper layers of bacterial cells and their magnetosome chains. The number of  
161 bacterial layers in this study was estimated to be about five, and the UV transmittance regarding  
162 the number of layers are shown in Table S2.

163 One limitation of this study is the use of freeze-dried cells that are not metabolically active.  
164 A temperature control system that can better reflect the metabolic and physiological changes of  
165 active bacterial cultures is crucial for future research. Moreover, laboratory-based simulation  
166 chambers with fully controllable environmental conditions are also important in future studies  
167 and should attempt to focus broad spectrum UV radiation rather than a single wavelength.  
168 Considering that Earth's lower near space is an exemplary analog to the present-day Martian  
169 surface and the atmosphere of Venus [2, 12], the survivability of MTB in this region suggests  
170 that MTB can be valuable candidates for astrobiology research. Further studies on MTB

171 exposure, not only to near space but also to the environments around space stations (e.g., the  
172 China's Space Station and the International Space Station) should be highly anticipated.

173

#### 174 **Conflict of interest**

175 The authors declare that they have no conflict of interest.

176

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#### 189 **Author contributions**

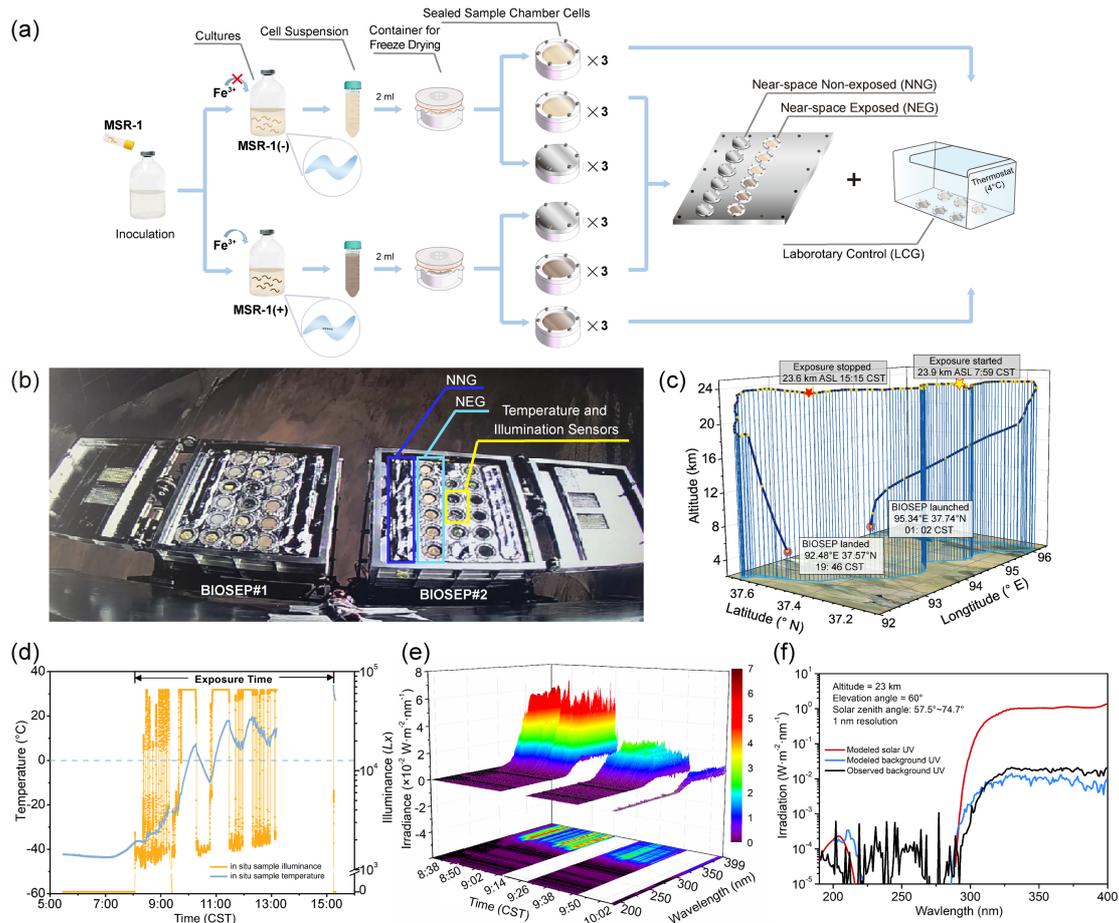
190 Wei Lin and Jia Liu conceived the project, designed the experiments, interpreted the results and  
191 wrote the manuscript with contributions from all authors. Jia Liu, Wensi Zhang, Kuang He and  
192 Li Liu performed the culture experiments, sample preparation, TEM observation, cell activation,  
193 rock magnetic experiments and data analysis. Chao Wang and Yuanda Jiang performed the  
194 environmental data experiments including pre-experiments using BIOSEP. Fei He provided UV  
195 data and contributed to the analysis. Shijiao Ma, Jiesheng Tian and Ying Li assisted with the  
196 culture experiments of MSR-1. Greig A. Paterson, Tongwei Zhang, Lanxiang Tian, Yong Wei  
197 and Yongxin Pan contributed to data interpretation and manuscript proofreading. All authors  
198 read and approved the final manuscript.

199

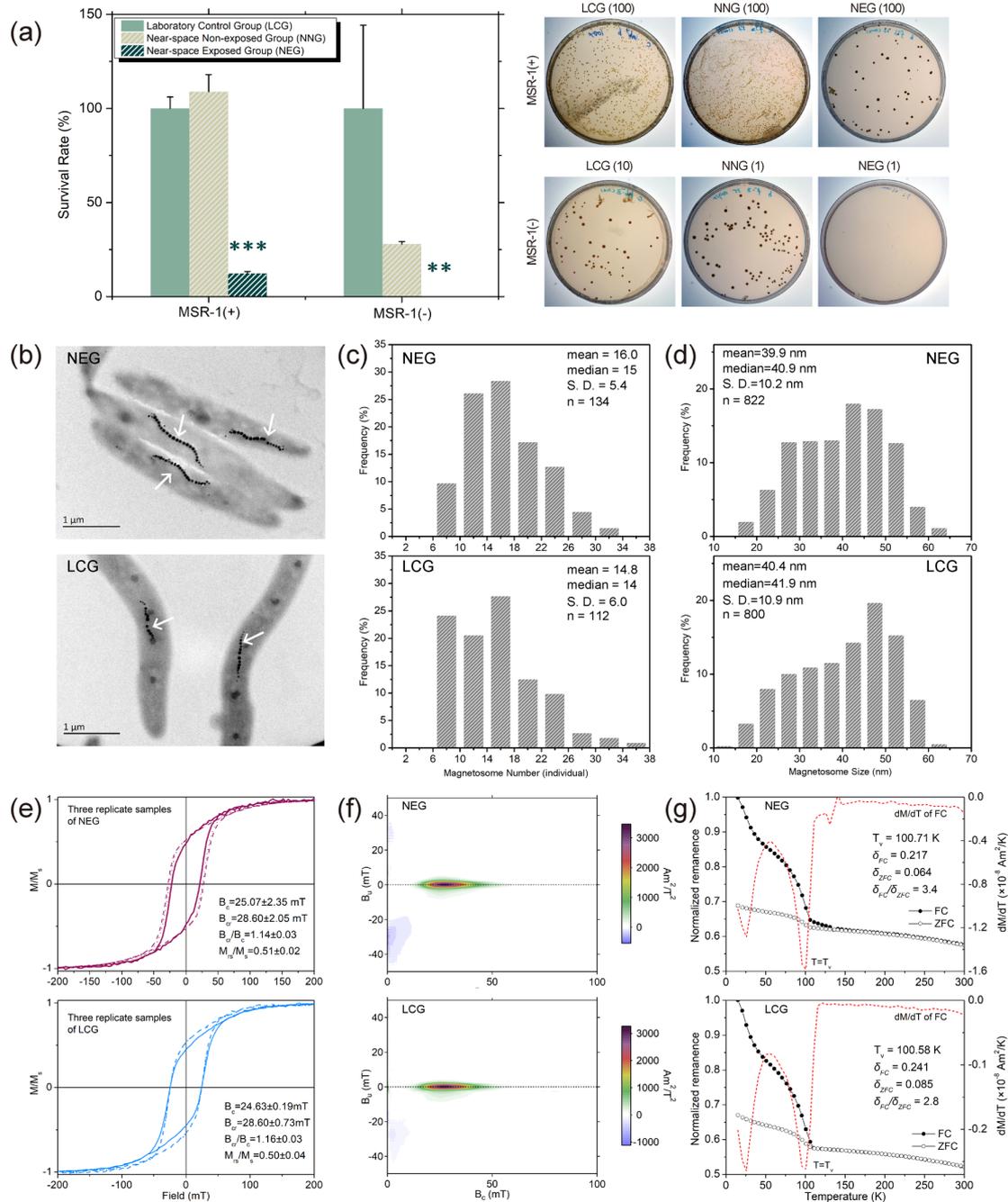
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**Figure 1.** Near-space exposure experiments of *Magnetospirillum gryphiswaldense* strain MSR-1 (MSR-1). **(a)** Scheme of sample preparation process. Culture medium with or without ferric citrate was used to obtain bacterial samples with (MSR-1(+)) and without (MSR-1(-)) magnetosomes. Sample chamber cells were divided into three groups: near-space exposed group (NEG), near-space shielded non-exposed group (NNG) and laboratory control group (LCG), each containing triplicate samples. **(b)** Image of two BIOlogical Samples Exposure Payloads (BIOSEP) at ~23 km in the lower near space. The chamber cells containing MTB samples are placed in the first (NNG) and second (NEG) columns from left to right in BIOSEP#2. **(c)** Travel map of the HH-20-7 flight mission. **(d)** Temperature and solar illumination measured by sensors parallel to the samples for inferring the direct and effective exposure time. The low-illumination regions represent the BIOSEP with its back to the Sun where the UV radiation for samples is negligible. Temperature gradually increases with the duration of continuous exposure. The temperature and solar illumination recording were interrupted during 13:11-15:14 CST. **(e)** Diffuse UV data recorded by UltraViolet Spectrometer (UVS). The wavelength recorded ranged from 191 to 399 nm with a spectral resolution of 1 nm. It should be noted that the UVS had a relatively small field of view and was mounted at a fixed pitch angle of 30° to monitor the diffuse UV radiation rather than the direct solar UV radiation. **(f)** The MODerate resolution atmospheric TRANsmission (MODTRAN) model show that the modeled diffuse radiation spectrum (blue line) is generally in consistent with the observation (black line), and the modeled direct solar UV spectrum (red curve) is approximately two orders of magnitude higher, mostly contributed from the significant enhancement in UVA.



**Figure 2.** Survival rates, magnetosome features and rock magnetic properties of *M. gryphiswaldense* MSR-1. **(a)** Left: the freeze-dried cells of MSR-1 with magnetosomes (MSR-1(+)) gain a survival rate of ~12.32% after near space exposure while cells without magnetosomes (MSR-1(-)) are completely inactivated after exposure. Survival rates were determined as  $C_i / C_c$ , where  $C_i$  is the CFU in respective experimental groups and  $C_c$  is the CFU in the laboratory controls. The error bars represent the standard deviation of three duplicates. \*\* / \*\*\* Significant changes in survival rates with  $P < 0.005$  /  $P < 0.001$  (Table S1). Right: representative plates for counting colony forming units (CFU) of MSR-1. Numbers in parentheses represent the sample dilution folds. **(b)** Transmission electron microscope images of NEG and LCG MSR-1. White arrows indicate magnetosomes. **(c)** Histograms of magnetosome number per cell of NEG and LCG MSR-1. S.D. represents the standard deviation. **(d)** Histograms of magnetosomal crystal size of the two groups. **(e)** Room-temperature hysteresis loops of each group are normalized by the value of saturation magnetization ( $M_s$ ). **(f)** First-order reversal curve (FORC) diagrams of the representative samples of the two groups. **(g)** Low-temperature magnetic measurements of the representative samples of the two groups.

## Supplementary materials

# Survival of the magnetotactic bacterium *Magnetospirillum gryphiswaldense* exposed to Earth's lower near space

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## **Materials and methods**

### **Strain description and culture condition**

*Magnetospirillum gryphiswaldense* strain MSR-1 (MSR-1), originally isolated from a eutrophic river nearby Greifswald, Germany, is one of the cultivable representatives of MTB [1]. The culture medium LA2 for MSR-1 was modified according to [2, 3] to achieve both high yields of bacterial cells and magnetosomes. The LA2 medium was made with (per liter of deionized water) 2 ml sodium L-lactate 60% solution, 0.34 g NaNO<sub>3</sub>, 0.1 g KH<sub>2</sub>PO<sub>4</sub>, 0.15 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 2.38 g HEPES, 0.1 g yeast extract, 3 g soy bean peptone, 0.05 g sodium thioglycolate, 0.5 ml mineral elixir [3] and 60 µM ferric citrate. MSR-1 was incubated in flasks sealed with sand-cored silicone stoppers for 24 hours at 30°C (shaking at 100 rpm). MSR-1 incubated in this condition were used as the strain MSR-1(+) with intracellular magnetosomes. In addition, the ferric citrate-free LA2 medium was used to incubate MSR-1 cells without magnetosomes (MSR-1(-)).

### **Sample preparation**

Prior to boarding the payload, MSR-1 were freeze-dried to reduce damage from the freeze-thaw process during flight. The cultures were firstly centrifuged at 3000 g for 5 min to remove the supernatant. Next, the remaining pellet was resuspended with a lyophilization protectant (5% trehalose solution), and 2-ml aliquots of bacterial suspensions were transferred to sterilized sample chamber cells within the vented sealing film-packed glass containers. Finally, after being pre-frozen at -80°C for 10 hours, MSR-1 samples were freeze-dried in vacuum using an Alpha 1-4 LSC plus Christ lyophilizer (Christ, Germany). All these operations were carried out under strictly sterile conditions.

### **Experimental design**

For the exposure experiment, both MSR-1(+) and MSR-1(-) were divided into three groups: the near-space exposed group (NEG), the near-space non-exposed group (NNG) and the laboratory control group (LCG). Each group contained three replicates (Fig. 1a). Chamber cells

were sealed from the outside environment with constant pressure to remove the effects of pressure and desiccation. Both the NEG and NNG samples were launched into near space with the BIOlogical Samples Exposure Payload (BIOSEP): NEG were exposed to solar radiations through UV-grade quartz glass (Tangsinuo, China) that enabled >85% transmission of UV at 200 nm and >91% transmission of UV at 210-280 nm, while NNG were shielded from solar radiations by opaque aluminum lids (Fig. S2). As the control, LCG were stored at 4°C in the laboratory. All these samples were prepared from the same batch.

### **UV radiation measurement**

The strength of UV (191-399 nm) radiation at the float altitude was measured by the UltraViolet Spectrometer (UVS, with a temporal resolution of 3 seconds). With a relatively small field of view, UVS was mounted at a fixed pitch angle of 30° to monitor the diffuse UV radiation instead of the direct solar UV radiation. The data acquisition had lasted for 1 hours and 30 minutes. The diffuse radiation dose was 2.86 J/m<sup>2</sup> for vacuum ultraviolet (VUV, 191-200 nm), 47.16 J/m<sup>2</sup> for UVC (200-280 nm), 588.53 J/m<sup>2</sup> for UVB (280-320 nm) and 6213.88 J/m<sup>2</sup> for UVA (320-400 nm). Considering the transmission efficiency (>85% for VUV and >91% for UVC) of the UV-grade quartz glass of each sample chamber cell, the diffuse doses of VUV and UVC received by MTB samples were 2.43 J/m<sup>2</sup> and 42.92 J/m<sup>2</sup>, respectively. Since the balloon could not maintain its orientation, the illumination angle of the sample changed with the balloon rotation during the flight, causing the light intensity value to fluctuate randomly. The low-illumination region is around 10<sup>3</sup> Lx representing the BIOSEP with its back to the Sun where the direct UV radiation for samples is negligible. Therefore, we estimated the direct exposure time of samples to be 5 hours 18 minutes. Accordingly, the estimated diffuse UV radiation dose received by samples during the direct exposure was  $\sim 2.42 \times 10^4$  J/m<sup>2</sup> (8.59 J/m<sup>2</sup> for VUV,  $1.52 \times 10^2$  J/m<sup>2</sup> for UVC,  $2.08 \times 10^3$  J/m<sup>2</sup> for UVB and  $2.20 \times 10^4$  J/m<sup>2</sup> for UVA). According to the MODerate resolution atmospheric TRANsmiission (MODTRAN) model [4], the direct total solar UV spectrum at an altitude of 23 km near Dachaidan was approximately two orders of magnitude higher than the diffuse radiation spectrum and was estimated to be  $\sim 10^6$  J/m<sup>2</sup>, mostly contributed from the significant enhancement in UVA. For the UVC band, the diffuse radiation dose was comparable to the direct solar radiation dose. For the UVB band, the direct solar

radiation dose was  $\sim 7.26 \times 10^4 \text{ J/m}^2$ , which was about 35 times higher than the diffuse radiation dose.

### **Activation and enumeration**

After returning from near space, the sample chamber cells were wiped with 75% alcohol and disassembled under strictly sterile conditions. 2 ml of the LA2 medium were used to resuspend the freeze-dried MSR-1 samples. The suspensions were then 10-fold serially diluted to a final dilution factor of 1/100. A required volume of the suspensions was spread over the LA2 agar and incubated for 168 hours at 30°C. The number of survivors in each sample was estimated by colony-forming unit (CFU) enumeration, calculated as  $C = N \times d / V$ , where  $N$  is the average colony count,  $d$  is the dilution factor and  $V$  is the volume of the bacterial suspensions spread. Survival rates were determined as  $C_i / C_c$ , where  $C_i$  is the CFU in respective experimental groups and  $C_c$  is the CFU in the laboratory controls.

### **Transmission electron microscopy (TEM) analysis**

Sterilized cell scrapers were used to gently collect the colonies from the culture plates and prevent defacing the agar. About 1  $\mu\text{L}$  of bacterial colonies was diluted with Milli-Q water and deposited onto carbon-coated copper grids for TEM observation. Images of bacteria were captured using a JEOL JEM2100 TEM (Tokyo, Japan) operating at a 200-kV accelerating voltage. The number and crystal size of magnetosomes within MSR-1 were measured using ImageJ (v.1.50b) [5].

### **Rock magnetic measurement**

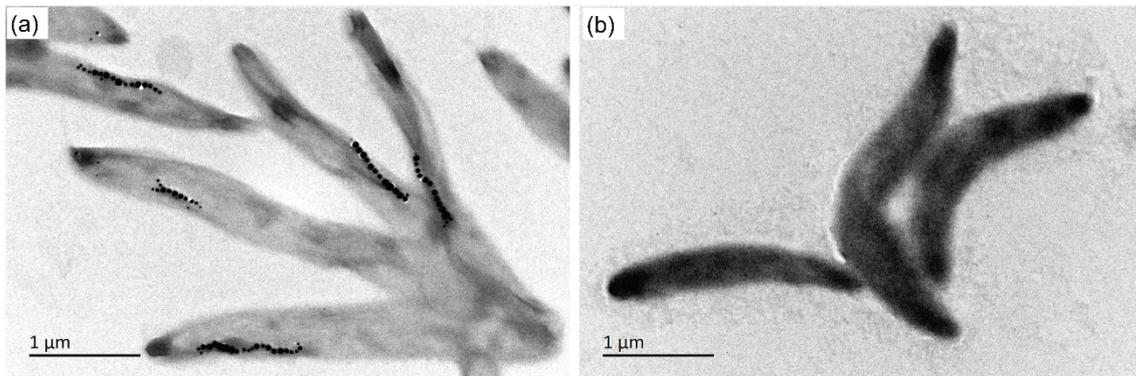
Pure bacterial colonies collected from the culture plates were transferred into non-magnetic capsules, followed by the centrifugation at 12000 g for 1 minute and the air-drying in an anaerobic glove box to avoid possible oxidation. Hysteresis loop, backfield curve and first-order reversal curve (FORC) of each sample were determined using a PMC Micromag 3900 vibrating sample magnetometer (VSM, Princeton Measurements Corporation, Princeton, NJ, USA). The hysteresis loops were measured with a maximum field of 500 mT, a measurement step of 4.0 mT and an averaging time of 500 ms. The saturation magnetization ( $M_s$ ), saturation remanence ( $M_{rs}$ ), and coercivity ( $B_c$ ) of the magnetic bacteria were obtained from hysteretic

measurements, and both  $M_s$  and  $M_{rs}$  were normalized to the maximum value of  $M_s$ . The  $B_{cr}$  value was retrieved from the backfield curve. For each sample, a total of 150 first-order reversal curves (FORCs) were determined with a saturation field of 1 T, a field step of 1.46 mT and an averaging time of 300 ms. The FORC diagrams were calculated using the FORCinel (v.3.06) software [6]. The smoothing factor of each FORC diagram was:  $S_{c,0} = 4$ ,  $S_{c,1} = 5$ ,  $S_{b,0} = 4$ ,  $S_{b,1} = 5$ , and  $\lambda_c = \lambda_b = 0.1$  [7]. The low-temperature measurements were conducted on one representative sample of each of the three groups using a SQUID MPMS XL-5 magnetic property measurement system (Quantum Design, San Diego, CA, USA). For zero-field cooled (ZFC) curve, after each sample was zero-field cooled from 300 K to 15 K, a saturation isothermal remanent magnetization (SIRM) was obtained at 15 K in a 2.5-T field before warming back to 300 K in a zero field. For field cooled (FC) curve, after each sample was cooled in a 2.5-T field from 300 K to 15 K, the sample was warmed from 15 K to 300 K in a zero field. For both ZFC and FC curves, the magnitude of SIRM was measured at 5 K intervals.

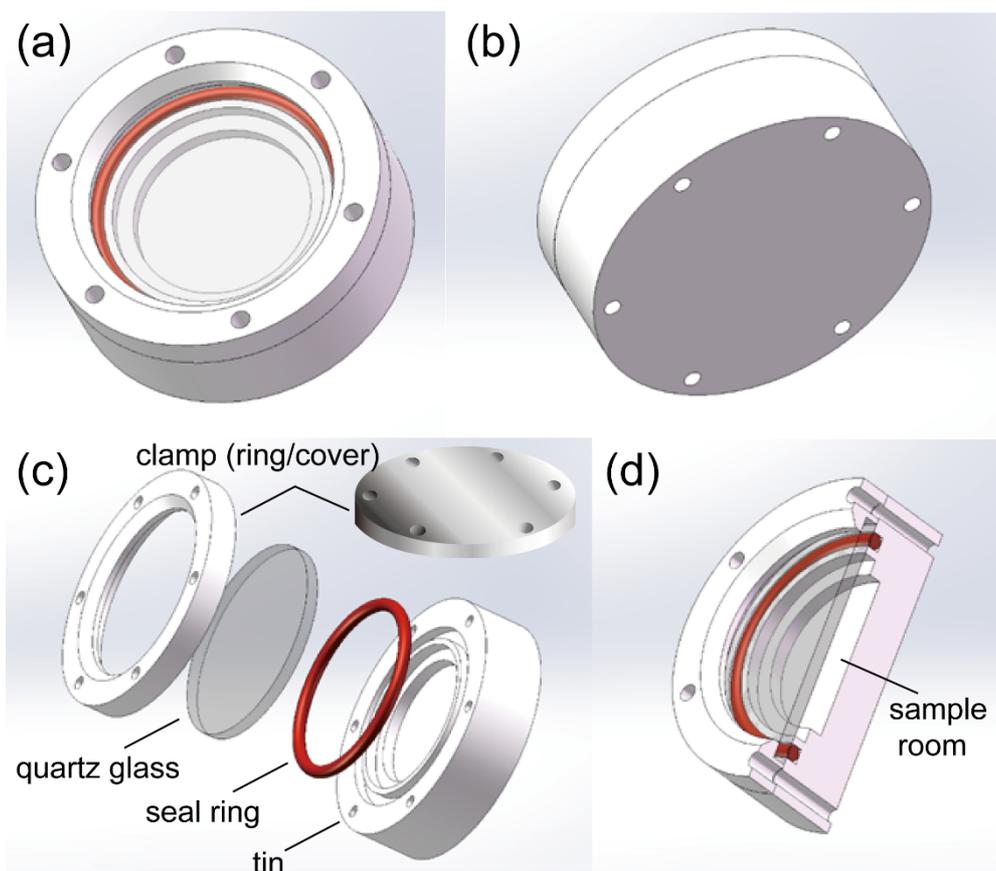
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## Supplementary figures and tables



**Figure S1.** Two groups of *Magnetospirillum gryphiswaldense* strain MSR-1 (MSR-1) used in this study: **(a)** MSR-1(+) with intracellular magnetosomes and **(b)** MSR-1(-) without intracellular magnetosomes. The proportion of cells with magnetosomes accounted for about 85% of the total cells in MSR-1(+) group and less than 1% in MSR-1(-) group as revealed by TEM observation.



**Figure S2.** Schematic diagrams of sample chamber cells. (a) Front view and (b) rear view of a chamber cell. (c) Exploded view of a sample chamber cell illustrating the difference in clamps between the exposed group and the non-exposed group. (d) Cross-sectional view of a chamber cell.

**Table S1.** Numbers of *Magnetospirillum gryphiswaldense* strain MSR-1 (MSR-1) survived in near space-exposed group (NEG), near-space non-exposed group (NNG) and laboratory control group (LCG) as estimated by CFU enumeration.

	MSR-1(+) LCG (CFU)	MSR-1(+) NNG (CFU)	MSR-1(+) NEG (CFU)	MSR-1(-) LCG (CFU)	MSR-1(-) NNG (CFU)	MSR-1(-) NEG (CFU)
duplicate1	2.20×10 <sup>6</sup>	2.03×10 <sup>6</sup>	2.20×10 <sup>5</sup>	0.80×10 <sup>4</sup>	~4.00×10 <sup>3</sup>	0
duplicate2	1.90×10 <sup>6</sup>	~2.20×10 <sup>6</sup>	2.60×10 <sup>5</sup>	1.20×10 <sup>4</sup>	4.20×10 <sup>3</sup>	0
duplicate3	2.00×10 <sup>6</sup>	2.40×10 <sup>6</sup>	2.70×10 <sup>5</sup>	2.30×10 <sup>4</sup>	3.80×10 <sup>3</sup>	0
statistic	2.03±0.12×10 <sup>6</sup>	2.21±0.15×10 <sup>6</sup>	2.50±0.22×10 <sup>5</sup>	1.43±0.63×10 <sup>4</sup>	4.00±0.16×10 <sup>3</sup>	0
rate	100.00%	108.69%	12.32%	100.00%	27.97%	0%

**Table S2.** The transmitted UVB radiation intensity (peaking at 297 nm with initial intensity of 4.1 W/m<sup>2</sup>) through different layers of the MSR-1 cells.

	1 layer	2 layers	3 layers	4 layers	5 layers
MSR-1(+)	2.36 W/m <sup>2</sup>	0.9 W/m <sup>2</sup>	0.56 W/m <sup>2</sup>	0.31 W/m <sup>2</sup>	0.11 W/m <sup>2</sup>
MSR-1(-)	2.35 W/m <sup>2</sup>	0.89 W/m <sup>2</sup>	0.59 W/m <sup>2</sup>	0.47 W/m <sup>2</sup>	0.29 W/m <sup>2</sup>