1 **Cold adapted desiccation-tolerant bacteria isolated from polar soils presenting**
2 **high resistance to anhydrobiosis**

3 *Running title:* Cold adapted desiccation-tolerant bacteria isolated from polar soils

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15 **Abstract**

16 Life on Earth is strictly dependent on liquid water. In polar terrestrial environments,
17 water exists in solid state during almost the entire year. Polar microorganisms have not
18 only to adjust their metabolism to survive at subzero temperatures, but also need to cope
19 with extremely dry conditions. We investigated the presence of desiccation-adapted
20 bacteria in Arctic permafrost and Antarctic surface soils and characterized their
21 survivability to dryness. We selected desiccation tolerant cells by treating the soils with
22 chloroform prior to cultivation, in order to mimic the stress of low water activity for
23 long periods. From over 1000 colonies from different samples, 23 unique strains were
24 selected and identified as members of phyla *Firmicutes*, *Proteobacteria* and

25 *Actinobacteria*. About 60% of the strains survived after 50 days in anhydrobiosis. The
 26 competence to withstand desiccation varied between close related strains isolated from
 27 different locations, bringing the question if environmental conditions may play a role in
 28 the observed desiccation tolerance. Survivability was also affected by the solution in
 29 which the cells were suspended before drying; R2B medium being more protective than
 30 water. This is the first time that chloroform was used to select desiccation tolerant
 31 microorganisms from polar soils. The collection of polar microorganisms described
 32 herein opens the possibility of further experiments aiming to investigate the resistance
 33 mechanisms of polar anhydrobionts. Desiccation tolerance is fundamental to the
 34 survivability of microorganisms to the space environment and at the surface of thin-
 35 atmosphere planets like Mars. Therefore, the selected strains may open a road to better
 36 understand the limits of cold adapted life on Earth and beyond, and compare
 37 mechanisms of resistance with anhydrobionts from divergent extreme environments.

38 **Keywords:** Desiccation, Psychrotrophs, Bacteria, Antarctica, Arctic, Extremophiles

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45 **Introduction**

46 Life as we know it cannot thrive without water; it can survive through dry seasons
 47 (Stevenson et al., 2015; Chaplin, 2006). In polar terrestrial environments, water exists in
 48 solid state during almost the entire year. Still, microbial communities in these regions
 49 are diverse, even in less dynamic ecosystems such as the permafrost (Steven et al.,
 50 2006; Jansson and Tas, 2014). This state of water stress is known as anhydrobiosis – a
 51 state of extreme metabolism reduction and physiological adaptations (Billi and Potts,
 52 2002). The study of the physiology of anhydrobionts (one of the terms used to address
 53 desiccation tolerant species) depends on strains isolation. It came to our attention a
 54 culture method for isolation of desiccation resistant microorganisms, from soils
 55 subjected to seasonal droughts, using chloroform (Narvaez-Reinaldo et al., 2010). This
 56 methodology exploits the effect of bacterial protective measures taken during
 57 anhydrobiosis that not only prevent damage related to desiccation, but also to exposure
 58 to organic solvents (Manzanera et al., 2004; Vilchez et al., 2008). It is unknown if
 59 microorganisms endemic to cold regions have the same drought tolerance and survival
 60 mechanisms like other well-studied anhydrobionts; and whether this would be a valid
 61 method for isolation of polar microorganisms. Experiments using desiccated
 62 microorganisms vary, not only regarding the experimented strains, but also in the
 63 solution that the cells are suspended before drying. Commonly used solutions are saline
 64 solutions of different concentrations, culture medium and water. It is not usually
 65 addressed in these experiments the effect of the drying solution in bacteria survivability,
 66 although protective solutions can induce desiccation resistance (Julca et al., 2012).

67 The understanding of the limits for cellular life on Earth and beyond – including
 68 water absence – is under the realm of astrobiology. Experiments performed at the
 69 International Space Station and in simulation chambers on Earth, investigate the effects

of environmental factors found in outer space (e.g. pressure, desiccation, temperature, UV and radiation) in algae, lichens and bacteria (Horneck et al., 2001; Mancinelli, 2015; Rabbow et al., 2009; Olsson-Francis and Cockell, 2010; Meessen et al., 2015). In most, if not all, of these experiments, desiccation and low temperature tolerance preconditions microbial survival – which underscores the importance of studying cold adapted anhydrobionts. The likelihood of extant microbes in comets or meteorites (central to the Panspermia hypothesis) is also dependent on life tolerance to extremely dry and cold conditions (Clark, 2001). On Mars, microbial life is still a possibility; its subsurface soil contains water ice, and liquid water (if only ephemeral) any microbial life that might exist must cope with desiccation (Boynton et al., 2002; Ojha et al., 2015).

This study aims were: (1) to test an isolation methodology for desiccation resistant polar microorganisms (from soil samples from the Arctic and Antarctica); (2) to assess each strain's desiccation tolerance over time (up to 50 days) and the drying solution influence (water vs. culture medium R2B 10%); (3) to investigate the relationship between strain's phylogeny and its habitat to the measured desiccation tolerance.

Materials and Methods

Soil samples

Soil samples were collected from two regions in Antarctica – soil exposed by glacier retreat (Baranowski Glacier, King George Island) and volcanic soil from Deception Island – and from one region in the Canadian High Arctic (Table 1). Sampling in Antarctica was made during 2008/2009 summer; sampling in the Arctic was made during July 2010. Antarctic soils were collected from the surface (no more than 10 cm deep) with sterile materials and kept frozen (-20 °C to -50 °C) in sterile plastic bags until usage in 2013. Arctic sample were collected on polygonal permafrost terrain located near the McGill Arctic Research Station (MARS); a core was taken as

95 previously described and kept frozen until usage (Wilhelm et al., 2012), and from it
96 three soil depths were analyzed (Table 1).

97 **Table 1** - Soil samples used for the isolation of desiccation resistant microorganisms.

Sample	Description	Sampling sites	Coordinates
Dec85	Border of Deception's caldera (5 °C)	Deception Island	62°55'21.55"S
		Antarctica	60°38'7.19"O
Dec92	Border of geothermal lake (10 °C)	Deception Island	62°58'50.00"S
		Antarctica	60°34'24.00"O
Dec94	Border of geothermal lake (6 °C)	Deception Island	62°58'46.31"S
		Antarctica	60°34'31.65"O
Bas01	Distance from glacier: 0 m	Baranowsky	62°11'53.42"S
		Antarctica	58°26'53.19"O
Bas06	Distance from glacier: 189 m	Baranowsky	62°11'52.87"S
		Antarctica	58°26'40.19"O
Bas12	Distance from glacier: 473 m	Baranowsky	62°11'52.56"S
		Antarctica	58°26'20.62"O
ArcA	Active Layer - Depth: 25 cm	Axel Heiberg	79°24'57.90"N
		Arctic	90°45'48.12"O
ArcM	Transient Layer - Depth: 50 cm	Axel Heiberg	79°24'57.90"N
		Arctic	90°45'48.12"O
ArcP	Permafrost - Depth: 100 cm	Axel Heiberg	79°24'57.90"N
		Arctic	90°45'48.12"O

98 *Strain isolation*

99 In order to mimic the selective pressure exerted by the absence of water for a
100 prolonged time, 5 grams of air-dried soil was incubated with 10 mL of ≥ 99.8%

101 chloroform for 30 minutes at room temperature in sterile conditions, the first step of our
 102 isolation and selection of desiccation-tolerant bacteria (Fig. 1). This step was performed
 103 according to the methodology established by Narvaez-Reinaldo et al. (2005) with minor
 104 changes. After incubation, samples were air dried for 30 minutes until complete
 105 chloroform evaporation. Treated soils were then stirred (not shaken) with 10 mL R2B
 106 medium (EMD Chemicals Inc.) for 30 minutes. A total of 100 μ L of the resulting
 107 solution was inoculated in R2A and TSA media (Difco Laboratories), and incubated at
 108 30 °C and 0 °C until colony appearance.

109

110 **Figure 1** - Strain selection diagram.

111 *Isolates identification*

112 Aiming to select strains with unique phylotypes, we used the BOX-PCR
 113 fingerprinting technique, excluding the isolates with similar DNA amplification profile,
 114 as previously described (Martin et al., 1992). With the formerly selected isolates, part of

the 16S rRNA gene was amplified for phylogenetic study, using the forward primer 27F (5' AGAGTTTGATCMTGGCTCAG 3') and the reverse primer 1492R (5' TACGGYTACCTTGTTACGACTT 3'), following standard PCR methods (Goodfellow and Stackebrandt, 1991). Sequencing reaction was made with the primer 27F, yielding sequences with 700pb length. Sequences were submitted to the GenBank genetic sequence database (Submission ID: SUB1160085). Sequence quality was verified by Phred software (Ewing et al., 1998) and aligned against the SILVA database, using the SINA Web-aligner. The alignment was combined with 30 neighboring sequences from SILVA database using the ARB MERGE tool (Pruesse et al., 2012). ARB was used for reconstruction of Maximum Likelihood (ML) phylogenetic trees (Ludwig et al., 2004) with RAxML (Randomized Accelerated Maximum Likelihood), a random starting tree and GTR+C model. Support values for internal branches of the ML tree were obtained by bootstrapping (1000 replicates).

Isolates nomenclature carries information of its soil of origin (e.g. ArcA30R01), its isolation temperature (e.g. ArcA30R01), culture medium (ArcA30R01) and a differentiation number (e.g. ArcA30R01, ArcA30R02, etc.).

Desiccation experiments

Isolates were grown in TSA or R2A plates, at room temperature, until colony formation up to 4 weeks. Two variables were tested; desiccation solution and desiccation time.

Desiccation solution: One colony of approximately 1 mm² was suspended in 100 µL of ultrapure water or R2B medium 10% (Proteose Peptone: 0.5 g/L; Glucose: 0.5 g/L; Magnesium Sulfate Heptahydrate: 0.1 g/L; Sodium Pyruvate: 0.3 g/L; Yeast Extract: 0.5 g/L; Casein Acid Hydrolysate: 0.5 g/L; Soluble Starch: 0.5 g/L; Dipotassium Phosphate: 0.3 g/L). Two µL of the cell solution was then deposited on a

sterile circular glass slide (5 mm²) and air-dried at room temperature (Relative humidity = 45 ± 4%). The untreated control was not allowed to dry.

Desiccation Time: Desiccation tolerance was examined by incubating the previously desiccated cells in a silica-gel exsiccator (Relative humidity = 5% ± 1%) for 0, 1, 5 and 50 days.

Survival rate determination

After the desiccation treatment, dry cells were suspended thoroughly with 10 µL of their correspondent desiccation solution (ultrapure water or R2B 10%), plated in TSA or R2A and incubated at room temperature in sterile conditions until colony appearance. Ten-fold serial dilutions were made to obtain a manageable number of colony forming units per plate (30 ≤ CFU ≤ 300 per plate). Plates were made in triplicates for each dilution; we calculated the data mean and the standard deviation (SD). Strain survivability was determined from the ratio N / N₀, where: N = Colony-forming units (CFU) after desiccation treatment; N₀ = Control group, CFU without treatment.

Deinococcus radiodurans R1 (Anderson et al., 1956) and *Escherichia coli* K-12 strain MG1655 (Blattner et al., 1997) were used as a positive and negative control for desiccation tolerance, respectively. In addition, strains from Antarctica – *Exiguobacterium antarcticum* B7 (Carneiro et al., 2012) – and the Arctic – *Planococcus halocryophilus* OR1 (Mykytczuk et al., 2012) – were also tested. All strains described in this study will be made available upon request. Survival rate comparisons of each isolate were done using Kruskal-Wallis test (p<0.05, Bonferroni correction).

Results

Isolation and phylogenetic study of strains

Colonies grew from all chloroform treated soil samples, except from two soil samples from Antarctica. We isolated strains from all incubation temperatures and culture media from the Arctic soils, but not from Antarctica. For example, from Deception Island, a region of volcanic activity, we only obtained colonies at 30 °C and TSA medium. From Antarctic soils exposed by the Baranowski glacier retreat, colonies grew at 30 °C and 5 °C, but only in TSA. We isolated around 1000 strains from all sites; CFU/g of the treated soil samples ranged between 10^2 and 10^3 (Table 2).

Table 2 - Number of Colony Forming Units per gram of soil (CFU.g⁻¹) isolated from chloroform treated soil samples.

Sample	TSA 5 °C	TSA 30 °C	R2A 5 °C	R2A 30 °C
Dec85	0	33	0	0
Dec92	0	0	0	0
Dec94	0	606	0	0
Bas01	0	472	0	0
Bas06	0	0	0	0
Bas12	568	0	0	0
ArcA	173	126	13	40
ArcM	693	186	66	126
ArcP	543	320	53	53

We selected around 100 isolates for BOX amplification screening, 23 of which were selected by their unique BOX-PCR amplification profile for identification by partial 16S rRNA gene sequencing. Strains were part of the Phylum: Actinobacteria (1

176 strain, Deception Island), Proteobacteria (4 strains, Deception Island) and Firmicutes
177 (18 strains from all the sampling sites) (Fig. 2).

178
179 **Figure 2** - Phylogenetic tree based on gene 16S rRNA sequences showing the
180 relationship between the desiccation-resistant isolates. The scale bar indicates 0.03
181 substitutions per nucleotide position. Bootstrap values (%), higher than 60 are shown in
182 the nodes.

183 *Influence of suspension solution in desiccation tolerance*

184 Six strains did not show resistance to desiccation under any circumstance:
185 ArcP0513, ArcA05T10, ArcA05T02 – strains closely related to the *Sporosarcina* genus;
186 ArcA30R03, ArcA30T09, ArcM30T12 – of the *Paenibacillus* genus. All strains
187 presented a significantly higher survival rate when desiccated at R2B medium 10%
188 (Fig. 3 and 4). Strains isolated from Deception Island were highly sensitive when
189 desiccated in water suspension. Similarly, reference strains only showed desiccation

Figure 4 - Survival of isolates after 1, 5 and 50 days of desiccation. One colony was suspended in a solution of ultrapure water, deposited on a glass slide and desiccated in an exsiccator. After treatment, we suspended the cells in H₂O. Error bars show the standard deviations for three replicates.

Desiccation tolerance within bacterial families

Desiccation tolerance was not specific to a particular group (Table 3). Nevertheless, we found that the most resistant strains were within the Family *Micrococcaceae*, *Planococcaceae* and *Paenibacillaceae*. Gram-negative bacteria (*Moraxellaceae*) were more susceptible to desiccation, especially when desiccated in water suspension. Interestingly, strains from the same genera *Paenibacillus* and *Sporosarcina*, but isolated from different samples, showed different desiccation tolerance (Table 3).

218 **Table 3** - Summary of strain tolerance to desiccation for each phylum and family.

Phylum	Family	Strain	Survival rate H ₂ O				Survival rate R2B			
			1 day	5 days	50 days	Signif.	1 day	5 days	50 days	Signif.
Firmicutes	Planococcaceae	ArcP5T13	0	0	0	-	0	0	0	-
		ArcA5T10	0	0	0	-	0	0	0	-
		ArcA5T02	0	0	0	-	0	0	0	-
		Dec8530T02	9,6E-02	4,8E-02	9,5E-03	*	2,6E-02	2,4E-02	2,6E-03	ns
		Bas1205T08	4,8E-03	2,9E-03	1,0E-03	ns	1,3E-03	9,8E-04	2,9E-04	ns
		Bas1205T05	2,0E-03	4,3E-03	2,4E-03	*	5,9E-04	1,2E-03	2,3E-04	ns
		Dec8530T01	1,4E-02	0,0E+00	0,0E+00	ns	2,1E-03	1,5E-03	0,0E+00	*
		Bas1205T17	6,6E-04	7,6E-05	0,0E+00	ns	3,8E-04	1,9E-04	1,1E-04	ns
	Paenibacillaceae	ArcM30T12	0	0	0	-	0	0	0	-
		ArcP30T02	4,2E-02	5,2E-02	1,9E-02	ns	2,0E-01	4,3E-01	2,2E-01	ns
		ArcP30T13	1,5E-02	3,0E-02	6,6E-02	*	7,5E-02	1,1E-01	1,5E-01	ns
		ArcP30T20	5,5E-02	5,6E-02	2,4E-02	ns	2,3E-02	4,8E-02	1,9E-02	ns
		Bas0130T01	2,8E-04	1,7E-04	0,0E+00	ns	5,2E-04	2,2E-04	1,6E-04	ns
		Bas0130T06	2,4E-04	7,0E-04	0,0E+00	*	3,4E-03	2,1E-03	1,6E-03	ns
		ArcA30R01	1,1E-01	6,6E-02	3,2E-01	*	5,3E-02	6,8E-02	1,7E-01	ns
		ArcA30R03	0	0	0	-	0	0	0	-
		ArcP30T13	1,5E-02	3,0E-02	6,6E-02	*	7,5E-02	1,1E-01	1,5E-01	ns
		ArcA30T09	0	0	0	-	0	0	0	-
Actinobacteria	Micrococcaceae	Dec8530T04	9,1E-01	3,9E-01	1,7E-03	*	8,8E-01	7,5E-01	3,2E-01	ns
Proteobacteria	Moracellaceae	Dec9230T05	5,6E-04	0,0E+00	0,0E+00	ns	3,7E-01	3,9E-02	7,4E-04	*
		Dec9230T03	3,2E-04	0,0E+00	0,0E+00	ns	7,5E-01	2,6E-01	4,6E-03	*
		Dec9230T07	1,4E-04	0,0E+00	0,0E+00	ns	1,4E-01	1,7E-01	0,0E+00	*
		Dec9230T01	2,0E-04	0,0E+00	0,0E+00	ns	4,3E-01	8,8E-01	0,0E+00	*

219 ^a N / N₀: N = Colony-forming units (CFU) after desiccation treatment; N₀ = Control group, CFU without

220 treatment, Signif. = comparisons were done with Kruskal-Wallis test (p<0.05, Bonferroni correction).

221 Discussion

222 *Chloroform as a selective agent*

223 Although, desiccation resistance accompanied the chloroform-selected strains
 224 (with some exceptions); the strains are being primarily selected by their resistance to
 225 organic solvents. The selection of desiccation resistant bacteria using organic solvents is
 226 a parallelism. The presented methodology is especially useful for its relativity
 227 quickness, and effectiveness. A comparison with a standard isolation method was made
 228 in the original report of the methodology, but studies using more traditional isolation
 229 procedures or other selective agents are encouraged. Regarding the exceptions, six of
 230 the isolated microorganisms that survived the chloroform treatment did not show any
 231 tolerance to desiccation. We hypothesize that some cells, when attached to soil particles
 232 conglomerates, may be protected against chloroform exposure and water loss.
 233 Additionally, chloroform exposure time also plays a part in the endurance of non-
 234 desiccation-tolerant microorganisms (Narvaez-Reinaldo et al., 2010). Chloroform may
 235 take more than 30 minutes to damage some cells even when they are not fully resistant
 236 to organic solvents or desiccation. Still, some isolates related to the ones presented in
 237 our work were previously described as desiccation resistant in independent experiments.
 238 In our study, we isolated the strain Dec8530T04 part of the phylum Actinobacteria and
 239 the *Micrococcaceae* family. One closely related species, *Micrococcus luteus*, was
 240 shown to be highly desiccation-tolerant (Merrick and Bruce, 1965). Another component
 241 of this phylum, *Arthrobacter siccitolerans*, was isolated by the same methodology using
 242 organic solvents as an anhydrobiosis-resistant selection method and proved highly
 243 resistant to desiccation (SantaCruz-Calvo et al., 2013). However, in a recent study,
 244 *Psychrobacter cryohalolentis* K5 – isolated from a Siberian cryopeg in Russia –
 245 appeared to be especially sensitive to the desiccation process. This strain was subjected

246 to experiments simulating conditions analogous to the Mars surface, the survival of the
247 exposed cells were up to six orders of magnitude below the positive control (Smith et
248 al., 2009). *Psychrobacter*, here isolated exclusively from Deception Island, have often
249 been isolated from various cold environments, including ice, soil, and permafrost of
250 Antarctica and Siberia, as well as deep-sea sediment samples (Ayala-del-Rio et al.,
251 2010).

252 *Phylogeny study*

253 The identified DNA sequences unveiled a prevalence of gram-positive isolates of
254 the phylum *Firmicutes* within the prospected strains. This finding is consistent with the
255 established knowledge, which indicates that these bacteria, although not part of a
256 monophyletic group, are among the most studied bacteria that have anhydrobiosis
257 resistance (Garcia, 2011). The main distinguishing feature of these bacteria is the
258 presence of many layers of peptidoglycan (a polymer consisting of carbohydrates and
259 amino acids) forming a cell wall external to the cell cytoplasmic membrane, which
260 could favor water and small solute retention and prevent membrane collapse (Madigan
261 et al., 2010). This phylum is also capable of forming spores (or endospores) – a dormant
262 state that can resist for a long time to chemical and environmental challenges – allowing
263 the organism to survive in harsh environmental conditions (Cano and Borucki, 1995). In
264 our isolation protocol, we fixed the exposure to chloroform at 30 minutes aiming to
265 isolate both sporulating and non-sporulating bacteria; longer exposure periods have a
266 tendency to select only spores (Narvaez-Reinaldo et al., 2010). Although we did not
267 directly observed spore formation, this mechanism may be responsible for the resilience
268 of strains related to *Sporosarcina* and *Paenibacillus*. The isolation of spore-forming and
269 non-spore-forming bacteria opens the way for future studies aiming to understand
270 different mechanisms of resistance. Non-spore-forming bacteria have other means to

271 protect the cells from DNA damage and protein oxidation – consequences of water loss
272 – like the synthesis of non-reducing disaccharides, accumulation of antioxidants and
273 expression of specialized proteins like LEA proteins, recombinases, chaperones and
274 peroxidases (Fredrickson et al., 2008; Chakrabortee et al., 2007; Tunnacliffe et al.,
275 2001).

276 For some closely related isolates, but selected from different soil samples, the
277 desiccation tolerance results varied significantly. This could be the result of minute
278 genetic variations leading to a considerable improvement on desiccation resistance; but
279 also raised the question that, at least in some cases, the isolates geographical origin
280 rather than phylogeny was determinant to desiccation tolerance. Physiological trait
281 variations could be the result of heritable information that goes beyond DNA sequence,
282 shaped by the physicochemical characteristics of the environment, and the root to a
283 unique desiccation conditioning when compared to strains of different origin and the
284 "lab-adapted" controls (Casadesús and Low, 2006).

285 *Desiccation medium influence*

286 Survival rates were higher when cells were dried in the presence of the low
287 nutrient solution R2B diluted ten times. This experiment intended to observe the
288 influence of a low nutrient medium and its salt concentration compared to pure water in
289 the desiccation resistance of different strains. Inorganic salts present in the formula
290 conferred protection against osmotic stress, which might have been crucial to the
291 survivability of strains sensitive to osmotic shock. This can explain the low survivability
292 of the reference controls *P. halocryophilus*, *E. antarcticum* (both adapted to high
293 salinity environments), when suspended in water prior to the test. All used soil samples
294 were under the influence of the marine environment, especially in Antarctic samples
295 collected close to the shore. Suspension in pure water added osmotic stress to the cells.

296 Some molecules can act as stabilizer and protective agents during the anhydrobiosis
 297 state (Julca et al., 2012; Garcia, 2011). Sodium pyruvate – present in the R2B
 298 formulation and a source of energy for aerobic and anaerobic metabolisms – could also
 299 have a protective ability against reactive oxygen species (ROS) that threats desiccated
 300 cells (Giandomenico et al., 1997; Franca et al., 2007). We believe that a wider range of
 301 salt concentrations and media formulations would be necessary to understand the effect
 302 of this variable in desiccation survivability. The use of pure water in experiments
 303 seeking to understand the effect of other environmental challenges in desiccated cells
 304 may not be the best option.

305 *Significance to astrobiology*

306 NASA Astrobiology Strategy regards the understanding of microbial limits of life,
 307 and its physiological adaptations, as one of the key challenges to be dealt with in
 308 astrobiology research (Hays, 2015; Des Marais et al., 2008). Within this framework, we
 309 believe the isolates described herein to be useful additions for use in future
 310 investigations to further our knowledge regarding the survival limits of life beyond
 311 Earth. In experiments seeking to understand the survivability of life to extraterrestrial
 312 conditions, extreme desiccation caused by vacuum exposure is one of the main
 313 environmental stressors. The Biomex mission, for instance, underway at the
 314 International Space Station uses the EXPOSE facility to expose microorganisms to the
 315 Low Earth Orbit and simulated Mars environmental conditions (Rabbow et al., 2012).
 316 On Earth, simulation chambers expose organisms to extreme desiccation and freeze
 317 thaw cycles in a multitude of combinations of environmental stressors (Olsson-Francis
 318 and Cockell, 2010; Lage et al., 2012). Further studies could explore the unique
 319 physiology of polar microorganisms; understand if cold survival mechanisms has a
 320 direct influence in the survivability to space environment and compare their resistance

321 with microorganisms prone to desiccation originated from different extreme
322 environments. The ability to withstand desiccation was even described in thermophilic
323 microorganisms, opening the possibility to compare the convergence of adaptations in a
324 contrasting thermal environment (Beblo et al., 2009).

325 In conclusion, we found that the proposed isolation methodology was able to
326 isolate cold-adapted desiccation tolerant bacteria from assorted polar soils – with some
327 isolates presenting a considerable resistance even after 50 days of anhydrobiosis. The
328 solution on which the cells were dried also influenced the results; R2B medium being
329 more protective than water. This is the first time that this methodology was applied to
330 isolate cold-adapted, desiccation tolerant microorganisms from the Arctic and
331 Antarctica. This study brought forward isolates of interest to future astrobiology
332 projects seeking to understand the limits of life and looking to compare its physiology
333 with anhydrobionts from different extreme environments.

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