EMPIRICAL TESTING OF CRYOCONITE GRANULATION: ROLE OF CYANOBACTERIA IN THE FORMATION OF KEY BIOGENIC STRUCTURE DARKENING GLACIERS IN POLAR REGIONS

Running head: Formation of cryoconite granules

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52 **Abstract**

53 Cryoconite, the dark sediment on the surface of glaciers, often aggregates into oval or irregular
54 granules serving as biogeochemical factories. They reduce a glacier's albedo, act as biodiversity
55 hotspots by supporting aerobic and anaerobic microbial communities, constitute one of the
56 organic matter (OM) sources on glaciers and are a feeder for micrometazoans. Although
57 cryoconite granules have multiple roles on glaciers, their formation is poorly understood.
58 Cyanobacteria are ubiquitous and abundant engineers of cryoconite hole ecosystems. This study
59 tested whether cyanobacteria may be responsible for cryoconite granulation as a sole biotic
60 element. Incubation of Greenlandic, Svalbard and Scandinavian cyanobacteria in different
61 nutrient availability and substratum for the growth (distilled water alone, and water with either
62 quartz powder, furnaced cryoconite without OM or powdered rocks from glacial catchment)
63 revealed that cyanobacteria bind mineral particles into granules. The structures formed in the
64 experiment resembled those commonly observed in natural cryoconite holes: they contained
65 numerous cyanobacterial filaments protruding from aggregated mineral particles. Moreover, all
66 examined strains were confirmed to produce extracellular polymeric substances (EPS), which
67 suggest that cryoconite granulation is most likely due to EPS secretion by gliding cyanobacteria.
68 In the presence of water as the only substrate for growth, cyanobacteria formed mostly carpet-
69 like mats. Our data empirically prove that EPS-producing oscillatorialean cyanobacteria
70 isolated from the diverse community of cryoconite microorganisms can form granules from
71 mineral substrate and that the presence of the mineral substrate increases the probability of the
72 formation of these important and complex biogeochemical microstructures on glaciers.

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74 ***Key index words*:** Alcian blue staining, biogenic aggregations, cryoconite granules,
75 extracellular polymeric substances, glacial ecosystems, glacier cyanobacteria, Oscillatoriales,
76 *Phormidesmis*, *Microcoleus*, scanning electron microscopy

77 ***Abbreviation*:**

78 EPS, extracellular polymeric substances

79 OM, organic matter

80 SEM, scanning electron microscopy

INTRODUCTION

The significance of cryoconite, the dark sediment on the glacier surface, for the functioning of the glacial ecosystems has been discussed in the literature for decades (Cook et al., 2015, Kohshima et al., 1993, Rozwalak et al., 2022, Takeuchi et al., 2001). It substantially reduces albedo of the glacier surface and speeds-up ice melting (Kohshima et al., 1993, Li et al., 2019) which results in formation of cryoconite holes – freshwater ecosystems in the ice surface (Fountain et al., 2004, Zawierucha et al., 2018). In some regions, sediments aggregate into spherical and/or irregular forms called cryoconite granules (Rozwalak et al., 2022, Takeuchi et al., 2001), which support aerobic and anaerobic microbial communities (Segawa et al., 2020). They have great importance for the biogeochemical function of the glacier surface, sustaining biodiversity hotspots and serving as refuges and feeders for microorganisms on glaciers (e.g. Segawa et al., 2020, Zawierucha et al., 2018, Žárský et al., 2013). Such aggregates significantly darken the surface of ice and trigger warming of the glacial surface (Leidman et al., 2020, Takeuchi et al., 2001).

It has been hypothesised that cryoconite granules are formed as a result of microbial activity in the cryoconite (Rozwalak et al., 2022). Fundamental papers of Takeuchi et al. (2001, 2010) and Langford et al. (2010, 2014) suggested that extracellular polymeric substances (EPS), secreted by cyanobacteria and other EPSs producers (e.g. heterotrophic bacteria, microalgae), are responsible for cryoconite granulation. EPSs are organic polymers, constituted by polysaccharides, proteins, nucleic acids, and amphiphilic substances such as (phospho)-lipids (Wingender et al., 1999). The role of the microbial EPS is crucial in many ecosystems from the biological soil crust (soil ecosystem) to algal blooms (aquatic ecosystem) (Liu et al., 2018, Rossi et al., 2018). The excretion of EPSs is an important physiological process that promotes cell adhesion and cohesion, improves the resilience to freezing and thawing, helps the accumulation of minerals and nutrients and also protects the microorganisms from UV-

radiation (Rossi et al., 2018). Moreover, due to great cohesive properties, the aggregation of organisms and minerals acts as a refuge against consumers (like protozoan predation). In addition, organisms in symbiotic aggregates can benefit through the mutual use of nutrients, as shown in the example of cryoconite granules, where heterotrophic bacteria use compounds produced by cyanobacteria, while cyanobacteria can use those produced by heterotrophic bacteria inside the granules (Segawa et al., 2020). Therefore, the role of EPSs seems to be crucial for the biogeochemistry of cryospheric habitats (Nagar et al., 2021, Segawa et al., 2020, Takechi et al., 2001). Hence, it has been suggested that cyanobacteria abundant on glaciers are a substantial element shaping the structure of cryoconite (Rozwalak et al., 2022, Takeuchi et al., 2010). Despite the crucial, multilevel role of cryoconite granules, experimental studies on cryoconite granulation are surprisingly scarce. A laboratory experiment conducted by Musilova et al. (2016) showed the great importance of light and nutrients to cryoconite granule formation by the microbial community. In turn, Langford (2012) revealed that lake isolates of Antarctic filamentous cyanobacteria (*Leptolyngbya* cf. *antarctica*, *Wilmottia murrayi*) can use mineral grains to form granules when a nutrient-rich liquid medium is applied.

In this study, we tested whether Arctic filamentous cyanobacteria isolated directly from cryoconite sediments can granulate cryoconite under poor nutrient levels and at low temperatures – the environment that reflects semi-natural conditions in the cryoconite hole. In our empirical test on cryoconite granulation, several oscillatoriallean cyanobacteria isolates were inoculated into the plastic wells filled with distilled water and different types of mineral substrates. Using powdered form of substrates, we reflected the transfer of tiny mineral substrates by wind to the glacier surface, thereby we were able to track cryoconite granulation phenomenon from the starting point. During several months of incubation, cultures were monitored for physical structure formation. We also checked if our model cyanobacterial strains

130 are able to produce EPSs, which is recognized as a main factor triggering the microbial
131 aggregation and cryoconite granulation.
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134 METHODS
135 *Cyanobacteria strains and culturing technique.* Cyanobacteria were obtained from cryoconite
136 collected from four localities in the Arctic: Longyearbreen Glacier in Svalbard (78° 10' N, 15°
137 31' E), Russel Glacier (67° 9' N, 50° 0' W) and Dark Zone (67° 04' N, 49° 23' W) in South-West
138 Greenland, and Storglaciären Glacier (67° 54′ N, 18°34′E) in Sweden. Samples were collected
139 with pipettes to Whirl-Pak bags or falcon tubes and then frozen and transported to the laboratory
140 and stored at -20°C. The plastic bags and falcon tubes were sterile and have not been opened
141 during the transport or in the field in order to avoid contamination. Single filaments (without
142 any attached microalgae) were isolated from cryoconite using a serial dilution technique with
143 microcapillaries (Andersen & Kawachi, 2005). As cryoconites contain various algae species
144 and heterotrophs (Perini et al., 2022, Poniecka et al., 2020, Zawierucha et al., 2018), we used
145 precise technique for isolation and culturing solely cyanobacteria._Before the isolation of
146 cyanobacterial filaments, cryoconite material was vortexed to separate cyanobacteria from
147 mineral granules. Then, single filaments were rinsed several times in imipenem antibiotic
148 solution (10 μg mL^{-1}) to remove potential bacterial contaminations (Ferris & Hirsch, 1991).
149 When an isolate was found to be contaminated with bacteria, re-isolation of filaments was
150 conducted. Purity of the cultures was assessed in dense cultures using an inverted phase contrast
151 microscope Leica DM IL LED (Leica Microsystems, Wetzlar, Germany). When microscopy
152 inspections of the cultures showed a lack of spherical, rod-shaped, curved objects or biofilms,
153 they were recognized as pure. Stock isolates were grown in well plates with wells filled with 2
154 mL of WC medium (Guillard and Lorenzen 1972). Cultures were stored in a walk-in incubator

under defined temperature (3°C) and light intensity (33-44 µmol photons m^{-2} s^{-1}). After about seven months, we had four pure, viable and successfully growing isolates. Taxonomic identification of strains was based on a combination of molecular marker (16S rRNA gene sequencing; for methods of DNA extraction, amplification and phylogeny see supporting information (Appendix 1)) and microscopic observations of filaments and diagnosis of their key morphological features according to Komárek & Anagnostidis (2005), Teneva et al. (2023), Strunecký et al. (2011), Strunecký et al. (2013).

Experimental design. Inocula of each strain (10 filaments per a single inoculum) were transferred into the wells of 24-well plates containing either 1 mL of distilled water alone (control treatment) or different types of mineral particles with 1 mL of distilled water: 1) cryoconite from each sampled glacier (sterilised in 550°C to remove organic matter [OM]), 2) quartz powder (Ø: mean = 7.38 µm, SD = 4.75 µm). Greenlandic strains were additionally incubated with 3) 1 ml of distilled water and ground-up rocks (Ø: mean = 4.27 µm, SD = 2.58 µm) – a local type of minerals occurring nearby the Russell Glacier catchment. The rocks were collected during the Greenlandic expeditions, and we decided to use these rocks as a potential substrate for cyanobacterial growth. Mineral dust is delivered to the ice surface from local Greenlandic sources (Nagatsuka et al., 2016), thus this material is an analogue to other minerals wind-blown on the ice surface. Each experimental group was replicated four times. Minerals were distributed in wells to cover the whole bottom and form a thin layer. Plates contained only distilled water and various substrata mimicking poor nutritional conditions on the glacier surface. Quartz powder contains only silica (SiO_2), rocks from Greenland were composed of gneiss and granitoids. Finally, furnaced cryoconite from each investigated glacier contained original mineral substratum present on the glacier during sampling.

179 Apart from single-strain experimental cultures on various mineral substrates, we also
180 distinguished an additional group – a mixture of two Greenlandic strains – designed to test
181 whether the competition between cyanobacteria influences the rate of granule formation. The
182 detailed experimental setup is presented in Fig. 1. All cultures were incubated for 6 months
183 under the same temperature and light intensity as the ones that the stock cultures were
184 maintained in. Cyanobacterial growth was detectable microscopically after three months of
185 incubation. During the further incubation period, well plates were gently shaken at least once
186 per week to mimic natural water flow disturbance on Arctic glaciers. The time-lapse imaging
187 using microscope OLYMPUS SZ and QuickPHOTO Camera 3.0 software was conducted every
188 week for the next three months until the end of the experiment. During the microscopic imaging,
189 the formation of mats, granules and their behaviour (transition from mats to granules) were
190 noted. Regular or irregular spherical or oval shaped structures were classified as granules. In
191 turn, the thick "carpet-like" structures were identified as mats.
192
193 *Cyanobacterial EPS staining procedure*. Cyanobacterial trichomes were stained with Alcian
194 Blue dye, which is commonly used for detection of polysaccharides in cyanobacteria (e.g. Perez
195 et al., 2018, Sohm et al., 2011). The 2 mL culture volume of each strain were collected in
196 Eppendorf tubes and centrifuged for 5 min at 15 000 rpm (Rotina 380R centrifuge, Hettich,
197 Germany). Subsequently, the supernatant was removed, and the pellet was diluted with 1 mL
198 of distilled water and re-suspended in a water column by 2 min vortex-shaking. After that, 0.5
199 mL of the Alcian Blue solution (1% in H2O, w/v) was added to the tubes. After 15 min
200 incubation, cyanobacterial biomass was centrifuged and rinsed three times with distilled water.
201 Cyanobacterial trichomes were then observed under the inverted microscope Leica DM IL LED
202 (Leica Microsystems, Wetzlar, Germany) equipped with a digital camera Jenoptik Gryphax
203 NAOS (Jenoptik Optical Systems, Jena, Germany).

Statistical analysis. The difference in the probability of mats and granules formations between treatments was tested with binomial Generalized Linear Mixed Models with the logit link function. Models tested the difference in probability of granules formation between substrates (separately for granules and mats formation models) with a cyanobacterial strain as a random intercept effect. The impact of the number of strains per well (one or two) on the probability of granules and mat formation was tested with the substratum included as a random intercept effect. The models were implemented with *glmmTMB* packaged (v1.0.2.1; Brooks et al. 2017) in R 3.6.3 software (R Core Team 2020). To assess whether the implemented models have significantly better goodness of fit than the null model, we compared them separately with a respective null model using the Chi-square test. The R script and the data are available in supporting information (Appendix 2).

RESULTS AND DISCUSSION

In total, we isolated four monoclonal cyanobacterial strains from four glaciers in the Arctic. All of them represent Oscillatoriales (supporting information, Figure S1, Appendix 3). Three strains were identified with a DNA analysis as *Phormidesmis priestleyi* (strain 101, Storglaciären Glacier, Sweden, GenBank accession number OR120247; strain 102, Dark Zone, South West Greenland, GenBank accession number OR120248; strain 103, Russel Glacier, South West Greenland, GenBank accession number OR120249). Key morphological features of our *Ph. priestleyi* strains are as follows: mats of brownish, greenish or yellowish colour; filaments occurring solitarily and in irregular aggregations; filaments straight and pliant with visible fine sheaths; cells inside the trichomes appear barrel-shaped, their width is less than their length, ranging from 2.5 – 3.1 µm (strain 101), 2.6 – 3.9 µm (strain 102), 2.3 – 3.5 µm (strain 103); terminal cells with rounded end without calyptra (Fig. 2, left panel). These features match

229 the characteristics of *Ph. priestleyi* (Komárek et al., 2009). The next strain (104, Longyearbreen,
230 Spitsbergen, Svalbard, GenBank accession number OR120250) was identified with DNA
231 analysis as *Microcoleus autumnalis*). Microscopic observations revealed that filaments of this
232 strain are usually straight, rarely slightly curved (only in the case of long pieces), occurring
233 solitarily or weakly aggregated/entangled with each other, and enclosed with fine or distinct
234 sheaths. Strain 104's cell width is usually larger than its length, ranging from 5.0 to 8.1 µm. Its
235 apical cells are sometimes slightly elongated and rounded at the end. The calyptra is weakly
236 expressed. During microscopic inspection of fresh and old cultures, we did not find multiple
237 trichomes in a common sheath – the feature characteristic for *Microcoleus vaginatus* (Strunecký
238 et al., 2013), but not obligatorily present in strains (Teneva et al., 2023). Taxonomic revision
239 of the genus *Microcoleus* is needed, especially given the high diversity and wide distribution
240 of strains and the existence of numerous lineages in which new species could be present
241 (Stanojković et al., 2022).

242 Both *Ph. priestleyi* and *M. autumnalis* have been earlier reported from the cryosphere
243 (Chrismas et al., 2015, 2016, Strunecký et al., 2010, 2013) and can produce EPS (Chrismas et
244 al., 2015, 2016, Mugnai et al., 2018, Pajdak-Stós et al., 2001, Vicente Garcia et al., 2004).
245 Alcian blue staining of cyanobacterial filaments from examined strains confirmed the presence
246 of sheaths surrounding the filaments in all strains. The sheath of *Phormidesmis priestleyi* strain
247 101 (Fig. 2A) was closed at the ends and visible only when empty, while in the *Ph. priestleyi*
248 strain 102 (Fig. 2B), the non-lamellar sheath was strictly adherent to the trichomes. In the *Ph.*
249 *priestleyi* strain 103, the sheath is wavy and sometimes lamellar (Fig. 2C). *Microcoleus*
250 *autumnalis* strain 104 is characterized by a well-defined sheath (Fig. 2D). In case of strains 102,
251 103 and 104, observation of trichomes stained with Alcian Blue revealed the presence of slime
252 outside the sheath. As we can clearly see in Fig. 2B, the deposition of the slime can differ along
253 the trichome.

Mats were formed by cyanobacteria at a similar rate when incubated in water alone or with the addition of a substratum (sterilised cryoconite – OM free, quartz powder or grinded rocks, the latter two representing silt fraction), with no difference between substrate types (Fig. 3A; χ^2 = 2.247, df = 3, p = 0.523). However, the probability of granule formation was significantly higher in water with some substratum than without it (Fig. 3B; χ^2 = 6.603, df = 2, p = 0.037);no differences were found between substrate types. The formation of artificial granules from North-West Greenland is shown in Fig. 4. Mats and other artificial granules are presented in supporting information (Appendix 4). There was no difference in the probability of granules or mats formation between groups with one or two mixed strains (granules: χ^2 = 0.591, df = 1, p = 0.442; mats: χ^2 = 0.057, df = 1, p = 0.811).

We observed that the surface of granules was usually overgrown by cyanobacteria filaments (Fig. 5). Their presence in and on granules was also visible on SEM photos: particles in the granules were stuck together, though in some cases we documented cyanobacterial filaments protruding beyond the cryoconite granules. These filaments were often attached to the bottom of the SEM stub of the sample as well. The SEM images confirmed the presence of the EPS slime surrounding the filaments produced by *M. autumnalis* strain 104 (Fig 6A). *Ph. priestleyi* strain 102 (Fig. 6B) showed filament bundles that appeared as a blanket over the surface of granules, which had porous structure and interspaces. In the SEM picture of *Ph. priestleyi* strains 102 and 103, the cyanobacterial extracellular secretion acts as a gluing agent, forming organic bridges and binding filaments together (Fig. 6C). Such structures like EPS bridges have earlier been found by Román et al. (2021) in crusts formed by EPSs of soil cyanobacterium *Nostoc commune*.

Recently, Rozwalak et al. (2022) as well as Park & Takeuchi (2021) discussed potential factors controlling the formation of cryoconite granules. The main hypothesised factor was the presence of cyanobacteria with the ability to secrete EPS with a different level of gelification.

279 Here, we provide the first evidence that the formation of cryoconite granules on simulated
280 glacier surface conditions (cyanobacteria kept with minerals in distilled water at a low
281 temperature) is driven by the presence of cryoconite cyanobacteria strains that produce EPS.
282 Their proliferation and activity (gliding on mineral particles or squeezing between them) result
283 in binding fine mineral particles into larger and larger granules. Direct evidence on extracellular
284 polymeric substances (EPSs) produced by the examined strains corroborates the hypothesis that
285 cryoconite granulation phenomenon is due to the cyanobacterial EPS that promote the mineral
286 grains aggregation. Although we have observed few spherical structures in treatments without
287 substratum in strain 101 from Storglaciären Glacier, they did not form cemented structures but
288 rather consortia of integrated cyanobacteria filaments. At the same time, the richness of
289 nutrients originating from grain material (grinded rocks from glacial catchment, cryoconite
290 sterilised at 550°C, quartz powder) does not influence the probability of granules formation.
291 These results suggest that glacier cyanobacteria are highly resilient to extremely low nutrient
292 content, and grow in both water and water mixed with mineral particles. Nevertheless, we
293 cannot exclude some minor transfer of nutrients during inoculation of cyanobacterial filaments
294 from stock cultures into the wells at the beginning of the experiment, as WC medium (that stock
295 cultures were reared in) is rich in nutrients. However, for the inoculation of cyanobacteria, we
296 used very fine microcapillaries of ~10 μm diameter, minimising the amount of medium
297 transferred into the wells. Hence, the enrichment of experimental wells with extra nutrients was
298 at most negligible.

299 The presence of the substrate is positively related to granules formation. Rozwalak et al.
300 (2022) found that cryoconite granules are more frequent on glaciers in the northern hemisphere
301 and are less common in the southern hemisphere, even though cyanobacteria in Antarctica
302 produce EPS. The results of our study indicate that these differences may stem, at least in part,
303 from the fact that cryoconite holes investigated by authors in the southern hemisphere consisted

of gravel fraction rather than silt (Rozwalak et al., 2022); that may impede formation of granules. Fine mineral particles are usually lighter, thereby they can be transported with gliding cyanobacterial filaments, probably until their mass does not exceed critical gliding capability of a given cyanobacterial filament. The stickiness of cyanobacterial EPS is more suitable for fine mineral particles aggregations. Therefore, small minerals may also perform the role of a scaffold for cyanobacteria. Additionally, in a nutrient-poor environment, cyanobacteria can start biological weathering (Zawierucha et al., 2019). For example, the attachment of cells to the mineral surface is an important factor in microbial-mediated dissolution of rocks in glacial forefield (Frey et al., 2010). As was shown by Uetake et al. (2016), there is a strong correlation between mineral and cyanobacteria abundance on glaciers. The silt and sand mineral supply on glaciers is, therefore, a fundamental factor for cyanobacterial growth and consequently for cryoconite granules formation as was proved here experimentally. Neither other algae species nor grazing of animals on glaciers is required for the formation of granules (Langford, 2012, this study); however, we cannot exclude that the presence of other microorganisms may boost cryoconite granulation. Although non-EPS producing benthic cyanobacteria are rather rare in polar ecosystems, their role in potential control over the ecosystem should also be identified.

CONCLUSIONS

The results of our study are the first evidence that EPS-producing oscillatorialean cyanobacteria as a single biotic element are responsible for cryoconite granulation phenomenon that occurs commonly in cryoconite holes on glaciers. The presence of sheath and EPS slime in the examined cyanobacterial strains and SEM documentation of mineral particles bonded with cyanobacterial trichomes and EPS, indicate that the ability of cryoconite microorganisms to produce EPS is most likely a key factor in the cryoconite granules formation. Future studies

should focus on the quantification of different EPS fractions (sheath and slime) to further expand our knowledge on the role of EPSs in cryoconite granules formation.

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AUTHOR CONTRIBUTIONS

ŁW, EP and KZ - conceptualisation, EP, KZ - collection of material, ŁW, EP - culturing of cyanobacteria, ŁW – staining of cyanobacterial EPS and visualization. ŁW, JB, PK, KZ - laboratory experiments, analysis and visualisation. AP – performing molecular analysis. MKD – performing phylogenetic analyses. ŁW, KZ - writing draft of manuscript, ŁW, JB, PK, NT, AP, MKD, GM, and KZ - wrote, corrected and approved final version.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY STATEMENT

All data generated during and/or analysed during the current study are available in the manuscript and supplementary materials.

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Figures and captions.

525 Figure 1. The experimental design. Inocula of each strain (10 filaments per single inoculum)
526 were transferred into the wells of 24-well plates containing either 1 mL of distilled water
527 alone (control treatment) or different types of mineral particles with 1 mL of distilled
528 water: 1) cryoconite from each sampled glacier, 2) quartz powder. Greenlandic strains
529 were additionally incubated with 3) 1 mL of distilled water and ground up rocks.
530 Scheme created with BioRender.com
531 Figure 2. Cyanobacteria strains used in the experiment: A) *Phormidesmis priestleyi* strain 101
532 (Storglaciären Glacier, Sweden), B) *Phormidesmis priestleyi* strain 102 (Dark Zone,
533 South West Greenland), C) *Phormidesmis priestleyi* strain 103 (South West Greenland,
534 Russel Glacier), D) *Microcoleus autumnalis* strain 104 (Longyearbreen, Spitsbergen,
535 Svalbard). Photos represent specimens of strains fixed with Lugol (left panel) or stained
536 with Alcian blue to reveal EPS. EPS is visible as blue layers surrounding trichomes or
537 empty sheaths. Scale bar on each photo is 10 µm.
538 Figure 3. Probability of granules and mat formation between treatments. The estimates are
539 presented by horizontal lines on a linear scale, while the 95% confidence interval is
540 represented by the grey band. The model outputs, presented in tables, illustrate the
541 estimates on a response scale.
542 Figure 4. Time laps of the treatment: quartz with cyanobacteria, strain 102 (*Ph. priestleyi*,
543 Dark Zone, South West Greenland). The pictures were taken weekly for twelve weeks.
544 Figure 5. View of artificial cryoconite granules and filaments of *Ph. Priestleyi*. A) mixed
545 strains 102 and 103 + rocks. The arrows indicates cyanobacteria filaments while
546 asterisk the cemented granule. B) strain 103 + rocks. Dotted line divides the granule
547 into two layers – surface covered by filaments and cemented part (most likely anoxic).
548 Figure 6. Granules (biogenic structures) in SEM. A) strain 104 (*M. autumnalis*,
549 Longyearbreen, Spitsbergen, Svalbard) + cryoconite (sterilised in 550°C to remove

organic matter), view of the formed granule; B) strain 102 (*Ph. priestleyi*, Dark Zone, South West Greenland), view of the mat of glued filaments. C) strain 102 (*Ph. priestleyi*, Dark Zone, South West Greenland; strain 103, *Ph. priestleyi* South West Greenland, Russel Glacier) + quartz, view of the granule with *Phormidesmis* filaments. Meaning of the symbols: asterisk – solid and cemented part of the granule, arrowheads – cyanobacterial filaments. All scale bars are in μm.

Appendix 1: Molecular methods: 1. Extraction of cyanobacterial DNA, 2. PCR amplification and purification of a product, 3. Molecular identification and phylogeny.

Appendix 2: The R script and the data are available in supporting information.

Appendix 3: Blast search of the most similar sequences.

Appendix 4: Mats and other artificial granules formed during incubation.

Figure S1: Maximum likelihood phylogenetic tree of Cyanobacteria, using Tamura 3-parameter algorithm and 1000 bootstrap resampling. The values next to the nodes represent the bootstrap support.

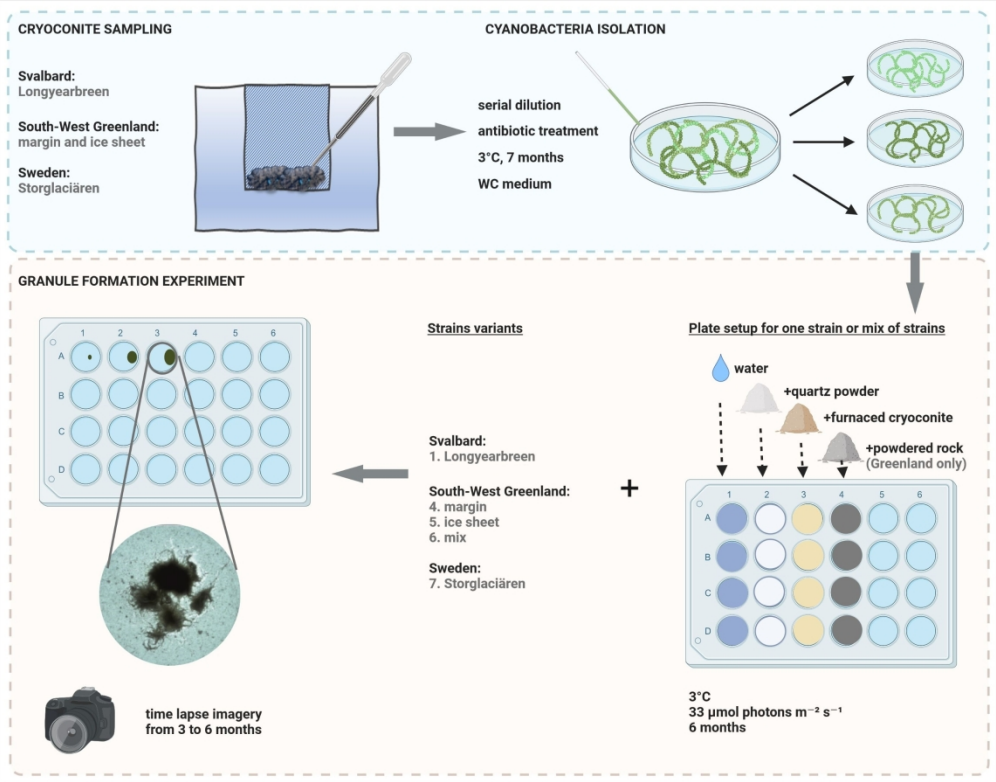
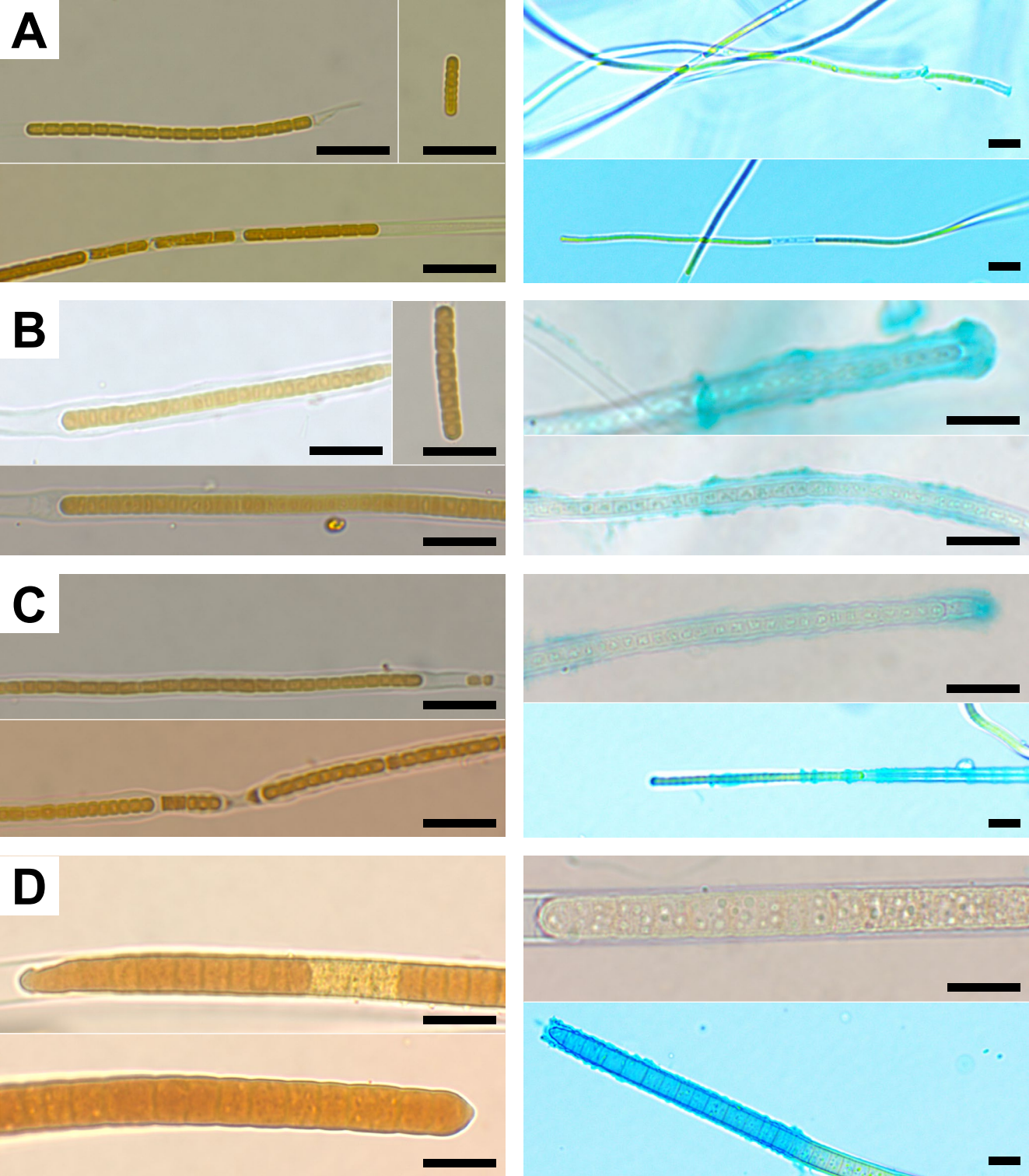


Figure 1. The experimental design. Inocula of each strain (10 filaments per single inoculum) were transferred into the wells of 24-well plates containing either 1 mL of distilled water alone (Control treatment) or different types of mineral particles with 1 mL of distilled water: 1) cryoconite from each sampled glacier, 2) quartz powder. Greenlandic strains were additionally incubated with 3) 1 mL of distilled water and ground up rocks. Scheme created with BioRender.com

Figure 2. Cyanobacteria strains used in the experiment: (a) *Phormidesmis priestleyi* strain 101 (Storglaciären Glacier, Sweden), (b) *Phormidesmis priestleyi* strain 102 (Dark Zone, South West Greenland), (c) *Phormidesmis priestleyi* strain 103 (South West Greenland, Russel Glacier), (d) *Microcoleus autumnalis* strain 104 (Longyearbreen, Spitsbergen, Svalbard). Photos represent specimens of strains fixed with Lugol (left panel) or stained with Alcian blue to reveal EPS. EPS is visible as blue layers surrounding trichomes or empty sheaths. Scale bar on each photo is 10 μm.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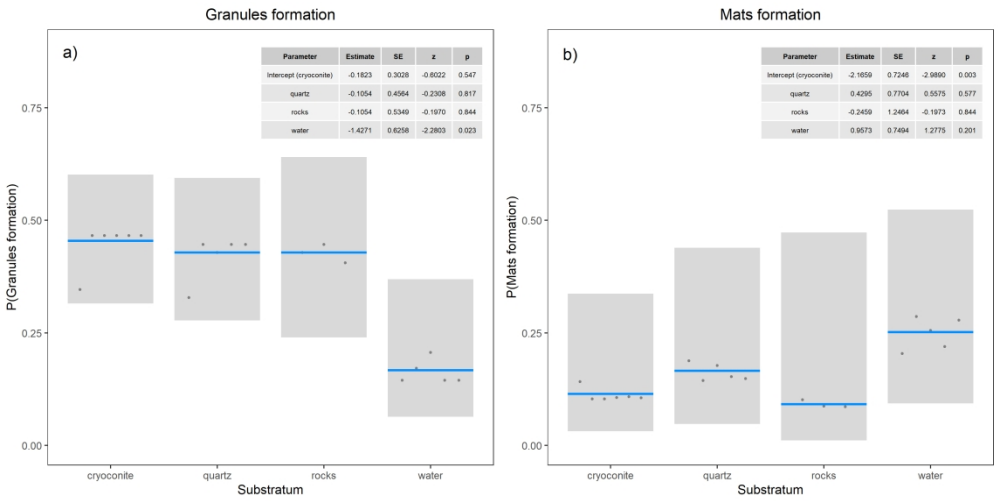

Figure 3. Probability of mat and granule formation between treatments. The estimates are presented by horizontal lines on a response scale, while the 95% confidence interval is represented by the gray band. The model outputs, presented in tables, illustrate the estimates on a linear scale.

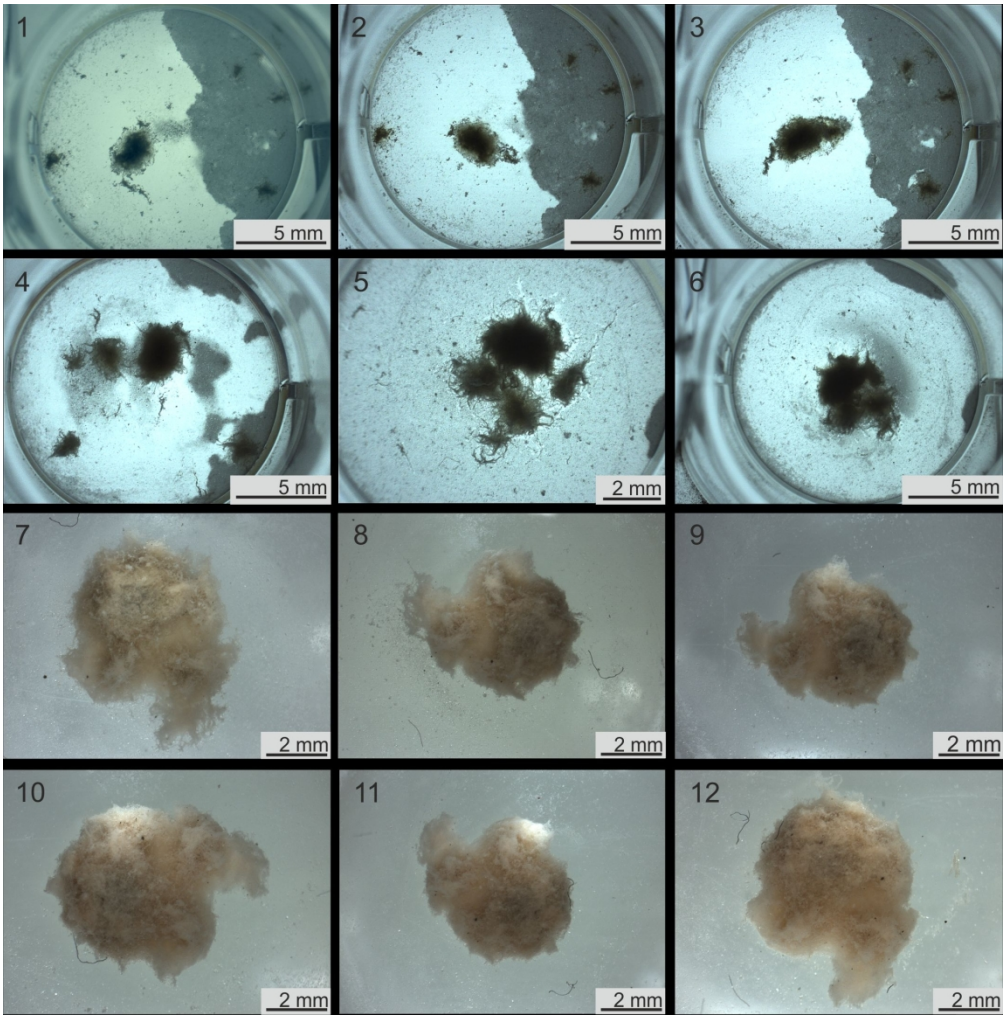

Figure 4. Time lapse of the treatment: quartz with cyanobacteria, strain 102 (*Ph. priestleyi*, Dark Zone, Southwest Greenland). The pictures were taken weekly for 12 weeks.

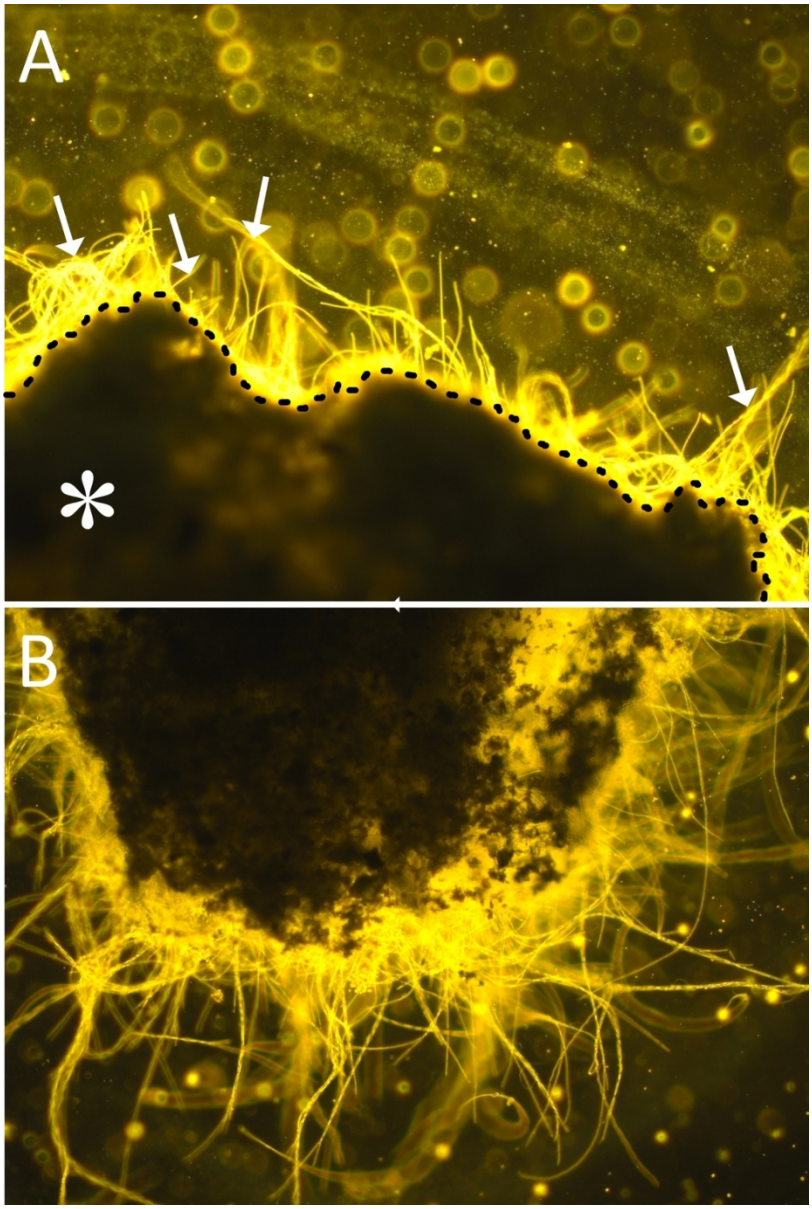

Figure 5. View of artificial cryoconite granules and filaments of *Phormidesmis priestleyi*. (a) Mixed strains 102 and 103+rocks. The arrows indicate cyanobacteria filaments while the asterisk indicates the cemented granule. (b) Strain 103 + rocks. The dotted line in (a) divides the granule into two layers—surface covered by filaments and cemented part (most likely anoxic).

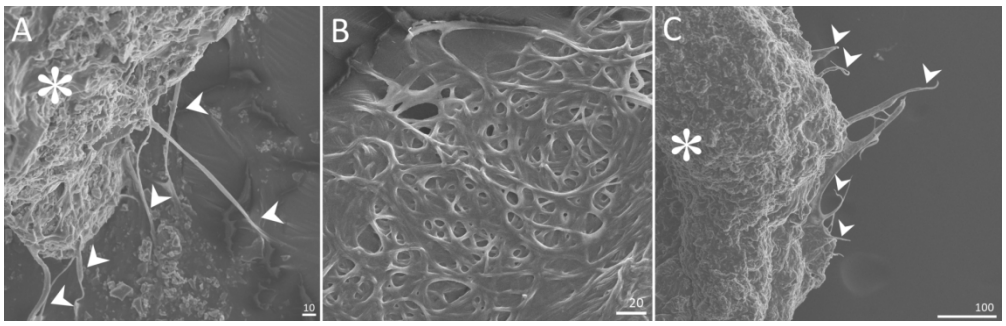

Figure 6. Granules (biogenic structures) in SEM. (a) strain 104 (*Microcoleus autumnalis*, Longyearbreen, Spitsbergen, Svalbard) + cryoconite (sterilized in 550°C to remove organic matter); view of the formed granule; (b) strain 102 (*Phormidesmis priestleyi*, Dark Zone, South West Greenland); view of the mat of glued filaments. (c) strain 102 (*Ph. priestleyi*, Dark Zone, Southwest Greenland; strain 103, *Ph. priestleyi* South West Greenland, Russel Glacier) + quartz; view of the granule with *Phormidesmis* filaments. Meaning of the symbols: asterisk—solid and cemented part of the granule; arrowheads—cyanobacterial filaments. All scale bars are in μm.