Cryoconite holes on frozen lakes as source of interesting extremophilic and extremotolerant organisms

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Abstract

The key thought of this short communication is biodiversity evaluation of the photo-autotrophic inhabitants of lake cryoconite holes and point out their potencial as extremo-tolerant or even extremophilic organisms that deserve attention. Cyanobacterial diversity of these environments was investigated in cryoconites holes of 2 permanently and 1 seasonally ice-covered lakes at James Ross Island. Twelve species from different taxonomic groups, both coccal and filamentous (with and without heterocytes) cyanobacteria, were determined using a light microscope and morphological features of autorophic micro-organisms. The results suggested relatively high diversity in such extreme environments and also indicated potential of lake cryoconites to serve as a reservoir of organism that can have special protection properties.

Key words: cryoconite holes, cyanobacteria, extreme environment, diversity, adaptation

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Introduction

Polar regions constitute extremely cold environments providing a lot of exceptional aquatic ecosystems. Fact, which makes Antarctic ecosystems different from the other ecosystems around the world (outside polar region) is their dependence on the phase change of water between solid and liquid. Life in these areas as well as in others functioning aquatic ecosystems is reliant on liquid water. Therefore, Antarc-

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tic aquatic ecosystems are driven by freeze-thaw cycles (Howard-Williams et Hawes 2007).

One of the biologically interesting ecosystems of polar region are cryoconite holes. These ecosystems, named “cryoconites” by A. E. Nordenskjöld (Foreman et al. 2007), are created on the ice surfaces by localized melting that is caused by the reduced albedo associated with the progressive accumulation of dark-coloured particles (Gerdel et Drouet 1960, Takeuchi et al. 2001, Takeuchi 2002, Edward et al. 2011). During the summer season, they form unique environments containing liquid water with materials released from the particles and especially from the melted ice (Christner et al. 2003). As described in Bertilsson et al. (2013), ice cover has substantial impact on the availability of microbial resources due to accumulation of nutrients deposited from atmosphere into the ice cover. Thus, ice cover represents extensive nutrient source during melting, which strongly supports development of microbial communities. Such rich source of nutrients is not just important for development of life in cryoconites, but for all water supplied ecosystem in polar regions.

Cryoconite holes in the polar areas contain complex microbial communities (Mueller et Pollard 2004). Säwström et al. (2002) suggested that the constituents of the microbial life forms are abundant in material on the cryoconite bottoms than in the other parts of holes. Despite the low temperature in these ecosystems, microbial activity of cryoconite holes may be exceptionally high (Anesio et al. 2007). As demonstrated by previous studies (Hodson et al. 2005, Anesio et al. 2009, Sattler et al. 2010), phototrophic primary producers, especially cyanobacteria, dominate in cryoconites. However, there can be also such organisms as heterotrophic bacteria, fungi, green algae, diatoms, rotifers, tardigrades and nematodes (Mueller et al. 2001, Christner et al. 2003, Mueller et Pollard 2004).

Cryoconite holes are found on glaciers in both Antarctica and the Arctic (Säwström et al. 2002) but they are also present on permanently frozen lakes. Presence of these structures has been documented from Antarctica (Sattler et al. 2010) exclusively due to the too low number of permanently frozen lake in the Arctic. It is caused by warmer climatic conditions in northern polar region which are associated with global climatic change. Cryoconites in Antarctic permanently frozen lake represent an unexplored part of cryoconite research. Nevertheless, they could constitute very interesting environments inhabited by different extremophilic or extremotolerant organisms which could be missing in glacier´s cryoconites because character of these two seemingly similar ecosystems is distinct.

The main aim of this paper is to indicate which cyanobacteria prosper in lake cryoconites at James Ross Island (JRI) and point out potential of cryoconites as source of organisms which cope and prosper in low temperatures (to 1.5°C) with an emphasis on cryoconites created on permanently or seasonally frozen lakes on JRI. Generally, research of cryoconites has increased in last few decades, however, biological information about this ecosystem is still scarce. Attention is devoted to biodiversity, and biogeochemistry, in particular. However, cryoconite hole reaserch is still developing and more specialized studies are required. For example, results from disciplines such as physiology or molecular biology are still very limited. Moreover, itis well known that cyanobacteria from extreme environment are very good material for protein´s engineering and other biotechnologically oriented disciplines.

Material and Methods

Site description

Fieldwork was undertaken on the Ulu Peninsula, which represent northern part of James Ross Island (JRI), where is located Czech Antarctic station - Johann Gregor Mendel Station (63° 48´ 02´´ S, 57° 52´ 57´´ W). Mean daily air temperatures above 0°C occur only in December and January, with fluctuation in the range from -5°C to 10°C (Láska et al. 2011, Hrbáček et al. 2015).

Three lakes were investigated (Fig. 1): two of them (sites 1 and 2) are located close to Halozetes Valley and the third one (site 3) is placed on eastern side under the Lachman crags. Lakes at Halozetes Valley were frozen more than 1 year and a large number of cryoconites were observed in all of them. Lake under Lachman crags was partially thawed, but there were numerous cryoconites at its frozen parts. Moreover, lakes at Halozetes Valley (sites 1 and 2) were largely covered with snow throughout the summer season 2015. However, snow cover was decreased on the shores of the lakes due to melting of snow on interface snow/rock.

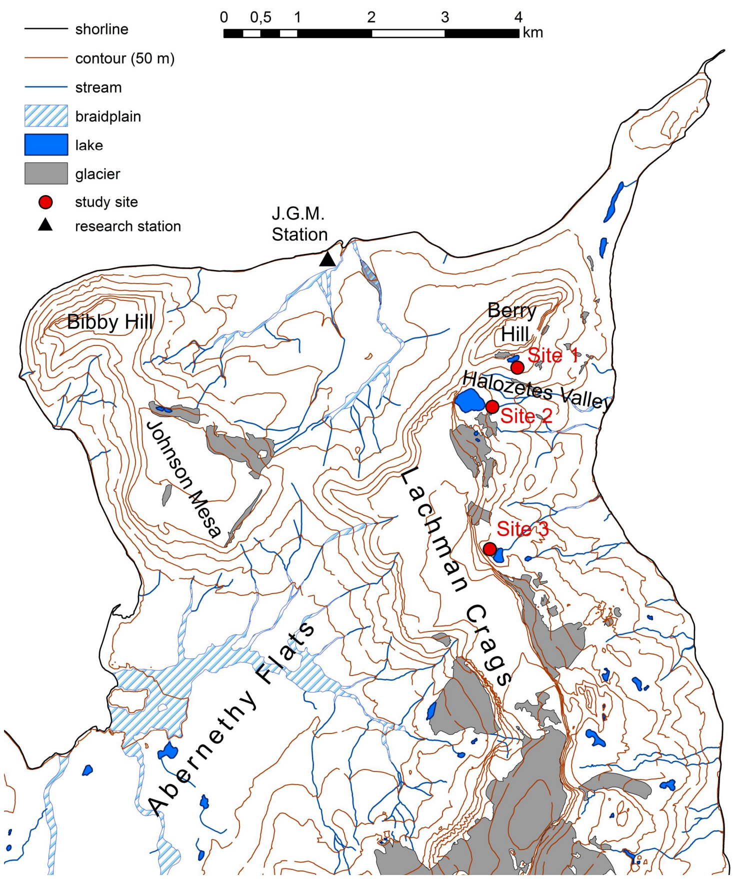

Fig. 1. Study area showing sampling sites (1, 2 and 3) in the northern part of James Ross Island (Czech Geological Survey 2009).

Sample collection

During an expedition to JRI in January-February 2015, there were collected three samples of cryoconites from three different lakes, when in each site was sampled 1 cryoconite hole. Physico-chemical properties of sampled cryoconited holes were measured by HI98129 pH/Conductivity/ TDS Tester (Hanna Instruments, Czech Republic), which are shown in Table 1. Values of temperature and conductivity clearly documented extremity of these exceptional ecosystems. The samples were taken to a sterile flask and stored into the freezer. The samples were transported to the Czech Republic in thermo-cooling box with cooling pads and then were frozen (-20°C). Morphological determination of samples was carried out after two months.

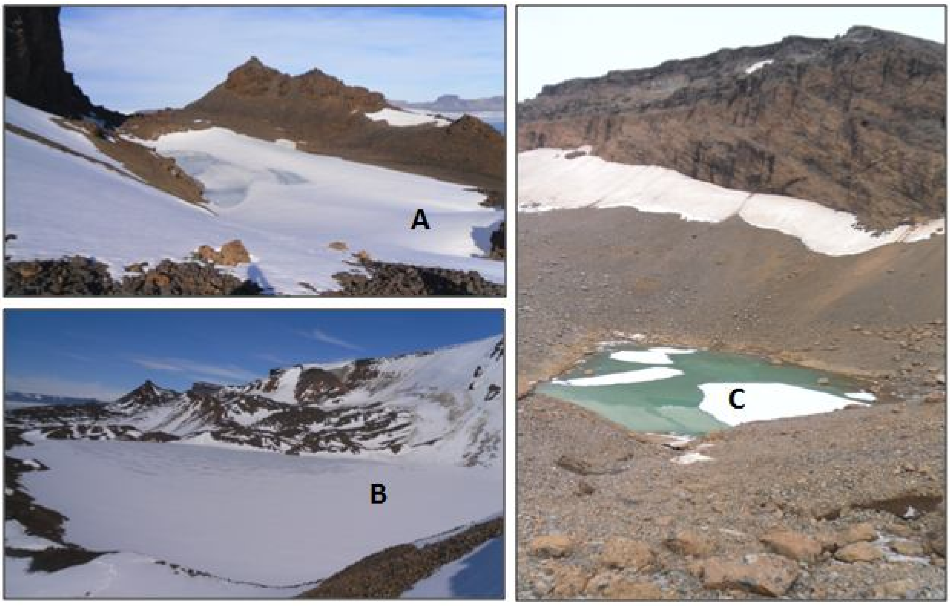

Fig. 2. Sampling areas. All study sites where cryoconites communities were collected. A – Site 1; B – Site 2; C – Site 3.

Morphological determination

Taxonomic analyses were carried out by Microscope Olympus CX21 (Olympus America INC., New York, USA). As auxiliary literature Anagnostidis et Komárek (1985, 1988), Komárek et Anagnostidis (1989) were used as well as others supplementary papers from the Antarctic literature (Komárek et al. 2008, Skácelová et Barták 2014). The common morphological traits were used for cyanobacterial classification our samples. Shape, width and length of intercalary cell and shape of end cells, presence or absence sheath, heterocytes, calyptras, necridic cells and position of thylakoids in cells, were assessed for filamentous cyanobacteria. For coccal cyanobacteria observed parameters included especially number of cells, their organisation and presence of sheath. All of observed cyanobacteria were classified to genus level.

Lake	GPS	T (°C)	C (µS)	pH	Species
Site 1	63° 48′ 49′′ S 57° 50′ 02′′ W	0.3	190	8.33	*Geitlerinema* sp., *Nostoc* sp., *Phormidium* sp. 1
Site 2	63° 49′ 10′′ S 57° 50′ 33′′ W	0.5	97	9.02	*Leptolyngbya* sp., encapsuled *Nostoc* sp., *Geitlerinema* sp., *Chroococcus* sp.
Site 3	63° 50′ 14′′ S 57° 50′ 22′′ W	1.3	59	8.7	*Leptolyngbya* sp., *Microcoleus* sp., *Phormidium* sp. 1, *Phormidium* sp. 2, *Phormidium* sp. 3, *Chroococcus* sp. 2, *Chroococcidiopsis* sp.

Table 1. Cyanobacterial diversity and physicochemical parameters of cryoconites at sampling time.

Results

Cyanobacterial communities of three investigated cryoconites from 2 constantly frozen lakes and 1 seasonally lake were studied for biodiversity. Selected physicochemical parameters were recorded (*see* Table 1). The pH values in cryoconites hole were slightly alkaline and ranged between 8.33 and 9.02. The highest temperature of cryoconites was recorded at site 3, where temperature reached 1.3°C and the lowest temperature was recorded at site 1, exactly 0.3°C.

Cyanobacterial diversity of water supplied ecosystem from James Ross Island has already been reported in other studies (*e.g.* Komárek et al. 2008, Skácelová et Barták 2014). However, this is the first report focused on cryoconite holes and their cyanobacterial communities from James Ross Island.

In the collected samples, 13 cyanobacterial species from all three morphological types were found. The species compositions in samples from site 1 and site 2 were similar, while cyanobacterial community of third sampling site (cryoconite) was different. The following genera were identified in cryoconite from site 1: *Nostoc* sp. 1, *Phormidium* sp. 1 (*see* Fig. 3), and *Geitlerinema* sp. 1. The genera in cryoconite hole from site 2 included *Leptolyngbya* sp., *Nostoc* sp. 2, *Chroococcus* sp. 1 and *Geitlerinema* sp. 2. The highest species richness was found in sample from site 3, containing *Phormidium* sp. 1, *Leptolyngbya* sp., *Phormidium* sp. 2, *Phormidium* sp. 3, *Microcoleus* sp., *Chroococcus* sp. 2, *Chroococcidiopsis* sp. All species were classified to the genus since no genomic study was carried out in this work and morphological traits are insufficient for reliable classification. Therefore, detailed study of taxonomic composition of cryoconite holes at James Ross Island with completed molecular taxonomic data should be performed in the future.

Both heterocytous and non heterocytous filamentous types of cyanobacteria were present in all studied samples. Coccoid type was present only in cryoconites hole from second and third sampling site. Higher abundance of coccoid cyanobacteria was recorded in sampling site 3. The most abundant filamentous cyanobacteria were from the genera *Leptolyngbya, Phormidium*, heterocytous *Nostoc* and the most abundant coccoid cyanobacteria was genus *Chroococcus*.

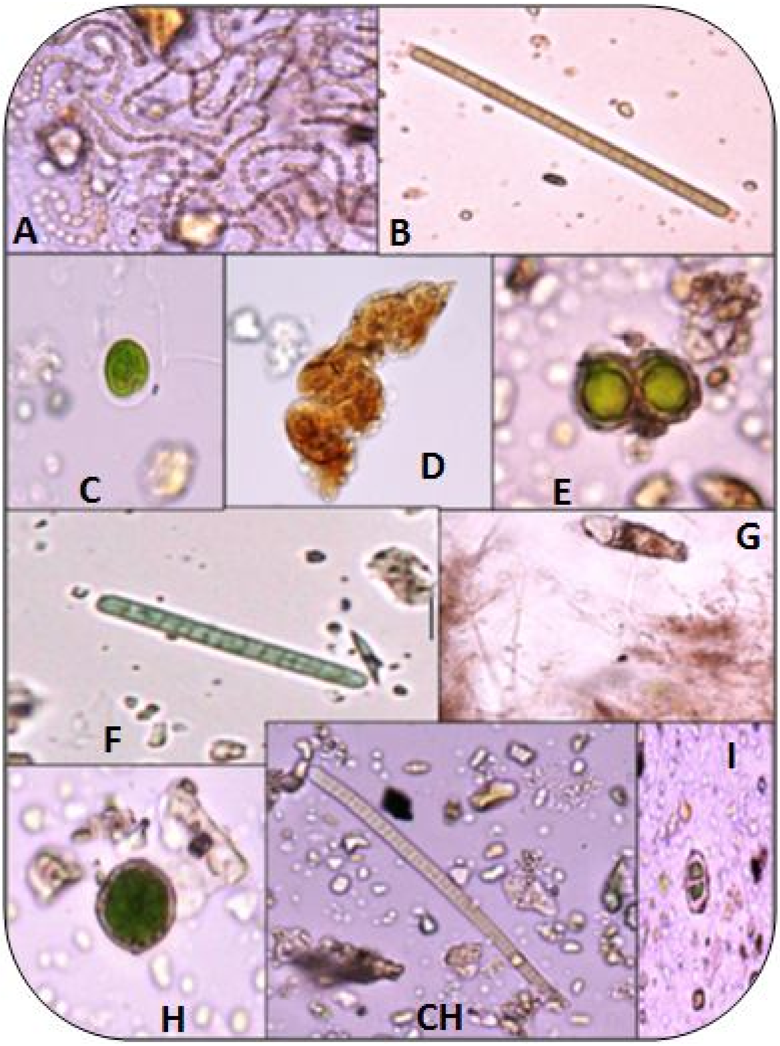

Fig. 3. Cyanobacteria present in lake cryoconites at James Ross Island. A - *Nostoc* sp., B - *Phormidium* sp. 1, C - *Chroococcus* sp. 1, D - encapsuled *Nostoc* sp., E - *Aphanocapsa* sp., F - *Phormidium* sp. 2, G - *Leptolyngbya* sp., H - *Chroococidiopsis* sp., CH - *Phormidium* sp. 3, I - *Chroococcus* sp. 2

Discussion

Even though permanently ice-covered Antarctic lakes belong to extremes environments, there is a natural environment for a lot of organisms, including cyanobacteria. Such lakes provide life space for physiologically and ecologically complex microbial community able of photosynthesis, nitrogen fixation and decomposition (Priscu et al. 1998). In summer season, organisms such as algae or cyanobacteria move in water column due to mixing of water. In the same period, up to 40% of the total ice cover volume can be liquid water (Adams et al. 1998, Priscu et al. 1998) and layer of ice-cover on edge of the lake can be very thin or also missing. Therefore, this period can serve for microlimate-dependent colonization of cyanobacteria and other organisms to cryoconites from lake water thus increase the diversity of cryoconites.

As shown in Komárek et al. (2008), ecological, ecophysiological, biochemical or molecular studies of cyanobacterial diversity should be based on molecular analyses, however morphological analysis can also provide important information. Therefore, this paper reported only morphological data in accordance with the main aims of this work.

Comparison of cyanobacterial communities of cryoconite holes with other studied aquatic ecosystem at James Ross Island (Komárek 2007, Komárek et al. 2008, Strunecký et al. 2012, Skácelová et Barták 2014) showed that most species observed in this paper are identical with those found in other ecosystems on James Ross Island. This fact can be explained by various ways of transport from other places well-supplied bz water during summer season. There are several transport ways how organisms get into the cryoconites. The cells of unicellular organisms can be distributed by wind or transfered by meltwater and deposited in cryoconites (Stibal et al. 2006). In this study, filamentous cyanobacteria, Oscillatoriales and Nostocales, in particular, are dominant species. As stated by Vonnahme et al. (2015), these organisms and coccal heterotrophic bacteria act as ecosystem engineers within the cryoconites because they are able to form substrate for growth of diverse groups of organisms. Although, still exist a very low number of papers from Antarctica focused on cryoconites and no paper focused on cynobacterial diversity of cryoconites from permanently frozen lakes, general comparison of diversity with other area from Antarctica is possible. Porazinska *et al.* (2004) published data focused on biodiversity of cryoconites from McMurdo Dry Valley glaciers. In McMurdo Dry Valleys were observed members of 2 genuses (*Nostoc, Chroococcus*) same as at James Ross Island. On the other hand, most of observed genuses were distinct in both areas. Distinct genuses observed in McMurdo Dry Valley were Chlorococcus, Crinalium, Oscillatoria and Spirulina. The result suggests that cyanobacterial diversity of McMurdo Dry Valley glaciers cryoconites is distinct against cyanobacterial diversity of cryoconoties at James Ross Island. One of main reasons of this distribution is different climatic conditions in McMurdo Dry Valley (continental Antarctica) and at James Ross Island (maritime Antarctica) which is obvious from work Speirs et al. (2012) and Láska et al. (2011).

Study focused on adaptation and acclimation mechanisms of photosynthetic microorganisms to permanently cold environment is summarized in review of Morgan-Kiss et al. (2006). The basic ones are maintenance of membrane fluidity with using reorganisation of lipid composition (Hazel et Williams 1990, Russel 1990, Bartlett 1999), production of cold-adapted enzymes (Sato 1995, Russel 2000) and effective utilization of electron transport chain in thylakoid membrane (Glazer 1994, Sidler 1994, Green et Durnford 1996, Cooley et al. 2000). Cyanobacteria in cryoconites must be endowed with such protective mechanisms to survive there. It is well known that cyanobacteria are endowed with their ability to rapidly change genome as a reaction to new life condition. Some representatives of genera observed in this paper are known for their adaptation to a life in cryoconites. As shown by Vonnahme *et al.* (2015), *Leptolyngbya* sp. and *Phormidium* sp. are known for their capability to protect other organisms from physical stress (Takeuchi et al. 2001). Futhermore, *Nostoc* sp. can develop big colonies as protection against environmental stresses and also can storage large amount of nitrogen through heterocytes (Li et Gao 2007). However, very low number of papers focused on physiology and molecular protective mechanisms of cryoconites photoautotrophs. Cryoconites are very extreme environment, where organisms must face a lot of stresses such as nutrient stress or freeze-thaw cycles and especially temperatures not exceeding 1.5°C. Thus it can be assumed that cyanobacteria of cryoconite holes could evince differences compared to those which live in favourable conditions. For example, production of new biotechnologically interesting proteins and other compounds, signaling pathways of protective mechanisms or differences in physiological response could be explored.

In conclusion, presence of relatively diverse group of cyanobacteria in this extreme environment shows that study of cryoconite holes on frozen lake certainly deserves detailed research. Adaptations of cyanobacteria to this habitat are obvious, but there is very little information about their protective mechanisms, signaling and physiological response that can provide new interesting findings about this exceptional ecosystems.

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