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Ubiquity of ice nucleation in lichen – possible atmospheric implications

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Ice nucleation has previously been described in only a few lichens from a single location. Here we greatly extend this work and suggest that in lichens ice nucleation is a water harvesting adaption. Fifty-seven lichen samples from a variety of widespread locations were tested for ice nucleation by differential scanning calorimetry (DSC). Samples initiated freezing in the range –5.1° to –20°C and the median freezing temperature was –7.2°C. The vapour pressure difference between ice and water is significant at this temperature, and so ice grows at the expense of water (Bergeron–Findeisen process). Therefore, the ability to form ice at these temperatures provides a useful water-harvesting mechanism for lichens. Ice nucleation appears to be ubiquitous in lichens and is more likely to be associated with the mycobiont and may influence atmospheric processes.

Liquid water can be supercooled to well below the melting point of 0°C without freezing. Pure water does not freeze until the temperature drops to –38°C or even –42°C for very small samples (Franks 1985, Debendetti and Stanley 2003). When pure water freezes homogeneously it requires the random arrangement of water molecules to realign into the hexameric pattern of an embryo critical for further water deposition and crystal growth (Vali 1996). When water freezes above –38°C this is termed heterogeneous freezing and is reliant upon the presence of ice nucleating particles in the water. These catalyse the phase transition at much higher temperatures by aiding the alignment of water molecules to form the ice embryo (Kajava and Lindow 1993, Vali 1996). All ice, other than that which forms at high altitudes and in polar regions, is a product of heterogeneous freezing.

There are many sources of ice nuclei (IN), including minerals (Mason 1975), inorganic and organic molecules (Fukuta 1966) and biological particles (Szyrmer and Zawadzki 1997, Möhler et al. 2007). However, the ice nuclei which induce freezing at the highest temperatures are bacterial (Morris et al. 2004). Ice nucleation in bacteria appears to be limited to a small number of plant epiphytes which use specific proteins to induce freezing in order to damage plants and so gain nutrients (Lindow 1983). These include *Pseudomonas syringae* and *Pantoea agglomerans* (formerly *Erwinia herbicola*) which can nucleate water to form ice at temperatures as high as –1.3°C (Lindow et al. 1989).

Although some species of fungi and lichen are known to catalyse the freezing of water at high temperatures, the extent and basis of eukaryotic ice nucleating abilities have been less fully explored. Of 15 lichen species previously tested for ice nucleation activity, 13 were active at temperatures above –8°C (Kieft 1988), and when axenic cultures of each partner were analysed, the most efficient ice nucleation was seen in the mycobionts (Kieft and Ahmadjian 1989).

We have tested a much greater variety of lichen for ice nucleation activity and have demonstrated that ice nucleation is a ubiquitous feature. We suggest that lichen produce ice nuclei in order to obtain additional water and the ice nucleation activity is likely to reside in the mycobiont as this is the partner in direct contact with the atmosphere.

Material and methods

Samples were either collected by the authors into sterile containers and, if damp, allowed to dry before analysis, or provided by professional lichenologists. The lichen samples were collected from the UK, the Faroe Islands, Norway, Ethiopia, southeast Australia and Antarctica.

Ice nucleation was determined using differential scanning calorimetry (DSC 822e, Mettler Toledo, Leicester, UK). Approximately 0.1 mg of sample immersed in 10 µl of molecular grade water (Sigma, Gillingham, Dorset, UK) was contained within a sealed aluminium crucible. This was then cooled progressively from 0°C to –30°C (1°C min^{-1}). When the sample froze, the release of latent heat was detected by an array of thermocouples below the crucible. The warmest temperature of freezing of water controls was –22°C. To determine if the activity was biological in origin, heat stability (after incubation at 90°C for 10 min) was used. To establish if it was bacterial, the effect of exposure to 5 mg ml^{-1} lysozyme (Sigma, Gillingham, Dorset, UK) for 4 h at 37°C was tested.

Results

The highest onset temperature at which freezing was induced by each sample is given in Table 1 and typical DSC traces shown in Fig. 1. Fifty seven lichen samples, representing 40 identified species, were tested. The ice nucleating temperature of the lichens ranged from –5.1°C

Table 1. Threshold ice nucleation temperatures (°C) for lichen. Freezing was detected by the output of latent heat using differential scanning calorimetry.

Lichen	Temperature	Source
Buellia frigida	-7.2	Antarctica
Unidentified	-6.3	Australia
Unidentified	-7.1	Australia
Unidentified	-7.4	Australia
Unidentified	-13.6	Australia
Cladonia pyxidata	-5.2	UK
Physcia tenella	-5.2	UK
Physcia tenella	-5.2	UK
Xanthoria candelaria	-7.0	UK
Candelariella vitellina	-6.5	UK
Farnoldia jurana	-6.3	UK
Stereocaulon evolutum	-5.5	UK
Xanthoria calcicola	-6.0	UK
Parmelia omphalodes	-6.2	UK
Ramalina subfarinacea	-7.0	UK
Stereocaulon vesuvianum	-7.0	UK
Parmelia saxatilis	-6.0	UK
Lecanora gangaleoides	-6.5	UK
Lasallia pustulata	-6.5	UK
Cladonia coniocraea	-7.0	UK
Evernia prunastri	-5.6	UK
Pertusaria hymenea	-5.1	UK
Parmotrema perlatum	-5.5	UK
Cladonia chlorophaea	-5.6	UK
Lepraria sp.	-5.7	UK
Usnea sp.	-6.4	UK
Xanthoria parietina	-7.0	UK
Protoblastenia incrustans	-8.2	UK
Candellariella vitellina	-7.2	UK
Parmelia saxatalis	-7.7	UK
Cladonia coniocraea	-8.0	UK
Physciae adscendens	-7.1	UK
Pertusaria hemisphaerica	-7.6	UK
Imshaugia aleurites	-10.0	UK
Rhizocarpon geographicum	-10.9	UK
Evernia prunastri	-10.0	UK
Porpidia sp.	-12.7	UK
Leptogium sp.	-12.5	UK
Xanthoria parietina	-12.6	UK
Clauzadea immersa	-14.4	UK
Caloplaca sp.	-13.2	UK
Cladonia sp.	-14.2	UK
Stereocaulon vesuvianum	-17.7	UK
Xanthoria parietina	-17.8	UK
Aspicilia contorta	-20.0	UK
Unidentified	-8.6	Ethiopia
Unidentified	-8.6	Ethiopia
Unidentified	-11.7	Ethiopia
Unidentified	-6.0	Faroe Islands
Parmelia saxatilis	-7.0	Faroe Islands
Cladonia rangiferina	-7.2	Norway
Nephroma arcticum	-7.3	Norway
Cetrariella delisei	-7.6	Norway
Stereocaulon alpina	-7.6	Norway
Flavocetraria nivalis	-9.0	Norway
Solorina crocea	-9.5	Norway
Thamnolia vermicularis	-10.4	Norway

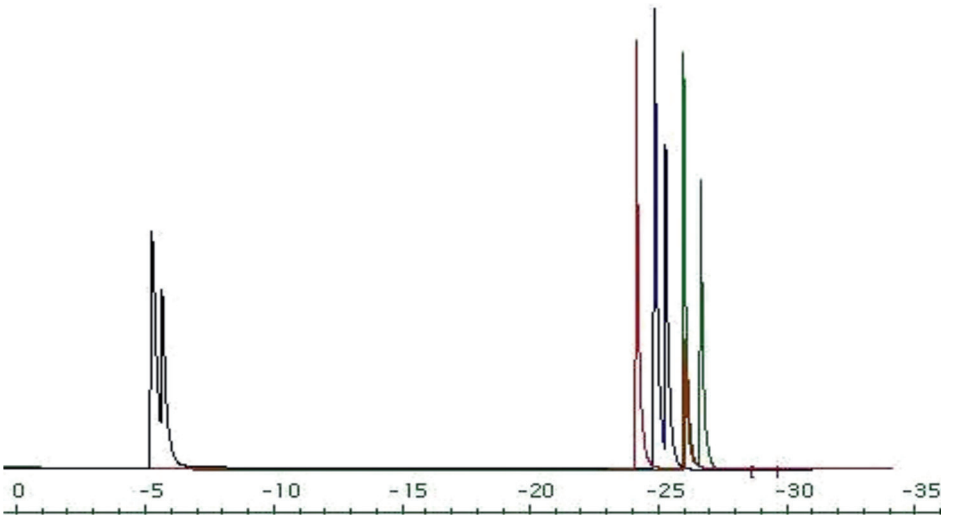

Figure 1. DSC trace for two lichen (freezing at around –5°C) and five water controls (freezing at around –25°C). Each peak represents a single analysis and is created by the latent heat produced as the sample freezes.

to –20.0°C. However, 74% ice nucleated at temperatures above –10°C and this is reflected by the median of –7.2°C (average –8.6°C ± 3.4 SD). The overall distribution of ice nucleation temperatures is illustrated in Fig 2. There is no obvious correlation with location.

Five lichen species were heated for 10 min at 90°C prior to testing, and all showed reduced ice nucleation activity (by 5°C). However, the same lichens showed resistance to treatment with lysozyme. This enzyme destroys bacterial IN by disrupting the cell wall and suggests the ice nucleation associated with lichen is biological but not bacterial in origin.

Discussion

The ice nucleating ability of a wide range of lichen species from different locations was examined. All contained ice nuclei, freezing water at temperatures 18–33°C higher than would occur with homogenous freezing (–38°C). This extends the range of lichens tested and suggests that ice nucleation is ubiquitous in lichen. Although biological ice nucleation has been mainly studied in bacteria (Morris and Sands 2012), there is an increasing awareness of the relevance to atmospheric process of all biogenic ice nuclei active at temperatures warmer than about –15°C (Després et al. 2012 for a comprehensive tabulation). One major difference between prokaryotic and eukaryotic ice nuclei is the temperatures at which they are active. In bacteria ice nucleation can occur at temperatures up to –1.3°C, although the mean upper temperature of nucleation of 67 *Pseudomonas syringae* isolates tested by Morris et al. (2008) was –3.5 ± 0.7°C (± SD). This higher temperature of activity is because bacterial ice nuclei are thought to have evolved to obtain nutrients by damaging plant cells. Therefore there is a selective advantage in triggering nucleation at the highest possible temperatures. This is because, in frost-sensitive plants, the freezing of leaves supercooled by only a degree or two below 0°C will kill the tissue (Rajashekar et al. 1983). By contrast, ice nucleation for water acquisition operates over a wider and lower temperature range. The mechanism works because the vapour pressure over ice is lower than the vapour pressure over water at the same temperature. Once ice has formed, e.g. by the nucleation of dew, the net movement of water vapour is from the water to the growing ice crystal. By initiating freezing at temperatures at which the ice growth rates are maximal (the peak is at –14.2°C but it operates at ≈70% of maximum at –5°C, Byers 1965) lichen make best use of this water gathering mechanism, which was overlooked by previous authors (Kieft 1988, Kappen 1993). A small amount of water on the thallus, once it is frozen, promotes further deposition of water vapour which freezes on contact. So although the process is initiated by immersion freezing the extra water is acquired by the deposition mode (from vapour direct to ice). When the temperature increases the ice melts and the water is available for metabolism. We also characterised the lichen ice nuclei in terms of properties which are often used to recognise ice nuclei of bacterial origin, all of which display heat and lysozyme sensitivity. The lichen ice nuclei are susceptible to heat which indicates they are biological (Christner et al. 2008). However, as all known bacterial ice nuclei are susceptible both to lysozyme and heat treatments above 30°C (Pouleur et al. 1992) the resistance of lichen IN to lysozyme, as well as the accompanying 37°C incubation included with this treatment, suggests the ice nucleating activity was not of bacterial origin (Henderson-Begg et

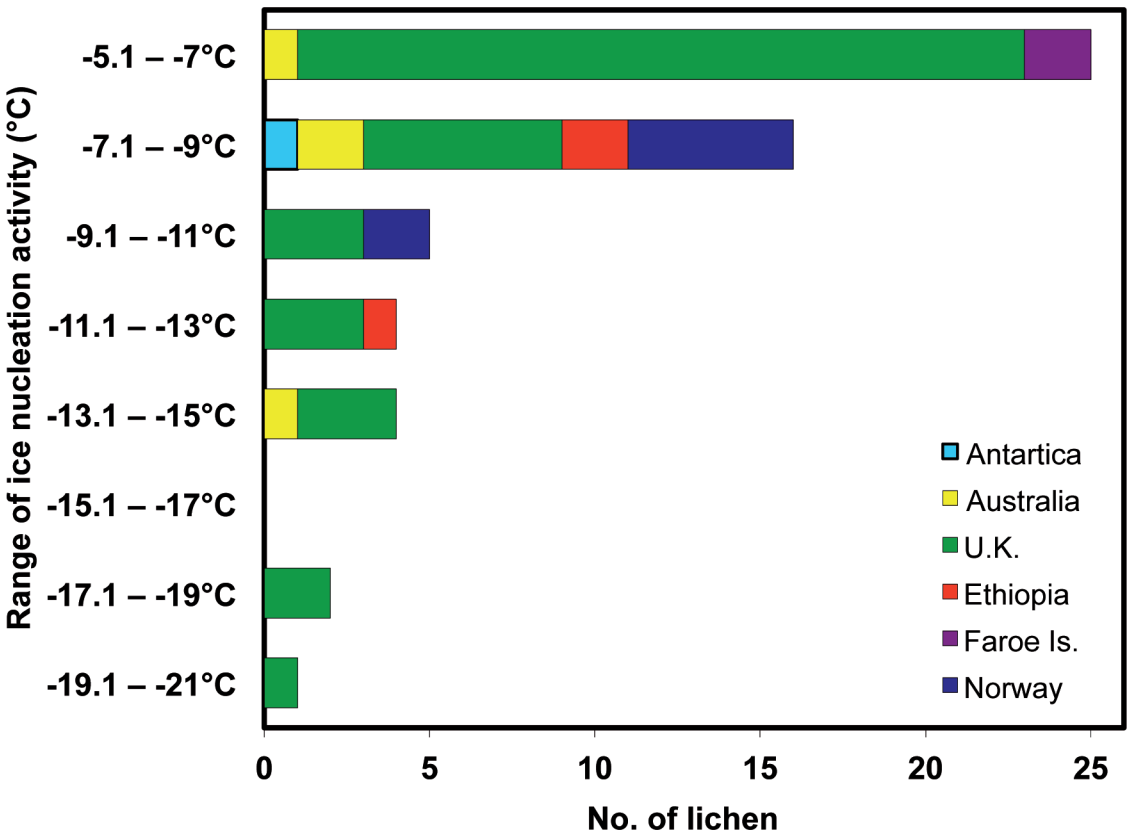

Figure 2. The distribution of ice nucleation temperatures of lichen by geographical region.

al. 2009). This demonstrates the effect was not due to ice nucleating bacteria on the lichen surface and agrees with the work of Kieft (1988). In addition it is thought that ice nuclei which are active above -10°C are unlikely to be of mineral origin (Georgakopoulos et al. 2009).

We intend to carry out further work to focus on the analysis of individual species across space and season and confirming the suggestion by Kieft and Ahmmadjian (1989) that the mycobiont is responsible for the ice nucleation activity.

Estimates of lichen biomass are notoriously difficult, but Margulis (1998) has stated that worldwide there is 10^{14} tonnes. By initiating freezing at the temperature at which the vapour pressure difference between water and ice is at its maximum, lichen provide exactly the type of nuclei which will drive the Bergeron–Findeisen process (Pruppacher and Klett 1997). Therefore lichen-derived IN, when airborne, are candidiates for initiating ice formation leading to precipitation. In an aerobiological monitoring programme carried out on Signy Island in the Maritime Antarctic, lichen soredia (an asexual reproductive unit composed of algae or cyanobacteria surrounded by fungal hyphae) were the most abundant airborne propagules with a size range of 30 to 100 µm (Marshall 1996). Tormo et al. (2001) also found lichen soredia in urban air in Spain. We are currently sampling cloud water, precipitation and air to show that lichen-derived IN are present in the atmosphere and involved in atmospheric processes.

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