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Supplement of

Peltigera lichen thalli produce highly potent ice-nucleating agents

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Comparison of the IN-activity of *Peltigera* and non-*Peltigera* lichens

Fig. S1 compares the IN-activity of four lichens, *P. herrei, S. globosus, B. fuscescens,* and *C. squamosa*, measured by Eufemio et al. (2023) with the IN-activity of *Peltigera* lichens measured in the current study.

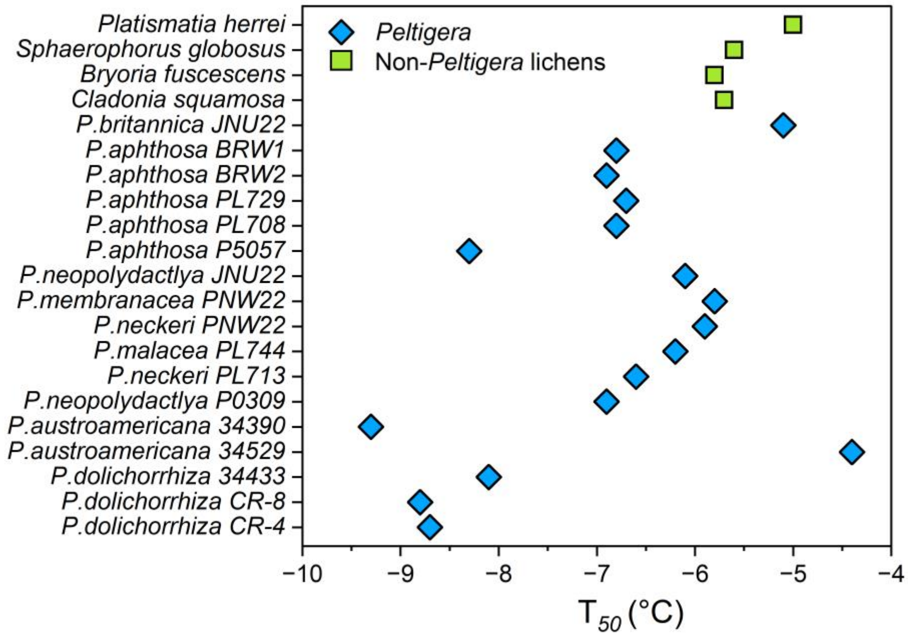

Fig. S1. Highest recorded ice nucleation activity of the *Peltigera* lichens measured in the current study (represented by blue diamonds) and the four most active non-*Peltigera* lichens (*P. herrei, S. globosus, B. fuscescens,* and *C. squamosa*) measured by Eufemio et al., 2023 (shown by green squares). Shown are the T_{50} values, where T_{50} is defined as the temperature at which 50% of the sample droplets are frozen.

Modeling the underlying distribution of *P. britannica* JNU22 freezing temperatures

The heterogeneous underlying-based (HUB) method and numerical code were used to interpret the heterogeneous ice nucleation temperatures obtained from the cumulative freezing spectra (N_m) of *P. britannica* JNU22 (Fig. 2A). This analysis implements a stochastic optimization procedure that fits the cumulative spectra with a linear combination of Gaussian subpopulations. The resulting differential spectra reproduces the distribution of ice nucleation temperatures and allows for the characterization of the underlying IN classes (de Almeida Ribeiro et al., 2023).

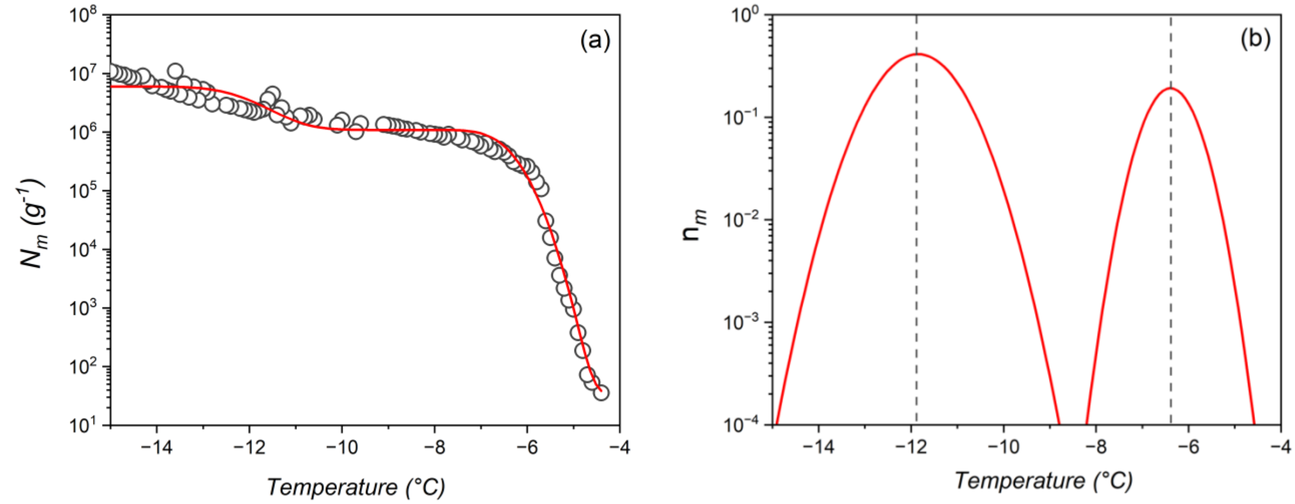

Fig. S2. Freezing experiments of aqueous extracts containing lichen INs from *P. britannica* JNU22. (a) Cumulative number of INs per unit mass of *P. britannica* JNU22 (N_m). The red line depicts the optimized cumulative spectrum obtained through the HUB-backward code. (b) Differential spectrum that represents the underlying distribution of heterogeneous freezing temperatures that produces the cumulative freezing spectrum. The grey dashed lines indicate the temperatures that give the modes of the distribution. The mode, spread, and weight of the class 1 IN subpopulation are -6.39°C, 0.47°C, and 22.4%, respectively. For the class 2 IN subpopulation, the mode, spread, and weight are -11.85°C, 0.75°C, and 77.6%.

Heat treatments

Fig. S3 shows the ice nucleation activity of *Peltigera* IN solutions after incubation at 98°C for 1 hour. The ice nucleation activity was determined using the Vali-type assay immediately after heating. We find that all the *Peltigera* lichens show a decrease in ice nucleation activity after exposure to 98°C.

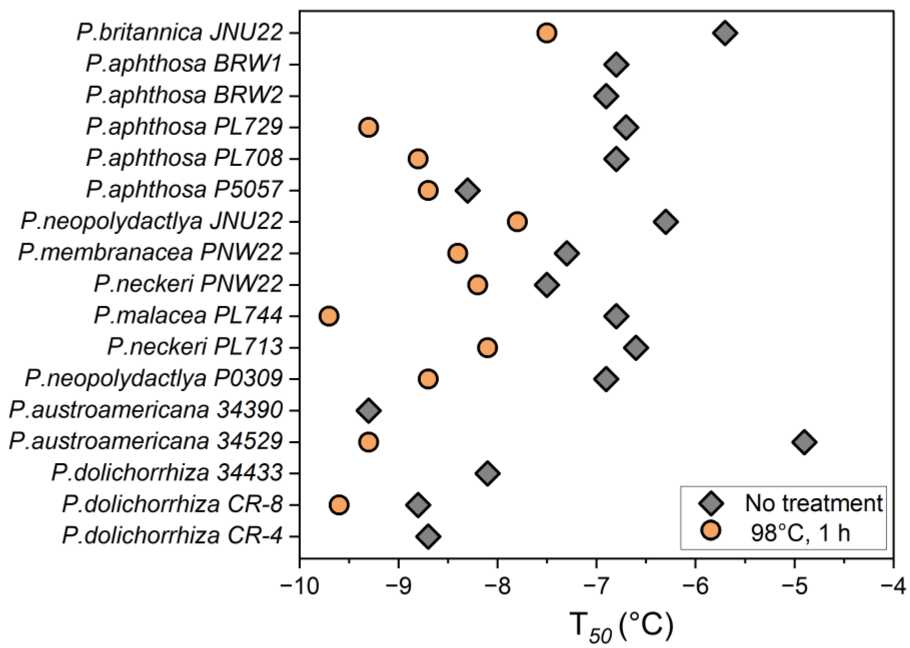

Fig. S3. Effects of high-temperature treatment on the IN-activity of *Peltigera* lichen extracts determined using a Vali-type assay. Untreated and heated extracts are represented by gray diamonds and orange circles, respectively. Lichens for which only the gray diamond is shown were not measured for IN-activity after heating.

Optical microscopy of selected cultures isolated from *P. britannica* JNU22

Optical microscopy (Zeiss Axioscope 5, Carl Zeiss Microscopy GmbH, Jena, Germany) was used to visually identify the pure cultures isolated from the thalli of *P. britannica* JNU22.

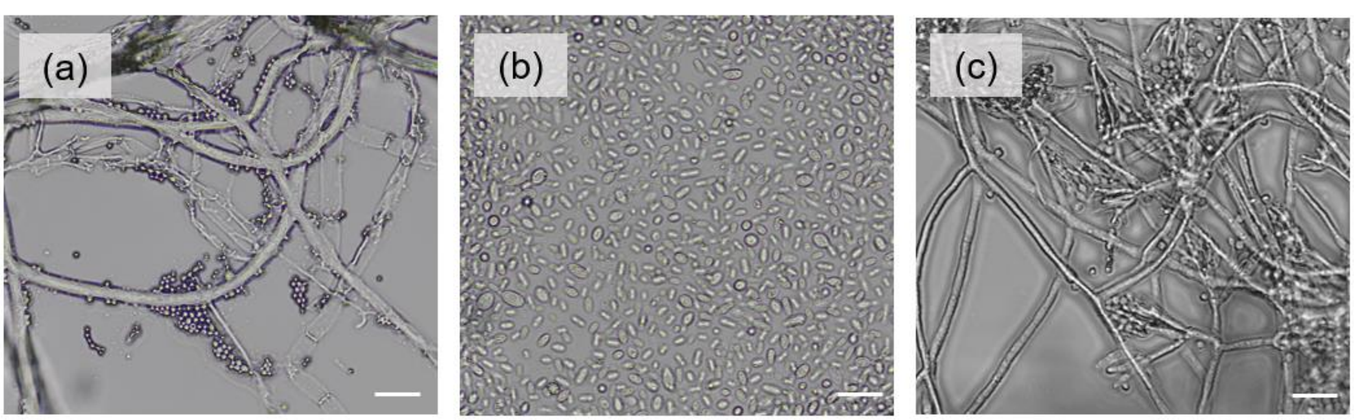

Fig. S4. Optical microscopy at 400x magnification showing (a) L01-tf-B03, identified as a filamentous fungus, (b) L01-tf-B01, identified as a yeast, and (c) L01-tf-A01, identified as a filamentous fungus. Scale bar = 200 µm.

Isolation of IN-active lichen components

Aqueous extracts of *P. britannica* JNU22-derived cultures were tested for ice nucleation activity. Droplet freezing assays show a variation of 4.4°C between the T_{50} of the most active and least active cultures.

Table S1. Ice nucleation activity of aqueous extracts (10 mg mL^{-1}) from cultures isolated from *P. britannica* JNU22 determined using a Vali-type (initial) droplet freezing assay. Cultures are listed from highest to lowest ice nucleation activity.

Subculture	Initial T_{50} (°C)
L01-tf-B03	-5.2
L01-ts-A05	-6.5
L01-ts-A02	-6.8
L01-ts-A03	-6.8
L01-ts-A06	-6.9
L01-ts-A01	-6.9
L01-ts-A04	-7.1
L01-ts-B02	-7.2
L01-ts-A07	-7.3
L01-ts-B01	-7.3
L01-ts-B03	-7.4
L01-tf-A01	-8.3
L01-tf-B02	-8.3
L01-tf-B01	-9.6

IN-activity of the culture L01-tf-B03 isolated from *P. britannica* JNU22

The freezing spectra shown in Fig. S5 corresponds to Fig. 3 in the main text. Shown in Fig. S5 are the total number of INs active for the culture L01-tf-B03 and alive *Pseudomonas syringae* strain Cit7 at starting concentrations of 1 mg mL^{-1}. We find that at the lowest measured dilution, L01-tf-B03 contains $10^{13} g^{-1}$ INs, while in contrast, the cumulative number of *P. syringae* INs is $10^{10} g^{-1}$.

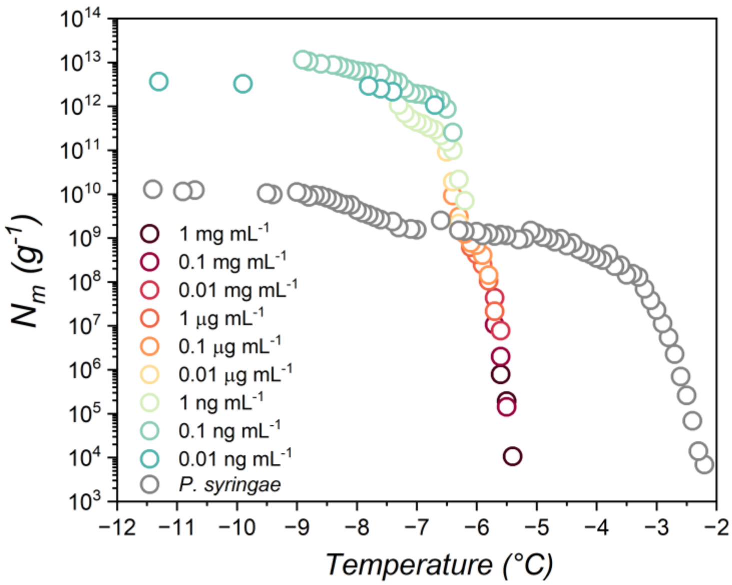

Fig. S5. Dilution effects on the IN-activity of L01-tf-B03 and *P. syringae* as shown by the cumulative number of INs per unit mass (N_m) of sample. The symbol colors of L01-tf-B03 indicate data from different dilutions and are identical to the uncolored dilutions shown for *P. syringae.*

Supplemental References

Eufemio, R. J., de Almeida Ribeiro, I., Sformo, T. L., Laursen, G. A., Molinero, V., Fröhlich-Nowoisky, J., Bonn, M., and Meister, K.: Lichen species across Alaska produce highly active and stable ice nucleators, Biogeosciences, 20, 2805–2812, 2023.

de Almeida Ribeiro, I., Meister, K., and Molinero, V.: HUB: a method to model and extract the distribution of ice nucleation temperatures from drop-freezing experiments, Atmos. Chem. Phys., 23, 5623–5639, https://doi.org/10.5194/acp-23-5623-2023, 2023.