Supplement of

Peltigera lichen thalli produce highly efficient ice nucleating agents

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Heat treatments

Table S1 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.

Table S1. Ice nucleation activity of undiluted *Peltigera* lichen extracts determined using a Vali-type (initial) assay. Effects of high temperature treatments on IN activity were determined. Extracts labeled N/A were not measured.

***Peltigera* Species**	**Initial T_{50} (°C)**	**Heat Treated (°C)**
P. britannica JNU22	-5.7	-7.5
P. aphthosa BRW1	-6.8	N/A
P. aphthosa BRW2	-6.9	N/A
P. aphthosa PL729	-6.7	-9.3
P. aphthosa PL708	-6.8	-8.8
P. aphthosa P5057	-8.3	-8.7
P. neopolydactyla JNU22	-6.3	-7.8
P. membranacea PNW22	-7.3	-8.4
P. neckeri PNW22	-7.5	-8.2
P. malacea PL744	-6.8	-9.7
P. neckeri PL713	-6.6	-8.1
P. neopolydactyla P0309	-6.9	-8.7
P. austroamericana 34390	-9.3	N/A
P. austroamericana 34529	-4.9	-9.3
P. dolichorrhiza 34433	-8.1	N/A
P. dolichorrhiza CR-8	-8.8	-9.6
P. dolichorrhiza CR-4	-8.7	N/A

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 S2. 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

Freezing efficiency of the culture L01-tf-B03 isolated from *P. britannica* JNU22

The freezing spectra shown in Fig. S1 corresponds to Fig. 3 in the main text. Shown in Fig. S1 are the total number of INs active for the lichen 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 $10^{10} g^{-1}$. The relatively low concentration of active bacterial INs highlights the potency of L01-tf-B03 INs compared to *P. syringae* INs.

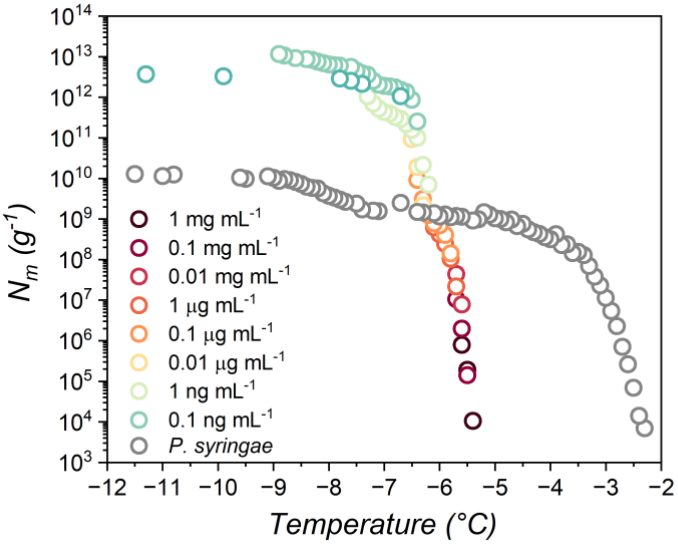

Figure S1. Dilution effects on the IN-activity of L01-tf-B03 and *P. syringae* as shown by the cumulative number of INs per unit mass (N_m) of sample. The symbol colors of L01-tf-B03 indicate data from different dilutions and are identical to the uncolored dilutions shown for *P. syringae*.