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Cultivable halotolerant ice-nucleating bacteria and fungi in coastal precipitation

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SUPPLEMENTARY FIGURES AND TABLES LEGEND

Supplementary Table S1. Identity and characteristics of precipitation and aerosol isolates. Aerosol sampling period labels begin with "A" to distinguish from precipitation sampling periods 1-11 (see Table S3 for precipitation and aerosol sampling times). Taxonomy is denoted by results of BLAST from 16S rRNA gene sequences, percent identity to BLAST assignments, and membership to taxonomic divisions. Isolate identifiers derive from results of multiple sequence alignment analysis (Fig. S2). "IN onset temperature" refers to the first temperature at which isolates exhibited significant IN behavior above the background of the ZoBell media in which they were suspended. 18S fungal sequences were obtained from 16S primers due to coamplification (see Methods Sec. 2.2). Duplicate isolates that were derived from the same sampling period were not featured in Fig. 3a or Fig. 4a and were not counted toward the 14 total identified ice nucleating isolates, but each duplicate was tested for its IN ability and results are shown here.

Supplementary Table S2. Cloud characteristics during 11 precipitation events over the sampling site: SIO Pier (32.8662 °N, 117.2544 °W). Cloud base and top altitudes were estimated using the RH product of the High-Resolution Rapid Refresh real-time atmospheric model, and three altitudes within the cloud were used as particle release points for FLEXPART back-trajectories (see Figure 6.1): cloud top, cloud base, and middle (halfway between). Regions where RH (Relative Humidity) > 95 % were considered for all events except Sampling Period 4 and 5, when a criterion of > 90 % was applied. The hourly products closest in time to the precipitation sampling period were used. When cloud depth and altitudes changed over the sampling period, the lowest cloud base and highest cloud altitude were selected as release points for FLEXPART back-trajectories.

Supplementary Table S3. Sampling periods for precipitation and aerosol samples collected on SIO Pier (32.8662 °N, 117.2544 °W). Synoptic weather conditions for each precipitation event were determined using National Weather Service Weather Prediction Center Surface Analysis Satellite Composite products.

Supplementary Table S4. Results of taxonomic assignment of SILVA Incremental Aligner (SINA) using the SILVA databases determined from 16S rRNA gene sequences.

Supplementary Table S5. Summary of ice nucleation behavior of the 9 isolates that were tested for ice nucleation in the absence of media and resuspended in filtered autoclaved seawater (FASW). In comparison with ice nucleation behavior above ZoBell background levels, ice nucleation behavior was generally enhanced in media-free isolates, except in the case of SSA45, *Psychrobacter* sp. The first temperature at which ice nucleation is significant above background was determined by applying the criterion described in Methods. See Methods and Figure S6 for details of media-free freezing experiment.

Supplementary Figure S1. INP concentrations at -20 °C in aerosol collected at the Scripps Institution of Oceanography Pier (32°52'01.4"N 117°15'26.5"W) during and between precipitation events. INP concentrations in aerosol are represented by blue circles, and pink circles indicate background INP levels in field blanks. Dark blue bars indicate cumulative precipitation over a 24-hr period. (a) Highlighted in grey are three sampling days during which aerosol samples were available immediately before, during and after precipitation events. (b) Precipitation events over March 6 and 7, 2016 (left grey region in Fig. S5a) are magnified for better visibility. (c) The precipitation event from May 5 to May 6, 2016 (right grey region in Fig. S5a) is magnified for better visibility. For all three periods, INP concentrations in aerosol decrease during precipitation events, indicating sweepout of INPs by hydrometeors.

Supplementary Figure S2. Controls of IN measurements. (a) IN activity of 11 ZoBell media blanks. (b) IN activity of selected media–free isolate with a dilution factor of 1 (undiluted). Filtered autoclaved seawater (FASW) was used for resuspension of the isolates for INP measurement. (c) IN activity of selected media–free isolates with a dilution factor of 5. Some isolates were diluted with FASW to decrease opacity such that freezing events could successfully be detected by the camera, and the IN spectra of the isolates and FASW are scaled by the same dilution factor for analysis of the isolate's ice nucleation ability beyond its media (Methods Sec. 2.4). Only INP concentrations that were significantly enhanced above FASW ($p < 0.005$, see Methods Sec. 2.4) are shown in (b) and (c).

Supplementary Figure S3. IN spectra of the 14 IN isolates and their respective ZoBell media samples, with IN spectra of both ZoBell and isolates scaled by the isolates' dilution factor. All isolates were diluted with additional ZoBell to decrease opacity such that freezing events could be detected with the camera. Only INP concentrations that were significantly enhanced above ZoBell ($p < 0.005$) are shown. See Methods section for description of the criterion applied to determine significant ice nucleation behavior above the background ZoBell levels.

Supplementary Figure S4. INP concentrations per liter air sampled for 7 aerosol samples collected at Ellen Browning Scripps Memorial Pier at Scripps Institution of Oceanography (SIO) (32.8662 °N, 117.2544 °W) between March and May 2016. The blue shaded region represents the composite spectrum of INP concentrations observed in a range of marine and coastal environments including the Caribbean, East Pacific and Bering Sea as well as laboratory-generated nascent sea spray (DeMott et al., 2016)*. INP spectra of the three samples from which IN isolates were derived (A1, A2, A5, see Table 1 and S1) are outlined in black. While the INPs observed in aerosol samples compare with INP concentrations in marine environments at warmer temperatures (DeMott et al., 2016), concentrations are enhanced at moderate to cold temperatures indicating terrestrial sources may have additionally contributed.

*DeMott et al., 2016 data has been updated with a completed dataset for the ICE-T study, as shown in (Yang et al., 2020).

Supplementary Figure S5. 10-day back-trajectories from the SIO Pier (32.8662 °N, 117.2544 °W, 8m above MLLW) during the 3 aerosol sampling periods from which IN isolates originated, A1, A2, and A5 (see Tables 1, S1). FLEXPART back-trajectories were used to estimate potential aerosol sources. Shown are the particle centroids of back-trajectories. Origins of particles in the 10-day simulation are shown to range from 4000 m over Russia to 3000 m over the Northeast Pacific. FLEXPART results suggest a dominance of marine particle sources to aerosol samples.

Supplementary Figure S6. Image of aerosol (red box) and precipitation (yellow box) isolates on agar plates. Isolates derived from cultivation-based isolation.

Supplementary Figure S7. Taxonomic distributions of precipitation (a) and aerosol (b) isolates. 83% of the unique families and genera identified in aerosol were common to those found in precipitation.

Supplementary Figure S8. Phylogenetic relationships of isolates (in bold) related to Actinobacteria reference sequences. The environmental source of the reference sequences (based on NCBI metadata) is indicated in grey. Isolates with ice nucleating properties are shaded in yellow; bootstrap values (n=500) are indicated at nodes; scale bar represents changes per positions.

Supplementary Figure S9. Phylogenetic relationships of isolates (in bold) related to Firmicutes reference sequences. The environmental source of the reference sequences based on NCBI metadata is indicated in grey. Isolates with ice nucleating properties are shaded in yellow; bootstrap values (n=500) are indicated at nodes; scale bar represents changes per positions.

Supplementary Figure S10. INP concentrations observed in 14 halotolerant isolates derived from precipitation and aerosol samples, normalized to culture OD (590 nm). Sample numbers in the legend indicate the precipitation or aerosol sample from which the isolate was derived (see Table S3). Datapoints corresponding to isolates from aerosol are outlined in black. Error bars indicate 95% confidence intervals (see Methods Sec. 2.4). Only freezing activity that was significantly enhanced ($p < 0.005$) above ZoBell growth media is shown.

Supplementary Figure S11. Relationship of isolates within the same OTUs using multiple sequence alignments of 16S rRNA gene sequences. Multiple sequence alignments were used to generate phylogenetic trees. The resulting branch distances were used to label isolates within the same OTU. Distances > 0.1 were given a new number. This division was further subdivided by distances > 0.01 which were given a unique letter. Distances < 0.01 were considered possible duplicates. 18S fungal sequences were obtained from 16S primers due to coamplification (see Methods Sec. 2.2). Freezing temperatures are shown in yellow boxes to indicate isolates with detected ice nucleation activity.

The data set supporting this manuscript is hosted by the UCSD Library Digital Collections (https://doi.org/10.6075/J0GQ6W2Z).

Table S1

Isolate ID	Isolate	Sampling Period	IN Onset Temperature °C	BLAST Identity	% Identity	Phylum	Class	Order	Family
Iso1	*Rhodoturula* sp.	1	n/a	*Rhodotorula mucilaginosa*	99%	Basidiomycota	Urediniomycetes	Sporidiales	Sporiodiobolaceae
Iso2	*Cryptococcus* sp. 1	1	-9.25	*Cryptococcus aureus*	100%	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae
Iso3	*Arthrobacter* sp.	2	n/a	*Arthrobacter sp. (A. luteolus, A.citreus)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
Iso5	*Brevibacterium* sp. 1a	2	n/a	*Brevibacterium sp. (B.linens)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae
Iso4	*Curtobacterium* sp. 1	2	n/a	*Curtobacterium sp. (C. pusillum, C. flaccumfaciens, C. oceanosedimentum)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso6	*Lysobacter* sp.	2	n/a	*Lysobacter concretionis*	99%	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae
Iso10A	*Cryptococcus* sp. 2	3	n/a	*Cryptococcus sp. (C. flavescens, C. aureus)*	99%	Basidiomycota	Tremellomycetes	Tremellales	Tremellaceae
Iso10B	*Paenibacillus* sp. 1	3	-14.75	*Paenibacillus sp.*	100%	Firmicutes	Bacilli	Bacillales	Paenibacillaceae
Iso9	*Bacillus* sp. 2a	4	n/a	*Bacillus sp. (B. baekryungensis, B. hwajinpoensis)*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
Iso8	*Brevibacterium* sp. 1b	4	-2.25	*Brevibacterium sp. (B. luteolum)*	97%	Actinobacteria	Actinobacteria	Actinomycetales	Brevibacteriaceae
Iso20	*Bacillus* sp. 1b	5	n/a	*Bacillus sp. (B. baekryungensis, B. hwajinpoensis)*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
Iso12	*Microbacterium* sp. 1	5	n/a	*Microbacterium esteraromaticum*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso19	*Microbacterium* sp. 1	5	n/a	*Microbacterium esteraromaticum*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso7	*Microbacterium* sp. 2a1	5	n/a	*Microbacterium esteraromaticum*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso36A	*Citricoccoccus* sp.	6	n/a	*Citricoccoccus sp. (C. muralis)*	97%	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
Iso40	*Curtobacterium* sp. 2	6	n/a	*Curtobacterium sp.*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso41	*Curtobacterium* sp. 2	6	n/a	*Curtobacterium flaccumfaciens*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso35A	*Planococcus* sp. 2a2	6	n/a	*Planococcus sp.(P. maritimus, P. plakortidis, P. rifietoesis)*	100%	Firmicutes	Bacilli	Bacillales	Planococcaceae
Iso36B	*Planococcus* sp. 2a2	6	n/a	*Planococcus sp.*	100%	Firmicutes	Bacilli	Bacillales	Planococcaceae
Iso37	*Pseudomonas* sp. 1	6	n/a	*Pseudomonas sp. (P. synxantha, P. grimontii, P. extremaustralis)*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Pseudomonadaceae
Iso35B	*Pseudomonas* sp. 2a1	6	n/a	*Pseudomonas veronii*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Pseudomonadaceae
Iso34	*Pseudomonas* sp. 2a2	6	n/a	*Pseudomonas sp. (P. synxantha, P. grimontii, P. extremaustralis)*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Pseudomonadaceae
Iso39	*Pseudomonas* sp. 2a3	6	n/a	*Pseudomonas sp. (P. synxantha, P. mucidolens, P. grimontii)*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Pseudomonadaceae
Iso38B	*Psychrobacter* sp. 1b1	6	n/a	*Psychrobacter sp. (P. maritimus)*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
Iso38A	*Psychrobacter* sp. 1c1	6	n/a	*Psychrobacter sp.*	99%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
Iso32Ap	*Microbacterium* sp. 2a2	7	n/a	*Microbacterium esteraromaticum*	99%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso33By	*Microbacterium* sp. 2a4	7	n/a	*Microbacterium esteraromaticum*	99%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso32B	*Planococcus* sp. 1	7	-12.25	*Planococcus maritimus*	99%	Firmicutes	Bacilli	Bacillales	Planococcaceae
Iso33A	*Planococcus* sp. 2a1	7	n/a	*Planococcus sp.*	99%	Firmicutes	Bacilli	Bacillales	Planococcaceae
Iso33Bp	Unknown	7	n/a	Unknown *Microbacterium sp.*	89%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Iso31	*Bacillus* sp. 1a1	8	-14.5	*Bacillus halmapalus*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
Iso29	*Pantoea* sp. 1a	8	-17	*Pantoea sp. (P. agglomerans, P. ananatis)*	100%	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
Iso30	*Pantoea* sp. 1a	8	-16.75	*Pantoea sp. (P. agglomerans , P. ananatis)*	100%	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
Iso21	*Cellulosimicrobium* sp. 1a1	9	-14	*Cellulosimicrobium sp. (C. funkei, C.cellulans, C. marinum)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae
Iso22	*Cellulosimicrobium* sp. 1a2	9	-15	*Cellulosimicrobium sp. (C. funkei, C.cellulans, C. marinum)*	99%	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae
Iso24B	*Cellulosimicrobium sp.* 1a3	9	n/a	*Cellulosimicrobium sp. (C. funkei, C.cellulans, C. marinum)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae
Iso24A	*Metschnikowia* sp.	9	-16.5	*Metschnikowia sp. (M. zobellii, M. krissii, M. reukaufii)*	99%	Ascomycota	Saccharomycetes	Saccharomycetales	Metschnikowiaceae
Iso23	Unknown	9	-13.25	Unknown *Arthrobacter sp.*	86%	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae
Iso27	*Cellulosimicrobium* sp. 1a3	10	-14.75	*Cellulosimicrobium sp. (C. funkei, C.cellulans, C. marinum)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae
Iso28	*Cellulosimicrobium* sp. 1a3	10	-14.5	*Cellulosimicrobium sp. (C. funkei, C.cellulans, C. marinum)*	100%	Actinobacteria	Actinobacteria	Actinomycetales	Promicromonosporaceae
Iso49	*Psychrobacter* sp. 1b2	11	-13.75	*Psychrobacter sp. (P. pulmonis, P. faecalis)*	99%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
SSA42	*Idiomarina* sp.	A1	-14.25	*Idiomarina fontislapidosi*	100%	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae
SSA14	*Bacillus* sp. 1a2	A2	n/a	*Bacillus sp. (B. aquimaris, B. vietnamensis)*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
SSA15	*Pantoea* sp. 1b	A2	n/a	*Pantoea sp. (P. ananatis, P. stewartii, P. agglomerans)*	100%	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
SSA17	*Pantoea* sp. 1b	A2	n/a	*Pantoea sp. (P. ananatis, P. stewartii, P. agglomerans)*	100%	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
SSA18	*Pantoea* sp. 1b	A2	n/a	*Pantoea sp. (P. ananatis, P. stewartii, P. agglomerans)*	100%	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae
SSA16	*Psychrobacter* sp. 2a	A2	-17.5	*Psychrobacter sp.*	93%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
SSA43	*Bacillus* sp. 2b2	A3	n/a	*Bacillus sp. (B. muralis)*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
SSA44A	*Paenibacillus* sp. 1	A4	n/a	*Paenibacillus sp. (P. tundrae, P. amyloyticus, P. agarideverons, P. taichungensis)*	100%	Firmicutes	Bacilli	Bacillales	Paenibacillus
SSA46	*Bacillus* sp. 2b1	A5	n/a	*Bacillus halmapalus*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
SSA45	*Psychrobacter* sp. 1c2	A5	-14	*Psychrobacter sp.*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
SSA47	*Bacillus* sp. 2b1	A6	n/a	*Bacillus halmapalus*	100%	Firmicutes	Bacilli	Bacillales	Bacillaceae
SSA48	*Psychrobacter* sp. 1a	A6	n/a	*Psychrobacter sp. (P. pulmonis, P. faecalis)*	96%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae
SSA26	*Microbacterium* sp 1	A7	n/a	*Microbacterium esteraromaticum*	99%	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
SSA25	*Psychrobacter* sp. 2b	A7	n/a	*Psychrobacter sp. (P. pulmonis, P. faecalis)*	100%	Proteobacteria	Gammaproteobacteria	Pseudomondales	Moraxellaceae

Table S2

Period	Local date	Local time start	Local time stop	UTC date	UTC time	RH criteria	Temp (K)	Pressure (mb)	Geopotential height (m)
1	3/6/2016	9:07	10:07	3/6/2016	17:07 – 18:07	>95%	283 – 274	950 – 750	800 – 2000
2	3/7/2016	18:30	19:30	3/8/2016	2:30 – 3:30	>95%	275 – 268	850 – 750	1700 – 3000
3	3/11/2016	16:20	17:20	3/12/2016	00:20 – 1:20	>95%	282 – 275	950 – 800	500 – 2200
4	3/12/2016	8:20	9:14	3/12/2016	16:20 – 17:14	> 90%	280 – 278	925 – 900	1000 – 1100
5	3/29/2016	23:35	0:35	3/30/2016	6:35 – 7:35	> 90%	270, 275	800, 900	2000, 700
6	4/7/2016	8:15	11:00	4/7/2016	15:15 – 18:00	> 95%	278 – 270	1000, 750 – 650	2200 – 4000
7	4/7/2016	12:07	13:07	4/7/2016	19:07 – 20:07	> 95%	275 – 265	750 – 600	2000 – 4000
8	5/5/2016	22:59	23:59	5/6/2016	6:00 – 7:00	> 95%	275 – 273	825 – 750	1100 – 2100
9	5/6/2016	2:59	3:59	5/6/2016	10:00 – 11:00	>95%	282 – 270	875 – 700	1000 – 3000
10	5/6/2016	4:59	5:59	5/6/2016	13:00 – 14:00	> 95%	275 – 273	825 – 675	2000 – 3000
11	5/6/2016	8:59	9:59	5/6/2016	17:00 – 18:00	> 95%	275 – 270	825 – 750	1800 – 2800

Table S3

Precipitation

Sampling Period	Local Date	Local Time	UTC Date	UTC time	Number of Isolates	General characteristics
1	3/6/2016	9:07 – 10:07	3/6/2016	17:07 – 18:07	2	frontal rain
2	3/7/2016	18:30 – 19:30	3/8/2016	2:30 – 3:30	4	convective, local updraft rain
3	3/11/2016	16:20 – 17:20	3/12/2016	00:20 – 1:20	2	frontal rain from decaying atmospheric river
4	3/12/2016	8:20 – 9:14	3/12/2016	16:20 – 17:14	2	warm, low cloud rain
5	3/29/2016	23:35 – 0:35	3/30/2016	6:35 – 7:35	4	scattered, low coastal clouds, lack of dynamical system
6	4/7/2016	8:15 – 11:00	4/7/2016	15:15 – 18:00	7	frontal rain from tropical moisture source
7	4/7/2016	12:07 – 13:07	4/7/2016	19:07 – 20:07	4	frontal rain from tropical moisture source
8	5/5/2016	22:59 – 23:59	5/6/2016	6:00 – 7:00	2	pre-frontal rain, meso-scale system
9	5/6/2016	2:59 – 3:59	5/6/2016	10:00 – 11:00	3	post-frontal rain, meso-scale system
10	5/6/2016	4:59 – 5:59	5/6/2016	13:00 – 14:00	1	post-frontal rain, meso-scale system
11	5/6/2016	8:59 – 9:59	5/6/2016	17:00 – 18:00	1	post-frontal rain, meso-scale system

Aerosol

Sampling Period	Local Date	Local Time	UTC Date	UTC time	Number of Isolates
A1	3/5/2016	10:40 – 12:15	3/5/2016	18:40 – 20:15	1
A2	3/7/2016	14:58 – 16:52	3/7/2016	22:58 – 23:52	5
A3	3/8/2016	11:34 – 14:42	3/8/2016	19:34 – 22:42	1
A4	3/28/2016	09:09 – 12:22	3/28/2016	16:09 – 19:22	1
A5	4/28/2016	11:45 – 13:22	4/28/2016	18:45 – 20:22	2
A6	5/5/2016	12:00 – 14:23	5/5/2016	19:00 – 21:23	2
A7	5/6/2016	14:15 – 16:37	5/6/2016	21:15 – 23:37	2

Table S4

Sequence_BLAST IDENTITY	sequence score	bp score	identity	quality	startpos	stoppos	ecolipos	bps	SILVA Lowest Common Ancestor Taxonomy
ISO1_Rhodotorula_mucilaginosa	0.984494	91	98.951	98	11889	27653	513	572	Eukaryota;Opisthokonta;Nucletmycea;Fungi;Dikarya;Basidiomycota;Pucciniomycotina;Microbotryomycetes;Sporidiobolales;
ISO2_Cryptococcus_aureus	0.994595	85	99.811	99	13141	26978	524	529	Eukaryota;Opisthokonta;Nucletmycea;Fungi;Dikarya;Basidiomycota;Agaricomycotina;Tremellomycetes;Tremellales;
ISO3_Arthrobacter_sp	0	97	100	100	13141	27150	524	366	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;
ISO4_Curtobacterium_sp	0	92	100	100	13141	27638	524	381	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Curtobacterium;
ISO5_Brevibacterium_sp_linens	0.992084	98	98.9071	99	13143	27150	525	365	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Brevibacteriaceae;Brevibacterium;
ISO6_Lysobacter_sp	0.980307	101	95.1219	98	13128	27149	519	369	Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Luteimonas;
Iso7_Microbacterium_esteraromaticum	0.99227	95	99.729	99	13141	27155	524	369	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO8_Brevibacterium_sp_luteolum	0.915246	71	93.266	91	10367	25293	508	294	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Brevibacteriaceae;Brevibacterium;
ISO9_Bacillus_sp	0.99212	95	99.455	99	13132	27152	521	367	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
ISO10A_Cryptococcus_sp	0.989263	99	99.4545	98	14958	28464	555	550	Eukaryota;Opisthokonta;Nucletmycea;Fungi;Dikarya;Basidiomycota;Agaricomycotina;Tremellomycetes;Tremellales;
ISO10B_Paenbacillus_sp	0	89	100	100	13141	27173	524	374	Bacteria;Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus;
ISO12_Microbacterium_esteraromaticum	0	104	100	100	13870	28434	535	380	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
SSA13_Paracoccus_marcusii	0	102	100	100	13874	28438	537	378	Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Paracoccus;
SSA14_Bacillus_sp	0.992029	118	99.7215	99	14962	28438	557	359	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
SSA15_Pantoea_sp	0.981347	96	98.9848	98	13874	28475	537	394	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Pantoea;
SSA16_Psychrobacter_sp	0.919103	105	93.8548	91	14958	28431	555	357	Unclassified;
SSA17_Pantoea_sp	0	111	100	100	14286	28449	545	371	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Pantoea;
SSA18_Pantoea_sp	0.981347	96	98.9848	98	13874	28475	537	394	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Pantoea;
ISO19_Microbacterium_esteraromaticum	0	102	100	100	13861	28434	533	382	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO20_Bacillus_sp	0.994949	99	98.9529	99	13874	28451	537	381	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
ISO21_Cellulosimicrobium_sp	0.987932	118	99.4475	98	14962	28450	557	362	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Promicromonosporaceae;Cellulosimicrobium;
ISO22_Cellulosimicrobium_sp	0.753773	74	83.0409	75	14955	34478	553	535	Unclassified;
ISO23_Unknown	0.568338	33	67.8571	56	21333	26161	668	166	Unclassified;
ISO24_Cellulosimicrobium_sp	0	103	100	100	13870	28438	535	382	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Promicromonosporaceae;Cellulosimicrobium;
ISO24A_Metschikowia_sp	0.967483	76	95.8716	96	16525	28476	652	436	Eukaryota;Opisthokonta;Nucletmycea;Fungi;Dikarya;Ascomycota;Saccharomycotina;Saccharomycetes;Saccharomycetales;
ISO24B_Cellulosimicrobium_sp	0	103	100	100	13870	28438	535	382	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Promicromonosporaceae;Cellulosimicrobium;
SSA25_Psychrobacter_sp	0	100	100	100	13870	28450	535	382	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
SSA26_Microbacterium_esteraromaticum	0.97394	93	98.4962	97	13869	28476	535	399	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO27_Cellulosimicrobium_sp	0	102	100	100	13869	28438	535	383	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Promicromonosporaceae;Cellulosimicrobium;
ISO29_Pantoea_sp	0.968582	117	97.8022	96	14952	28438	552	364	Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Pantoea;
ISO31_Bacillus_sp	0	118	100	100	14965	28434	559	355	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
ISO32Ap_Microbacterium_esteraromaticum	0.991482	89	99.7015	99	13874	26858	537	335	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO32Ay_Microbacterium_esteraromaticum	0.991369	88	99.7151	99	13146	26919	526	350	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO32B_Planococcus_sp_maritimus	0.992269	94	98.9218	99	13143	27163	525	371	Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;
ISO33A_Planococcus_sp	0.996495	101	99.2188	99	13860	28438	533	384	Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;
ISO33BpR_Unknown	0.75437	63	79.661	75	13146	25494	526	295	Unclassified;
ISO33By_Microbacterium_esteraromaticum	0.988208	95	99.4595	98	13151	27165	528	370	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Microbacterium;
ISO34_Pseudomonas_sp	0	100	100	100	13870	27655	535	372	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;
ISO35A_Planococcus_sp	0	119	100	100	14978	28438	564	353	Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;
ISO35B_Pseudomonas_veronii	0.989209	92	99.6416	98	15875	26919	595	278	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;
ISO36A_Citriococcus	0.946717	84	96.793	94	13151	26856	528	342	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Micrococcaceae;Citricoccus;
ISO36B_Planococcus_sp	0.983258	118	98.892	98	14961	28449	556	360	Bacteria;Firmicutes;Bacilli;Bacillales;Planococcaceae;
ISO37_Pseudomonas_sp	0	98	100	100	13146	27152	526	363	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;
ISO38A_Psychrobacter_sp	0.996442	93	99.2063	99	13143	27638	525	378	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
ISO38B_Psychrobacter_sp	0	98	100	100	13128	27150	519	369	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
ISO39_Pseudomonas_sp	0.996323	115	99.7245	99	14948	28434	551	363	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;
ISO40_Curtobacterium_sp	0.974701	108	98.3957	97	14965	28476	559	374	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Curtobacterium;
ISO41_Curtobacterium_sp	0.99151	92	99.7207	99	13143	26988	525	357	Bacteria;Actinobacteria;Actinobacteria;Micrococcales;Microbacteriaceae;Curtobacterium;
SSA42_Idiomarina_sp	0.991886	87	99.7159	99	13143	26976	525	352	Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae;Idiomarina;
SSA43_Bacillus_sp	0.996471	92	99.4751	99	13141	27645	524	381	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
SSA44A_Paenbacillus_sp	0	96	100	100	13874	27638	537	366	Bacteria;Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus;
SSA44B_Paenbacillus_sp	0	112	100	100	14973	27645	561	342	Bacteria;Firmicutes;Bacilli;Bacillales;Paenibacillaceae;Paenibacillus;
SSA45_Psychrobacter_sp	0.992322	95	99.1914	99	13132	27157	521	371	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
SSA46_Bacillus_sp	0	90	100	100	13143	27584	525	378	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
SSA47_Bacillus_sp	0.992261	95	99.7283	99	13134	27152	522	368	Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;
SSA48_Psychrobacter_sp	0.937055	78	95.9459	93	13151	25502	528	296	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
ISO49_Psychrobacter_sp	0.984997	98	98.9796	98	11887	27645	512	392	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;
									57 species (93%)
									4 unclassified (7%)

Table S5

Isolate	Species	ZoBell		FASW	
		Ice nucleation above background?	First Significant Freezing Temperature (°C)	Ice nucleation above background?	First Significant Freezing Temperature (°C)
Iso3	*Arthrobacter* sp. (*A. luteolus*, *A.citreus*)	no	n/a	yes	-15.25
Iso4	*Curtobacterium* sp. (*C. pusillum*, *C. flaccumfaciens*, *C. oceanosedimentum*)	no	n/a	yes	-13
Iso5	*Brevibacterium* sp. (*B. linens*)	no	n/a	yes	-11.75
Iso30	*Pantoea* sp. (*P. agglomerans*, *P. ananatis*)	yes	-16.75	yes	-15.5
SSA42	*Idiomarina fontislapidosi*	yes	-14.25	yes	-9.75
SSA16	*Psychrobacter* sp.	yes	-17.5	yes	-15.5
SSA18	*Pantoea* sp. (*P. ananatis*, *P. stewartii*, *P. agglomerans*)	no	n/a	yes	-11.5
SSA45	*Psychrobacter* sp.	yes	-14	no	n/a
SSA26	*Microbacterium esteraromaticum*	no	n/a	no	n/a

Figure S1

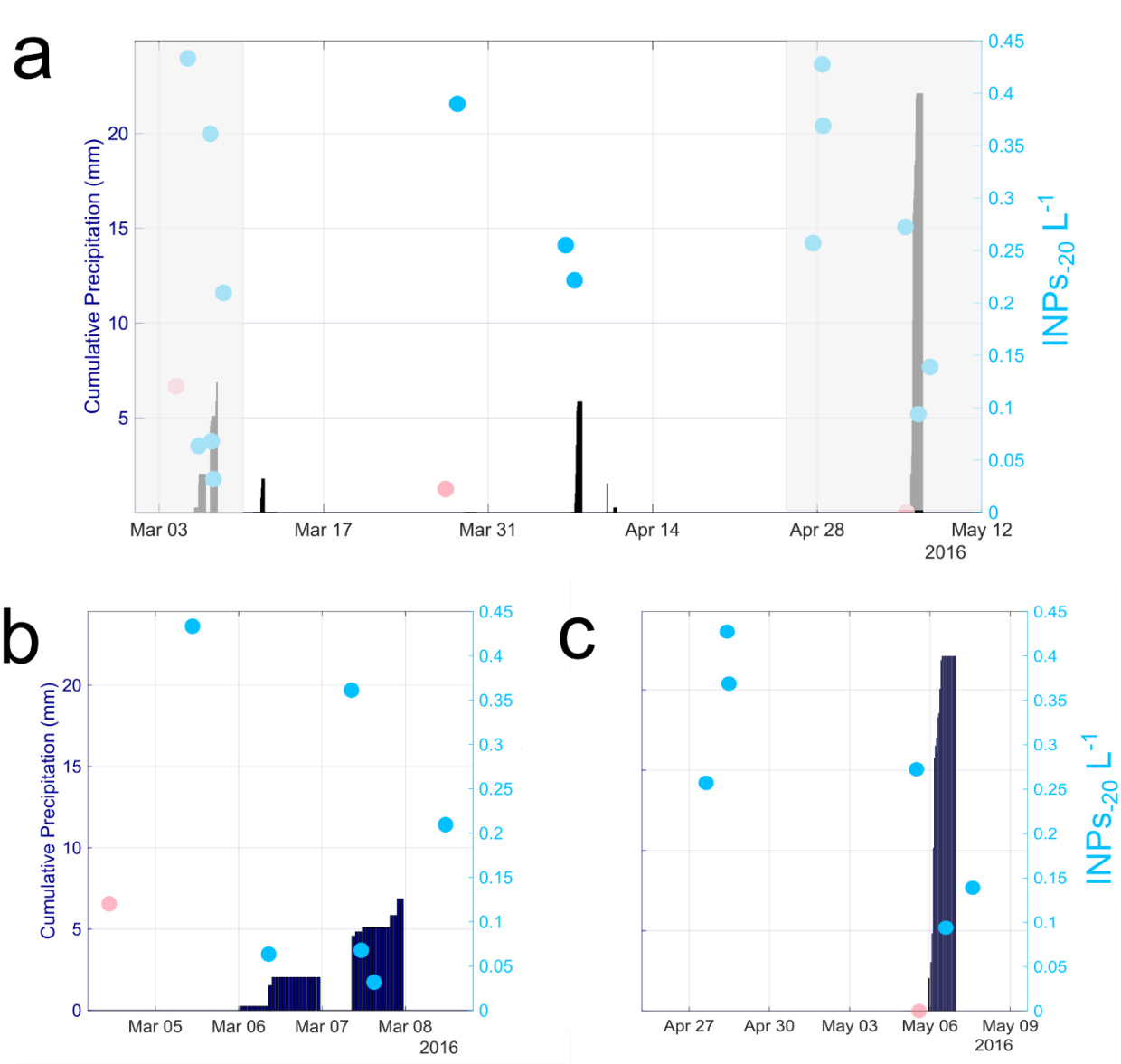

a
b
c
Cumulative Precipitation (mm)
INPs-20 L-1
Mar 03
Mar 17
Mar 31
Apr 14
Apr 28
May 12
2016
Mar 05
Mar 06
Mar 07
Mar 08
Apr 27
Apr 30
May 03
May 06
May 09

Figure S2

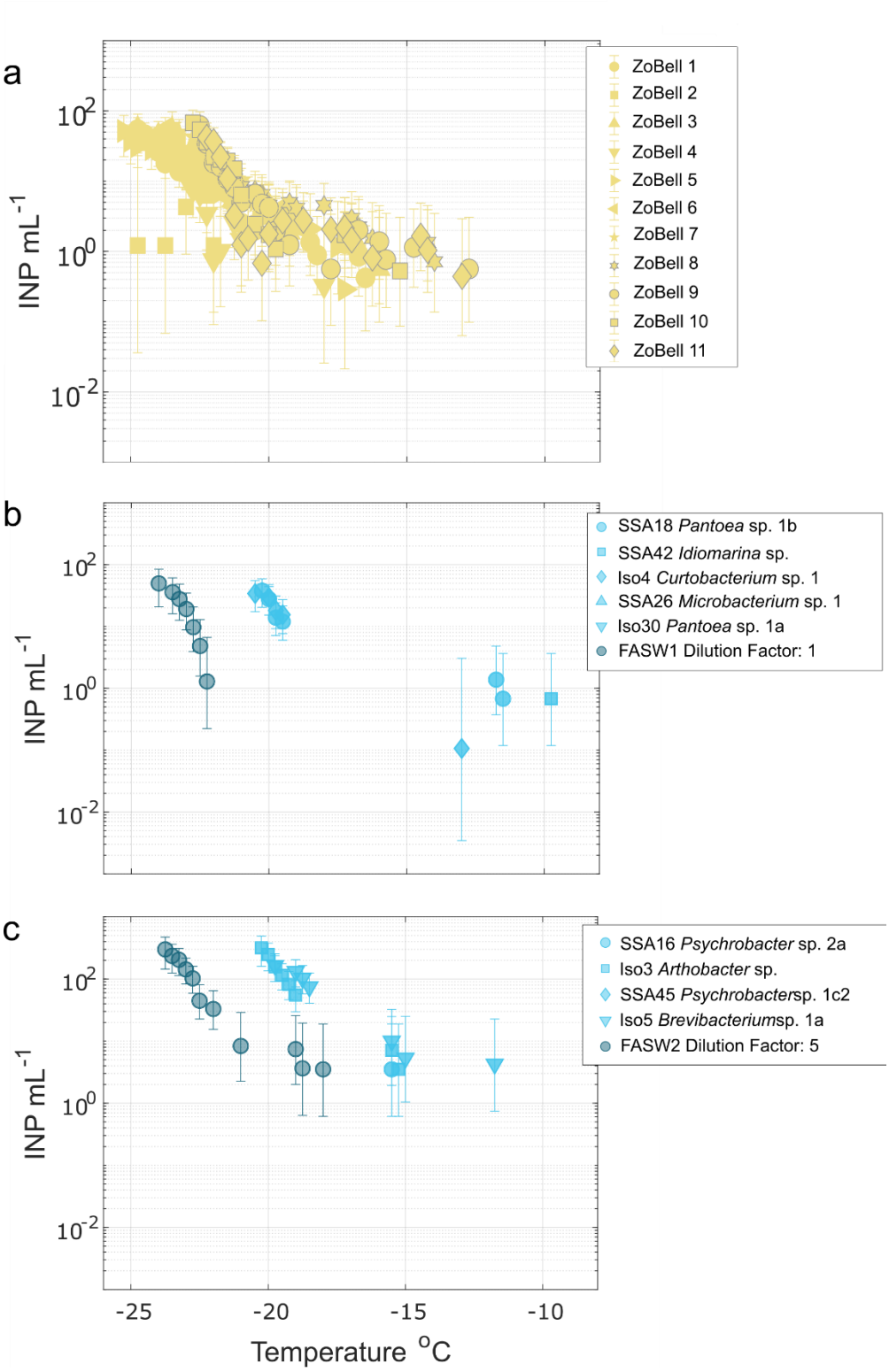

a
b
c
INP mL-1
10^2
10^0
10^-2
ZoBell 1
ZoBell 2
ZoBell 3
ZoBell 4
ZoBell 5
ZoBell 6
ZoBell 7
ZoBell 8
ZoBell 9
ZoBell 10
ZoBell 11
SSA18 Pantoea sp. 1b
SSA42 Idiomarina sp.
Iso4 Curtobacterium sp. 1
SSA26 Microbacterium sp. 1
Iso30 Pantoea sp. 1a
FASW1 Dilution Factor: 1
SSA16 Psychrobacter sp. 2a
Iso3 Arthobacter sp.
SSA45 Psychrobactersp. 1c2
Iso5 Brevibacteriumsp. 1a
FASW2 Dilution Factor: 5
-25
-20
-15
-10
Temperature °C

Figure S3

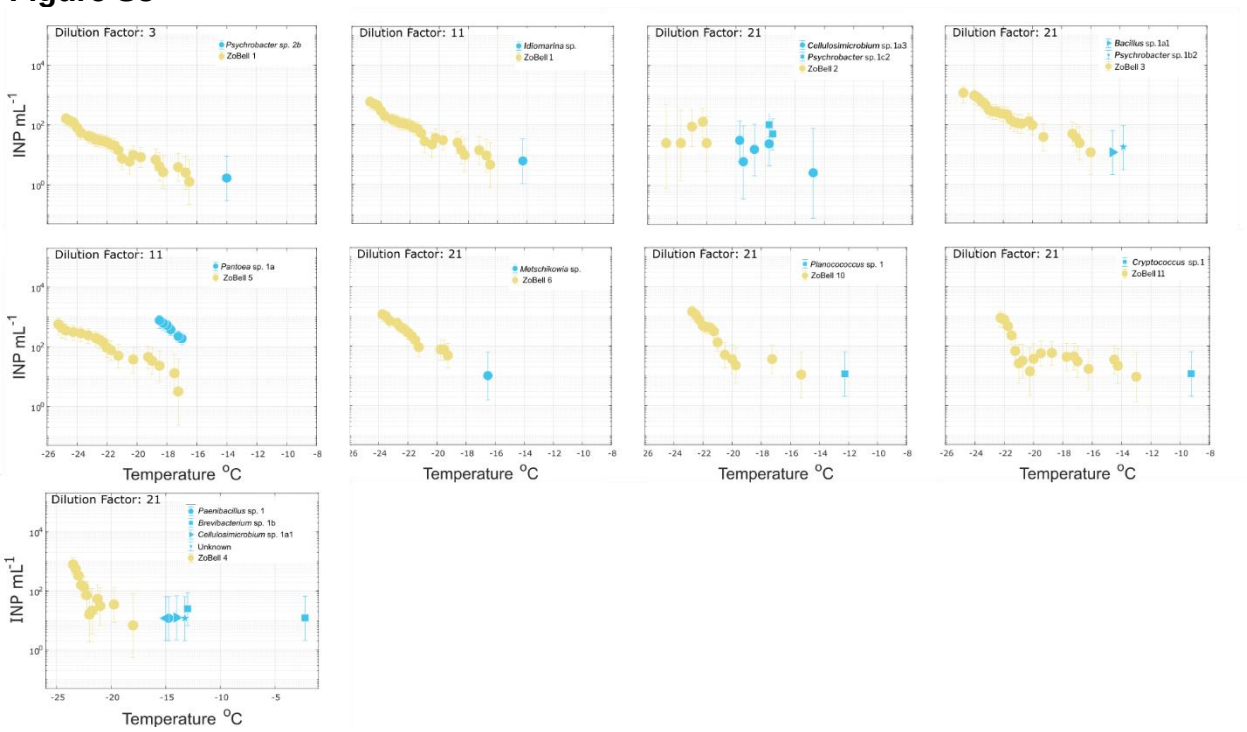

Dilution Factor: 3
Psychrobacter sp. 2b
ZoBell 1
Dilution Factor: 11
Idiomarina sp.
ZoBell 1
Dilution Factor: 21
Cellulosimicrobium sp. 1a3
Psychrobacter sp. 1c2
ZoBell 2
Dilution Factor: 21
Bacillus sp. 1a1
Psychrobacter sp. 1b2
ZoBell 3
Dilution Factor: 11
Pantoea sp. 1a
ZoBell 5
Dilution Factor: 21
Metschikowia sp.
ZoBell 6
Dilution Factor: 21
Planococcus sp. 1
ZoBell 10
Dilution Factor: 21
Cryptococcus sp. 1
ZoBell 11
Dilution Factor: 21
Paenibacillus sp. 1
Brevibacterium sp. 1b
Cellulosimicrobium sp. 1a1
Unknown
ZoBell 4
INP mL-1
Temperature °C

Figure S4

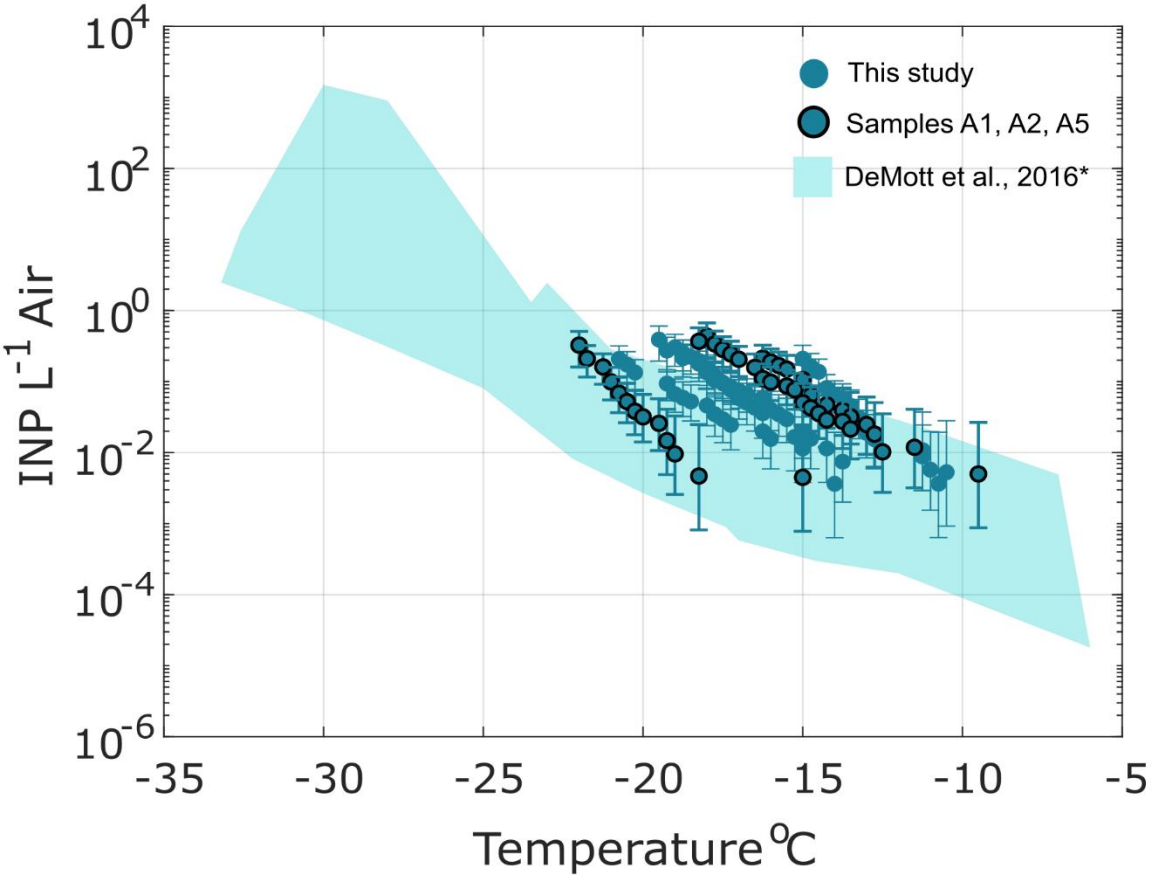

This study
Samples A1, A2, A5
DeMott et al., 2016*
INP L-1 Air
Temperature °C
10^4
10^2
10^0
10^-2
10^-4
10^-6
-35
-30
-25
-20
-15
-10
-5

Figure S5

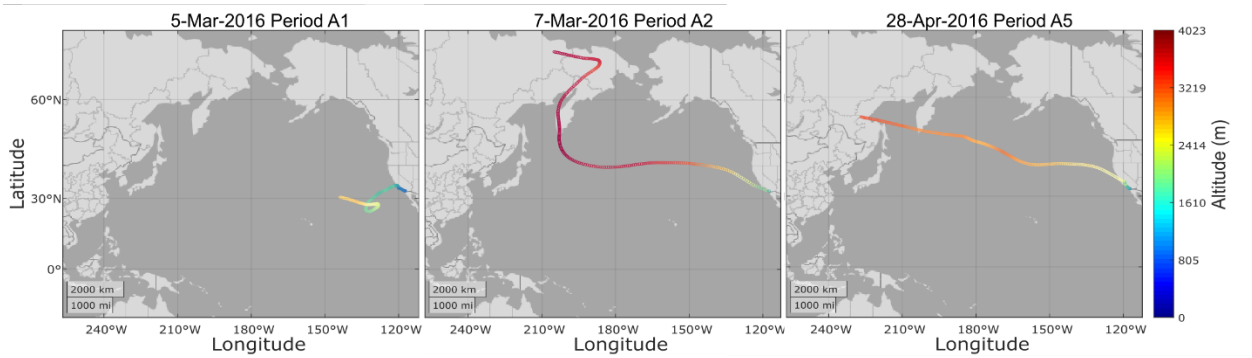

5-Mar-2016 Period A1
7-Mar-2016 Period A2
28-Apr-2016 Period A5
Latitude
60°N
30°N
0°
2000 km
1000 mi
240°W
210°W
180°W
150°W
120°W
Longitude
Altitude (m)
4023
3219
2414
1610
805
0

Figure S6

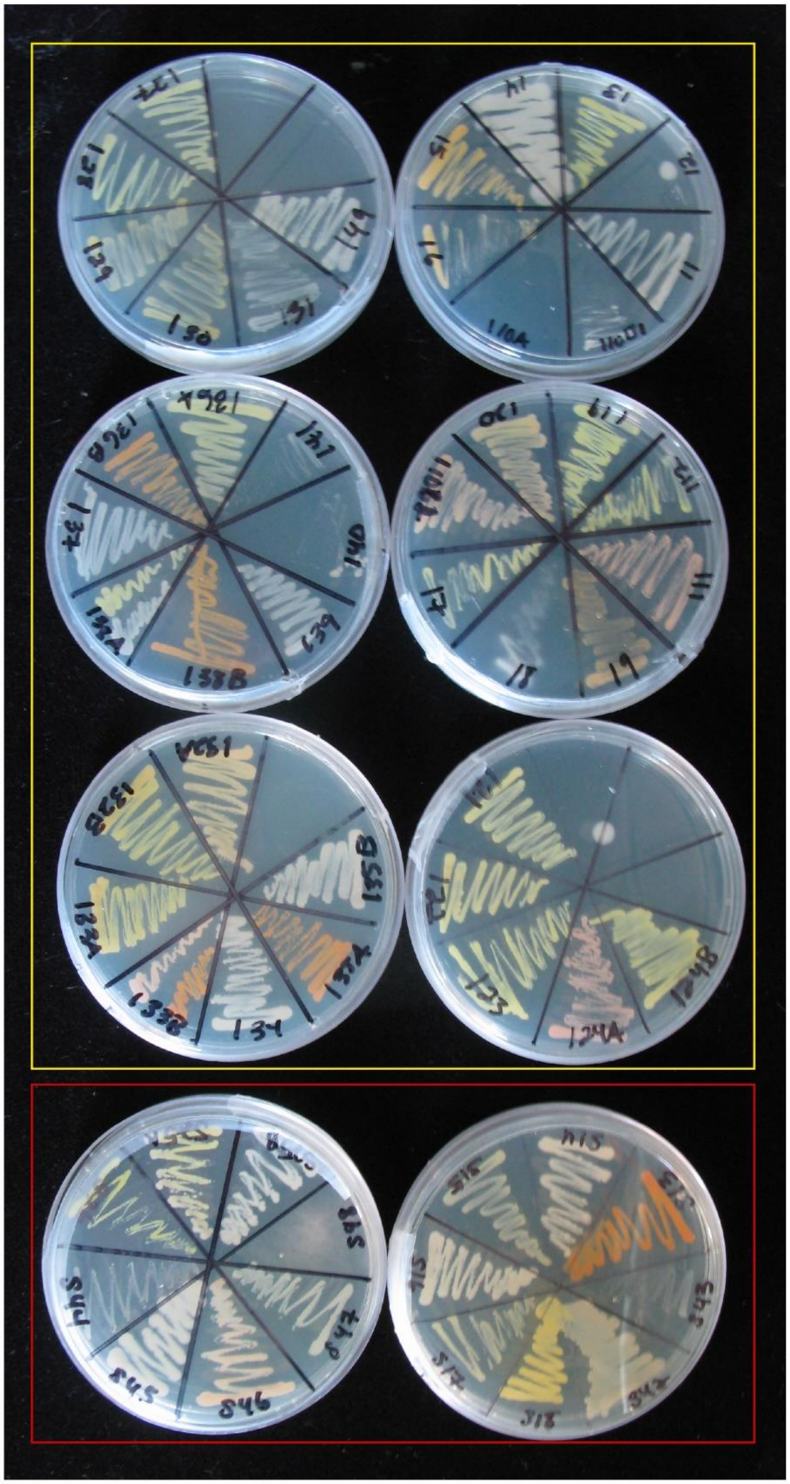


Figure S7

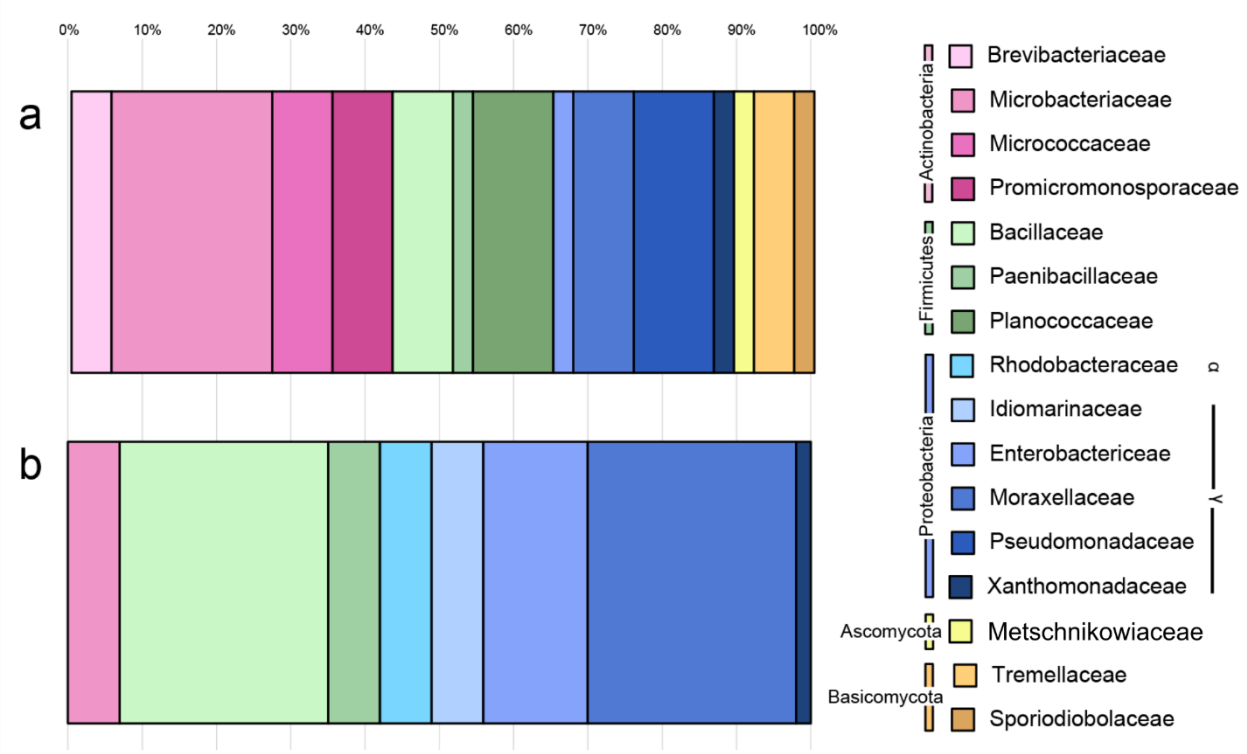
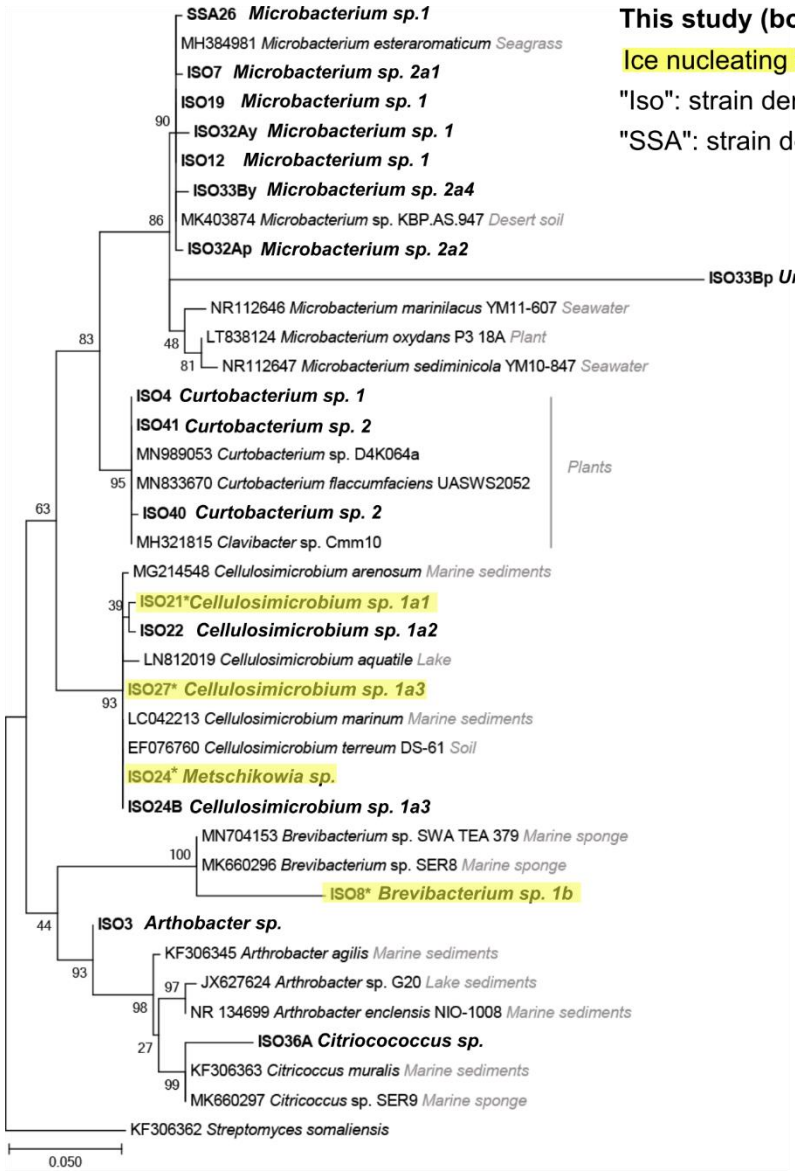

0%
10%
20%
30%
40%
50%
60%
70%
80%
90%
100%
a
b
Actinobacteria
Firmicutes
Proteobacteria
Ascomycota
Basicomycota
Brevibacteriaceae
Microbacteriaceae
Micrococcaceae
Promicromonosporaceae
Bacillaceae
Paenibacillaceae
Planococcaceae
Rhodobacteraceae
Idiomarinaceae
Enterobactericeae
Moraxellaceae
Pseudomonadaceae
Xanthomonadaceae
Metschnikowiaceae
Tremellaceae
Sporiodiobolaceae
α
γ

Figure S8



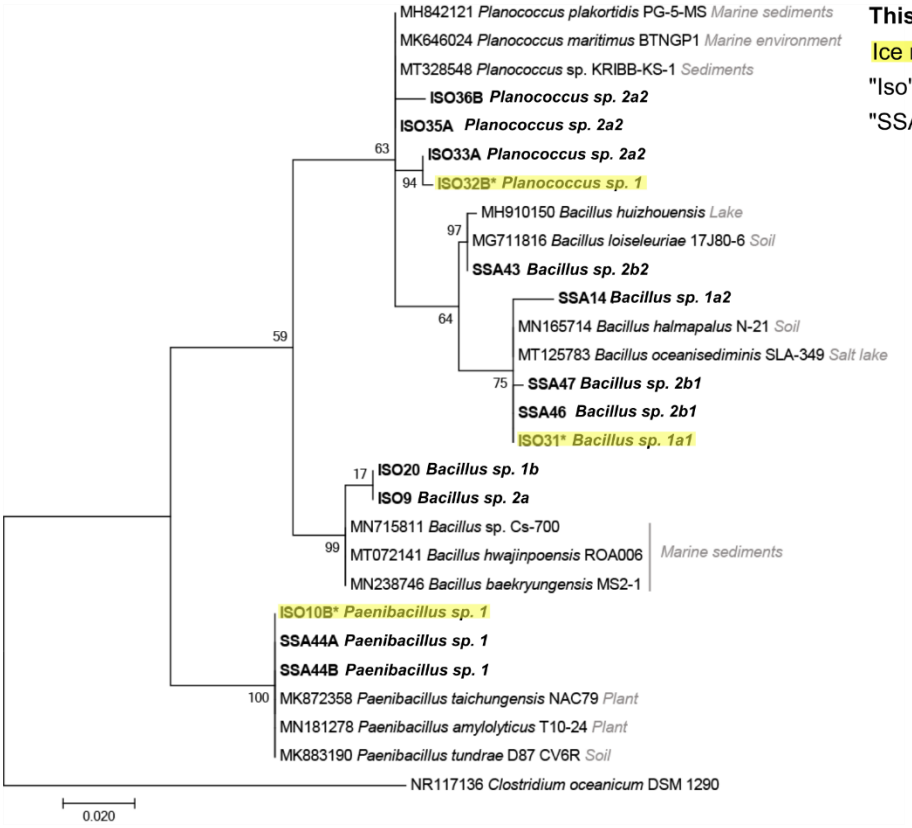
This study (bold)

Ice nucleating strains

"Iso": strain derived from precipitation

"SSA": strain derived from aerosol

Figure S9



This study (bold)

Ice nucleating strains

"Iso": strain derived from precipitation

"SSA": strain derived from aerosol

Figure S10

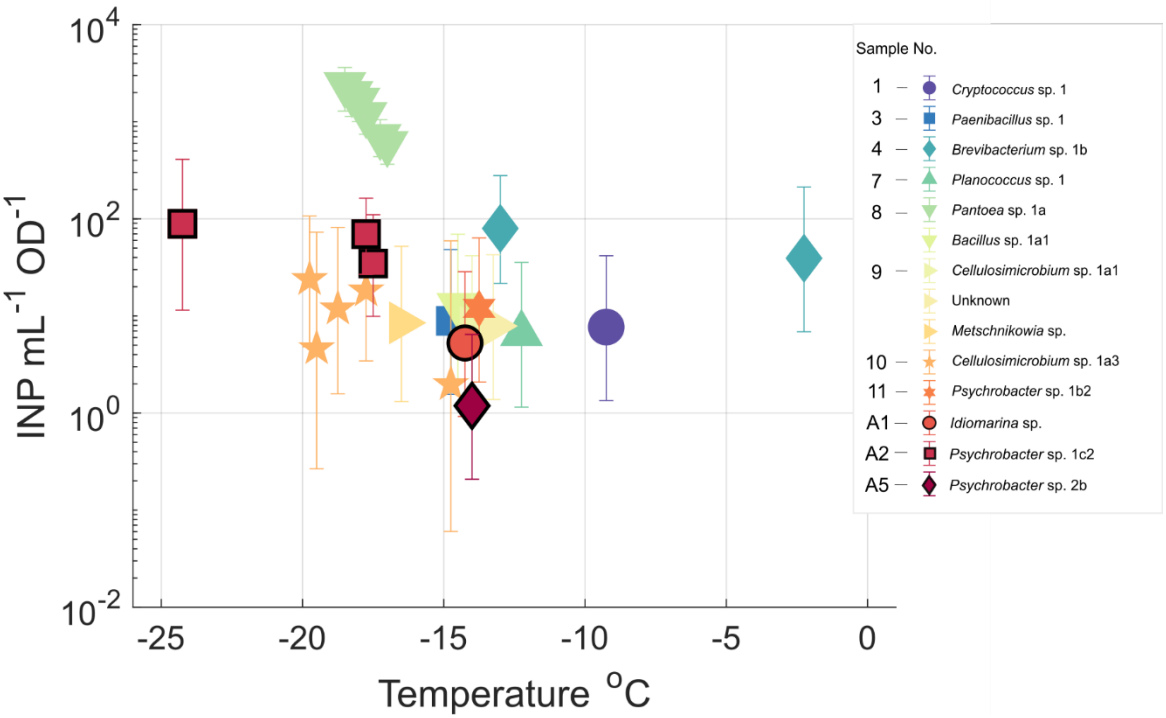
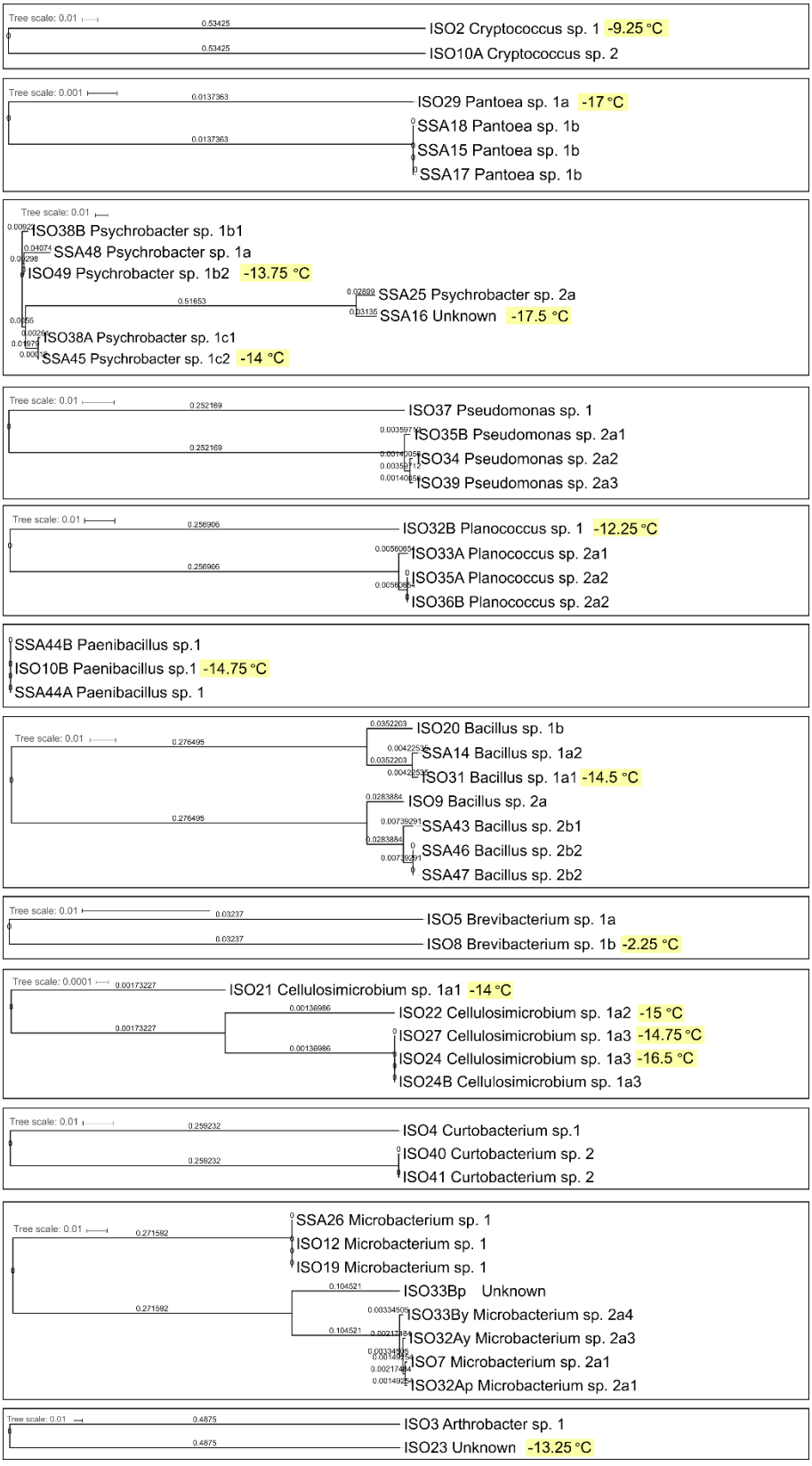

INP mL-1 OD-1
10^4
10^2
10^0
10^-2
-25
-20
-15
-10
-5
0
Temperature °C
Sample No.
1 – Cryptococcus sp. 1
3 – Paenibacillus sp. 1
4 – Brevibacterium sp. 1b
7 – Planococcus sp. 1
8 – Pantoea sp. 1a
Bacillus sp. 1a1
9 – Cellulosimicrobium sp. 1a1
Unknown
Metschnikowia sp.
10 – Cellulosimicrobium sp. 1a3
11 – Psychrobacter sp. 1b2
A1 – Idiomarina sp.
A2 – Psychrobacter sp. 1c2
A5 – Psychrobacter sp. 2b

Figure S11


Tree scale: 0.01
0.53425
0.53425
ISO2 Cryptococcus sp. 1 -9.25 °C
ISO10A Cryptococcus sp. 2
Tree scale: 0.001
0.0137363
0.0137363
ISO29 Pantoea sp. 1a -17 °C
SSA18 Pantoea sp. 1b
SSA15 Pantoea sp. 1b
SSA17 Pantoea sp. 1b
Tree scale: 0.01
ISO38B Psychrobacter sp. 1b1
SSA48 Psychrobacter sp. 1a
ISO49 Psychrobacter sp. 1b2 -13.75 °C
0.51653
SSA25 Psychrobacter sp. 2a
SSA16 Unknown -17.5 °C
ISO38A Psychrobacter sp. 1c1
SSA45 Psychrobacter sp. 1c2 -14 °C
Tree scale: 0.01
0.252169
0.252169
ISO37 Pseudomonas sp. 1
ISO35B Pseudomonas sp. 2a1
ISO34 Pseudomonas sp. 2a2
ISO39 Pseudomonas sp. 2a3
Tree scale: 0.01
0.256906
0.256906
ISO32B Planococcus sp. 1 -12.25 °C
ISO33A Planococcus sp. 2a1
ISO35A Planococcus sp. 2a2
ISO36B Planococcus sp. 2a2
SSA44B Paenibacillus sp.1
ISO10B Paenibacillus sp.1 -14.75 °C
SSA44A Paenibacillus sp. 1
Tree scale: 0.01
0.276495
0.276495
ISO20 Bacillus sp. 1b
SSA14 Bacillus sp. 1a2
ISO31 Bacillus sp. 1a1 -14.5 °C
ISO9 Bacillus sp. 2a
SSA43 Bacillus sp. 2b1
SSA46 Bacillus sp. 2b2
SSA47 Bacillus sp. 2b2
Tree scale: 0.01
0.03237
0.03237
ISO5 Brevibacterium sp. 1a
ISO8 Brevibacterium sp. 1b -2.25 °C
Tree scale: 0.0001
0.00173227
0.00173227
0.00136986
0.00136986
ISO21 Cellulosimicrobium sp. 1a1 -14 °C
ISO22 Cellulosimicrobium sp. 1a2 -15 °C
ISO27 Cellulosimicrobium sp. 1a3 -14.75 °C
ISO24 Cellulosimicrobium sp. 1a3 -16.5 °C
ISO24B Cellulosimicrobium sp. 1a3
Tree scale: 0.01
0.259232
0.259232
ISO4 Curtobacterium sp.1
ISO40 Curtobacterium sp. 2
ISO41 Curtobacterium sp. 2
Tree scale: 0.01
0.271592
0.271592
0.104521
0.104521
SSA26 Microbacterium sp. 1
ISO12 Microbacterium sp. 1
ISO19 Microbacterium sp. 1
ISO33Bp Unknown
ISO33By Microbacterium sp. 2a4
ISO32Ay Microbacterium sp. 2a3
ISO7 Microbacterium sp. 2a1
ISO32Ap Microbacterium sp. 2a1
Tree scale: 0.01
0.4875
0.4875
ISO3 Arthrobacter sp. 1
ISO23 Unknown -13.25 °C