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ORIGINAL PAPER

Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria

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Abstract The diversity and physiological characteristics of culturable bacteria associated with lichens from different habitats of the Arctic and Antarctica were investigated. The 68 retrieved isolates could be grouped on the basis of their 16S rRNA gene sequences into 26 phylotypes affiliated with the phyla *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, and *Firmicutes* and with the classes *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. Isolates belonging to the *Alphaproteobacteria* were the most abundant, followed by those belonging to *Actinobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Deinococcus-Thermus*. Phylogenetic analysis showed that approximately 21 % of the total isolates represented a potentially novel species or genus (≤97 % sequence similarity). Strains belonging to the genera *Sphingomonas*, *Frondihabitans*, *Hymenobacter*, and *Burkholderia* were recovered from lichen samples from both geographic locations, implying common and important bacterial functions within lichens. Extracellular protease activities were detected in six isolates, affiliated with *Burkholderia*, *Frondihabitans*, *Hymenobacter*, *Pseudomonas*, and *Rhodanobacter*. Extracellular lipase activities were detected in 37 isolates of the genera *Burkholderia*, *Deinococcus*, *Frondihabitans*, *Pseudomonas*, *Rhodanobacter*, *Sphingomonas*, and *Subtercola*. This is the first report on the culturable bacterial diversity present within lichens from Arctic and Antarctica and the isolates described herein are valuable resources to decode the functional and ecological roles of bacteria within lichens. In addition, the low similarity (≤97 %) of the recovered isolates to known species and their production of cold-active enzymes together suggest that lichens are noteworthy sources of novel bacterial strains for use in biotechnological applications.

Keywords Lichens · Endophytes · Cultivation · Polar areas · Cold-adapted enzymes

Introduction

Lichens are a symbiotic microsystem between fungi and photosynthetic algae or cyanobacteria (Kappen 2000). Most lichens are extremely tolerant to desiccation, low temperature surviving for months to years in a state of cryptobiosis, and UV radiation, because many of their diverse secondary metabolites act as UV filters (Grube and Berg 2009). Although the mechanisms by which lichens tolerate extreme stress have been poorly investigated, it is generally assumed that it relies on the physiological integration of the symbionts (Selbmann et al. 2010). As a successful life strategy for survival under extreme or unfavorable conditions, lichen symbiosis may partially explain the wide distribution of lichens in all terrestrial ecosystems of the planet, including extreme environments such as polar and alpine areas as well as deserts, and their ability to colonize a wide range of substrates within these habitats (Kappen 2000).

Lichens also provide stable microenvironments for bacterial colonization and the presence of lichen-associated bacteria as an additional and integral component of lichen symbiosis has been accordingly proposed (Selbmann et al.

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Table 1 Lichens used in this study, sampling sites, and number of bacterial isolates recovered from each lichen

Sample information			Sampling information			Isolation
Sample no.	Sample ID[a]	Scientific name	Latitude	Longitude	Locality	Bacterial isolates (n)
LYM100206-16	A	*Usnea* sp.	62°14.063′S	58°46.921′W	Antarctic	5
LYM100206-36	B	*Cladonia borealis*	62°13.563′S	58°47.014′W	Antarctic	10
LYM100206-39	C	*Psoroma* sp.	62°13.563′S	58°47.014′W	Antarctic	26
KEH100713-04	D	*Stereocaulon* sp.	78°54.738′N	11°57.278′E	Arctic	7
KEH100713-07	E	*Cladonia borealis*	78°54.726′N	11°57.167′E	Arctic	7
KEH100713-08	F	*Umbilicaria* sp.	78°54.720′N	11°57.086′E	Arctic	4
KEH100713-11	G	*Cetraria* sp.	78°54.733′N	11°57.784′E	Arctic	1
KEH100717-01	H	*Cladonia* sp.	78°54.661′N	11°56.695′E	Arctic	3
KEH100717-06	I	*Ochrolechia* sp.	78°54.684′N	11°56.927′E	Arctic	5

[a] Sample ID was designated from A to I

2010; Bates et al. 2011). In previous studies, high bacterial diversity and abundance in lichens were revealed by culture-dependent or culture-independent approaches, although the bacterial phylogenetic groups that predominated differed according to the applied methods or lichen specimens used (Cardinale et al. 2006, 2008; Grube and Berg 2009; Selbmann et al. 2010; Bates et al. 2011). Several putative functional roles of bacteria in lichens, such as nitrogen fixation, defense against lichen pathogens and feeders, degradation of lichen thalli to facilitate biomass mobilization, and growth-promoting effects by the production of hormones, have been suggested (Grube and Berg 2009). However, the functional roles of lichen-associated bacteria are still largely unknown.

Microbial strains have been isolated from polar areas with harsh environments, characterized by low nutrient concentrations, low temperatures, extreme variability in day length, and strong solar UV radiation exposure. Because of their adaptive properties, these strains are valuable resources in ecological studies and in biotechnology applications (Lee et al. 2012). Among the distinct habitats of polar areas for microbial residence, lichens, as stable habitats for bacterial colonization, have not been studied, in contrast to sea ice, permafrost, oceanic water, and lakes. Since endophytes are considered to be highly promising, but as yet barely exploited microbial resources (Wang and Dai 2011), the discovery of novel bacterial strains from lichens would similarly be of great interest. Thus, in this study, we present the taxonomic affiliations of bacterial isolates recovered from Antarctic and Arctic lichens to gain insights into the nature of the culturable bacterial diversity within these extreme environments. In addition, the physiological characteristics of the isolates were investigated. Our results provide further evidence that lichens from polar areas provide microniches for novel and psychrophilic bacterial strains with potential applications in biotechnology processes.

Materials and methods

Sample collection and sampling sites

Three samples, *Usnea* sp., *Cladonia borealis,* and *Psoroma* sp., were collected from King George Island, Antarctica, and six samples, *Stereocaulon* sp., *Cladonia borealis*, *Umbilicaria* sp., *Cetraria* sp., *Cladonia* sp., and *Ochrolechia* sp., from Svalbard Archipelago, in the Arctic Ocean, all in 2011 (Table 1 and Fig. S1). A chisel was used to obtain the samples, which were transported at room temperature for 2–3 days to the laboratory in Korea, where they were preserved at 4 °C until use.

Isolation of bacterial strains

Washing method for isolation of putative endophytic bacteria from Antarctic and Artic lichens was different. Antarctic lichens were isolated by immersing the specimens in 1 ml of sterile distilled water in Petri dishes for 1 min and repeating this step four times (Liba et al. 2006). For Arctic lichens, the specimens were washed for 10 min in 1 ml of 0.85 % NaCl by vortexing in a Multi-EP tube vortexer (FinePCR, Gunpo, Korea) followed by centrifugation at 10,000 rpm (Eppendorf, USA) for 5 min, discarding the supernatant. The process was repeated four times. After the final wash, the samples were crushed in a TissuLyzer II containing steel beads (Qiagen, Germany) twice for 2 min. One hundred microliters of the final suspension was then spread on R2A (BD, Sparks, MD) solid media for oligotrophic bacteria isolation, ISP 4 (BD) solid media for isolation of Actinobacterial strains, and MY (BD) solid media for fungi and bacteria together. The plates were incubated at 4 °C for 10–48 days until fungal growth did not disturb bacterial growth. After the incubation, bacterial colonies from the agar plates were picked on the basis of their morphology and subcultured in fresh agar medium three or

more times, until pure isolates were obtained. Pure cultures of the bacterial isolates were deposited in the Polar and Alpine Microbial Collection of the Korea Polar Research Institute (PAMC, Lee et al. 2012).

Physiological characterization

Cell suspensions were prepared by adding a half-full loop (5 mm diameter) of cells from the agar plates to 200 µl of 0.85 % NaCl with vigorous shaking using a vortex mixer. Two hundred microlitre of the suspension was then transferred to 96-well plates for replica plating. The optimal growth temperature and the production of extracellular proteases and lipases were determined according to the methods of Lee et al. (2012). For the former, cell suspensions were replica plated with a 96-pin replicator (VP-408B, V&P Scientific, San Diego, CA) and then incubated for 7 days at 4, 10, 15, 20, 25, 30, or 37 °C. Growth was evaluated by scoring the size and turbidity of the colonies, as described by Lee et al. (2012). Protease and lipase secretion was examined by replica plating of the cell suspensions onto 0.1× R2A plates supplemented with 1 % skim milk (BD) or 1 % tributyrate (Sigma, St. Louis, MO), respectively. Most of the isolates grew well during 7 days incubation. Thus, the plates were incubated for 7 days at 4, 10, and 20 °C, respectively. Enzyme secretion was scored based on the ratio of colony size to the width of the clear zone surrounding the colony.

Identification of bacterial isolates

Bacterial strains were identified based on sequence similarities and phylogenetic analyses of their 16S rRNA gene sequences. The 16S rRNA gene was PCR-amplified from a single colony of pure cultures with two universal primers, 27F; 5′-AGA GTT TGA TCM TGG CTC AG-3′ and 1492R; 5′-GGT TAC CTT GTT ACG ACT T-3′, as described by Lane (1991). PCR was carried out using the method described by Lee et al. (2012). The PCR products were purified using the LaboPass PCR purification kit (Cosmogenetech, Seoul, Korea) and sequenced with the same primers used for amplification. The sequence of the 16S rRNA gene was compared with that of type strains available in the EzTaxon-e database (Kim et al. 2012) to find closely related species and to choose reference sequences for the phylogenetic analyses. Phylogenetic trees were reconstructed by the neighbor-joining method (Saitou and Nei 1987) based on the distance matrix generated according to the Kimura's two-parameter model (Kimura 1980) and using phydit version 3.2 (http://plaza.snu.ac.kr/~jchun/phydit/). The confidence level of the tree topology was evaluated by bootstrap analysis using 1,000 sequence

Table 2 Distribution of bacterial isolates according to lichen

Phylum or class	Species name[a]	Bacterial isolates (n)	Sample ID[b]
Actinobacteria	*Frondihabitans* sp.	3	A, B, D
	Frondihabitans sucicola	6	A, B, E
	Microbacteriaceae sp. [1]	1	C
	Microbacteriaceae sp. [2]	3	D
	Nakamurella panacisegetis	1	C
	Streptomyces anulatus	1	C
	Subtercola boreus	2	D, E
Bacteroidetes	*Hymenobacter* sp. [1]	3	C
	Hymenobacter sp. [2]	1	D
Deinococcus-Thermus	*Deinococcus* sp.	1	A
Firmicutes	*Paenibacillus* sp.	2	C
Alphaproteobacteria	*Acetobacteraceae* sp.	2	B
	Aurantimonas sp.	1	A
	Methyloferula sp.	1	C
	Sphingomonas glacialis	1	I
	Sphingomonas paucimobilis	3	B
	Sphingomonas sp. [1]	1	A
	Sphingomonas sp. [2]	2	C
	Sphingomonas sp. [3]	5	F, G, I
	Sphingomonas sp. [4]	2	B
Betaproteobacteria	*Burkholderia sordidicola*	15	C, D, E, F, H, I
Gammaproteobacteria	*Pseudomonas* sp.	1	F
	Psychrobacter sp.	1	B
	Rhodanobacter sp. [1]	1	C
	Rhodanobacter sp. [2]	6	C
	Rhodanobacter sp. [3]	2	C

[a] Species name was determined when the isolate formed a monophyletic group with reference species and had a 98.5 % or higher similarity

[b] Sample represents the lichens from which the bacterial strains were isolated. Sample ID was coded as follows: A: *Usnea* sp., B: *Cladonia borealis*, C: *Psoroma* sp., D: *Stereocaulon* sp., E: *Cladonia borealis*, F: *Umbilicaria* sp., G: *Cetraria* sp., H: *Cladonia* sp., and I: *Ochrolechia* sp. Specimens A–C were collected from King George Island, Antarctica, and D–I from Svalbard Archipelago, in the Arctic Ocean

replications. The species affiliation of a bacterial isolate was determined when the isolate formed a monophyletic group with the reference species and the sequence similarity was 98.5 % or higher. The sequences were deposited NCBI GenBank under the accession numbers KJ606780–KJ606847.

Results

Identification of the isolates

Sixty-eight bacterial isolates, affiliated with 26 phylotypes of *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*, were recovered from nine lichen samples (Table 2 and Fig. 1). The largest number of isolates and the most diverse genera/families were recovered from *Psoroma* sp. collected from Antarctica (26 and 9, respectively; Tables 1 and 2). The largest group in terms of the number of genera recovered was the phylum *Actinobacteria*, with five genera/families of bacteria, followed by *Alphaproteobacteria* (4) and *Gammaproteobacteria* (3).

Isolates belonging to *Alphaproteobacteria* (26.5 %) were the most abundant, followed by *Actinobacteria* (25.0 %), *Betaproteobacteria* (22.1 %), *Gammaproteobacteria* (16.2 %), *Bacteroidetes* (5.9 %), *Firmicutes* (2.9 %), and *Deinococcus-Thermus* (1.5 %) (Table 2). Forty-four isolates were assigned to the genera *Aurantimonas* (1), *Methyloferula* (1), *Sphingomonas* (14), *Burkholderia* (15), *Pseudomonas* (1), *Psychrobacter* (1), and *Rhodanobacter* (9), and the family *Acetobacteraceae* (2) of *Proteobacteria*. Seventeen isolates belonged to the phylum *Actinobacteria*, represented by the genera *Frondihabitans* (9), *Nakamurella* (1), *Streptomyces* (1), and *Subtercola* (2), and by the family *Microbacteriaceae* (4). There were four isolates from the phylum *Bacteroidetes*, which was represented by the genus *Hymenobacter*. From the genus *Paenibacillus* of *Firmicutes*, two isolates were obtained and from the genus *Deinococcus* of *Deinococcus-Thermus* one isolate. The overall similarity of the bacterial isolates to the known type strains ranged from 93.7 % to 100 % (Table 3). Fourteen isolates with $\leq$ 97 % similarity to 16S rRNA gene sequences of known strains belonged to the genera *Hymenobacter*, *Paenibacillus*, *Aurantimonas*, *Methyloferula*, and *Sphingomonas* or to the families *Acetobacteraceae* and *Microbacteriaceae*, indicating the existence of many candidate novel species.

Bacterial isolates affiliated with *Frondihabitans*, *Hymenobacter*, *Sphingomonas*, and *Burkholderia* were obtained from lichen samples from both the Arctic and Antarctica, while strains of other genera were isolated from lichen samples only from one or the other location (Table 2). Isolates of *Burkholderia* were recovered from six of the nine lichen samples, the exceptions being *Cetraria* sp. from the Arctic and *Usnea* sp. and *Cladonia borealis* from Antarctica. *Sphingomonas* isolates were likewise isolated from six lichen samples, the exceptions in this case being *Stereocaulon* sp., *Cladonia borealis*, and *Cladonia* sp. from the Arctic. These results demonstrate the bi-polar distribution of the bacterial isolates within lichens. Isolates of *Frondihabitans* were commonly recovered from *Cladonia borealis* regardless of the lichen's geographic origin (Table 3).

Physiological characteristics

The growth temperature range of the 58 isolates and their production of extracellular proteases or lipases were determined. Most of the isolates (64.7 %) produced yellow, lemon, orange, pink, or red pigments recognizable with the unaided eye (Table 3). As the temperature increased from 4 °C to 20 °C, the number of strains able to grow increased from 22 to 56. Most of the isolates grew well between 10 °C and 20 °C but no strains grew at 37 °C (Fig. 2).

Extracellular protease activities were detected in six isolates, affiliated with *Microbacteriaceae*, *Hymenobacter*, *Burkholderia*, *Pseudomonas*, and *Rhodanobacter* (Table 3 and Fig. S2a). Three isolates were isolated from the Arctic lichens *Stereocaulon* sp. and *Umbilicaria* sp. and three from the Antarctic lichen *Psoroma* sp. Among them, *Pseudomonas* sp. PAMC 26590 had extracellular protease activity at 4 °C. Three isolates had proteolytic activity at 10 °C and five isolates at 20 °C (Table 3 and Fig. S2a).

In contrast to the few extracellular protease producers, 37 isolates showed extracellular lipase activities, 22 isolated from Arctic lichens and 15 from Antarctic lichens (Table 3). The isolates were mostly affiliated with *Burkholderia* (40.5 %), *Sphingomonas* (27.0 %), and *Frondihabitans* (18.9 %) but also with *Pseudomonas*, *Rhodanobacter*, *Deinococcus*, and *Subtercola* (Table 3 and Fig. S3). None of the isolates exhibited lytic activity at 4 °C while 29 and 37 isolates showed extracellular lipase activity at 10 °C and 20 °C, respectively. High extracellular lipase activity (score $\geq$3) was determined in 26 isolates (Table 3 and Fig. S2a).

Discussion

In this study, diverse bacterial isolates affiliated with *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria* were recovered. The same distinct bacterial lineages were retrieved from previous studies of bacterial diversity in lichens (González et al. 2005; Liba et al. 2006; Cardinale et al. 2008; Bates et al. 2011), indicating the existence of several ubiquitous taxa across

Table 3 Taxonomic assignments, physiology of the bacterial isolates, and their origin

Phylum or class (no. of isolates)	Species name	Similarity (%)	Closest known species	Accession no.	Growth temperature range (°C)	Protease[a]			Lipase[a]			Pigment charactristics	PAMC No.	Accession no.	Sample ID[b]
						4 °C	10 °C	20 °C	4 °C	10 °C	20 °C				
Actinobacteria (17)	*Microbacteriaceae* sp. [1]	98.3	*Glaciihabitans tibetensis*	KC256953	nd	nd	nd	nd	nd	nd	nd	nd	26555	KJ606838	C
	Frondihabitans sucicola	99.1	*Frondihabitans sucicola*	JX876867	10–20	0	0	0	0	0	3	Lemon	26565	KJ606839	B
	Frondihabitans sucicola	99.0	*Frondihabitans sucicola*	JX876867	4–30	0	0	0	0	2	4	Lemon	26579	KJ606845	E
	Frondihabitans sucicola	99.0	*Frondihabitans australicus*	DQ525859	4–30	0	0	0	0	2	4	Lemon	26586	KJ606846	E
	Frondihabitans sucicola	99.1	*Frondihabitans sucicola*	JX876867	4–30	0	0	0	0	2	4	Lemon	26587	KJ606847	E
	Frondihabitans sucicola	99.0	*Frondihabitans sucicola*	JX876867	4–25	0	0	0	0	2	2	Yellow	26588	KJ606844	E
	Frondihabitans sp.	98.7	*Frondihabitans sucicola*	JX876867	4–25	0	0	0	0	0	0	Yellow	26612	KJ606840	A
	Frondihabitans sucicola	99.0	*Frondihabitans sucicola*	JX876867	4–25	0	0	0	0	0	0	Yellow	26613	KJ606843	A
	Frondihabitans sp.	98.7	*Frondihabitans sucicola*	JX876867	10–20	0	0	0	0	0	1	Lemon	26614	KJ606841	B
	Microbacteriaceae sp. [2]	97.0	*Frondihabitans australicus*	DQ525859	10–25	0	0	0	0	0	0	White	26615	KJ606835	D
	Frondihabitans sp.	98.7	*Frondihabitans sucicola*	JX876867	10–25	0	0	0	0	0	0	White	26616	KJ606842	D
	Microbacteriaceae sp. [2]	97.0	*Frondihabitans australicus*	DQ525859	4–25	0	0	0	0	0	1	Ivory	26629	KJ606833	D
	Microbacteriaceae sp. [2]	97.0	*Frondihabitans australicus*	DQ525859	4–25	0	2	4	0	0	0	Red	26630	KJ606834	D
	Nakamurella panacisegetis	99.3	*Nakamurella panacisegetis*	HE599560	15–20	0	0	0	0	0	0	White	26572	KJ606832	C
	Streptomyces anulatus	100.0	*Streptomyces anulatus*	DQ026637	nd	nd	nd	nd	nd	nd	nd	nd	26508	KJ606831	C
	Subtercola boreus	99.7	*Subtercola boreus*	AF224722	4–30	0	0	0	0	2	4	Lemon	26580	KJ606836	E
	Subtercola boreus	99.7	*Subtercola boreus*	AF224722	4–25	0	0	0	0	0	0	Yellow	26576	KJ606837	D
Bacteroidetes (4)	*Hymenobacter* sp. [1]	97.0	*Hymenobacter ginsengisoli*	JN090860	10–20	0	0	0	0	0	0	Red	26553	KJ606780	C
	Hymenobacter sp. [1]	97.0	*Hymenobacter ginsengisoli*	JN090860	nd	nd	nd	nd	nd	nd	nd	Red	26554	KJ606781	C
	Hymenobacter sp. [1]	96.8	*Hymenobacter ginsengisoli*	JN090860	15–25	0	0	0	0	0	0	Red	26570	KJ606782	C
	Hymenobacter sp. [2]	94.1	*Hymenobacter glaciei*	GQ454806	4–25	0	0	4	0	0	0	Pink	26628	KJ606783	D
Deinococcus-Thermus (1)	*Deinococcus* sp.	98.0	*Deinococcus marmoris*	AJ585986	10–25	0	0	0	0	0	1	Red	26562	KJ606784	A

Table 3 continued

Phylum or class (no. of isolates)	Species name	Similarity (%)	Closest known species	Accession no.	Growth temperature range (°C)	Protease[a]			Lipase[a]			Pigment charactristics	PAMC No.	Accession no.	Sample ID[b]
						4 °C	10 °C	20 °C	4 °C	10 °C	20 °C				
Firmicutes (2)	*Paenibacillus* sp.	94.4	*Paenibacillus gansuensis*	AY839866	nd	nd	nd	nd	nd	nd	nd	nd	26516	KJ606786	C
	Paenibacillus sp.	94.0	*Paenibacillus gansuensis*	AY839866	15–20	0	0	0	0	0	0	nd	26517	KJ606785	C
Alphaproteobacteria (18)	*Aurantimonas* sp.	97.0	*Aureimonas phyllosphaerae*	JQ346806	15–25	0	0	0	0	0	0	Yellow	26543	KJ606801	A
	Acetobacteraceae sp.	96.1	*Gluconacetobacter takamatsuzukensis*	AB778531	10	0	0	0	0	0	0	Baby pink	26568	KJ606803	B
	Acetobacteraceae sp.	96.1	*Gluconacetobacter takamatsuzukensis*	AB778531	10	0	0	0	0	0	0	Baby pink	26569	KJ606804	B
	Methyloferula sp.	93.7	*Methyloferula stellata*	ARWA01000001	15–20	0	0	0	0	0	0	Pink	26571	KJ606802	C
	Sphingomonas paucimobilis	99.8	*Sphingomonas paucimobilis*	U37337	4–20	0	0	0	0	0	0	Ivory	26502	KJ606787	B
	Sphingomonas sp. [1]	97.9	*Sphingomonas aquatilis*	AF131295	nd	nd	nd	nd	nd	nd	nd	nd	26530	KJ606800	A
	Sphingomonas paucimobilis	99.8	*Sphingomonas paucimobilis*	U37337	10–20	0	0	0	0	0	2	White	26546	KJ606788	B
	Sphingomonas paucimobilis	99.8	*Sphingomonas paucimobilis*	U37337	10–25	0	0	0	0	0	0	White	26548	KJ606789	B
	Sphingomonas sp. [2]	97.2	*Sphingomonas asaccharolytica*	Y09639	10–25	0	0	0	0	0	2	Yellow	26556	KJ606792	C
	Sphingomonas sp. [4]	97.1	*Sphingomonas oligoaromativorans*	FJ434127	10–25	0	0	0	0	0	0	Yellow	26560	KJ606791	B
	Sphingomonas sp. [2]	97.3	*Sphingomonas cynarae*	HQ439186	10–25	0	0	0	0	0	2	Yellow	26561	KJ606793	C
	Sphingomonas sp. [4]	97.0	*Sphingomonas oligoaromativorans*	FJ434127	10–25	0	0	0	0	1	3	Lemon	26567	KJ606790	B
	Sphingomonas glacialis	99.9	*Sphingomonas glacialis*	GQ253122	10–25	0	0	0	0	2	3	Yellow	26605	KJ606799	I
	Sphingomonas sp. [3]	99.1	*Sphingomonas glacialis*	GQ253122	4–30	0	0	0	0	2	3	Orange	26608	KJ606796	I
	Sphingomonas sp. [3]	99.1	*Sphingomonas glacialis*	GQ253122	4–25	0	0	0	0	2	3	Dark orange	26617	KJ606798	F
	Sphingomonas sp. [3]	98.4	*Sphingomonas oligophenolica*	AB018439	4–25	0	0	0	0	2	2	Dark orange	26618	KJ606794	F
	Sphingomonas sp. [3]	99.2	*Sphingomonas glacialis*	GQ253122	4–30	0	0	0	0	2	3	Orange	26621	KJ606795	G
	Sphingomonas sp. [3]	99.1	*Sphingomonas glacialis*	GQ253122	4–30	0	0	0	0	2	2	Orange	26625	KJ606797	I
Betaproteobacteria (15)	*Burkholderia sordidicola*	98.9	*Burkholderia sordidicola*	AF512827	4–30	0	2	0	0	2	3	Ivory	26506	KJ606828	C
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	Ivory	26507	KJ606824	C
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	Ivory	26510	KJ606826	C

Table 3 continued

Phylum or class (no. of isolates)	Species name	Similarity (%)	Closest known species	Accession no.	Growth temperature range (°C)	Protease[a]			Lipase[a]			Pigment charactristics	PAMC No.	Accession no.	Sample ID[b]
						4 °C	10 °C	20 °C	4 °C	10 °C	20 °C				
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	Ivory	26537	KJ606827	C
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26575	KJ606817	D
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26577	KJ606823	E
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26582	KJ606822	E
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26592	KJ606821	F
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26606	KJ606818	I
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26607	KJ606816	I
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26609	KJ606820	H
	Burkholderia sordidicola	98.8	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	White	26610	KJ606825	H
	Burkholderia sordidicola	98.9	*Burkholderia sordidicola*	AF512827	10–30	0	0	0	0	2	3	Ivory	26611	KJ606819	H
	Burkholderia sordidicola	98.8	*Burkholderia udeis*	AY154367	10–30	0	0	0	0	2	3	Ivory	26509	KJ606829	C
	Burkholderia sordidicola	98.8	*Burkholderia udeis*	AY154367	10–30	0	0	0	0	2	3	Lemon	26633	KJ606830	C
Gammaproteobacteria (11)	*Pseudomonas* sp.	99.6	*Pseudomonas graminis*	Y11150	4–30	3	4	4	0	1	2	nd	26590	KJ606806	F
	Psychrobacter sp.	98.8	*Psychrobacter alimentarius*	AY513645	nd	nd	nd	nd	nd	nd	nd	nd	26498	KJ606805	B
	Rhodanobacter sp. [1]	98.4	*Rhodanobacter umsongensis*	FJ821731	nd	nd	nd	nd	nd	nd	nd	nd	26505	KJ606807	C
	Rhodanobacter sp. [2]	97.8	*Rhodanobacter ginsengisoli*	EF166075	nd	nd	nd	nd	nd	nd	nd	nd	26515	KJ606814	C
	Rhodanobacter sp. [3]	98.1	*Rhodanobacter ginsengisoli*	EF166075	4–20	0	0	0	0	0	3	Ivory	26518	KJ606812	C
	Rhodanobacter sp. [3]	98.1	*Rhodanobacter ginsengisoli*	EF166075	10–20	0	0	0	0	0	0	Yellow	26519	KJ606813	C
	Rhodanobacter sp. [2]	97.7	*Rhodanobacter ginsengisoli*	EF166075	10–25	0	0	0	0	1	2	Lemon	26538	KJ606809	C
	Rhodanobacter sp. [2]	97.8	*Rhodanobacter ginsengisoli*	EF166075	4–25	0	0	4	0	0	0	Yellow	26551	KJ606810	C
	Rhodanobacter sp. [2]	97.8	*Rhodanobacter ginsengisoli*	EF166075	4–25	0	0	4	0	0	0	Yellow	26552	KJ606811	C
	Rhodanobacter sp. [2]	97.7	*Rhodanobacter ginsengisoli*	EF166075	nd	nd	nd	nd	nd	nd	nd	nd	26557	KJ606808	C

Table 3 continued

Phylum or class (no. of isolates)	Species name	Similarity (%)	Closest known species	Accession no.	Growth temperature range (°C)	Protease[a]			Lipase[a]			Pigment charactristics	PAMC No.	Accession no.	Sample ID[b]
						4 °C	10 °C	20 °C	4 °C	10 °C	20 °C				
	Rhodanobacter sp. [2]	97.6	*Rhodanobacter ginsengisoli*	EF166075	nd	nd	nd	nd	nd	nd	nd	nd	26558	KJ606815	C

[a] Enzyme secretion was scored as follows: 0, no clear zone; 1, faint clear zone; 2, clear zone was evident and width of the clear zone was smaller than the radius of the colony; 3, width of the clear zone was larger than the radius and smaller than the diameter of the colony; and 4, width of the clear zone exceeded the diameter of the colony

[b] Sample represents the lichens from which the bacterial strains were isolated. Sample ID was coded as follows: A: *Usnea* sp., B: *Cladonia borealis*, C: *Psoroma* sp., D: *Stereocaulon* sp., E: *Cladonia borealis*, F: *Umbilicaria* sp., G: *Cetraria* sp., H: *Cladonia* sp., and I: *Ochrolechia* sp. Specimens A–C were collected from King George Island, Antarctica, and D–I from Svalbard Archipelago, in the Arctic Ocean

lichens. Especially, the predominance of alphaproteobacterial bacteria in the lichens has been well known regardless of the geographical origin of lichens. However, in previous studies that applied cultivation methods for bacterial community analysis in lichens, the alphaproteobacterial groups were poorly represented in the culturable fraction (Cardinale et al. 2006; Selbmann et al. 2010; Printzen et al. 2012) whereas bacterial strains belonging to the *Alphaproteobacteria* were predominant in our samples. Our findings are consistent with previous reports in which molecular techniques such as pyrosequencing and FISH analysis were used (Cardinale et al. 2008; Bates et al. 2011), in spite of the limitations of traditional culture-based methods. In addition, many putative novel isolates with ≤97 % similarity to 16S rRNA gene sequences of known strains were recovered from our samples. Thus, relatively simple cultivation methods can be used to isolate as-yet-undescribed taxa. These culturable isolates will likely expand our knowledge of previously unknown functions and physiologies of bacterial Operational Taxonomic Units (OTUs) detected by molecular techniques.

Some members of the *Alphaproteobacteria* are known by their common participation in symbiotic relationships in lichens (Cardinale et al. 2008; Hodkinson and Lutzoni 2009; Bates et al. 2011). In addition, nitrogenases are known to be ubiquitous in alphaproteobacterial bacteria indicating their participation in the nitrogen fixing (Grube and Berg 2009). Among the alphaproteobacterial isolates obtained in this study, 77.8 % belonged to the genus *Sphingomonas*. They were isolated from a variety lichens collected from the Arctic and Antarctica and included 10 strains with extracellular lipase activity. The genus *Sphingomonas* comprises plant-associated bacteria, some of which may promote plant growth and reduce plant diseases (Kim et al. 1998). The ability of *Sphingomonas* strains to degrade organic matter or xenobiotic substances and to fix nitrogen has been described (Ederer et al. 1997; Copley 2000; Asker et al. 2007; Videira et al. 2009). That many of the *Sphingomonas* strains recovered in this study are extracellular lipase producers and were isolated from diverse lichen specimens suggests their involvement in nutrient availability within a lichen, by contributing to the hydrolysis of major organic compounds. Colonization by heterotrophic bacteria that produce enzymes enabling macromolecular degradation may be of great advantage for lichens exposed to severely restricted nutrient supplies (Liba et al. 2006).

Besides *Sphingomonas*, bacterial taxa of *Frondihabitans*, *Hymenobacter*, and *Burkholderia* were recovered across lichen samples from both the Arctic and Antarctica, and most of them produced extracellular lipases. The exception was strains of *Hymenobacter*. Members of this genus form pink to red-pigmented colonies and these pigments may confer resistance to UV radiation by acting as an UV shield

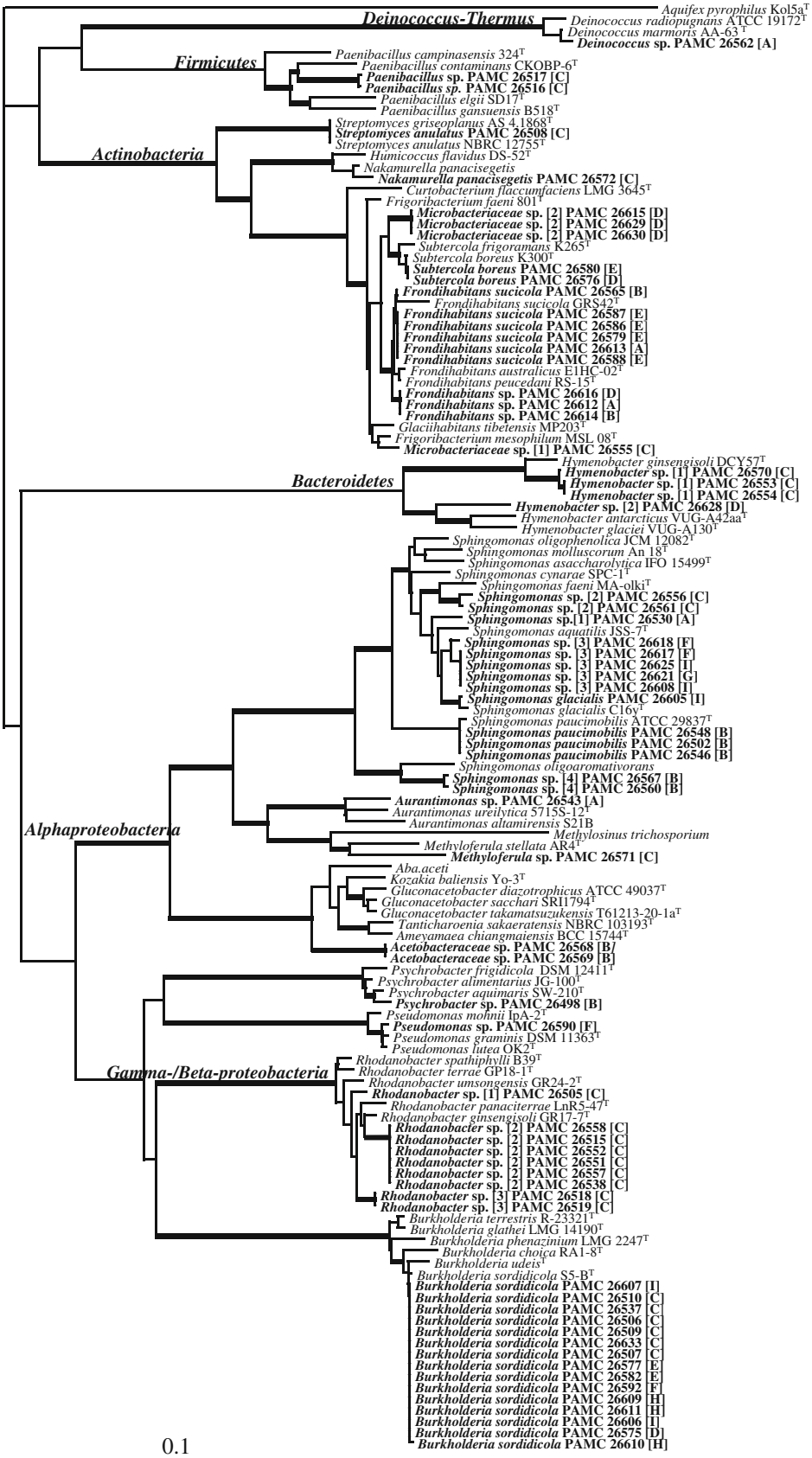

Fig. 1 Neighbor-joining tree of isolates with closely related reference species based on 16S rRNA gene sequences. Isolates for each phylotype are indicated by *bold letters*. Branches supported by high bootstrap values (>70 %) are shown as *thick lines*

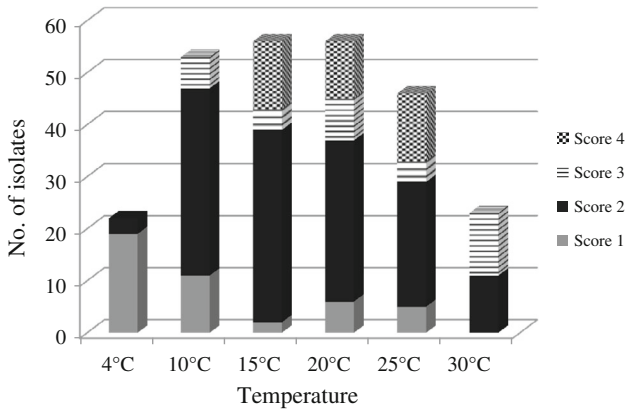

Fig. 2 Effect of temperature on bacterial growth. The degree of growth was scored from 1 to 4, with a higher number indicating better growth

absorbing maximum in the UV region (Fujii et al. 2010; Peeters et al. 2011; Singh and Gabani 2011). This property is particularly beneficial to lichens in polar areas, which are subjected to intense UV radiation. Strains of *Burkholderia* are common colonizers of temperate lichens and are well known as plant-associated bacteria that fix nitrogen and promote plant growth (Balandreau et al. 2001; Estrada-De Los Santos et al. 2001; Reis et al. 2004; Sessitsch et al. 2005; Selbmann et al. 2010). These known characteristics partially explain the wide distribution of these bacteria in lichens. Furthermore, the occurrence of related strains in lichens that are geographically distinct but nonetheless thrive in cold environments suggests similar strategies against freezing or low temperatures (Sheng et al. 2011).

In contrast to those strains with a wide distribution, strains of the genera *Nakamurella*, *Streptomyces*, *Deinococcus*, *Paenibacillus*, *Aurantimonas Methyloferula*, *Psychrobacter*, *Pseudomonas*, and *Rhodanobacter* and of the family *Acetobacteraceae* showed lichen-species-specific colonization, a phenomenon previously reported using molecular approaches (Cardinale et al. 2008; Grube and Berg 2009; Bates et al. 2011). Given the limitations of traditional cultivation methods, we could not elucidate the lichen species-specificity of lichen-associated bacteria. We can reasonably propose, however, that lichen-forming fungi produce diverse secondary metabolites that provide a selective environment, which in turn determines the phylotypes of the bacterial partners so as to promote survival.

Endophytes secrete extracellular enzymes that contribute to their colonization and growth (Wang and Dai 2011). In this study, bacterial isolates of the genera *Burkholderia*, *Deinococcus*, *Frondihabitans*, *Pseudomonas*, *Rhodanobacter*, *Sphingomonas*, and *Subtercola* produced extracellular proteases and/or lipases. Cold-active enzymes with high specific activities at low and moderate temperatures are extremely useful in a broad range of industrial, agricultural, and medical applications (Lee et al. 2012). Thus, in addition to their ecologically important roles in lichens, bacterial strains from polar lichens, including those with low similarity (≤97 %), that are able to synthesize and secrete cold-active enzymes are of biotechnological and commercial interest, as valuable sources of extracellular enzymes active at low temperatures.

The culturable fraction of bacteria by no means represents the complete bacterial diversity. However, as a first study of the culturable bacteria from Arctic and Antarctic lichens, the strains obtained in this study, all of them belonging to previously known major lineages (*Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Alphaproteobacteria*, and *Betaproteobacteria*), are also of interest in attempts to determine the functional and ecological roles of bacteria that colonize in lichens.

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