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SHORT NOTE

Polar and Alpine Microbial Collection (PAMC): a culture collection dedicated to polar and alpine microorganisms

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Abstract Microorganisms in polar areas may have important ecological roles in biogeochemical cycles and the food chain. They are adapted to polar environments by means of special physiological adaptation mechanisms that include cold-adapted enzymes and cryoprotectants such as exopolysaccharides. Culture collections for polar microorganisms can provide research resources for ecological and physiological studies. The Polar and Alpine Microbial Collection (PAMC) is a specialized culture collection for maintenance and distribution of polar and alpine microorganisms. A database system was developed to share important data fields with DarwinCore2 and Ocean Biogeographic Information System database schemas. Approximately 1,500 out of 5,500 strains maintained in PAMC have been identified and belonged primarily to the phyla *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*. Many of the microbial strains can grow at low temperature and produce proteases, lipases, and/or exopolysaccharides. PAMC provides search tools based on keywords such as taxonomy, geographical origin, habitat, and physiological characteristics. Biological materials and information provided by PAMC will be important resources for ecological and physiological studies on polar and alpine microorganisms.

Keywords Microorganisms · Biodiversity · Physiological characteristics

Introduction

Polar areas may have environments with low nutrient concentrations, low temperature, extreme variability in day length, and strong solar ultraviolet radiation exposure. Polar areas comprise distinct habitats such as sea ice, glacial ice, permafrost, tundra wetlands, oceanic water, and lakes (Reddy et al. 2009). Prokaryotes are dominant in polar areas and play crucial roles in biogeochemical cycles, food chains, and the mineralization of pollutants (Nichols et al. 1999). The concentration of small and easily metabolizable molecules is often low in polar environments because of the limited number and distribution of higher plants and limited residence time of some vertebrates; hence, extracellular enzymes secreted by cold-adapted microorganisms may play important ecological roles in the cycling of organic matters (Staley and Herwig 1993; Vazquez et al. 2004). Microorganisms such as bacteria, yeasts, filamentous fungi, and unicellular algae have developed diverse adaptation mechanisms that enable them to compensate for the deleterious effects of harsh environments (Gerday et al. 2000). Cold-active enzymes have high specific activities at low and moderate temperatures and are inactivated easily by a moderate increase in temperature. These properties can be extremely useful in a broad range of industrial, agricultural, and medical applications (Gerday et al. 2000). Exopolysaccharides from polar fungi and bacteria were suggested to function as a

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cryoprotectant in polar environments (Selbmann et al. 2002; Kim and Yim 2007). Thus, cold-adapted microorganisms have attracted the attention of the scientific community on account of their ability to produce cold-active enzymes and other materials.

Culture collections are important repositories of microbial diversity and are essential for the long-term availability of microbial strains and their genes (Nichols et al. 1999). There are 591 culture collections registered with the World Federation for Culture Collections (WFCC, http://wdcm.nig.ac.jp/wfcc). Some of these collections are specialized for microbial strains, and approximately 760,000 bacterial strains are maintained. However, most of the public culture collections hold only type cultures of identified species rather than groups of strains. This limits the biodiversity maintained in culture collections and restricts researchers to described microorganisms (Nichols et al. 1999). The Australian Collection of Antarctic Microorganisms (ACAM) is the only publicly accessible collection of microorganisms dedicated to microbial strains isolated from the Antarctic continent, sub-Antarctic islands, and the Southern Ocean and it holds about 400 isolates of heterotrophic bacteria (Nichols et al. 1999).

As the number of microbial strains isolated from polar and alpine areas increases and they are recognized as valuable resources in ecological studies and biotechnology, the necessity of a culture collection dedicated to polar and alpine microorganisms has increased. Thus, the Korea Polar Research Institute (KOPRI) established the Polar and Alpine Microbial Collection (PAMC) to share biodiversity information and bio-resources collected from polar and alpine areas with scientific and public communities. In this paper, we introduce the database and website structure, search tools, and microbial diversity maintained in PAMC (http://pamc.kopri.re.kr).

Collection, identification, and characterization of microbial strains

Most of the microorganisms maintained in PAMC were isolated by KOPRI scientists and some strains were deposited by university scientists. Usually, the microorganisms were isolated by plating on agar medium from terrestrial soil, sea water, marine sediment, cryoconite, and biotic materials, as described by Cho et al. (2008) and Lee et al. (2011). Pure cultures of bacterial isolates were preserved at −80 or −180 °C in 10–20 % glycerol. Bacterial strains were identified by sequence similarity and phylogenetic analysis of 16S rRNA gene sequences. Amplification, sequencing, and phylogenetic analyses were conducted as described by Lee et al. (2011). The growth temperature and production of extracellular enzymes such as protease and lipase were investigated by replica plating methods described by Lee et al. (2011). Briefly, the procedures were as follows. Bacterial cell suspensions were inoculated onto solid medium with a 96-pin replicator (VP-408B, V&P Scientific, San Diego, CA, USA) and incubated at 4, 10, 15, 20, 25, 30, or 37 °C for 3 days. Growth was evaluated by scoring as follows: 0, no growth; 1, the colony diameter was smaller than 4 mm and translucent; 2, the colony diameter was smaller than 4 mm and dense, or between 4 and 8 mm and translucent; 3, the colony diameter was between 4 and 8 mm, and dense; and 4, the colony diameter was larger than 8 mm. Protease and lipase activities were evaluated by the relative size of the clear zone compared with the colony size after incubation on solid medium supplemented with 1 % skim milk (Difco, Franklin Lakes, NJ, USA) for protease screening or 1 % tributyrate (Sigma, St. Louis, MO, USA) for lipase screening after 3 or 7 days incubation. 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. Production of exopolysaccharide (EPS) was recognized by ropy colonies (Macura and Townsley 1984).

Database structure

The PAMC database was constructed based on the DarwinCore2 schema (Wieczorek et al. 2012), which was used to construct the Global Biodiversity Information Facility (GBIF) database system (http://www.gbif.org) and Ocean Biogeographic Information System (OBIS) schema (http://iobis.org) which was modified from DarwinCore2 to manage marine biodiversity information. DarwinCore2 and OBIS schemas define name, requirement, data type, and description of data fields for taxonomy, geographical origin, and environmental parameters to enable biodiversity information to be shared more efficiently among databases (Table S1). In addition to these, data fields for physiological characteristics such as media and temperature for optimal growth and production of enzymes were added in PAMC database.

Overview of the PAMC

PAMC holds approximately 5,500 strains of microorganisms that originated from diverse habitats within Arctic,

Antarctic, and alpine areas. The major sources of microbial strains were marine sediments, terrestrial soil, cryoconite, sea water, biofilms formed on natural and artificial solid surfaces, and other biotic and abiotic samples (Fig. 1a). The samples were collected from the Kara Sea and Svalbard in the Arctic area, King George Island in the Antarctic, the European Alps, and other geographical areas (Fig. 1b). Groups of strains that belong to the same species are maintained in PAMC with locality and habitat information. Bacterial strains that belong to the same species may be variable in physiology and genomic structure (Jaspers and Overmann 2004), and ecotypes isolated from specific niches may have unique ecological functions (Moore et al. 1998; Prosser et al. 2007). Therefore, groups of strains that belong to the same species and are adapted to different environments are very useful for studies to understand adaptation to each niche and ecological functions. PAMC provides search tools to find microbial strains by taxonomy, geography, habitat, and physiological characteristics (Fig. S1a). By using search tools with multiple keys, users can specify the search conditions easily. PAMC provides full information about each strain, including taxonomic information from species to kingdom levels, geographical information (including longitudinal and latitudinal co-ordinates, locality, and elevation or depth), habitat information (including temperature, salinity, and host), and physiological characteristics (including growth temperature and extracellular enzyme production; see Fig. S1b). Currently, all of the strains are not publicly available. Biodiversity information for approximately 1,500 strains is available, and 500 strains are ready to distribute. However, more microbial strains and information will be made available after the validation process, including identification and preparation of delivery vials.

Microbial diversity maintained in PAMC

Approximately 1,500 strains out of the 5,500 strains maintained in PAMC were identified by phylogenetic analysis of 16S rRNA gene sequences and using a 98.5 % similarity cut-off for species recognition. Identification results revealed that PAMC strains belonged to *Actinobacteria*, *Bacteroidetes*, *Deinococcus-Thermus*, *Firmicutes*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Gammaproteobacteria*. The strains were classified into 42 families and 107 genera (Table 1). The major families that included a high number of microbial strains were *Bacillaceae*, *Flavobacteriaceae*, *Pseudoalteromonadaceae*, and *Pseudomonadaceae*. Regardless of the number of strains, the diversity of genera was high in the *Flavobacteriaceae*, *Enterobacteriaceae*, *Rhodobacteraceae*, and *Microbacteriaceae*. Phylogenetic analyses and sequence similarity searches showed that many of the microbial strains maintained in PAMC are candidates of novel species. This result implies that the PAMC is an excellent resource for taxonomic studies. Among PAMC strains, *Dasania marina* from Arctic marine sediment, *Sanguibacter antarcticus* from Antarctic sea sand, *Maribacter arcticus* from Arctic marine sediment, and *Actimicrobium antarcticum* from Antarctic coastal sea water were reported as novel species (Lee et al. 2007; Cho et al. 2008; Hong et al. 2008; Kim et al. 2011).

Physiological characteristics

Physiological characteristics such as growth temperature, production of extracellular enzymes or exopolysaccharide (EPS) were examined for approximately 2,900 strains. The number of microbial strains that could grow at 4 °C was

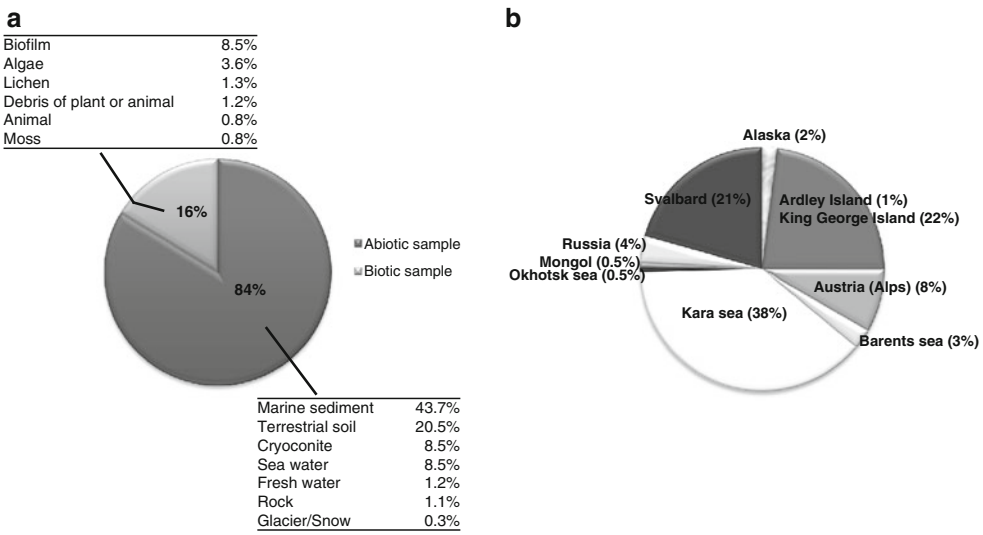

Fig. 1 Source of microbial strains. **a** Habitat. **b** Locality

Table 1 Overview of the strains held in the Polar and Alpine Microbial Collection

Phylum	Family	No. of genera	No. of strains
Actinobacteria (13.6 %)	*Brevibacteriaceae*	1	30
	Cellulomonadaceae	2	5
	Intrasporangiaceae	1	1
	Microbacteriaceae	8	57
	Micrococcaceae	2	91
	Nakamurellaceae	1	1
	Nocardioidaceae	2	11
	Sanguibacteraceae	1	8
	Streptomycetaceae	1	1
Bacteroidetes (13.3 %)	*Cyclobacteriaceae*	1	9
	Cytophagaceae	1	4
	Flavobacteriaceae	19	170
	Sphingobacteriaceae	3	17
Deinococcus-Thermus (0.1 %)	*Deinococcaceae*	1	1
Firmicutes (19.6 %)	*Aurantimonadaceae*	1	2
	Bacillaceae	5	202
	Carnobacteriaceae	3	5
	Paenibacillaceae	1	59
	Planococcaceae	4	27
Alphaproteobacteria (3.9 %)	*Acetobacteraceae*	1	2
	Beijerinckiaceae	1	1
	Bradyrhizobiaceae	1	1
	Erythrobacteraceae	1	1
	Phyllobacteriaceae	1	1
	Rhodobacteraceae	9	25
	Sphingomonadaceae	2	29
Betaproteobacteria (5.6 %)	*Burkholderiaceae*	1	30
	Chromatiaceae	1	1
	Colwelliaceae	2	4
	Comamonadaceae	3	5
	Oxalobacteraceae	4	45
Gammaproteobacteria (43.9 %)	*Alteromonadaceae*	2	10
	Enterobacteriaceae	9	29
	Granulosicoccaceae	1	1
	Oceanospirillaceae	1	1
	Pseudoalteromonadaceae	1	168
	Pseudomonadaceae	2	382
	Psychromonadaceae	1	6
	Shewanellaceae	1	44
	Unclassified Pseudomonadales	1	1
	Vibrionaceae	1	2
	Xanthomonadaceae	2	17

445 (15.3 %) and the number increased to 20 °C and then decreased at higher temperatures (Fig. 2). Many of the microbial strains produced extracellular protease, lipase, or

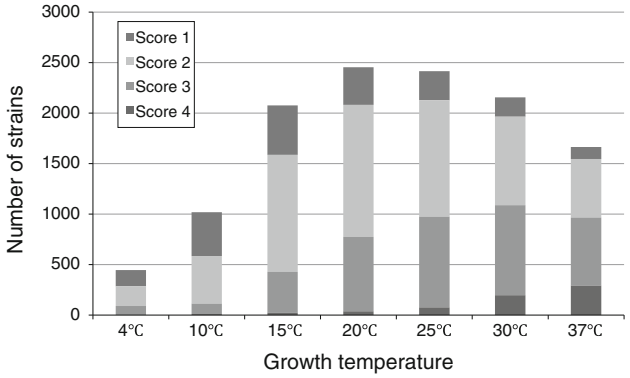

Fig. 2 Effect of temperature on growth. The scores represent the degree of growth from 1 to 4. A high number implies better growth

EPS (Fig. 3). The number of strains that produced extracellular enzymes or EPS was higher at 20 °C than at 10 °C (Fig. 3). Among these strains, 68 and 161 strains showed strong protease activities (scores 3 or 4) at 10 and 20 °C, respectively. Thirty-four and 59 strains showed strong lipase activities (scores 3 or 4) at 10 and 20 °C. Among the microbial strains that were identified and for which examination of physiological characteristics was conducted, species of *Arthrobacter*, *Bacillus*, *Janthinobacterium*, *Olleya*, *Pseudoalteromonas*, and *Pseudomonas* were the major taxa that produced extracellular protease, lipase, or EPS (Table 2). Studies on the cold-active protease, lipase, or EPS in PAMC microbial strains that were isolated from biofilms in Arctic marine environments, enrichment cultures of Arctic terrestrial and marine samples, and alpine cryoconite samples have been reported (Lee et al. 2005; Kim and Yim 2007; Kim et al. 2010a, b; Lee et al. 2011).

Concluding remarks

As a specialized culture collection for polar and alpine microorganisms, PAMC makes every effort to maintain strains for sustainable use and supply authentic strains to academic or industrial communities. PAMC will try to expand the microbial collection by isolation of bacterial and fungal strains from diverse habitats and geographical locations using a variety of isolation methods and by accepting deposition of microbial strains from domestic and international scientific communities. PAMC will also provide users with straightforward, unrestricted, and permanent access to accurate and up-to-date information about the microbial strains. PAMC follows the regulations contained in the Convention on Biological Diversity (CBD) and Bonn Guidelines on the utilization and benefit sharing

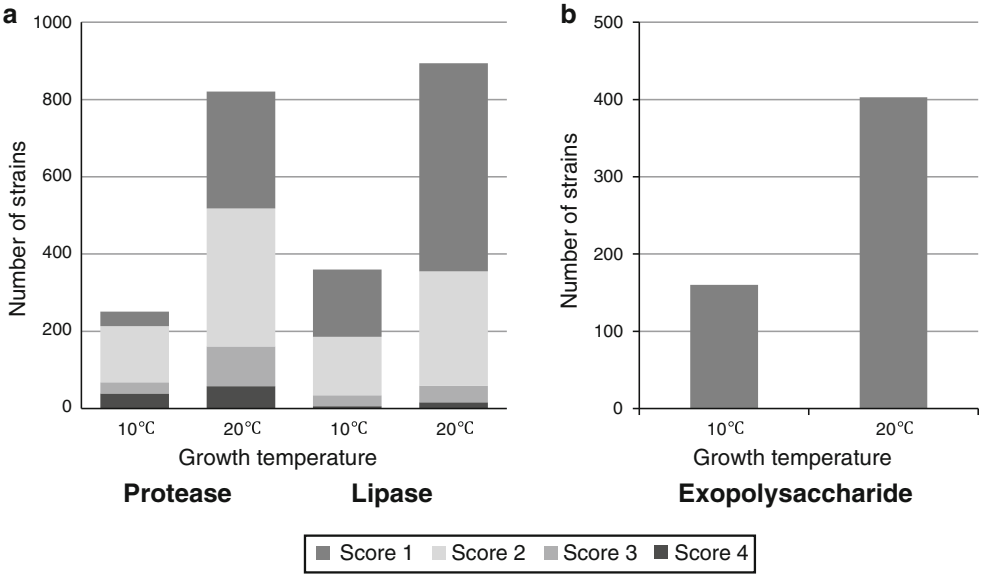

Fig. 3 Production of extracellular enzymes and exopolysaccharides. **a** The number of strains that produce protease and lipase. A high number implies stronger enzyme activity. **b** The number of strains that produce exopolysaccaride

Table 2 Taxa and number of strains that showed extracellular protease, lipase, and exopolysaccharide activity

Taxa	No. of strains					
	Protease		Lipase		Exopolysaccharide	
	10 °C	20 °C	10 °C	20 °C	10 °C	20 °C
Alteromonas	1	1	1	1	0	0
Arthrobacter	15	20	15	15	0	0
Bacillus	1	78	1	42	26	43
Brevibacterium	0	3	0	7	3	6
Burkholderia	0	0	0	15	0	0
Carnobacterium	1	1	1	1	0	0
Celeribacter	0	0	0	0	1	0
Cellulophaga	2	4	3	1	0	0
Cryobacterium	0	0	0	1	0	0
Enterobacteriaceae	4	4	6	1	0	0
Erythrobacter	0	0	0	1	0	0
Flavobacterium	2	4	4	2	4	0
Frondihabitans	0	0	0	4	0	0
Glaciecola	0	0	3	3	0	0
Hymenobacter	0	1	0	0	0	0
Janthinobacterium	35	28	38	37	0	0
Maribacter	0	0	3	1	0	0
Mucilaginibacter	1	1	0	1	0	0
Olleya	13	13	21	23	0	0
Oxalobacteraceae	1	1	1	0	0	0
Paenibacillus	0	0	0	8	0	1
Paenisporosarcina	0	0	0	0	0	1
Pedobacter	1	0	1	2	0	0
Polaribacter	0	0	0	0	1	1
Pseudoalteromonas	23	20	16	17	0	1
Pseudomonas	142	141	134	132	3	2
Psychrobacter	1	1	0	11	8	0
Rhodanobacter	0	2	0	1	0	0

Table 2 continued

Taxa	No. of strains					
	Protease		Lipase		Exopolysaccharide	
	10 °C	20 °C	10 °C	20 °C	10 °C	20 °C
Rhodococcus	1	0	0	1	0	0
Shewanella	1	12	13	1	19	1
Sphingomonas	1	2	2	9	0	0
Sphingopyxis	0	1	1	1	1	0
Stenotrophomonas	0	2	0	4	0	1
Subtercola	0	1	0	1	0	0
Terribacillus	0	1	0	1	0	1
Zobellia	0	0	3	2	0	0

of genetic and biological resources. PAMC will also adhere to upcoming regulations regarding genetic and biological resources from the Antarctic area.

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