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Temperature preferences of bacteria isolated from seawater collected in Kandalaksha Bay, White Sea, Russia

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Abstract Fifty-two bacteria were isolated from seawater collected in Kandalaksha Bay, White Sea, Russia, and classified by 16S rDNA sequencing. Most of the strains belonged to ubiquitous microorganisms. *Pseudomonas* was the most abundant genus (21 strains), including species of *P. fluorescens*, *P. putida* and *P. syringae*. *Serratia* was also common (10 strains) with species *S. plymuthica* and *S. proteamaculans*. *Sphingobacterium*, *Flavobacterium* and *Pantoea* were less represented (5, 3 and 2 strains, respectively). The only typical bacterium of marine Arctic regions was *Shewanella baltica*. The strains were tested for their optimal growth temperature in the range 0–45°C. The majority appeared to be psychrotolerant (42%) or mesophilic-psychrotolerant (40%). In addition, one strain (*Bacillus pumilus*) showed a rather narrow mesophilic profile. No true psychrophilic bacteria were found. Most of the strains showed a classical curve with fast growth decrease above the optimum; some others displayed uncommon flat curves with scarce differences between maximum and minimum of growth in a wide range of temperatures. Moreover, few strains presented an unusual profile being, in relation to the optimum, more tolerant to high rather than low temperatures. Preferences of the Kandalaksha Bay strains are generally different from those reported in literature for the same species: optima were at lower temperatures and, sometimes, ranges were broader showing increased eurythermism. This could indicate adaptation to the wide temperature variations recorded in this peculiar environment.

Keywords Temperature preferences · Bacterial strains · Sub-extreme environment · Kandalaksha Bay · White Sea

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Introduction

Seas and oceans represent a pool of extremely interesting environments for ecological and biotechnological studies. So far, much of the attention, for this kind of investigations, had been focused on other microbial ecosystems. Thus, marine environments, in particular those presenting extreme conditions, remain largely unstudied and unexploited (Rothschild and Mancinelli 2001; Bai et al. 2006; Srinivas et al. 2009).

White Sea is an enclosed basin, located at the Arctic Circle, that can be considered as a sub-extreme environment (Pantyulin 2003). Kandalaksha Bay is an estuarine system showing big sea level differences during tide cycles causing strong mixing of water (Melnikov et al. 2003; Savvichev et al. 2003). In addition, the seasonal extremes runoff of freshwater, due to the various rivers and intense precipitations, contributes to its very peculiar hydrodynamics (Howland et al. 1999; Dolotov et al. 2005).

The coastal zone influenced by the tides (intertidal zone or littoral) is particularly interesting because all biogeochemical processes are most evident. This is especially true if tidal phenomena are very pronounced as in Kandalaksha Bay. Organisms in this zone need adaptation to variable environmental conditions in which factors, such as water availability, temperature and salinity, change frequently (Savvichev et al. 2004).

Temperature is one of the crucial abiotic environmental factor determining the development and seasonal dynamic of many organisms: this has been widely demonstrated for White Sea also (Ushakova 2003; Zubakha and Usov 2004; Kuts and Ismailov 2009). Moreover, investigation on the growth temperature preferences is often a mandatory step to proceed with further studies. Temperature fluctuations in White Sea and Kandalaksha Bay are quite broad, and in the littoral zone, they are even more evident (Savvichev et al. 2003). Winter is harsh and long (sea surface is covered with ice for 6–7 months per year) and weather conditions could be very unpredictable. Temperature in winter may fall to −40°C, even if sometimes warm Atlantic air raises the temperature to ca. 5°C, while water temperature is about −1° to −2°C. In summer, air could reach 30°C, albeit the average is in between 15 and 20°C, while seawater could reach 15°C on surface layers (Berger and Gorbushin 2001; Shaporenko et al. 2005; Vershinin et al. 2006). In these extremely variable conditions, adaptation to wide temperature changes could be a winning surviving strategy as reported for microorganisms isolated in other extreme environments (Zucconi et al. 1996; Zhang et al. 2007; Srinivas et al. 2009). Only a very limited number of studies are available on microorganisms from the White Sea and even less regard Kandalaksha Bay (Gorlenko et al. 1985; Dul'tseva et al. 1996; Rabold et al. 2006; Gorelova et al. 2009). In addition, most of these studies limit the investigation to the total number of bacteria in a determined environment (Savvichev et al. 2003; Savvichev et al. 2004; Kravchishina et al. 2008). To the best of our knowledge, no study concerning temperature adaptation of bacteria from White Sea had been carried out.

In this work, we investigated 52 strains isolated from seawater collected in various zones of Kandalaksha Bay, White Sea, Russia. Identification was based on 16S rDNA sequence analysis, and strain temperature preferences for growth were studied in the range 0–45°C.

Materials and methods

Collection of samples

Seawater was taken in various areas of Kandalaksha Bay during an 8-day sampling campaign in September 2008. The majority of samples were collected, at minimum tide level, in an intertidal zone pool and from the adjacent water surface using sterile containers. Others, from different offshore locations and depths (0.5, 2.5, 15, 70 m), were taken by scuba divers or boats using sterile Niskin bottles. Sterilization was carried out by repeated and alternating washing with boiling sterile water and 70% (v/v) ethanol.

Seawater was filtered on sterile membranes (0.22 μm, Millipore, USA), and bacterial cultures were obtained placing the membranes on Plate Count Agar (PCA, Difco, USA) plates and then incubated at 4, 15 and 25°C. During sampling, air temperature was in the range 6.8–13.5°C. Water temperature in the intertidal zone and nearby sea surface was in the range 6.6–11.3°C; water at 0.5 and 2.5 m depth was ca. 8.0°C, while at 15 and 70 m, temperature was 6.6 and −1°C, respectively.

Strain isolation and culture conditions

Pure cultures of isolates (ca. 500) were obtained on PCA by streak plate method. In order to discharge evident replicates of the same isolate, preliminary tests were carried out considering some morphological characteristics (shape, color, morphology, aspect and dimensions), simple biochemical tests (catalase and oxidase production) and Gram reaction.

Gram staining was carried out using a commercial kit (Merck, Germany). Bacteria were measured on stained specimens using a Leitz Laborlux 11 microscope bearing a micrometric ocular calibrated with a micrometric slide (Leitz Wetzlar, Germany). Catalase and oxidase tests were performed as previously described (Kovacs 1956; Whittenbury 1964). Tests allowed to select 52 different isolates that were maintained on PCA slants at 4°C and routinely sub-cultured.

Strain identification

Isolates, grown for 24 h on PCA plates, were used for genomic DNA extraction by thermal shock as reported by Selbmann et al. (2010). The amplification of the 16S rDNA sequence was performed in a reaction mixture (final volume 25 μl) containing 2× BioMix (BioLine GmbH, Germany), 15–20 ng/μl of DNA template and 5 pmol/μl of the following universal primers (Sigma-Aldrich, USA): fD1 (5′-CCG AAT TCG TCG ACA ACA GAG TTT GAT CCT GGC TCA G-3′), rD1 (5′-CCC GGG ATC CAA GCT TAA GGA GGT GAT CCA GCC-3′) and rP2 (5′-CCC GGG ATC CAA GCT TACGGCTACCTTGTTACGAC TT-3′) as reported by Weisburg et al. (1991); 63f (5′-CAGGCCTAACACATGCAAGTC-3′) and 1389r (5′-A CGGGCGGTGTGTACAAG-3′) as reported by Hongoh et al. (2003). The amplification was carried out as previously reported (Selbmann et al. 2010). The PCR products were visualized by electrophoresis on agarose gel and quantified in comparison with the Ladder GeneRuler™ 1 kb DNA (Fermentas, Lithuania). The products were purified using Nucleospin Extract kit (Macherey–Nagel, Germany). Sequencing reactions were performed by Macrogen sequencing service (Macrogen Inc., Korea).

Sequence assembly was done using the software Chromas (version 1.5 2009, Technelysium Pty Ltd, Australia). Sequences with high similarity available in NCBI GenBank were identified using BLAST search (Altschul et al. 1997; http://www.blast.ncbi.nml.nih.gov).

Determination of optimal growth temperatures

Optimal temperatures for the growth of the isolates were tested in the range 0–45°C on PCA plates by steps of 5 ± 0.5°C. Plates (diameter 90 mm) were inoculated in triplicate with inocula obtained pipetting 2 µl of bacterial suspension in sterile water (concentration was normalized spectrophotometrically at 600 nm). Plates were incubated at the different temperatures for 7 days, and growth was measured as the average increase of colony diameter. Plates were incubated for further 7 days to confirm the inability to grow at 0°C.

Results

Strain isolation

Table 1 reports the results of the preliminary tests carried out on all the isolates. The majority of the bacteria (ca. 90%) were Gram negative. As for catalase and oxidase, positive strains were ca. 96 and 46%, respectively. All the isolates showed a more or less evident rod shape with a wide range of dimensions (1.1–4.0 µm length; 0.4–1.0 µm width).

Strain identification

Isolates subjected to 16S rDNA sequence analysis were affiliated to the following genera: *Acinetobacter, Arthrobacter, Bacillus, Enterobacter, Exiguobacterium, Flavobacterium, Janthinobacterium, Microbacterium, Myroides, Pantoea, Pseudomonas, Rhodococcus, Serratia, Shewanella, Sphingobacterium and Stenotrophomonas.* All sequences matched with GenBank entries with similarities ranging from 96 to 100%.

Table 2 reports the GenBank accession number of isolates and their closest phylogenetic relatives. Only 20 strains out of 52 (ca. 38%), showing 99–100% identity with a single known microorganism, were identified at species level. All other isolates were identified at the genus level only: for most of them, affiliation was not possible because 98 and 99% of identity was recorded with various species of the same genus. In addition, 3 strains showed a lower percentage of identity (96–97%).

Out of the 47 Gram negative, 37 belonged to the phylum *Proteobacteria* being *Pseudomonas* and *Serratia*, both in

Table 1 Morphological and biochemical characteristic of the strains isolated from water samples collected in various sites of Kandalaksha Bay

Strain	Gram	Cat	Oxy	Dimensions (µm)	Colony aspect[a] (color/shape/edge/surface/elevation)	Sampling site[b]
KB01	–	+	–	0.7 ± 0.1/1.2 ± 0.1	R/C/E/W/U	2/3/5/6
KB02	–	+	+	0.5 ± 0.0/3.0 ± 0.1	Y/C/U/P/F	1/2/5/6
KB03	+	+	+	0.4 ± 0.2/1.5 ± 0.2	O/C/C/G/R	1/2
KB04	–	+	+	0.5 ± 0.0/4.0 ± 0.1	B/C/F/P/F	1/2
KB05	–	+	+	0.8 ± 0.2/1.3 ± 0.0	Y/C/E/G/C	1/2/5
KB06	–	+	+	0.8 ± 0.0/4.0 ± 0.3	W-G/I/U/S/R	1/2/5/6
KB07	–	+	+	0.8 ± 0.2/1.8 ± 0.2	W-G/C/U/S/C	1/2/3/6
KB10	–	+	–	1.0 ± 0.2/2.5 ± 0.1	W-G/I/U/R/R	1/2/5/6
KB11	–	+	+	1.0 ± 0.1/2.0 ± 0.0	W-O/C/E/W/C	1/2/3/5/6
KB12	–	+	–	0.5 ± 0.1/3.0 ± 0.4	Y/I/L/S/R	2/5
KB16	–	+	–	0.8 ± 0.1/1.5 ± 0.1	W/C/E/G/P	2/6
KB17	–	+	–	0.8 ± 0.3/1.6 ± 0.2	W/C/E/G/P	1/2/3/5/6
KB20	–	+	+	0.8 ± 0.1/2.0 ± 0.3	W/I/U/S/C	1/2/6
KB21	–	+	+	0.8 ± 0.0/1.5 ± 0.0	W/C/E/S/C	1/2/6
KB22	–	+	–	0.8 ± 0.2/1.5 ± 0.1	W/C/E/G/P	1/2
KB23	–	+	+	1.0 ± 0.2/1.8 ± 0.2	Y-G/I/U/S/C	1/2
KB24	–	+	+	0.8 ± 0.1/2.0 ± 0.1	W/C/E/S/C	1/2/4/6
KB25	–	+	–	1.0 ± 0.1/1.5 ± 0.1	W/C/E/G/P	1/2
KB30	–	–	–	0.8 ± 0.3/1.2 ± 0.1	W-O/I/U/G/C	1/2/5/6
KB31	–	+	+	0.8 ± 0.1/1.5 ± 0.2	Y/C/E/G/C	1/2
KB32	–	+	+	1.0 ± 0.2/1.3 ± 0.1	Y/C/E/G/P	2

Table 1 continued

Strain	Gram	Cat	Oxy	Dimensions (μm)	Colony aspect[a] (color/shape/edge/surface/elevation)	Sampling site[b]
KB33	–	+	+	0.9 ± 0.3/1.2 ± 0.0	Y/C/E/G/C	1/2/6
KB36	–	+	+	0.8 ± 0.1/2.0 ± 0.0	W-G/C/L/R/R	2
KB37	–	+	+	1.0 ± 0.0/2.0 ± 0.1	W/C/U/G/C	1/2/5/6
KB38	–	+	–	0.8 ± 0.1/1.5 ± 0.0	Y-G/C/E/R/R	1/2/6
KB39	–	+	–	0.8 ± 0.0/1.8 ± 0.2	W-O/I/U/S/R	2
KB40	–	+	–	1.0 ± 0.2/2.0 ± 0.4	W/I/U/G/C	1/2/5/6
KB42	–	+	–	1.0 ± 0.3/2.5 ± 0.2	W/C/U/R/R	1/2
KB43	–	–	–	0.5 ± 0.0/2.0 ± 0.0	Y/C/E/G/C	1/2
KB44	–	+	–	0.8 ± 0.0/2.0 ± 0.1	W-Y/I/E/S/R	1/2
KB45	–	+	–	1.0 ± 0.1/3.0 ± 0.6	W-Y/C/E/S/C	1/2/6
KB46	–	+	+	1.0 ± 0.2/2.0 ± 0.3	Y/C/E/R/R	1/2
KB47	–	+	+	0.8 ± 0.1/2.0 ± 0.2	Y-G/I/U/R/R	1/2/6
KB49	–	+	–	1.0 ± 0.1/1.8 ± 0.2	W/C/U/G/P	1/2/6
KB50	–	+	+	0.5 ± 0.1/2.0 ± 0.0	Y-G/I/L/R/R	1/6
KB51	–	+	+	1.0 ± 0.1/2.0 ± 0.3	V/I/L/W/R	1/2
KB52	–	+	–	0.8 ± 0.2/1.1 ± 0.1	W/I/L/R/R	1/2/6
KB54	+	+	–	1.0 ± 0.3/1.2 ± 0.4	W-O/I/U/D/U	1/2/6
KB56	–	+	–	1.0 ± 0.0/1.8 ± 0.0	W/C/U/G/P	1/2/6
KB57	+	+	–	1.0 ± 0.3/1.2 ± 0.1	Y/C/E/G/R	1/2/6
KB58	–	+	+	0.5 ± 0.1/4.0 ± 0.5	Y/C/U/P/F	1/2
KB59	+	+	–	0.5 ± 0.2/1.1 ± 0.3	Y/C/E/G/P	1/2/6
KB61	–	+	–	1.0 ± 0.3/2.0 ± 0.0	W/C/U/G/P	1/6
KB63	–	+	–	1.0 ± 0.2/3.0 ± 0.2	W-O/I/L/S/R	1/6
KB64	–	+	–	1.0 ± 0.4/3.0 ± 0.7	Y/C/E/G/R	1/4/6
KB66	+	+	+	0.5 ± 0.2/2.0 ± 0.1	W/I/C/D/R	1
KB68	–	+	–	1.0 ± 0.1/2.0 ± 0.0	W/C/E/G/R	4/6
KB71	–	+	+	1.0 ± 0.2/1.8 ± 0.2	W-R/I/U/G/P	2
KB72	–	+	+	1.0 ± 0.2/2.0 ± 0.1	R/C/E/W/U	5
KB73	–	+	–	1.0 ± 0.0/2.0 ± 0.6	W/I/L/G/R	5
KB75	–	+	–	1.0 ± 0.2/1.2 ± 0.2	W-T/C/E/R/F	1
KB76	–	+	–	1.0 ± 0.1/2.0 ± 0.4	Y/I/L/R/F	1

Cat catalase, *Oxy* oxidase

[a] Colony aspect is described according to the following parameters: color: *B* brown, *G* green, *O* orange, *R* red, *T* transparent, *V* violet, *W* white, *Y* yellow; shape: *C* circular, *I* irregular; edge: *C* curled, *E* entire, *F* filamentous, *L* lobate, *U* undulate; surface: *D* dry, *G* glistening, *P* powdery, *R* rough, *S* smooth, *W* wrinkled; elevation: *C* convex, *F* flat, *P* pulvinate, *R* raised, *U* umbonate

[b] Samples were collected in the following sites: 1 = intertidal zone pool (66°33′15″N; 33°05′47″E); 2 = water surface nearby the intertidal zone pool (66°33′15″N; 33°05′47″E); 3 = 0.5 m depth (66°32′21″N, 33°14′77″E); 4 = 70 m depth (66°32′21″N, 33°14′77″E); 5 = 2.5 m depth (66°32′29″N, 33°14′94″E); 6 = 15.5 m depth (66°32′30″N, 33°14′94″E)

the class *Gammaproteobacteria*, the most predominant genera with 21 and 10 strains, respectively. Among *Pseudomonas*, only 7 strains were identified at species level and belonged to *P. fluorescens* (KB06, KB11, KB20, KB24 and KB36), *P. syringae* (KB10) and *P. putida* (KB12 and KB76). Among *Serratia,* strains were affiliated to *S. plymuthica* (KB1) and *S. proteamaculans* (KB22, KB49 and KB56). The only typical bacterium of cold marine environments was KB30 identified as *Shewanella baltica* (*Gammaproteobacteria*). All other Gram negative were related to the phylum *Bacteroidetes* including 5 strains affiliated to the genus *Sphingobacterium* and 4 to *Flavobacterium*.

As for Gram positive, the few strains present were affiliated to the phyla *Actinobacteria* (KB54, KB57 and KB59) and *Firmicutes* (KB3 and KB66).

Optimal growth temperature

Table 3 shows the growth range and optima of the various strains; if available, comparison with literature is also

Table 2 Identification of the Kandalaksha Bay strains based on 16S rDNA analysis

Strain	Accession number	Closest phylogenetic relative	Identity
KB01	JF327440	*Serratia plymuthica*	99%
KB02	JF327441	*Flavobacterium* sp.	98%
KB03	JF327442	*Exiguobacterium oxidotolerans*	100%
KB04	JF327443	*Flavobacterium* sp.	97%
KB05	JF327444	*Sphingobacterium* sp.	99%
KB06	JF327445	*Pseudomonas fluorescens*	100%
KB07	JF327446	*Pseudomonas* sp.	99%
KB10	JF327447	*Pseudomonas syringae*	99%
KB11	JF327448	*Pseudomonas fluorescens*	99%
KB12	JF327449	*Pseudomonas putida*	99%
KB16	JF327450	*Serratia* sp.	100%
KB17	JF327451	*Serratia* sp.	99%
KB20	JF327452	*Pseudomonas fluorescens*	100%
KB21	JF327453	*Pseudomonas* sp.	98%
KB22	JF327454	*Serratia proteamaculans*	100%
KB23	JF327455	*Pseudomonas* sp.	99%
KB24	JF327456	*Pseudomonas fluorescens*	100%
KB25	JF327457	*Serratia* sp.	99%
KB30	JF327458	*Shewanella baltica*	100%
KB31	JF327459	*Myroides* sp.	96%
KB32	JF327460	*Sphingobacterium* sp.	99%
KB33	JF327461	*Sphingobacterium* sp.	99%
KB36	JF327462	*Pseudomonas fluorescens*	99%
KB37	JF327463	*Pseudomonas* sp.	99%
KB38	JF327464	*Pantoea agglomerans*	99%
KB39	JF327465	*Pseudomonas* sp.	99%
KB40	JF327466	*Pseudomonas* sp.	99%
KB42	JF327467	*Pseudomonas* sp.	99%
KB43	JF327468	*Stenotrophomonas* sp.	100%
KB44	JF327469	*Pseudomonas* sp.	99%
KB45	JF327470	*Sphingobacterium* sp.	100%
KB46	JF327471	*Sphingobacterium* sp.	99%
KB47	JF327472	*Pseudomonas* sp.	99%
KB49	JF327473	*Serratia proteamaculans*	99%
KB50	JF327474	*Pseudomonas* sp.	99%
KB51	JF327475	*Janthinobacterium lividum*	100%
KB52	JF327476	*Enterobacter* sp.	98%
KB54	JF327477	*Rhodococcus erythropolis*	100%
KB56	JF327478	*Serratia proteamaculans*	99%
KB57	JF327479	*Arthrobacter* sp.	100%
KB58	JF327480	*Flavobacterium* sp.	97%
KB59	JF327481	*Microbacterium oxydans*	100%
KB61	JF327482	*Serratia* sp.	100%
KB63	JF327483	*Pseudomonas* sp.	99%
KB64	JF327484	*Pantoea agglomerans*	100%
KB66	JF327485	*Bacillus pumilus*	100%
KB68	JF327486	*Pseudomonas* sp.	99%
KB71	JF327487	*Serratia* sp.	99%
KB72	JF327488	*Serratia* sp.	98%
KB73	JF327489	*Pseudomonas* sp.	99%
KB75	JF32749	*Acinetobacter* sp.	98%
KB76	JF327452	*Pseudomonas putida*	99%

reported for the identified species. Most of the strains (ca. 42%) showed the optimum at 30°C. In addition, the majority of them were able to grow in a rather broad range of temperature. The lowest optimum was recorded at 15°C for one strain only (KB75), while highest optimum was at 40°C (KB22 and KB49). Most of the strains (56%) were able to grow at 0°C, while only 25% grew at 45°C.

Figure 1 shows the temperature growth profile of the KB strains. Colony diameters at optima ranged between 6 and 70 mm.

Discussion

White Sea is a peculiar microcosm, having unique physical, chemical, geological and biological characteristics (Pantyulin 2003). Although it could supply very interesting and new ecological information, this environment is scarcely investigated from the microbiological point of view. Even though samples have been collected only in Kandalaksha Bay, this extensive work represents the first study concerning the growth temperature preferences of bacteria isolated from this environment.

As for strain identification, most of the isolates belong to *Gammaproteobacteria,* but other well-represented classes are *Sphingobacteria* and *Flavobacteria*. Thus, in our samples, *Proteobacteria* and *Cytophaga–Flavobacterium–Bacteroidetes* group (CFB) seem to be dominant, as already reported for other cold environments (Bai et al. 2006; Amato et al. 2007; Srinivas et al. 2009). As said, *Pseudomonas* and *Serratia* were the most representative genera of *Proteobacteria*: this is not surprising since these are well-known ubiquitous bacteria. The only typical bacterium of cold seas was the gram-negative rod *S. baltica,* already described in samples from the Baltic Sea (Ziemke et al. 1998; Vogel et al. 2005). Moreover, this seems to be the only true marine species isolated in our study: all other strains are spread both in terrestrial and marine environments. This result was quite unexpected, since others works, carried out on similar environments, demonstrated that marine bacteria were more recurrent (Bowman et al.

Table 3 Temperature preferences comparison between Kandalaksha Bay strains and strains of the same species reported in literature

Strain	Closest phylogenetic relative	KB strains		Literature		Citation
		Range °C	Optimum °C	Range °C	Optimum °C	
KB01	*Serratia plymuthica*	5–45	30	10–40	30	(Breed et al. 1948; Pradhan and Ingle 2007)
KB02	*Flavobacterium* sp.	0–30	20			
KB03	*Exiguobacterium oxidotolerans*	5–35	30	4–40	34	(Yumoto et al. 2004)
KB04	*Flavobacterium* sp.	0–30	20			
KB05	*Sphingobacterium* sp.	0–45	25–30			
KB06	*Pseudomonas fluorescens*	0–35	30	0–37	30	(Gugi et al. 1991; Jaouen et al. 2004)
KB07	*Pseudomonas* sp.	5–45	30			
KB10	*Pseudomonas syringae*	5–40	30	4–30	28	(Young et al. 1977)
					22	(Sundareswaran et al. 2010)
KB11	*Pseudomonas fluorescens*	0–35	30	0–37	30	(Gugi et al. 1991; Jaouen et al. 2004)
KB12	*Pseudomonas putida*	5–45	25	0–30	30	(Palleroni 1984; Kotturi et al. 1991)
KB16	*Serratia* sp.	0–40	20			
KB17	*Serratia* sp.	5–45	30			
KB20	*Pseudomonas fluorescens*	0–45	20	0–37	30	(Gugi et al. 1991; Jaouen et al. 2004)
KB21	*Pseudomonas* sp.	0–45	20			
KB22	*Serratia proteamaculans*	0–45	40	4–37	20–30	(Grimont et al. 1982; Old et al. 1983)
KB23	*Pseudomonas* sp.	0–35	30			
KB24	*Pseudomonas fluorescens*	0–40	25–30	0–37	30	(Gugi et al. 1991; Jaouen et al. 2004)
KB25	*Serratia* sp.	5–40	25			
KB30	*Shewanella baltica*	0–35	20	4–37	25	(Ziemke et al. 1998; Vogel et al. 2005)
KB31	*Myroides* sp.	5–30	25			
KB32	*Sphingobacterium* sp.	0–40	25			
KB33	*Sphingobacterium* sp.	0–40	30			
KB36	*Pseudomonas fluorescens*	0–40	25	0–37	30	(Gugi et al. 1991; Jaouen et al. 2004)
KB37	*Pseudomonas* sp.	0–45	25			
KB38	*Pantoea agglomerans*	5–35	30	5–45	30	(Gavini et al. 1989; Son et al. 2006)
KB39	*Pseudomonas* sp.	5–45	35			
KB40	*Pseudomonas* sp.	0–45	20			
KB42	*Pseudomonas* sp.	0–35	30			
KB43	*Stenotrophomonas* sp.	5–40	25			
KB44	*Pseudomonas* sp.	0–40	30			
KB45	*Sphingobacterium* sp.	5–40	30			
KB46	*Sphingobacterium* sp.	0–35	25			
KB47	*Pseudomonas* sp.	0–35	25			
KB49	*Serratia proteamaculans*	0–45	40	4–37	20–30	(Grimont et al. 1982; Old et al. 1983)
KB50	*Pseudomonas* sp.	0–35	30			
KB51	*Janthinobacterium lividum*	0–30	20	4–30	25	(De Ley et al. 1978; Sneath 1984)
KB52	*Enterobacter* sp.	10–40	25			
KB54	*Rhodococcus erythropolis*	5–30	25	10–40	25	(Tomioka et al. 1994)
KB56	*Serratia proteamaculans*	5–40	30	4–37	20–30	(Grimont et al. 1982; Old et al. 1983)
KB57	*Arthrobacter* sp.	5–40	30			
KB58	*Flavobacterium* sp.	0–30	25			
KB59	*Microbacterium oxydans*	10–40	30		30	(Schumann et al. 1999)
KB61	*Serratia* sp.	0–40	20			
KB63	*Pseudomonas* sp.	0–35	25			
KB64	*Pantoea agglomerans*	5–40	25	5–45	30	(Gavini et al. 1989; Son et al. 2006)
KB66	*Bacillus pumilus*	10–45	35	10–55	37	(Battan et al. 2007)

Table 3 continued

Strain	Closest phylogenetic relative	KB strains		Literature		Citation
		Range °C	Optimum °C	Range °C	Optimum °C	
KB68	*Pseudomonas* sp.	5–40	30			
KB71	*Serratia* sp.	5–40	30			
KB72	*Serratia* sp.	5–40	30			
KB73	*Pseudomonas* sp.	0–40	25			
KB75	*Acinetobacter* sp.	0–30	15			
KB76	*Pseudomonas putida*	5–40	20	0–30	30	(Palleroni 1984; Kotturi et al. 1991)

1997; Amato et al. 2007; Srinivas et al. 2009). Possible explication is the peculiar Kandalaksha Bay estuarine hydrodynamic characterized by strong contribution of freshwater carrying lot of microorganisms also from soil (Howland et al. 1999; Pantyulin 2003; Cobelo-García et al. 2006). On the other hand, the above considerations are valid for the microorganisms identified at species level only. In fact, other marine bacteria could be present among the strains that are still partially identified. Besides, *Flavobacterium* is well known as one of the most represented genera in marine environments being common also in sea ice (Hayes 1963; Bowman et al. 1997).

As for temperature preferences, microorganisms are traditionally grouped according to their ranges and optima for growth (Wiegel 1990). However, current definitions are not satisfactory to cover the huge microbial variability. As already reported by the early studies of Rouf and Rigney (1971), since the range of growth for certain species is from 0 to 55°C, simple consideration of minimal, optimal and maximal growth temperatures may not be sufficient to classify them as psychrophiles or mesophiles. Due to the increased attention to extreme environments and consequent discovery of peculiar microorganisms, showing uncommon adaptations to temperature variations, this question became progressively more crucial and has been largely debated (Gounot 1991; Rothschild and Mancinelli 2001; Helmke and Weyland 2004). Nevertheless, the most accepted definition for cold-adapted bacteria is still that of Morita (1975). Accordingly, psychrophiles were defined as those having an optimal temperature for growth at about 15°C, a maximum at 20°C and a minimum at 0°C or below. Microorganisms able to grow at about 0°C, but having maximum above 20°C, were considered as psychrotrophics: optimum is not mentioned (Morita 1975; Gounot 1986; Gounot 1991; Dalluge et al. 1997). However, the term psychrotrophic had been recently replaced with the most significant "psychrotolerant" (Helmke and Weyland 2004). Mesophilic microorganisms are often defined as growing in the range 10–50°C (Russell and Fukunaga 1990; Wiegel 1990) while optima are generally in the range of ca. 30–40°C. Lot of mesophilic microorganisms from cold environments, even if showing optima in the mesophilic range, grow well at about 0°C. These organisms had been defined as mesophilic-psychrotolerant and fall also in the broad group of psychrotolerant (Zucconi et al. 1996; Helmke and Weyland 2004; Wang and Zheng 2005). Thus, the definition of psychrotolerant, that, in principle, has been proven to be useful, could be somehow too generic and nonspecific because it would combine microorganisms with very different optima. Therefore, the cardinal temperatures for psychrotolerants should be reconsidered on the basis of increased numbers of cold-adapted microorganisms isolated from various habitats and in light of new ecological data (Helmke and Weyland 2004). Even if strict definitions are never exhaustive, we would prefer to define as psychrotolerant those bacteria that able to grow at about 0°C and having their optima in the range >15–≤25°C. In consequence, we would consider as mesophilic-psychrotolerant those bacteria growing at about 0°C with optima in the range >25–≤40°C.

It is also important to know what is the widest temperature range (temperature span) over which a single organism can grow. A wider temperature range makes an organism more versatile with regard to environmental changes, and it enables the organism to utilize a wider array of ecological niches (Wiegel 1990). Generally, organisms able to grow in a wide range of temperature are defined as eurythermics.

In our case, according to the above definitions, the majority of the strains were psychrotolerant (42%) or mesophilic-psychrotolerant (40%) and no real psychrophiles were detected (Table 3). Almost all the strains could be considered as eurythemics indicating adaptation to frequent and wide temperature variations such as those of Kandalaksha Bay (Howland et al. 1999; Pantyulin 2003; Savvichev et al. 2004; Shaporenko et al. 2005). Actually, psychrophiles, with their narrow range of growth temperature, are typical of rather stable environments (Zucconi et al. 1996; Bowman et al. 1997; Helmke and Weyland 2004). Even strain KB75 (*Acinetobacter* sp.), having optimum at 15°C, cannot be strictly attributed to this group because it can grow up to 30°C. Anyway, presence of

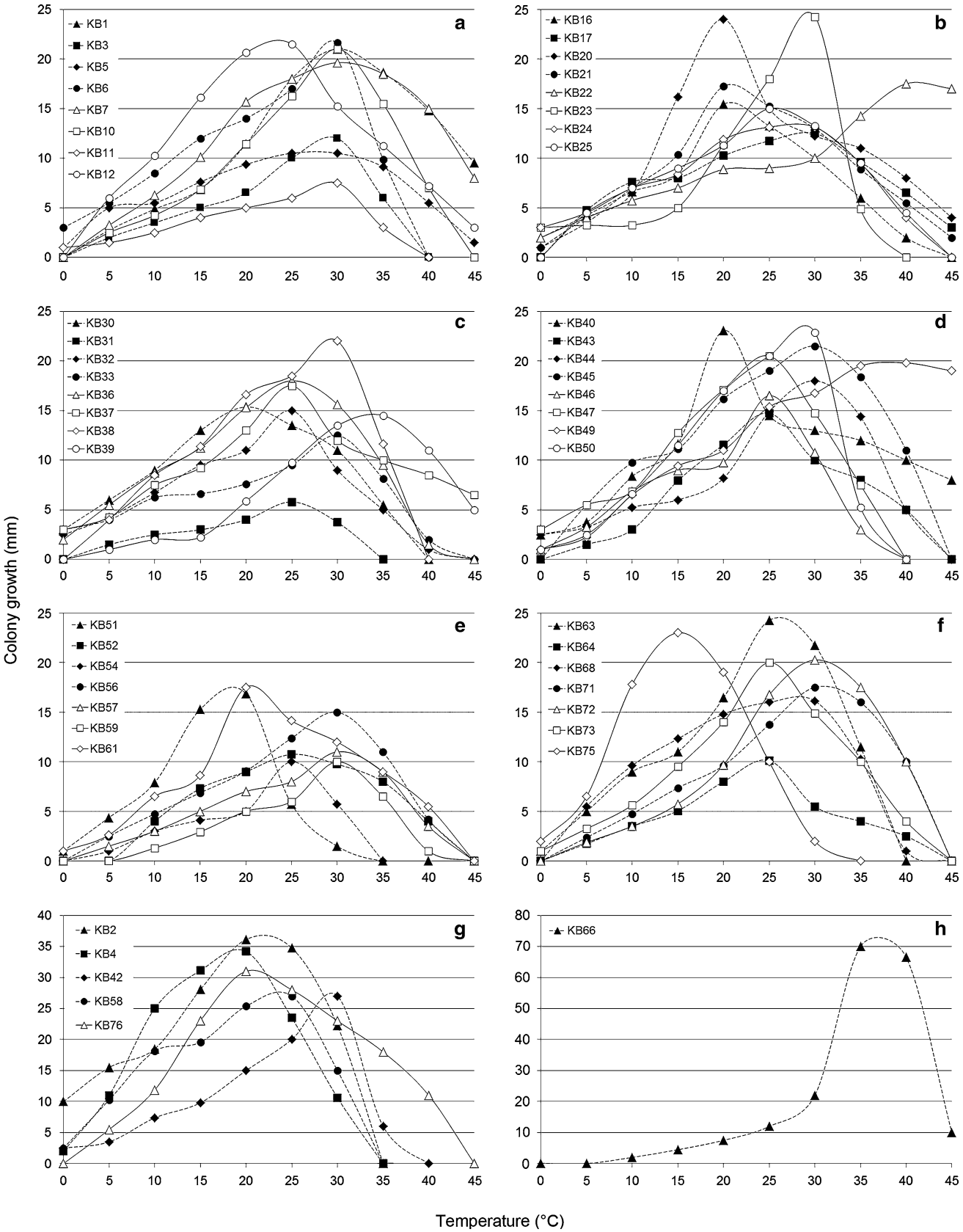

Fig. 1 Temperature profiles of KB strains cultivated in PCA in the range 0–45°C. Growth was measured as average increase of colony diameter. Data are mean of 3 replicates; SD was less than 10%

psychrotolerant microorganisms, rather than psychrophiles, in Arctic and Antarctic regions has been well documented (Zucconi et al. 1996; Ray et al. 1999; Shivaji et al. 2005). Finally, only KB66 showed a strictly mesophilic behavior.

Another important question to address is the growth curve profile. Normally, curves are characterized by an asymmetric shape: slope over the optimum is quite sharper than that below. In fact, a temperature rise of just 5°C above the optimum is often sufficient for strong growth inhibition, and a further increase of 5°C could lead to death. By contrast, below the optimum, microorganisms could survive well even with a decrease of 30–40°C. Growth at the optimum is generally much more pronounced than that at lowest and highest limits.

Growth profiles of KB bacteria are very diversified (Fig. 1) highlighting a composite community with different degrees of adaptation to environmental changing conditions (Berry and Foegeding 1997). Some strain (ca. 33%) had the above-described classic profile, and others showed uncommon flat and wide curves: they presented a limited growth, even at the optimum, but temperature span was quite extended. For example, KB11 grew between 0 and 35°C, but maximum colony diameter was ca. 7.5 mm only (Fig. 1a). In addition, few strains had a peculiar symmetric curve (e.g. KB75, Fig. 1f) with apparent similar response to cold and warm temperatures. Some other bacteria, such as KB61 and KB76, showed an unusual sharper growth decrease below rather than above the optimum (Fig 1e, g). Apparently, they were more tolerant to warm than to cold temperatures. This phenomenon has never been reported before.

As said, temperature spans were, in general, quite broad (ca. 35°C), and it was evident that many KB strains had wider ranges of growth if compared with same species described in literature; optima were also different. However, it is worth noting that studies concerning temperature preference of bacterial strains are not very common even for well-known species, and few data were available.

The availability of microorganisms showing a very broad eurythermism could be useful for biotechnological applications. For example, since microbial adaptation to temperature variations is often related to the production of enzymes, showing broad range of activity, these microorganisms could represent interesting source of new enzyems or enzymes showing very peculiar characteristics. The search of new extremozymes is one of the most important biotechnological field and, in general, the research is oriented to thermotolerant or to psychrotolerant biocatalysts. The discovery of enzymes with extremely broad temperature range of activity could have a very strong impact on specific industries.

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Amato P, Hennebelle R, Magand O, Sancelme M, Delort A-M, Barbante C, Boutron C, Ferrari C (2007) Bacterial characterization of the snow cover at Spitzberg, Svalbard. FEMS Microbiol Ecol 59:255–264

Bai Y, Yang D, Wang J, Xu S, Wang X, An L (2006) Phylogenetic diversity of culturable bacteria from alpine permafrost in the Tianshan mountains, northwestern China. Res Microbiol 157:741–751

Battan B, Sharma J, Dhiman SS, Kuhad RC (2007) Enhanced production of cellulase-free thermostable xylanase by *Bacillus pumilus* ASH and its potential application in paper industry. Enzym Microb Technol 41:733–739

Berger V, Gorbushin A (2001) Tolerance and resistance in gastropod mollusks *Hydrobia ulvae* and *H. ventrosa* from the White Sea to Abiotic Environmental Factors. Russ J Mar Biol 27:314–319

Berry ED, Foegeding PM (1997) Cold temperature adaptation and growth of microorganisms. J Food Prot 60:1583–1594

Bowman JP, McCammon SA, Brown MV, Nichols DS, McMeekin TA (1997) Diversity and association of psychrophilic bacteria in Antarctic sea ice. Appl Environ Microbiol 63:3068–3078

Breed R, Murray E, Hitchens A (1948) Bergey's manual of determinative bacteriology, 6th edn. The Williams & Wilkins Co., Baltimore

Cobelo-García A, Millward GE, Prego R, Lukashin V (2006) Metal concentrations in Kandalaksha Bay, White Sea (Russia) following the spring snowmelt. Environ Pollut 143:89–99

Dalluge JJ, Hamamoto T, Horikoshi K, Morita RY, Stetter KO, McCloskey JA (1997) Posttranscriptional modification of tRNA in psychrophilic bacteria. J Bacteriol 179:1918–1923

De Ley J, Segers P, Gillis M (1978) Intra- and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. Int J Syst Bacteriol 28:54–168

Dolotov YS, Filatov NN, Shevchenko VP, Nemova NN, Rimskii-Korsakov NA, Denisenko NV, Kutcheva IP, Boyarinov PM, Petrov MP, VKh Lifshitz, Platonov AV, Demina LL, Kukharev VI, Kovalenko VN, Zdorovennov RE, Rat'kova TN, Sergeeva OM, Novigatskii AN, Pautova LA, Filipieva KV, Nöthig EM, Loronzen C (2005) Monitoring tidal conditions in estuaries of the Karelian Coast of the White Sea. Water Res 32:611–628

Dul'tseva N, Dubinina GA, Lysenko AM (1996) Isolation of marine filamentous sulfur bacteria and description of the new species *Leucothrix thiophila* sp. nov. Microbiology 65:79–87

Gavini F, Mergaert J, Beji A, Mielcarek C, Izard D, Kersters K, De Ley J (1989) Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Int J Syst Bacteriol 39:337–345

Gorelova O, Kosevich IA, Baulina OI, Fedorenko TA, Torshkhoeva AZ, Lobakova ES (2009) Associations between the White Sea invertebrates and oxygen-evolving phototrophic microorganisms. Moscow Univ Biol Sci Bull 64:16–22

Gorlenko V, Puchkova NN, Demchev VV (1985) Photosynthetic microorganisms of supralittoral bath of White Sea. Dokl Biol Sci 5:66–72

Gounot AM (1986) Psychrophilic and psychrotrophic microorganisms. Cell Mol Life Sci 42:1192–1197

Gounot AM (1991) Bacterial life at low temperature: physiological aspects and biotechnological implications. J Appl Microbiol 71:386–397

Grimont PAD, Grimont F, Irino K (1982) Biochemical characterization of *Serratia liquefaciens sensu stricto*, *Serratia proteamaculans* and *Serratia grimesii* sp. nov. Curr Microbiol 7:69–74

Gugi B, Orange N, Hellio F, Burini JF, Guillou C, Leriche F, Guespin-Michel JF (1991) Effect of growth temperature on several exported enzyme activities in the psychrotrophic bacterium *Pseudomonas fluorescens*. J Bacteriol 173:3814–3820

Hayes PR (1963) Studies on marine *Flavobacteria*. J Gen Microbiol 30:1–19

Helmke E, Weyland H (2004) Psychrophilic versus psychrotolerant bacteria—occurrence and significance in polar and temperate marine habitats. Cell Mol Biol 50:553–561

Hongoh Y, Yuzawa H, Ohkuma M, Kudo T (2003) Evaluation of primers and PCR conditions for the analysis of 16S rRNA genes from a natural environment. FEMS Microbiol Lett 221:299–304

Howland R, Pantiulin AN, Millward GE, Prego R (1999) The hydrography of the Chupa Estuary, White Sea, Russia. Estuar Coast Shelf S 48:1–12

Jaouen T, De E, Chevalier S, Orange N (2004) Pore size dependence on growth temperature is a common characteristic of the major outer membrane protein OprF in psychrotrophic and mesophilic pseudomonas species. Appl Environ Microbiol 70:6665–6669

Kotturi G, Robinson CW, Inniss WE (1991) Phenol degradation by a psychrotrophic strain of *Pseudomonas putida*. Appl Microbiol Biotechnol 34:539–543

Kovacs N (1956) Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703

Kravchishina M, Mitzkevich I, Veslopolova E, Shevchenko V, Lisitzin A (2008) Relationship between the suspended particulate matter and microorganisms in the White Sea waters. Oceanology 48(6):837–854

Kuts V, Ismailov A (2009) Physiological and emission characteristics of the luminescent bacterium *Photobacterium phosphoreum* from the White Sea. Microbiology 78:554–558

Melnikov IA, Korneeva GA, Zhitina LS, Shanin SS (2003) Dynamics of ecological–biochemical characteristics of sea ice in coastal waters of the White Sea. Biol Bull Acad Sci USSR 30:164–170

Morita R (1975) Psychrophilic bacteria. Bacteriol Rev 39:144–167

Old D, Adegbola R, Scott S (1983) Multiple fimbrial haemagglutinins in *Serratia* species. Med Microbiol Immunol 172:107–115

Palleroni N (1984) Genus I. *Pseudomonas*. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore

Pantyulin AN (2003) Hydrological system of the White Sea. Oceanology 43:S1–S14

Pradhan N, Ingle AO (2007) Mineralization of phenol by a *Serratia plymuthica* strain GC isolated from sludge sample. Int Biodeter Biodegr 60:103–108

Rabold S, Gorlenko VM, Imhoff JF (2006) *Thiorhodococcus mannitoliphagus* sp. nov., a purple sulfur bacterium from the White Sea. Int J Syst Evol Micr 56:1945–1951

Ray MK, Sitaramamma T, Kumar GS, Kannan K, Shivaji S (1999) Transcriptional activity at supraoptimal temperature of growth in the antarctic psychrotrophic bacterium *Pseudomonas syringae*. Curr Microbiol 38:143–150

Rothschild LJ, Mancinelli RL (2001) Life in extreme environments. Nature 409:1092–1101

Rouf MA, Rigney MM (1971) Growth temperatures and temperature characteristics of *Aeromonas*. Appl Environ Microbiol 22:503–506

Russell NJ, Fukunaga N (1990) A comparison of thermal adaptation of membrane lipids in psychrophilic and thermophilic bacteria. FEMS Microbiol Lett 75:171–182

Savvichev A, Rusanov II, Yusupov SK, Bairamov IT, Pimenov NV, AYu Lein, Ivanov MV (2003) The process of microbial sulfate reduction in sediments of the coastal zone and littoral of the Kandalaksha Bay of the White Sea. Microbiology 72:478–489

Savvichev AS, Rusanov II, Yusupov SK, Pimenov NV, Lein AY, Ivanov MV (2004) The biogeochemical cycle of methane in the coastal zone and littoral of the Kandalaksha Bay of the White Sea. Microbiology 73:457–468

Schumann P, Rainey FA, Burghardt J, Stackebrandt E, Weiss N (1999) Reclassification of *Brevibacterium oxydans* (Chatelain and Second 1966) as *Microbacterium oxydans* comb. nov. Int J Syst Bacteriol 49:175–177

Selbmann L, Zucconi L, Ruisi S, Grube M, Cardinale M, Onofri S (2010) Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. Polar Biol 33:71–83

Shaporenko S, Koreneva GA, Pantyulin AN, Pertsova NM (2005) Characteristics of the ecosystems of water bodies separating from Kandalaksha Bay of the White Sea. Water Res 32:469–483

Shivaji S, Gupta P, Chaturvedi P, Suresh K, Delille D (2005) *Marinobacter maritimus* sp. nov., a psychrotolerant strain isolated from sea water off the subantarctic Kerguelen islands. Int J Syst Evol Microbiol 55:1453–1456

Sneath P (1984) Janthinobacteriurn De Ley, Segers and Gillis 1978, 164AL. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. The Williams & Wilkins Co., Baltimore, pp 376–377

Son H-J, Park G-T, Cha M-S, Heo M-S (2006) Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Bioresour Technol 97:204–210

Srinivas T, Nageswara Rao SSS, Vishnu Vardhan Reddy P, Pratibha MS, Sailaja B, Kavya B, Hara Kishore K, Begum Z, Singh SM, Shivaji S (2009) Bacterial diversity and bioprospecting for cold-active lipases, amylases and proteases from culturable bacteria of Kongsfjorden and Ny-Ålesund, Svalbard, Arctic. Curr Microbiol 59:537–547

Sundareswaran V, Singh A, Dube S, Shivaji S (2010) Aspartate aminotransferase is involved in cold adaptation in psychrophilic *Pseudomonas syringae*. Arch Microbiol 192:663–672

Tomioka N, Uchiyama H, Yagi O (1994) Cesium accumulation and growth characteristics of *Rhodococcus erythropolis* CS98 and *Rhodococcus* sp. strain CS402. Appl Environ Microbiol 60:2227–2231

Ushakova OO (2003) Combined effect of salinity and temperature on *Spirorbis spirorbis* L. and *Circeus spirillum* L. larvae from the White Sea. J Exp Mar Biol Ecol 296:23–33

Vershinin A, Moruchkov A, Morton SL, Leighfield TA, Quilliam MA, Ramsdell JS (2006) Phytoplankton composition of the Kandalaksha Gulf, Russian White Sea: *Dinophysis* and lipophilic toxins in the blue mussel (*Mytilus edulis*). Harmful Algae 5:558–564

Vogel BF, Venkateswaran K, Satomi M, Gram L (2005) Identification of *Shewanella baltica* as the most important H2S-producing species during iced storage of Danish marine fish. Appl Environ Microbiol 71:6689–6697

Wang X-W, Zheng R-Y (2005) *Chaetomium acropullum* sp. nov. (*Chaetomiaceae*, *Ascomycota*), a new psychrotolerant mesophilic species from China. Nova Hedwigia 80:413–417

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703

Whittenbury R (1964) Hydrogen peroxide formation and catalase activity in the lactic acid bacteria. J Gen Microbiol 35:13–26

Wiegel J (1990) Temperature spans for growth: Hypothesis and discussion. FEMS Microbiol Lett 75:155–169

Young JM, Luketina RC, Marshall AM (1977) The effects on temperature on growth in vitro of *Pseudomonas syringae* and *Xanthomonas pruni*. J Appl Microbiol 42:345–354

Yumoto I, Hishinuma-Narisawa M, Hirota K, Shingyo T, Takebe F, Nodasaka Y, Matsuyama H, Hara I (2004) Exiguobacterium oxidotolerans sp. nov., a novel alkaliphile exhibiting high catalase activity. Int J Syst Evol Microbiol 54:2013–2017

Zhang G, Ma X, Niu F, Dong M, Feng H, An L, Cheng G (2007) Diversity and distribution of alkaliphilic psychrotolerant bacteria in the Qinghai–Tibet plateau permafrost region. Extremophiles 11:415–424

Ziemke F, Hofle MG, Lalucat J, Rossello-Mora R (1998) Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *S. baltica* sp. nov. Int J Syst Bacteriol 48:179–186

Zubakha M, Usov N (2004) Optimum temperatures for common Zooplankton species in the White Sea. Russ J Mar Biol 30:293–297

Zucconi L, Pagano S, Fenice M, Selbmann L, Tosi S, Onofri S (1996) Growth temperature preferences of fungal strains from Victoria land, Antarctica. Polar Biol 16:53–61