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A glimpse of the diversity of complex polysaccharide-degrading culturable bacteria from Kongsfjorden, Arctic Ocean

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Abstract Cold-adapted, complex polysaccharide-degrading marine bacteria have important implications in biogeochemical processes and biotechnological applications. Bacteria capable of degrading complex polysaccharide substrates, mainly starch, have been isolated from various cold environments, such as sea ice, glaciers, subglacial lakes, and marine sediments. However, the total diversity of polysaccharide-degrading culturable bacteria in Kongsfjorden, Arctic Ocean, remains unexplored. In the study reported here, we tested 215 cold-adapted heterotrophic bacterial cultures (incubated at 4 and 20 °C, respectively) isolated from Kongsfjorden, for the production of cold-active extracellular polysaccharide-degrading enzymes, including amylase, pectinase, alginase, xylanase, and carboxymethyl (CM)-cellulase. Our results show that 52 and 41% of the bacterial isolates tested positive for extracellular enzyme activities at 4 and 20 °C, respectively. A large fraction of the bacterial isolates (37% of the positive isolates) showed multiple extracellular enzyme activities. Alginase and pectinase were the most predominantly active enzymes, followed by amylase, xylanase, and CM-cellulase. All isolates which tested positive for extracellular enzyme activities were affiliated to microbial class *Gammaproteobacteria*. The four genera with the highest number of isolates were *Pseudomonas*, followed by *Psychrobacter*, *Pseudoalteromonas*, and *Shewanella*. The prevalence of complex polysaccharide-degrading enzymes among the isolates indicates the availability of complex polysaccharide substrates in the Kongsfjorden, likely as a result of glacial melting and/or macroalgal load. In addition, the observed high functional/phenotypic diversity in terms of extracellular enzyme activities within the bacterial genera indicates a role in regulating carbon/carbohydrate turnover in the Kongsfjorden, especially by reducing recalcitrance.

Keywords Bacteria · Diversity · Culturable · Polysaccharides · Cold active enzymes · Kongsfjorden

Introduction

Kongsfjorden is an open glacial fjord located on the west coast of Spitsbergen, an island in the Svalbard archipelago, situated in the Arctic Ocean between mainland Norway and the North Pole (79°N, 12°E). It has been identified as a key European site for Arctic biodiversity monitoring (Hop et al. 2002) and is also suitable for exploring the impacts of possible climate changes (Włodarska-Kowalczuk and Węsławski 2001; Hop et al. 2002). Earlier studies in this fjord have focused on the physical characteristics of the environment (Svendsen et al. 2002), bacterial abundance and biomass (Jankowska et al. 2005), phytoplankton and zooplankton diversity (Hop et al. 2002), benthic microbial community (Srinivas et al. 2009), and marcobenthos (Wlodarska-Kowalczuk and Pearson 2004). However, in the recent past there have been reports on culturable (Srinivas et al. 2009; Choidash et al. 2012; Prasad et al. 2014) and non-culturable (Zeng et al. 2009; Piquet et al. 2010) bacterial diversity of the Kongsfjorden. These studies revealed that exceptionally diverse bacterial communities thrive in the perennially cold (<5 °C) water column of the Kongsfjorden. To date, cultivation-based studies have focused primarily on the bio-prospecting potential of the Kongsfjorden bacterial community with respect to the production of various cold-active enzymes, mostly amylases, lipases, and proteases (Srinivas et al. 2009; Chattopadhyay et al. 2013; Prasad et al. 2014). Despite these findings, however, little is known of the ability of the culturable Kongsfjorden bacterial

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community to produce extracellular cold-active polysaccharide-degrading enzymes other than amylases.

Kongsfjorden receives 5–10% of its total organic carbon of terrestrial origin from glacier melting, and about 90–95% of the organic carbon in the Kongsfjorden is contributed by primary production (Kuliński et al. 2014). However, Buchholz and Wiencke (2016) reported that macroalgae, in particular kelps, also contribute significantly to the pool of organic matter in the Kongsfjorden. It is important to note that high-molecular-weight carbohydrates or polysaccharides constitute a large proportion of the particulate organic matter (POM) and dissolved organic matter (DOM) in the ocean (Benner et al. 1992; lee et al. 2004). Marine microbial communities possess extracellular enzymes for hydrolyzing these high-molecular-weight substrates to yield sufficiently smaller substrates (<600 Da) for microbial uptake and to make them available for higher trophic levels (Azam et al. 1994; Arnosti 2011). Therefore, there is a strong possibility that complex polysaccharide-degrading cold-adapted bacteria will be present in the waters of Kongsfjorden.

The ability to produce cold-active enzymes is important for the growth and survival of microorganisms living in the permanently cold marine environment. Bacterial activity at low temperature requires higher levels of DOM compared to warmer environments (Pomeroy and Wiebe 2001). In addition, low temperature decreases the rate of chemical reactions and increases protein compactness, thereby affecting enzyme catalysis (Rasmussen et al. 1992). To overcome these challenges, cold-active enzymes have evolved high specific activity and a lower optimum temperature for activity (Feller et al. 1996). Thus, the detection of cold-adapted bacteria with cold-active polysaccharide-degrading enzymes is important for elucidating the mechanism of organic matter transformation in cold marine environments as well as to fulfill the needs of various industries (Georlette et al. 2004; Kumar et al. 2011). The aim of the study reported here was, therefore, to explore both the phylogenetic and functional/phenotypic (in terms of enzyme activity) diversity among polysaccharide-degrading cold-adapted culturable bacteria isolated from the Kongsfjorden in order to decipher their possible role in organic matter transformation.

Material and methods

Study area and sampling

Six different stations located in the Kongsfjorden were sampled, starting at the mouth of Kongsbreen and Kronebreen glaciers and continuing to the open ocean waters (Fig. 1). A

Fig. 1 Map of Kongsfjorden showing locations of the sampling stations (*red stars 1–6*)

Conductivity Temperature Depth (CTD) instrument was deployed at each station to determine depthwise variations in temperature, salinity, and fluorescence. Depthwise distribution of bacteria in Kongsfjorden is affected by the hydrological stratification and/or phytoplankton distribution. Moreover, bacterioplankton and phytoplankton generally tend to accumulate in the layers around the pycnocline in the Kongsfjorden (Jankowska et al. 2005). Therefore, in order to increase the probability of encountering diverse polysaccharide-degrading bacteria we collected 5-l water samples from the deep chlorophyll maxima (DCM) depths using Niskin bottles in June and July 2015. Immediately after collection, the water samples were filtered through a 0.22-μm pore-size polycarbonate membrane (Merck Millipore, Billerica, MA).

Isolation of heterotrophic bacteria

The bacterial population retained on the 0.22-μm pore-size polycarbonate membrane was cultivated. Specifically, immediately after filtration of a water sample, the filter paper was immersed in a flask containing 25 ml of Zobell Marine Broth (ZMB; Hi-Media, Mumbai, India) and incubated at 4 °C until visible bacterial growth, identified as increased turbidity, was recorded. At the end of the incubation period, 500 μl of the medium was preserved and transported back to the National Centre for Antarctic and Ocean Research (NCAOR) using standard protocols where individual bacterial cultures were then isolated. For this purpose all 500 μl of the preserved medium was emptied into a flask containing 10 ml of ZMB and incubated at 4 °C for 7 days. At the end of the incubation period 100 μl of the medium was appropriately serially diluted and spread-plated onto Zobell Marine Agar (ZMA). The plates were incubated at 4 °C until visible bacterial colonies formed. Morphologically distinct colonies were handpicked using a sterile loop and transferred onto ZMA slants.

Screening of bacterial isolates for cold-active extracellular polysaccharide-degrading enzyme activities

To study the activities of extracellular polysaccharide-degrading enzymes, such as amylase, pectinase, alginase, xylanase and CM-cellulase, we spot-inoculated bacterial isolates onto modified basal salt medium (MBM) supplemented with 0.5% (w/v) starch, 0.2% (w/v) pectin (poly-D-galacturonic acid methyl ester), 0.2% (w/v) sodium alginate, 0.5% (w/v) xylan from beechwood, and 0.5% (w/v) carboxylmethyl-cellulose (CMC). All polysaccharide substrates were procured from Hi-Media. The MBM contained (per liter) 2 g yeast extract, 0.3 g peptone, 15 g agar, 25 g NaCl, 0.75 g KCl, 7 g $MgSO_4$, 0.5 g NH_4Cl, 0.2 g $CaCl_2$, 7 ml 10% w/v K_2HPO_4, 3 ml 10% w/v KH_2PO_4, and 1 ml trace metal solution (Bhosle 1981). Prior to sterilization the pH of the MBM was adjusted to 7.2 using 1 N NaOH. After spot-inoculation of the bacterial isolates, the plates were incubated at 4 °C and 20 °C, respectively, for 7 days. The zone of clearance around bacterial colonies due to extracellular polysaccharide-degrading enzyme activities was observed by flooding the plates with appropriate reagents. Xylan- and CMC-containing plates were flooded with 1% Congo Red for 30 min followed by rinsing with 1.5 M NaCl (Khandeparker et al. 2011). Gram's iodine solution was used for detecting bacterial isolates which could hydrolyze starch, pectin (Sunnotel and Nigam 2002), and alginate (Sawant et al. 2015).

Genomic DNA extraction and PCR assay

Genomic DNA from bacterial cultures which tested positive for enzyme activity was extracted using the Purelink Genomic DNA Extraction kit (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The extracted DNA was stored in Purelink elution buffer at −20 °C until analyzed. The 16S rRNA gene from the genomic DNA was amplified using universal bacterial 16S primers 27 F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Lane 1991). PCR assays were carried out in 50-μl reaction mixtures containing 20 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM dNTPs, and 2.5 U Taq DNA polymerase. Amplifications were carried out in a thermocycler (model CFX 96; BioRad, Hercules, CA) with the following cycling program: an initial denaturation at 95 °C for 2 min followed by 29 cycles of 30 s at 95 °C, 30 s at 45 °C, and 2 min at 72 °C, with final extension for 10 min at 72 °C. Amplification was confirmed by 1% agarose gel electrophoresis. The amplified products were purified using The Purelink PCR Purification kit (Invitrogen).

Amplified ribosomal DNA restriction analysis and DNA sequencing

The PCR-amplified 16S rRNA gene products were digested separately with the restriction enzymes *Alu*I and *Hae*III. The resulting fragments were electrophoresed on 3% polyacrylamide gel, and the gel then stained with ethidium bromide and visualized under UV light. Amplified ribosomal DNA restriction analysis (ARDRA) profiles obtained on different gels were matched using a 100-bp ladder DNA (Invitrogen) as reference. On the basis of the ARDRA profiles, bacterial isolates were clustered into groups, with those isolates sharing similar restriction profiles placed together in a single group, and those isolates with distinct restriction patterns categorized into separate groups. Depending upon the phenotypic pattern (enzyme activities), a variable number of isolates, but always more than one isolate, were selected from each ARDRA group for 16S rRNA gene sequencing. The purified 16S rRNA gene fragments of each representative isolate were sequenced using an automated DNA sequencing system (Applied Biosystems, Foster City, CA).

Sequence analysis

The obtained 16S rRNA gene sequences were checked for the presence of chimera using the DISCPHER software toolset (http://decipher.cee.wisc.edu/; Wright et al. 2012). Non-chimeric sequences were compared with the NCBI database sequences using the MegaBLAST option of the BLAST (https://blast.ncbi.nlm.nih.gov/) algorithm (Altschul et al. 1990). In order to reliably identify the bacterial isolates to the genus level, we also used SeqMatch and classifier tools from Ribosomal Database Project Release 10 (http://rdp.cme.msu.edu/) (Wang et al. 2007). A total of 75 sequences were submitted to GenBank under accession numbers KX417120 to KX417194.

Phylogenetic tree

A phylogenetic tree of the representative sequences (1 sequence per ARDRA group) was constructed to visualize their respective relationship and affiliation with the closest relative type strain sequences from the NCBI database. The tree was constructed in MEGA-5 software using maximum composite likelihood as the substitution model, and bootstrap values were calculated using the neighbor-joining method with a resampling size of $n = 500$ (Tamura et al. 2011).

Results

Hydrography

Water column temperature decreased from 6.5 °C in the open ocean station (stn1) to 3.0 °C close to the glacier (stn6) (Table 1), and salinity ranged from 29 to 34.2 psu between the inner and outer fjord stations, respectively. The fluorescence signal decreased gradually from 6.7 mg m^{-3} at stn1 to 0.62 mg m^{-3} at stn6. In addition, the depth of the DCM decreased gradually from stn 1 (35 m) to stn 6 (5 m) in the Kongsfjorden (Table 1).

Screening for extracellular enzyme activities

A total of 215 bacterial isolates obtained from the six different stations located along the fjord axis were tested for various extracellular enzyme activities using the plate assay method. Of the total number of bacterial isolates assayed, 58% (125 isolates) were found to test positive for polysaccharide-degrading enzymes. At an incubation temperature of 4 °C, 52% (112 isolates) of all bacterial isolates tested positive for extracellular enzyme activities, whereas at 20 °C, 41% (88 isolates) of the total tested positive for extracellular enzyme activities. Among the bacterial isolates isolated, 17.8, 28.7, 41.3, 6.5, and 5.6% were able to hydrolyze starch, pectin, alginate, xylan and CM-cellulose, respectively, when incubated at 4 °C (Fig. 2a); at 20 °C, 22.6, 22.6, 28.8, and 26% of the total isolates were able to hydrolyze starch, pectin, alginate, and xylan, respectively (Fig. 2b). A relatively high percentage of bacterial isolates was able to degrade alginate, pectin, and starch, while the ability to hydrolyze xylan and CM-cellulose was restricted to few isolates. Interestingly, CM-cellulase activity in isolates was recorded exclusively at the 4 °C incubation temperature. These results indicate that the production of alginase and pectinase represent the main enzyme activities of isolates recovered from the Kongsfjorden.

Variation in enzyme activities among bacterial isolates according to location

The ability of the bacterial isolated to hydrolyze starch, pectin, alginate, xylan, and CM-cellulose varied with the location of the station (Fig. 3a, b). At the 4 °C incubation temperature, the ability to degrade pectin and alginate was significantly higher among isolates from stn3, stn4, and stn5 (Fig. 3a), while the ability to degrade xylan and CM-cellulose was expressed exclusively in bacterial isolates from stn6 (located near to Kongsbreen glacier; Fig. 3a). At the 20 °C incubation temperature, the ability to degrade xylan was expressed in isolates from stn1, stn4, stn5, and stn6 (Fig. 3b), which otherwise was exclusively observed in isolates from stn6 when incubated at 4 °C.

Table 1 Variations in temperature, salinity, fluorescence, and depth of the deep chlorophyll maxima according to water sampling station

Station No	Temperature (°C)	Salinity (psu)	Fluorescence (mg m^{-3})	DCM depth (m)
Stn1	6.5	34.2	6.7	35
Stn2	7.4	33	2.7	22
Stn3	5.5	33.2	2.8	21
Stn4	6.0	32	3.8	9
Stn5	4.3	29	1.0	6
Stn6	3.0	30.8	0.62	5

DCM Deep chlorophyll maxima

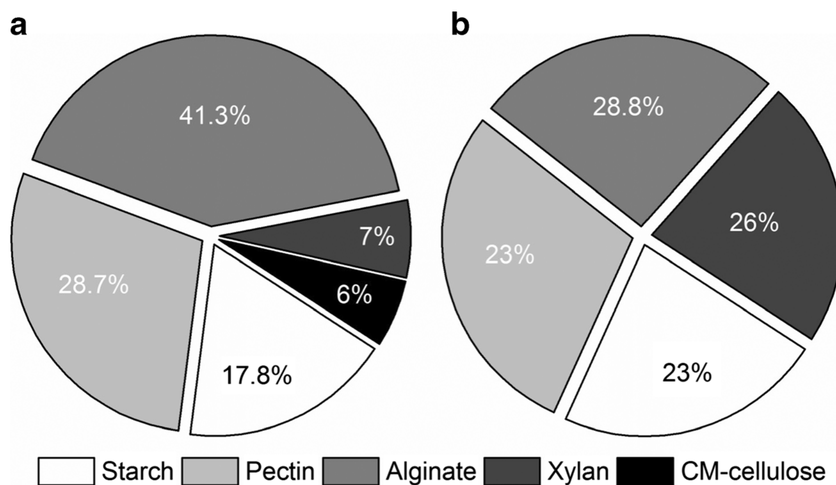

Fig. 2 Percentage of bacterial isolates from the total number of isolates that were able to hydrolyze starch, pectin, alginate, xylan, and carboxymethyl (*CM*)-cellulose at an incubation temperature of 4 °C (**a**) and 20 °C (**b**)

Multiple enzyme activities among bacterial isolates

Many bacterial isolates produced more than one extracellular cold-active polysaccharide-degrading enzyme. For example, at the 4 °C incubation temperature, one isolate (identified based on 16S rRNA gene similarity as *Pseudomonas extremaustralis*) tested positive for all five of the enzymatic activities screened, and two tested positive for four of these enzymatic activities; 36 isolates produced three of the hydrolytic enzymes tested, and 36 tested positive for two of these hydrolytic enzymes. At the 20 °C incubation temperature, none of the isolates tested positive for all five enzymatic activities screened, while nine tested positive for four of these; 21 isolates produced three of the hydrolytic enzymes tested, and eight tested positive for two of these. The percentage of isolates degrading additional substrates within the groups of isolates producing a particular enzymatic activity was calculated according to Tropeano et al. (2012), and the results are shown in Fig. 4a, b. More specifically, all of the isolates that tested positive for amylase were considered to have a value of 100%, and then the percentages of isolates capable of utilizing each one of the other substrates (pectin, alginate, xylan, CM-cellulose) were calculated. This analysis showed that the ability to degrade pectin and alginate was the most frequent enzymatic activity within the groups, followed by the ability to degrade starch, while the ability to degrade xylan and CM-cellulose was the least frequent among the isolated microbial groups. However, at the 4 °C incubation temperature, a large proportion of the isolates producing xylanase were also able to degrade CM-cellulose and vice-versa (Fig. 4a).

Diversity of polysaccharide-degrading bacterial isolates

The distribution of the 125 bacterial isolates which tested positive for extracellular enzyme activities among the different

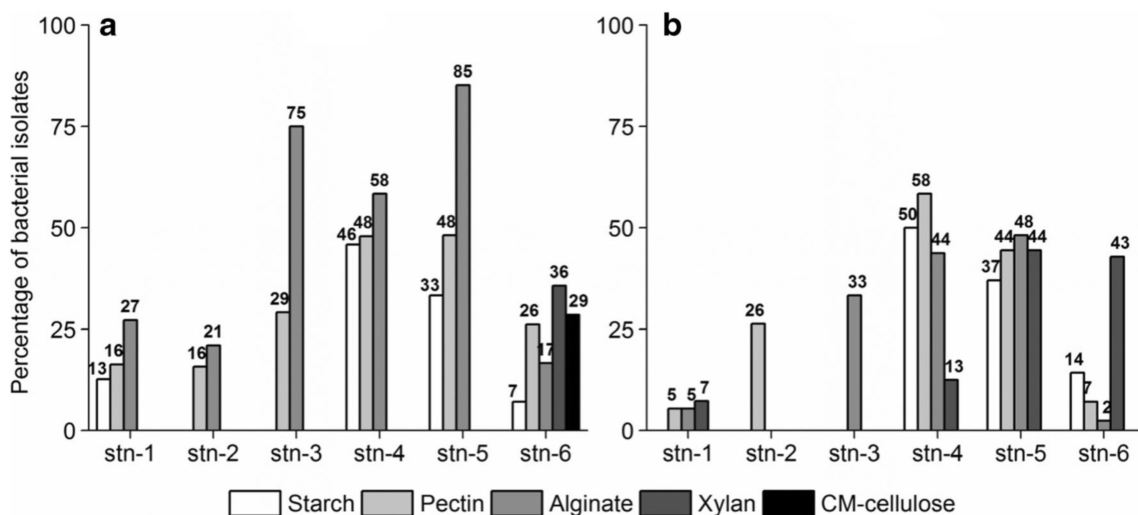

Fig. 3 Location-wise distribution of bacterial isolates degrading a specific substrate at an incubation temperature of 4 °C (**a**) and 20 °C (**b**). The percentage of bacterial isolates degrading particular substrate was calculated separately for each station (*stn*)

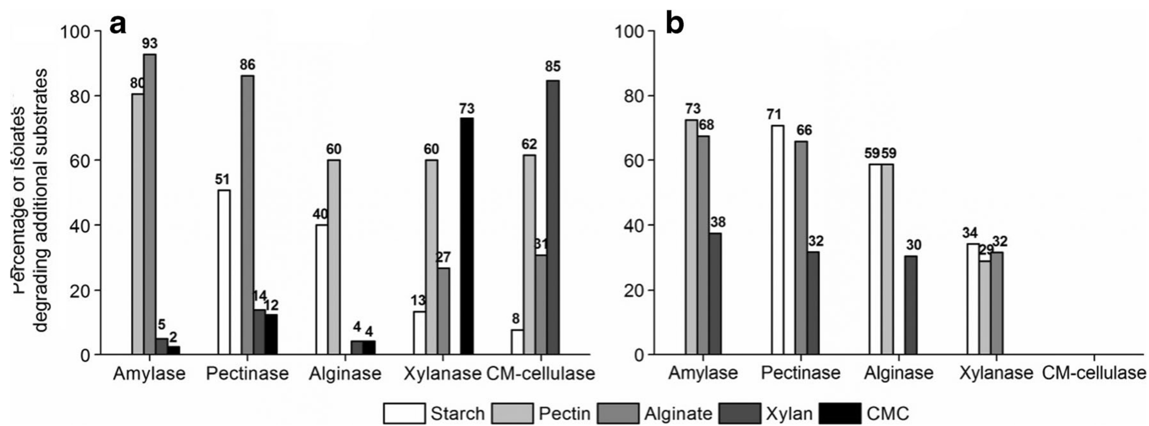

Fig. 4 Percentage of bacterial isolates able to degrade additional substrates within the groups of isolates producing a particular enzymatic activity at an incubation temperature of 4 °C (**a**) and 20 °C (**b**)

genera is shown in Table 2. The ARDRA analysis of these 125 bacterial isolates with two restriction enzymes showed 23 distinct cleavage patterns. A total of 75 representative isolates from all ARDRA groups were selected for 16S RNA sequencing (Table 2), and the results revealed that all of the sequences shared a high similarity to their nearest neighbor type strain sequences deposited in the databases. Most of the closest relative type strains are cold adapted and were isolated from spring and natural mineral water, Antarctic pond water, Antarctic coastal marine environment, diatoms, seaweed, seafood, Arctic sea-ice, and seawater (Table 2). The overall identity of 92% of the bacterial isolates with respect to their closest type strains was >98.8% (Table 2), while approximately 7.6% of the isolates showed a <98.7% similarity to the type strain. Previous studies reported that 98.7% of 16S rRNA gene sequence similarity can be used as the threshold for differentiating two species (Wayne et al. 1987; Stackebrandt and Ebers 2006; Kim et al. 2014). Thus, the isolates in our study most likely represent novel complex polysaccharide-degrading bacterial species. A phylogenetic tree of probable novel bacterial isolates is shown in Fig. 5. It should be noted that multiple ARDRA profiles were observed for the strains belonging to the same species (Table 2), possibly due to intra-species diversity (Chang et al. 2005). Interestingly, high functional/phenotypic diversity in terms of enzyme activities was observed among strains belonging to same genus and/or species (Table 2). Taxonomic affiliation of the representative isolates revealed that all strains belonged to the class *Gammaproteobacteria.* Four genera were identified, with the highest number of isolates belonging to genus *Pseudomonas* (50 representative isolates), followed by *Psychrobacter* (19 isolates), *Pseudoalteromonas* (8 isolates), and *Shewanella* (2 isolates). Our results clearly indicate that among all four of these genera, those isolates belonging to *Pseudomonas* genus were predominant in terms of demonstrating all extracellular enzyme activities tested at 4 °C and 20 °C. A large fraction the isolates in the Kongsfjorden which produce amylase, pectinase, alginase, xylanase and CM-cellulase belong to the genus *Pseudomonas*.

Discussion

The observed decrease in temperature in the upper layer water column from the outer fjord to the near glacier station indicates cooling of the inner Kongsfjorden. This cooling could be induced by the large influx of cold glacier meltwater into the inner fjord during the summer (Hop et al. 2002). The sharp lateral gradient in salinity decreasing towards near glacier locations indicate that glacier melt induced freshening of the inner fjord. The decrease in magnitude of fluorescence from the outer to inner Kongsfjorden could be attributed to reduced light penetration into the water column as a result of the increased input of turbid freshwater from glacier melting (Svendsen et al. 2002).

Extracellular enzyme activities of cold-adapted bacteria contribute essentially to the processes of nutrient turnover, biomass production, and litter decomposition (Hatha et al. 2013). Thus, the presence of a large number of bacterial isolates capable of producing various cold-active extracellular polysaccharide-degrading enzymes in our samples highlights their importance in carbohydrate/carbon turnover in the Kongsfjorden. In addition, the higher proportion of isolates showing extracellular enzyme activities at the 4 °C incubation temperature compared to the 20 °C incubation temperature suggests that the bacterial community adapted to the permanently cold waters of Kongsfjorden with lower temperature optima of their extracellular enzymes (Huston et al. 2000) and/or temperature-dependent production of extracellular enzymes (Buchon et al. 2000). Bacterial extracellular enzyme activities in most of aquatic environments depend upon the presence or concentration of corresponding substrates (polymers) (Münster et al. 1992). Moreover, it has been widely accepted that hydrolytic enzymes are induced by the presence of

Table 2 Distribution of the 125 bacterial isolates which tested positive for enzyme activities into groups based on amplified ribosomal DNA restriction analysis results, with their taxonomic affiliation and hydrolyzed substrates.

ARDRA groups	Laboratory reference number of isolates	Substrate hydrolyzed[a]	% Similarity	Closest relative type strains (accession number), isolation source
Group 1	1.39, 5.15, 5.16, **5.18**, 5.20, 5.23, 7.14, 7.26, **10.1**, 10.2, 10.5, 10.7, 10.8, 10.9, 10.11	A	99.6	*Pseudomonas orientalis* (AF064457), Lebanese spring water
	3.15, 5.12, 5.14, 5.22, **5.24**, 10.30, 10.32, **10.33**, 10.34	A^{4}	99.6	
	9.20, 9.26	S^{4}, P, A, X^{20}		
	10.26	P^{4}, A^{4}, X, CM^{4}	99.6	
	10.28	S, P, A^{4}, X	99.6	
	10.18	P, X	99.7	
	10.16	X	99.6	
	10.19, 9.15	X^{20}	99.6	
	7.25, 7.20	S, P^{20}, A	99.7	
	10.21	X^{20}, CM^{4}	99.6	
	10.29, 7.23	S, P, A^{4}, X^{20}	99.6	
Group 2	9.19, **9.24**, 9.23**, 9.25**, 9.27	S, P, A, X^{20}	99.8	*Pseudomonas antarctica* (AJ537601), Cyanobacterial mat in Antarctica pond
	7.24	S	99.6	
	9.3	P	99.9	
	3.13, 3.14,	P, A^{4}	99.8	
Group 3	**1.36**, 1.30	S^{4}, A^{4}	99.9	*Pseudomonas grimontii* (AF268029), French natural mineral water
	1.37, 1.40	P, A	99.9	
	1.38, 5.13, 5.17	P^{4}, A^{4}	99.9	
Group 4	**5.21**	A^{4}	99.8	*Pseudomonas extremaustralis* (AJ583501), Antarctic pond
	10.13	S, P,A^{4}, X, CM^{4}	99.3	
	5.7, 5.8, 5.10	P^{4}, A^{4}	99.6	
	10.22	P^{4}, X, CM^{4}	98.8	
	10.35	A^{20}		
Group 5	**5.9,** 5.11, 1.31	A^{4}	99.8	*Pseudomonas antarctica* (AJ537601), Cyanobacterial mat in Antarctica pond
	3.16	P	99.6	
	3.17	P^{20}	99.9	
	3.18	P^{20}, A^{4}	99.8	
	5.4	P^{4}, A^{20}	99.8	
	5.19	P^{4}, A^{4}	99.9	
	7.18, 10.42	S^{20}	99.8	
	1.25	P, A, X^{20}	99.6	
	7.17	S, P, A	99.9	
	9.21	S^{4}, P^{4}, A	99.9	
	9.18, 9.13	S^{20}, P, A, X^{20}	99.9	
Group 6	**1.33**	S^{4}, P, A^{4}	99.9	*Pseudomonas grimontii* (AF268029), French natural mineral water
	10.23	S, P^{4}, A	98.3	
Group 7	**7.19**	S	99.4	*Pseudomonas mandelii* (AF058286), French natural mineral water
	7.15	P^{20}, A^{4}	99.4	
	5.5	A^{20}		
Group 8	**7.27**, 7.36, 7.37, 7.48,	S, P, A	99.6	*Psychrobacter cryohalolentis* (AY660685), Sebarian permafrost
	7.35	S, P^{4}, A	99.6	
	7.7, 10.17	P^{4}, A^{4}, X^{20}	99.5	
Group 9	**7.4**	P^{4}, A, X^{20}	99.8	*Psychrobacter fozii* (AJ430827), Antarctica coastal marine environment
	7.22	S^{20}, A^{4}	99.7	
	9.22	S^{20}, P, A	99.6	
	10.24	S^{20}		
Group 10	**7.21**	S^{4}	99.3	

Table 2 (continued)

ARDRA groups	Laboratory reference number of isolates	Substrate hydrolyzed[a]	% Similarity	Closest relative type strains (accession number), isolation source
				Psychrobacter aquimaris (AY722804), Seawater South Sea
Group 11	**7.16**	S, P, A, X^{20}	99.4	*Psychrobacter fozii* (AJ430827), Antarctica coastal marine environment
	1.32	S^{4}, P, A^{4}	99.8	
	9.16	S, P, A, X^{20}	99.7	
	10.31	S^{20}, A^{4}, CM^{4}	99.6	
Group 12	**1.28**	P^{4}, A^{4}	99.9	*Pseudomonas grimontii* (AF268029), French natural mineral water
	1.29	S^{4}, P, A^{4}	99.9	
	10.15	P^{4}, X, CM^{4}	98.8	
Group 13	**10.14**	X, CM^{4}	99.3	*Pseudomonas orientalis* (AF064457), Lebanese spring water
	10.25	P^{4}, A^{4}, X^{20}, CM^{4}	99.4	
Group 14	**7.31,** 7.32, 7.34, 7.40, 7.41, 7.46	S, P, A	98.5	*Pseudoalteromonas mariniglutinosa* (AJ507251), Diatom associated
Group 15	**7.39**	A^{20}	98.6	*Pseudoalteromonas prydzensis* (U85855), Sea-ice
	7.42	S^{20}, P^{20}	98.8	
	7.33, 7.38	S,P,A	98.6	
Group 16	**7.6**	P, A, X^{20}	99.4	*Shewanella vesiculosa* (AM980877), Antarctic coastal marine environment
	7.28	P^{4}, A^{4}	99.3	
Group 17	**7.44**	S, P, A	99.5	*Pseudoalteromonas elyakovii* (AF082562), Seaweed associated
	7.45	A^{20}	99.8	
Group 18	**7.10**	P^{4}, A^{4}, X^{20}	98	*Pseudomonas mandellii* (AF058286), French natural mineral water
Group 19	**10.20,** 10.24	P^{4}, X, CM^{4}	99.4	*Pseudoalteromonas arctica* (DQ787199), Sea-ice and seawater
	10.27	X, CM^{4}	99.6	
Group 20	**9.17**	S, P, A, X^{20}	99.6	*Pseudomonas extremaustralis* (AJ583501), Antarctic pond
Group 21	**1.26**	S^{4}, A	99.6	*Psychrobacter luti* (AJ430828), Antarctic marine environment
Group 22	**1.27**	S^{4}, A^{4}	98.4	*Psychrobacter alimentarius* (AY513645), Seafood associated
Group 23	**1.35**	P, A, X^{20}	98.4	*Psychrobacter namhaensis* (AY722805), Seawater South sea Korea

The isolates selected for 16S rRNA gene sequencing are shown in bold. Underlined sequences are those with a similarity level of $<98.7\%$ and mostly likely represent novel species

ARDRA Amplified ribosomal DNA restriction analysis,

[a] *S* starch, *P* pectin, *A* alginate, *X* xylan, *CM* CM cellulose. Superscript [4] and [20] represent incubation temperatures of 4 °C and 20 °C incubation temperatures, respectively. No superscript represents positive results at both incubation temperatures

polymeric substrates (Chróst 1991; Vetter and Deming 1999; Yu et al. 2009). Thus, the predominance of xylanase and CM-cellulase activities in bacterial isolates from the near glacier location could be due to the presence of lignocellulose-rich organic matter of terrigenous origin, most likely contributed by glacial melting in the summer. Kuliński et al. (2014) reported that glacier melting contributes 5–10% of the total organic carbon of terrestrial origin into the Kongsfjorden. In addition, the dominance of alginate- and pectin-degrading bacteria in the middle and inner fjord locations could be associated with the degradation of macro-algal derived organic matter in the Kongsfjorden. Brown algae are the major source of dissolved and particulate alginate in ocean waters. Hop et al. (2012) reported the occurrence of endemic Arctic brown algae *Laminaria solidungula* in the middle and inner part of the Kongsfjorden. Recently, Buchholz and Wiencke (2016) estimated that brown alga *Alaria esculenta* loses its blade gradually at a rate of 3 ± 0.8% of the blade area per day, thereby contributing significantly to the pool of POM in the Kongsfjroden. Consequently, location-wise variation in the ability to degrade polysaccharide substrates among bacterial isolates signifies the presence of a wide variety of organic matter in these waters. It has been reported that the salinity of the sampling location has a negative impact on microbial extracellular enzyme activities (Park et al. 2006; Khandeparker et al. 2011). However, in the present study we

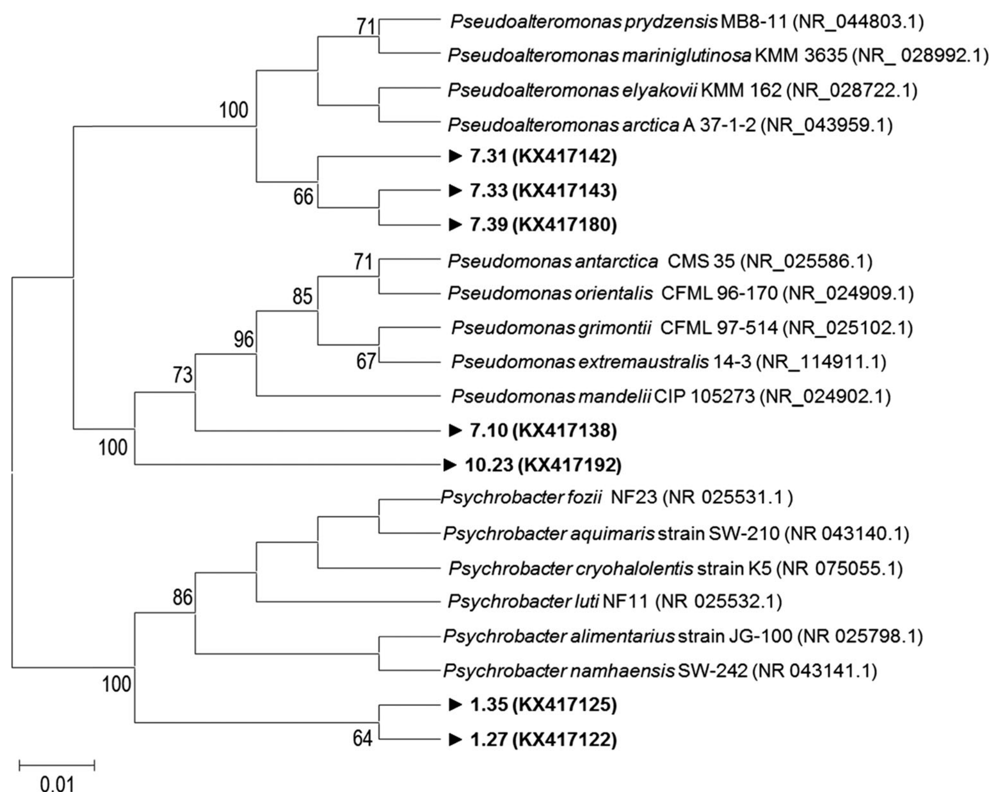

Fig. 5 Phylogenetic tree (maximum likelihood) showing evolutionary relationship of probable novel bacterial isolates with the type strains. Bootstrap values of >50% are given at the nodes (calculated with the neighbor-joining method). *Scale bars* Percentage of sequence divergence. The isolates are represented by the laboratory reference number along with genbank accession number (in parenthesis)

did not observed any negative correlation between percentage abundance of polysaccharide-degrading bacteria and the salinity of the sampling location. Nevertheless, Liu and Boone (1991) reported that increased concentrations of NaCl inhibit the growth of lignocellulose-degrading bacterial population. Therefore, it is possible that in this study decreased salinity due to glacial melting might have also contributed to the predominance of xylan- and CM-cellulose degrading bacteria in near glacier location.

The high proportions of isolates capable of producing multiple polysaccharide-degrading enzymes indicate the exceptional adaptation of the Kongsfjorden microbiota to flourish in a cold environment. The ability to produce multiple enzymes provides microorganisms with a higher chance to adapt and survive, which is particularly useful in the rapidly changing marine environment of the Kongsfjorden in terms of uncertainty in the availability of substrates for bacterial metabolism (Tropeano et al. 2012). In addition, the ability of several isolates to produce both xylanase and CM-cellulase has a huge potential for the conversion of lignocellulolytic biomass to bioethanol (Agustini et al. 2012). Interestingly, we observed high phenotypic variation, in terms of enzyme activities, among bacterial isolates belonging to a particular genus and/or species. This phenomenon is known as microbial phenotypic heterogeneity and is a non-genetic variation which does not involve changes in the genome level, but rather is a result of random alterations in the rates of protein synthesis (Veening et al. 2008; Davidson and Surette 2008) modulated by external environmental factors (Rossignol et al. 2009). Phenotypic heterogeneity allows a genetically identical bacterial population to cope with substrate limitations and fluctuations, thus ensuring their survival under highly variable environmental conditions (Ackermann 2015).

Our phylogenetic analyses of the polysaccharide-degrading bacteria obtained in this study reveal that they all belong to the class *Gammaproteobacteria.* This result is in accordance with earlier reports of a link between carbohydrate degradation in the marine environment and the dominance of members of the class *Gammaproteobacteria* (Edwards et al. 2010; Gifford et al. 2014). In our study, members of genera *Pseudomonas* and *Psychrobacter* were particularly abundant among the isolates which tested positive for the extracellular enzyme activities. This result not only suggests their role in carbohydrate degradation in the cold marine environment but also indicates the bio-prospecting potential of *Pseudomonas* and *Psychrobacter* isolates from Kongsfjorden for all of the extracellular enzymatic activities tested. The members of genus *Pseudomonas* demonstrate a great deal of metabolic diversity and consequently are able to colonize a wide range of niches (Palleroni 1984, 1992). They are found predominantly in the cultured bacteria community from various marine environments due to their ease of culture in vitro (Mancinelli 1984). Members of *Pseudomonas* and *Psychrobacter* genera have been reported to produce mainly cold-active lipase (Choo et al. 1998; Rashid et al. 2001; Alquati et al. 2002; Zhang and Zeng 2008; Dang et al. 2009; Dey et al. 2014)

and protease (Martınez-Rosales and Castro-Sowinski 2011). In this regard, our results are significant in that we demonstrate the potential of Kongsfjorden bacterial isolates belonging to these genera to produce cold-active complex polysaccharide-degrading enzymes. The closest type strains within the *Pseudomonas* genus (*P. orientalis*, *P. antarctica*, *P. grimontii*, *P. extremaustralis*, *P. mandellii*) were isolated from freshwater environments. Earlier studies from Svalbard, Arctic also reported the dominance of bacterial isolates belonging to *Pseudomonas* genus, particularly *P. orientalis* in the glacier ice core (Singh et al. 2015) and glacier meltwater stream (Reddy et al. 2009). Therefore, it appears that increased terrestrial and glacial inputs during the summer might have contributed to the observed dominance of members of *Pseudomonas* genus in our samples from Kongsfjorden. In addition, the presence of a few isolates in our samples having $< 98.7\%$ sequence similarity with the type strain sequence in the database indicates the possibility of having novel and/or unexplored culturable bacterial diversity, which probably contributes to complex carbohydrate degradation in the Kongsfjorden.

In conclusion, our results clearly demonstrate that Kongsfjorden bacterial isolates possess multiple enzyme activities as well as phenotypic heterogeneity for complex polysaccharides degradation, thereby ensuring their survival and growth in an Arctic environment characterized by the variability and availability of different polymeric or complex substrates. Enhanced terrestrial and glacial inputs may also contribute complex carbohydrate-degrading bacteria to the Kongsfjorden. These results have significant implications for understanding carbohydrate/carbon turnover in a glacially influenced, permanently cold marine coastal environment. Further, Kongsfjorden bacterial isolates could be harnessed for the production of cold-active polysaccharide-degrading enzymes for various industrial applications.

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