

Degradation properties of various macromolecules of cultivable psychrophilic bacteria from the deep-sea water of the South Pacific Gyre

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Abstract The deep-sea water of the South Pacific Gyre (SPG, 20°S–45°S) is a cold and ultra-oligotrophic environment that is the source of cold-adapted enzymes. However, the characteristic features of psychrophilic enzymes derived from culturable microbes in the SPG remained largely unknown. In this study, the degradation properties of 174 cultures from the deep water of the SPG were used to determine the diversity of cold-adapted enzymes. Thus, the abilities to degrade polysaccharides, proteins, lipids, and DNA at 4, 16, and 28 °C were investigated. Most of the isolates showed one or more extracellular enzyme activities, including amylase, chitinase, cellulase, lipase, lecithinase, caseinase, gelatinase, and DNase at 4, 16, and 28 °C. Moreover, nearly 85.6 % of the isolates produced cold-adapted enzymes at 4 °C. The psychrophilic enzyme-producing isolates distributed primarily in *Alteromonas* and *Pseudoalteromonas* genera of the *Gammaproteobacteria*.

Pseudoalteromonas degraded 9 types of macromolecules but not cellulose, *Alteromonas* secreted 8 enzymes except for cellulase and chitinase. Interestingly, the enzymatic activities of *Gammaproteobacteria* isolates at 4 °C were higher than those observed at 16 or 28 °C. In addition, we cloned and expressed a gene encoding an α -amylase (Amy2235) from *Luteimonas abyssii* XH031^T, and examined the properties of the recombinant protein. These cold-active enzymes may have huge potential for academic research and industrial applications. In addition, the capacity of the isolates to degrade various types of organic matter may indicate their unique ecological roles in the elemental biogeochemical cycling of the deep biosphere.

Keywords Cold-adapted enzymes · Deep-sea water · Screening · South Pacific Gyre (SPG) · α -Amylase

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L. Zhang and Y. Wang contributed equally to this work.

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Introduction

The deep sea is generally characterized by low temperature (except for volcano or hydrothermal vents), high pressure, and absence of light and nutrients. Microbes inhabiting these environments are usually extremophiles, and they account for nine-tenths of the deep-sea benthic biomass (Pfannkuche 1992). Among the microbes, bacteria are responsible for a significant proportion of heterotrophic respiration (Fang et al. 2015), and are an important component of marine ecosystems.

Marine microbes are the main components of food chains in marine ecosystems. The primary food source of the majority of benthic organisms that inhabit the ocean's aphotic zone and abyssal plains is thought to be particulate organic matter (POM), which originates primarily from the sedimentation of the ocean's primary surface production

(D'Hondt et al. 2002, 2004). Within the global carbon cycle, dissolved organic matter (DOM) represents one of the largest organic carbon pools (Hedges 1992; Hopkinson et al. 2002). Prokaryotes are the primary organisms that transform POM and DOM into inorganic nutrients and CO₂ (Ogawa et al. 1999). Previous studies have shown that *Bacteroidetes* have the ability to efficiently degrade biopolymers, such as protein and chitin, that constitute a substantial fraction of the DOM pool in the ocean (Hoppe 1983; Cottrel and Kirchman 2000). *Bacteroidetes* are believed to participate in the process of biopolymer degradation in the marine environment (Kirchman 2002).

Bacterial-based enzymatic degradation of POM/DOM is the first step in carbon and nitrogen recycling (Talbot and Micheline 1997). Therefore, psychrophilic isolates secreting various enzymes are thought to have crucial ecological roles in the decomposition of POM in the deep sea. However, enzymes that participate in the degradation of POM are relatively unknown.

Cold-active enzymes have been detected recently in cold-adapted microbes (Groudieva et al. 2004; Marshall 1997), which have attracted increased attention with their higher catalytic efficiencies at cold temperatures. They show ten times higher hydrolytic activities than their mesophilic counterparts at low temperatures (Feller and Gerday 2003; Margesin et al. 2005). Then, they are economically beneficial in relation to energy savings. Presently, these cold-adapted enzymes have been utilized in many agricultural and industrial fields, such as washing additives, leather processing, and food industries (Joseph et al. 2008). For example, psychrophilic cellulase is used in the textile industry for biopolishing and stone-washing (Gerday et al. 2000). Cold-active lipases can be used to develop various food flavors. Psychrophilic enzymes have significant applications not only in consumer-based industries, but also in bioremediation, biomass conversion, and molecular bio-technology (Lu et al. 2010). Therefore, the screening of enzymes from marine bacteria adapted to cold environments should be a good way to identify psychrophilic enzymes.

Our samples were previously obtained from the SPG during the integrated ocean drilling program (IODP) (<http://www.iodp.org/>). The SPG is the world's most typical ultra-oligotrophic oceanic area that has a low deposition rate (0.1–1 m per million years), which has been considered to be an ideal habitat for some extreme environmental microbes (D'Hondt et al. 2009). These indigenous extremophiles may prove to be the ideal resource of new functional genes and biologically active substances. However, the functional roles of those extremophiles still remain poorly studied.

Marine microbes may have evolved unique properties to adapt to their environment by producing various enzymes

with extreme activity and special characteristics. So far, some enzymes with particular features, such as psychrophilic activity or/and salinity tolerance, have been obtained from marine microbes (Qin et al. 2014a, b; Liu et al. 2011; Zhang and Zeng 2008). In this study, we screened for various exoenzymes from 174 isolates, and detected their abilities to degrade polysaccharides, proteins, lipids, and DNA at 4, 16, and 28 °C. In addition, an α -amylase from one of the isolates was biochemically studied.

Materials and methods

Station description and sampling

The sampling sites had variable water depths (3,738–5,697 m), and were located at 7 positions of the SPG, with sedimentation rates ranging from 0.017 to 0.178 (cm/kyr) (Table S1). The temperature at the seven sites was approximately 1–2 °C.

Mud samples were collected using an Advanced Piston Corer on the drilling research vessel *JOIDES Resolution* (<https://iodp.tamu.edu/tools/>) (D'Hondt et al. 2010, 2011). Deep water was extracted from the mud samples immediately after they were transported to the shipboard and then placed in sterilized (121 °C/15 min) centrifuge tubes and preserved at 4 °C. Strains were isolated on marine R2A agar (Fluka R2A; prepared with distilled seawater) (Suzuki et al. 1997) and marine 2216E agar (Zhang 2007). The plates were incubated at 4, 16, and 28 °C until colonies appeared. These colonies were re-plated at least 3 times to obtain pure cultures. Bacterial lawns were stored at –80 °C in sterile 0.9 % (w/v) NaCl with 15 % (v/v) glycerol.

Strains, plasmids, and cultural conditions

There were 174 culturable strains that were isolated from the 7 sites. The *Gammaproteobacteria* phylum represented the largest number of strains (143 isolates), whereas the *Betaproteobacteria* phylum represented the fewest, accounting for only 1 % of all strains (Li et al. 2014). The 16S rDNA sequences and their similarity analyses were performed according to a previous study (Li et al. 2014). *Luteimonas abyssii* XH031^T was isolated from the SPG. pET-24a (+) and *Escherichia coli* BL21 (Novagen) were the hosts for gene cloning and expression, respectively. Luria–Bertani (LB) broth supplemented with kanamycin (100 µg/mL) was used to incubate *E. coli* BL21 at 37 °C.

Screening for the extracellular hydrolytic enzymes

Starch, carboxymethyl cellulose, and chitin were used as substrates on diffusion agar plates to detect the extracellular

polysaccharide hydrolytic activities of these isolates with incubation at 4, 16, and 28 °C for 4–14 days. Amylase activity was screened using marine agar plates (per liter of NSW: 5 g of peptone, 1 g of yeast extract, 20 g of agar, and 0.1 g of FePO₄) supplemented with 0.2 % (w/v) soluble starch (Sangon, China) (Zhang 2007); the pH was adjusted to 7.6 after the starch was added. Gram's iodine solution (0.3 % I₂–0.6 % KI) was used to flood the starch agar plates to detect amylase producers. Strains with distinct transparent zones were identified as amylase producers. Extracellular chitinase activity was tested on marine agar plates by adding 0.1 L of chitin colloid (10 %, w/v) (Ocean University of China) per 1 L of medium (Zhang 2007). Typically, 1–2 weeks were required to incubate these strains, because chitin is difficult to degrade. The appearance of a transparent zone around a colony indicated that the strain was positive for chitinolytic activity. A cellulase activity screening was conducted on marine agar plates supplemented with sodium carboxymethyl cellulose (1 %, w/v; Sigma, USA) (pH 7.6). Hydrolysis activity was tested by Congo red staining (1 mg/mL). After the plates were covered in Congo red for 1–2 h, NaCl solution (1 mol/mL) was used to flood the plates for at least 1 h, and NaCl solution (1 mol/mL) was finally used to wash the plates repeatedly. The result was positive if a transparent zone appeared around a bacterial colony.

Casein and gelatin were used as substrates to screen protease producers. Caseinase activity was tested on double agar plates. The upper layer contained 0.5 L of NSW, 10 g of agar, 10 g of peptone, and 3 g of yeast extract, and the lower layer contained 0.5 L of distilled water, 10 g of agar, and 10 g of casein (Sigma, USA); the pH did not need to be adjusted. The gelatinase screening medium consisted of marine agar plates containing 15 g of gelatin per 1 L of medium (pH 7.6). Trichloroacetic acid (40 %, w/v) was used to detect caseinase and gelatinase activities. A clear zone appearing around colonies indicated the presence of caseinase activity or gelatinase activity. Conversely, the appearance of a white precipitate indicated that an isolate was negative (Zhang 2007).

Tween 20, 40, 80, and lecithin were used to screen lipase-positive producers. The medium consisted of marine agar plates with 0.05 % (v/v) Tween 20 (polyethylene glycol sorbitan monolaurate), Tween 40 (polyoxyethylene sorbitan monopalmitate), or Tween 80 (polyoxyethylene sorbitan monooleate) (Sigma, USA). When opaque halos appeared, those clones were regarded as positive. Lecithinase medium was comprised marine agar plates supplemented with 0.1 L of yolk suspension (10 %, v/v) per 1 L of medium. The appearance of a milky halo around a colony signified that the strain was positive (Zhang 2007).

DNase activity was detected using DNase test agar (Qingdao Hope Bio-technology Co., Ltd) according to the

manufacturer's instructions, in which distilled water was replaced by sterile seawater. HCl (1 M) solution was used to flood the plates to detect DNase producers; a clear transparent zone appearing around the colony indicated that the strain was positive.

Cloning and expression of α -amylase Amy2235 from *Luteimonas abyssi* XH031^T

The whole genome of *Luteimonas abyssi* XH031^T has been sequenced (Zhang et al. 2015) and the gene of Amy2235 was annotated in the genome. According to this, the PCR primers were designed by PRIMER 5.0. The forward primer: CGGAATTCATGACCACACCCCTGGTGG (*Eco*RI) and reverse primer: CCCTCGAGGCCCGGTTGCACCG (*Xho*I). PCR was performed with the following program for 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 67 °C for 30 s, and 72 °C for 3.5 min, with a final extension at 72 °C for 10 min. The PCR product was purified and digested with *Eco*RI and *Xho*I (Takala), and cloned into the corresponding sites of pET-24a (+), then it was transformed into *E. coli* BL21 for expression and purification.

Expression and purification of the recombinant α -amylase Amy2235

The recombinant was cultured overnight at 37 °C in LB medium with 100 μ g/mL kanamycin. Then, the culture was inoculated into 200 mL fresh LB broth to grow. Amy2235 induction was performed when the OD₆₀₀ reached 0.4–0.6 by adding filter-sterilized isopropyl- β -D-thiogalactopyranoside (IPTG) to a final concentration of 0.1 mM. The induction was carried out at 16 °C for 16–20 h. Cells were harvested by centrifugation at 6,000g for 10 min at 4 °C. The cell pellet was resuspended in 50 mM Tris–HCl buffer (pH 7.0) and disrupted by sonication. The lysates were centrifuged at 12,000g for 30 min before the supernatant was purified using Ni–NTA–agarose column. Then, SDS-PAGE (12 %) was performed to check the purity of the recombinant α -amylase Amy2235.

Effects of temperature and pH on enzyme activity and stability

To examine optimum temperature of enzyme activities, activities were measured at 0, 10, 16, 28, 37, 50, 60, and 80 °C in 50 mM Tris–HCl buffer (pH 8.0) with 1 % soluble starch. The enzyme thermostability was determined by pre-incubation at the above range of temperatures for 1 h in Tris–HCl (50 mM) buffer, and the residual enzyme activity was measured using standard enzyme assay.

Sequence analysis

Multiple sequence alignment of the amino acid sequences of Amy 2235 with α -glucosidases of *Xanthomonas* sp. Mitacek01 (WP_055249184.1), *Luteimonas* sp. FCS-9 (WP_047136483.1), and *Pseudoxanthomonas suwonensis* (WP_024868235.1) was performed using NCBI database (<http://www.ncbi.nlm.nih.gov/>) and basic local alignment search tool (BLAST). The search engine is at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>.

Results

Diversity of psychrophilic enzymes produced by isolates in deep-sea water

Different media were used to detect extracellular low-temperature enzymes. The sizes of the transparent zones or hydrolytic circles in the media indicated the activity strengths of a series of enzymes (Table S2). Typical images of transparent or hydrolytic zones produced by 10 types of culture media at 4 °C are shown in Fig. S1. Of the 174 isolates screened, there are 154 isolates displayed extracellular hydrolytic enzyme activities, and 149 isolates produced exoenzymes at 4 °C. Tween 20 lipase activity at 4 °C was most common; gelatinase positives took a considerable proportion of all strains (~50 % of the isolates), and the least was cellulolytic species. The number of isolates that were able to degrade Tween 20, Tween 40, and Tween 80 at 4 °C is 112, 80, and 77, respectively, most of them were *Gammaproteobacteria*. Starch, cellulose, and chitin-degrading species at 4 °C were 44, 6, and 19, respectively (Table 1). Isolates from the *Alphaproteobacteria* and *Betaproteobacteria* classes had no extracellular lipolytic activity. *Gammaproteobacteria* was predominant in terms of activities for all the enzymes (Table S3).

Table 1 Number of strains and their percentages with hydrolytic enzyme activities at 4 °C of 174 isolates from the deep-sea water

Enzymes detected at 4 °C	Number of strains	Percentage (%)
Tween 20 lipase	112	64.37
Tween 40 lipase	80	46.55
Tween 80 lipase	77	44.25
Chitinase	19	10.92
Cellulase	6	3.45
Amylase	44	25.29
DNase	22	12.64
Lecithinase	18	10.34
Caseinase	31	17.82
Gelatinase	87	50.0

The amylase producers at 4 °C were members of the *Gammaproteobacteria* (40 isolates), *Alphaproteobacteria* (1 isolate), *Bacteroidetes* (2 isolates), and *Firmicutes* (1 isolate). *Bacteroidetes* had three positive isolates, and both *Alphaproteobacteria* and *Firmicutes* had only one positive strain. Starch was not degraded by *Betaproteobacteria* or *Actinobacteria* representative. Cellulase positivity at 4 °C was demonstrated by only 6 isolates; four of which were members of the *Gammaproteobacteria*, whereas the other two were from *Firmicutes* and *Actinobacteria* (Table S3). Caseinase, gelatinase, chitinase, and lipase positivity was mainly distributed within the genus *Pseudoalteromonas* of the *Gammaproteobacteria* (Fig. 1). DNase positivity accounted for 22.4 % of all the strains; there were 22 DNase-positive bacterial strains at 4 °C, with 20 strains affiliated with *Gammaproteobacteria*, and the other two in *Firmicutes* and *Actinobacteria*. Most of the gelatinase positives were in *Gammaproteobacteria*. *Firmicutes* isolates accounted for a large proportion of the total (7 isolates). All 6 *Bacillus* isolates had the ability to secrete gelatinase (Table S3).

The pattern of extracellular enzymes production at 28, 16, and 4 °C by all of 174 isolates is given in Table S3. Tween 20 lipase positivity consisted of 86 isolates at 28 and 16 °C, and in 112 isolates at 4 °C. Tween 40 lipase positivity was recorded in 56, 60, and 81 isolates at 28, 16, and 4 °C, respectively. Sixty-nine, 79, and 77 isolates produced extracellular Tween 80 lipases at 28, 16, and 4 °C. The predominant lipase-producing bacteria were from *Pseudoalteromonas* and *Alteromonas* of the *Gammaproteobacteria*. In addition, one strain of *Marinilactibacillus* in *Firmicutes* demonstrated Tween 40 and 80 lipase activities at 4 °C, respectively. There was only one Tween 20 and one Tween 40 lipase producer at 4 °C, which was from *Microbacterium* in the *Actinobacteria* and *Aquimarina* of *Bacteroidetes*. Twenty-five isolates produced extracellular lecithinase at 28 °C, and there were 42 isolates with the ability at 16 °C, and 18 isolates at 4 °C (Table S3). All the lecithinase positives at 4 °C were distributed in 7 genera of the *Gammaproteobacteria* (Fig. 1).

A total of ten exoenzyme activities detected at 4 °C are summarized in Fig. 1. Among these isolates, species of *Gammaproteobacteria* were dominant (Li et al. 2014), and accounted for 82 % of the total. From the *Gammaproteobacteria*, *Pseudoalteromonas* spp. predominated with the ability to degrade 9 macromolecules but not cellulose; *Alteromonas* spp. were the second-most predominant species, secreting 8 enzymes but not cellulase or chitinase (Fig. 1).

Alphaproteobacteria did not have any enzyme activities at 4 °C, although one isolate of *Loktanella tamensis* could degrade starch (Table S3). *Gammaproteobacteria* strains had the highest activities of 10 types of exoenzymes at 4 °C (Fig. 2). Isolates of *Firmicutes* phyla accounted for

Fig. 1 Hydrolytic enzyme activities at 4 °C among all the isolates (174 strains) from the deep-sea water

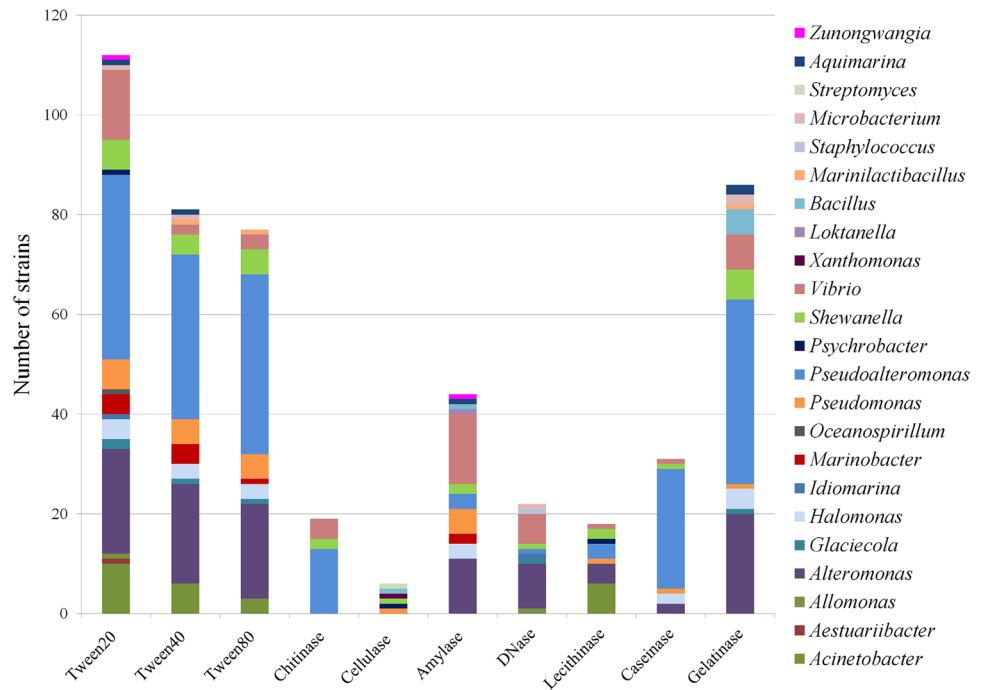
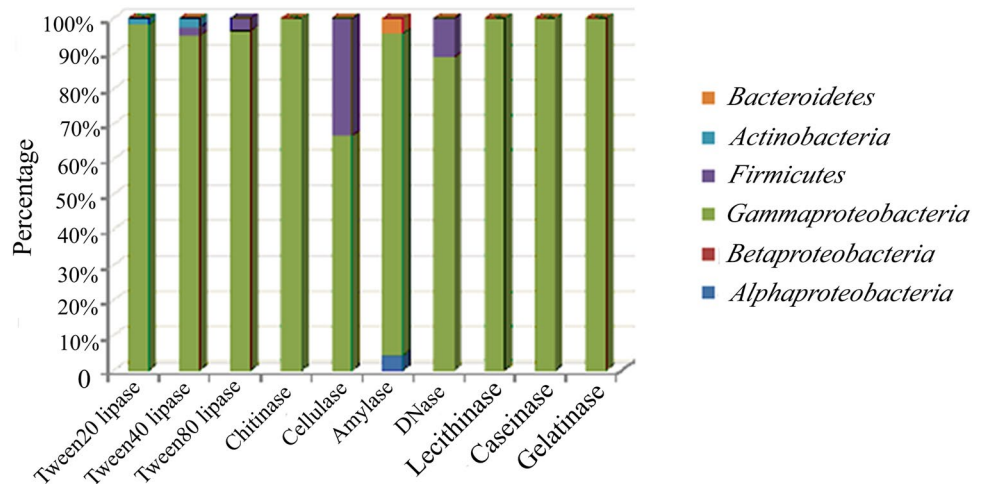


Fig. 2 Relative percentage of eleven types of extracellular hydrolytic enzymes with higher enzyme activities at 4 °C than at 28 or 16 °C



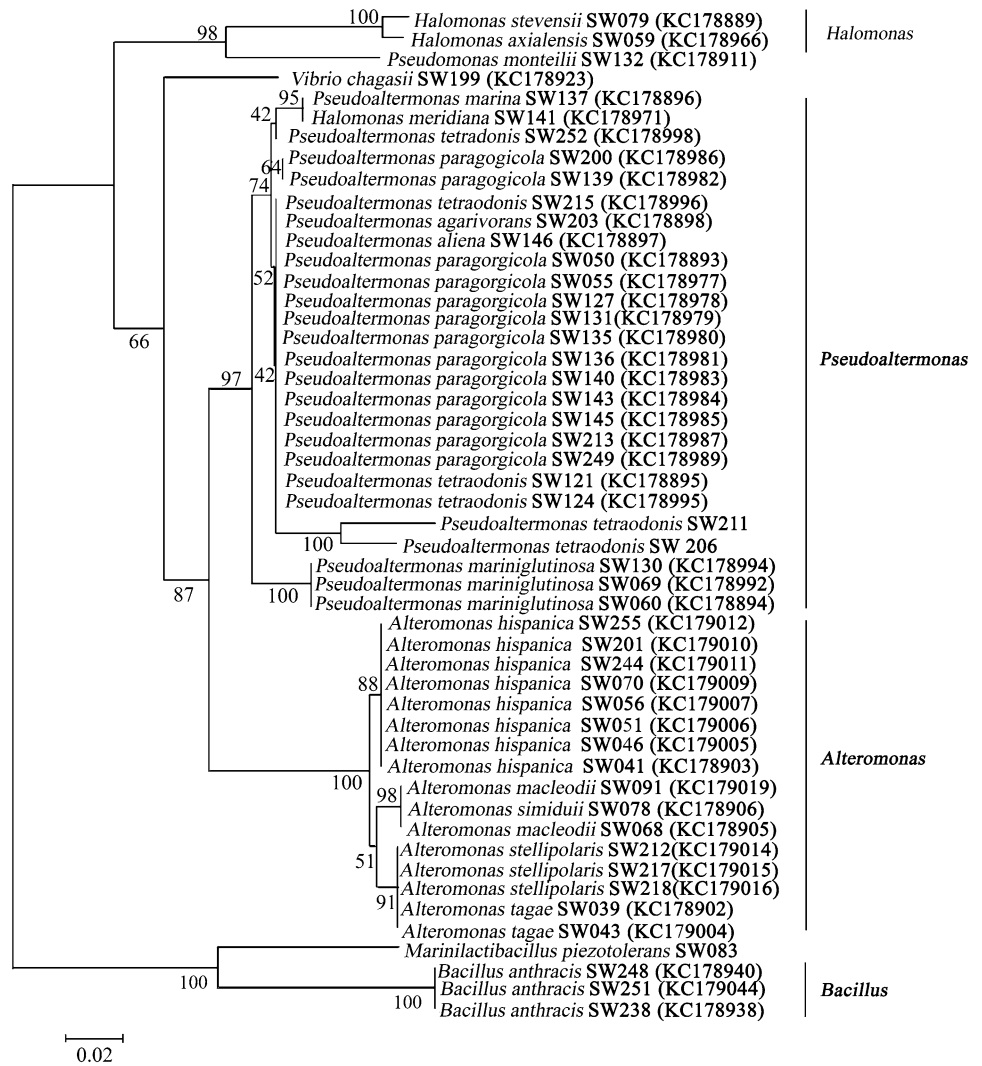
approximately 1/3 and 1/6 of cellulase and DNase positives, respectively, and their enzyme activities at 4 °C were higher than those at 28 and 16 °C (Fig. 2).

Diversity of protease-producing bacteria in the deep-sea water of the SPG

Casein and gelatin were used as substrates to detect protease activities. Caseinase and gelatinase positives amounted to 17.8 and 50 % of all the strains. Sixty-five isolates at 28 °C, 47 isolates at 16 °C and 31 strains at 4 °C produced caseinases, and 104 isolates at 28 °C, 97 isolates at 16 °C, and 87 strains at 4 °C produced gelatinases

(Table S3). At 4 °C, all the caseinase positives were distributed within *Gammaproteobacteria*, and most were strains of the *Pseudoalteromonas*. The gelatinase-positive strains were primarily distributed within *Gammaproteobacteria* (76 isolates), which accommodated 43.7 % of all isolates. The others were distributed in *Firmicutes* (7 isolates), *Actinobacteria* (2 isolates), and *Bacteroidetes* (2 isolates). Almost all the strains of the *Alteromonas*, *Pseudoalteromonas*, and *Shewanella* genera in *Gammaproteobacteria* had the ability to secrete gelatinases (Table S3). A neighbor-joining phylogenetic tree was constructed for protease-producing bacteria based on 16S rRNA gene sequences from the GenBank database (Fig. 3). Among all

Fig. 3 Phylogenetic tree of the protease-producing isolates recovered from the deep-sea water of seven sampling stations in the SPG by the neighbor-joining method, based on 16S rRNA gene sequences



the protease-producing isolates, with the exception of four strains (SW248, SW251, SW238, and SW083) that were Gram-positive bacteria belonging to the *Bacillus* genera of *Firmicutes*, the other isolates were primarily affiliated with the *Gammaproteobacteria* phylum and grouped in *Pseudoaltermonas*, *Alteromonas*, *Pseudomonas*, *Vibrio*, and *Halomonas*. By comparison, groups of *Pseudoaltermonas* (52 %) and *Alteromonas* (32 %) were the predominant protease-producing bacteria. The other genera producing proteases accounted for between 2.0 and 4.0 % of all strains. Moreover, three bacilli had the highest sequence identity (equal to 100 %), and they originated from the same sampling site (site U1371).

Gene cloning, expression, purification, and sequence analysis

The α -amylase gene of Amy2235, was successfully cloned from *Luteimonas abyssi* XH031^T. The gene is 1,626 bp in

length, which encodes a protein of 541 amino acids with a predicted molecular mass of 60.8 kDa and *pI* of 4.97. Conserved domains analysis indicated that Amy2235 had clear homology of α -amylase in GH13 family. By SDS-PAGE, the recombinant Amy2235 was purified to electrophoretic homogeneity with a single-band ~62.0 kDa, which was near to the predicted size (Fig. 4). Homology searches by protein blast indicated that Amy2235 (the accession number is WP_058835247) amino acid sequence showed 82, 78, and 75 % identities, respectively, to α -glucosidase from *Xanthomonas* sp. Mitacek01 (WP_055249184.1), *Luteimonas* sp. FCS-9 (WP_047136483.1), and *Pseudoxanthomonas suwonensis* (WP_024868235.1). Sequence blast of α -glucosidase of Amy2235 and other three are shown in Fig. S2.

Activity and thermostability assay

The activity of Amy2235 peaked at 50 °C. However, activity declined sharply above 50 °C, keeping only about 23 %

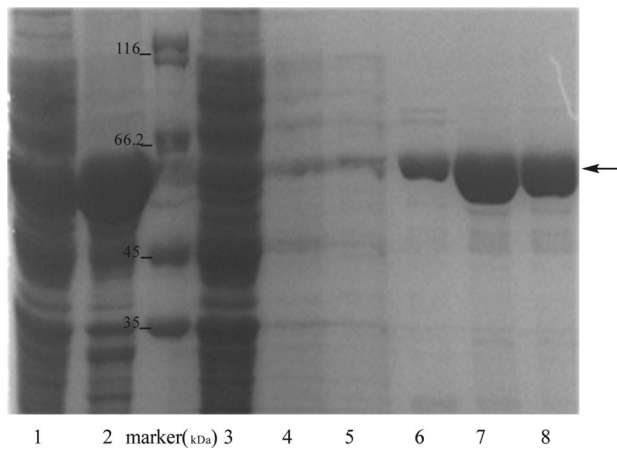


Fig. 4 SDS-PAGE analysis of Amy2235. SDS-PAGE analysis of recombinant α -amylases purification (As the *arrow* shows, the weight of recombinant α -amylases is about 62.0 kDa). 1 the supernatant of protein, 2 the sediment of protein, 3 the effluent of protein, 4 10 mM imidazole elution, 5 20 mM imidazole elution, 6 50 mM imidazole elution, 7 100 mM imidazole elution, 8 250 mM imidazole elution, Marker molecular weight marker

of its maximum activity at 60 °C. Moreover, the recombinant protein retained 18.7 and 36 % activity at 0 and 10 °C, respectively (Fig. 5a). Meanwhile, it was found that the enzymatic activity of Amy2235 retained 44.6 % optimum activity at 50 °C after 30 min, and it retained 45 % at 55 °C after 10 min (Fig. 5b), which showed that Amy2235 was, indeed, thermolabile. The comparison of the properties of Amy2235 with these of mesophiles and cold-adapted organism is shown in Table S4.

Discussion

Deep-sea water includes a large pool of organic matter that is primarily produced by the death and lysis of organisms that, in turn, become nutrient sources for extremophiles. Strains producing lipases and proteases may have principal roles in the mineralization of organic matter. Lipolytic activity was reported to be most widespread in the sea (Zaccone et al. 2002), which may be due to the degradation of some zooplankton components. As a matter of fact, except for proteins, lipids are the most significant zooplankton fraction. In this study, a large number of strains have been detected to produce lipases and protease (primarily gelatinase) (Fig. 1), showing that there are relatively higher concentrations of lipids and proteins in the deep-sea water of the SPG. The diversity of protease-producing bacteria revealed that organic nitrogen may be degraded in deep-sea water. In sub-Antarctic sediments, protease producers were distributed primarily among *Gammaproteobacteria* (Zhou et al. 2009), which is consistent with our

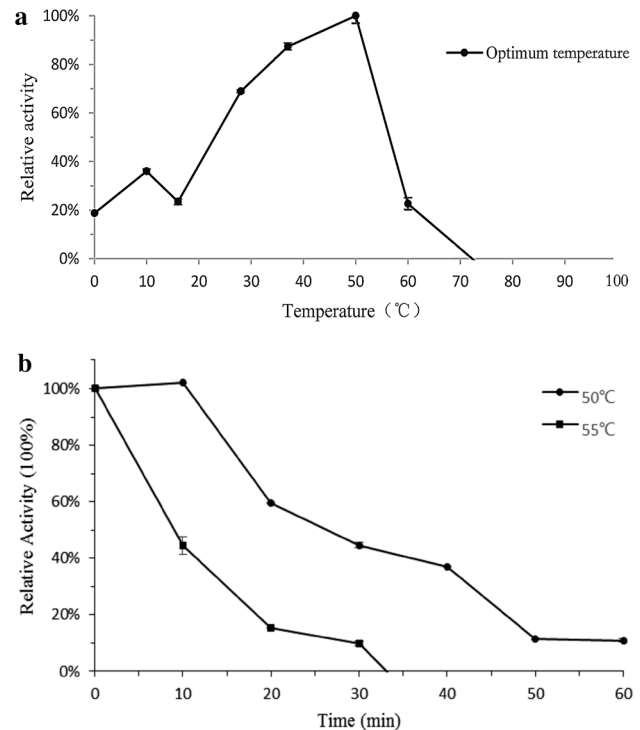


Fig. 5 Effects of temperature on enzyme activity and stability. **a** Effect of temperature on the Amy2235 activity (filled circle). **b** Thermostability of Amy2235 at 50 and 55 °C. The enzyme was pre-incubated in Tris-HCl (50 mM) buffer at the range of temperatures for 1 h. 50 °C (black circle); 55 °C (black squares)

results. Isolates that were responsible for polysaccharide hydrolysis were found to be distributed mainly among the *Alteromonas*, *Pseudomonas*, *Pseudoalteromonas*, *Vibrio*, and *Shewanella*, indicating that these genera may play a major role in decomposing phytoplankton detritus and exopolymeric substances. Chitin is the most plentiful polysaccharide in aquatic biosphere (Souza et al. 2011). The primary source may well be the dead bodies or detritus of marine planktonic crustaceans, suggesting that these isolates may directly participate in the mineralization process of euphausiid, amphipod, and copepod detritus (Chen et al. 2009). Certainly, chitinolytic microbes are ubiquitous in marine conditions. However, in this study, the average chitinolytic isolates at 4 °C accounted for only 11 % of the total, which was less than the expected (ca. 20 %). The main reason is, perhaps, that a large number of chitinolytic organisms (including all anaerobes) are unculturable or dormant in the initial isolation media. DNase positives were primarily distributed among *Alteromonas* and *Vibrio*, which could degrade extracellular DNA in the deep-sea ecosystem to provide C, N, and P sources for prokaryotic metabolisms. In the deep-sea environment, free-dissolved DNA is thought to comprise a crucial trophic resource that substantially contributes to C, N, and P cycling (Pinchuk

et al. 2008). A large number of strains isolated from the deep-sea water of the SPG could produce various cold-adapted enzymes, implying that in situ microbes may have evolved the genetic or physiological properties to degrade POM via the production extracellular cold-adapted enzymes. Because the extracellular enzymes in the deep sea are major drivers of nutrient cycling, these isolates producing cold-adapted enzymes may play a crucial role in the material circulation of the deep-sea biosphere, and they act as important ecological roles in the deep-sea microbial ecosystem.

At present, the idea that polymeric substrates can induce the production of hydrolytic enzymes has widely been accepted (Vetter and Deming 1999). The diversity of cold-adapted enzymes may signify that various polymeric substrates are present in the SPG. Moreover, the screening of multiple enzymes indicates that these extremophiles inhabiting in ultra-oligotrophic and freezing temperature deep sea still have a very active physiological and metabolic functions.

As far as we know, the optimum temperature of cold-active α -amylase from *Halobacillus* sp. MA-2, *Pseudoalteromonas* sp. MY1, and *Nocardiopsis* sp. 7326 were 50, 40, and 35 °C, respectively (Amoozegar et al. 2003; Zhang and Zeng 2008). The optimum temperature of Amy2235 is 50 °C. One of the best studied cold-active α -amylase from *Pseudoalteromonas haloplanktis* retained about 25 % of the activity at 5 °C (Feller et al. 1992, 1994). An isolate that is affiliated to *Actinobacteria* from Svalbard (Groudieva et al. 2004) and a soil isolate related to *Bacillus* (Mojallali et al. 2013) retained 20 and 13 % of the α -amylase activity at 0 °C, respectively. The α -amylase from a strain related to *Nocardiopsis* (Zhang and Zeng 2008) retained 25 % of its activity at 0 °C. The comparison (Table S4) showed that relative enzymatic activity of Amy2235 is slightly lower than AHA at low temperature, which may indicate the cold adaptation of Amy2235. In addition to α -amylase, many other cold-active enzymes, such as cellulase, chitinase, lipase, DNase, and protease et al. in XH031^T, are also worthy of further study. It is argued that the studies on the characteristics of these other cold-active enzymes may bring new advances in enzymology.

Cold-active enzymes have the extensive potential of biotechnological application in industrial fields, where cold temperatures are needed, such as wastewater treatment, additives of detergents for cold washing, bioremediation in contaminated cold environment, synthesis of active compounds under cold conditions, etc. (Gerday et al. 2000).

Although the results from the degradation of diverse macromolecules of bacterial origin are preliminary, this screening may provide a resource to discover novel biocatalysts. In addition, it may have a significant application potential in industries. Moreover, this study will set the

foundation for the development of new functional genes and the study of new metabolic pathways.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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