Structural characterization and ecological roles of a novel exopolysaccharide from the deep-sea psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913

Guokui Qin,¹ Lizhi Zhu,² Xiulan Chen,¹ Peng George Wang^{1,2} and Yuzhong Zhang¹

Correspondence
Yuzhong Zhang
zhangyz@sdu.edu.cn

¹State Key Lab of Microbial Technology, Marine Biotechnology Research Center, Shandong University, Jinan, 250100, P. R. China

²Department of Biochemistry, The Ohio State University, Columbus, OH 43210, USA

Pseudoalteromonas sp. SM9913 is a psychrotolerant bacterium isolated from deep-sea sediment. The structural characterization and ecological roles of the exopolysaccharide (EPS) secreted by this strain were studied in this work. The yield of the EPS increased as the culture temperature decreased in the range 30–10 °C, and it reached 5.25 g l⁻¹ (dry weight) under optimal growth conditions (15 °C, 52 h). EPS fraction was purified and its structure was identified by the combination of NMR spectra, high-resolution mass spectrometry (HRMS) analysis and methylation analysis. The ratio of the sugar units, the acetyl group and the ethoxyl group was close to 4 : 5 : 1. The major sugar unit of the EPS was 6-linked glucose (61.8 %); other sugar units present included terminal arabinofuranosyl (11.0 %) and glucopyranosyl (11.2 %) residues and a small amount of other sugar derivatives. Its structure was different from EPSs reported for other marine bacteria. Besides the structural elucidation of the EPS, its ecological roles were studied. This EPS could enhance the stability of the cold-adapted protease MCP-01 secreted by the same strain through preventing its autolysis. It could bind many metal ions, including Fe²⁺, Zn²⁺, Cu²⁺, Co²⁺. It was also a very good flocculating agent and could conglomerate colloidal and suspended particles. These results indicated that the EPS secreted by strain SM9913 might help this strain enrich the proteinaceous particles and the trace metals in the deep-sea environment, stabilize the secreted cold-adapted proteases and avoid its diffusion. This is believed to be the first report on the structure of the EPS secreted by a deep-sea psychrotolerant bacterium and its ecological roles. According to these results and other studies, a schematic diagram of the lifestyle of the deep-sea psychrotolerant strain SM9913 is suggested.

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INTRODUCTION

The deep sea is a major component of the planet's biosphere and represents 75 % of the total volume of the oceans. Current studies have focused on the extremophilic micro-organisms isolated from deep-sea hydrothermal vents (Staley & Gosink, 1999; Guezennec, 2002), and a variety of fascinating micro-organisms well adapted to these extreme environments have been investigated (Guezennec, 2003). Due to the attractive chemical and rheological properties of polysaccharides produced by these extremophilic micro-organisms, studies have been conducted to

investigate their potential application in biotechnology, environmental protection, the food industry, and so on (Kirschner & Woods, 2001; Collic-Jouault *et al.*, 2004). Although the structures of some exopolysaccharides (EPSs) from deep-sea hydrothermal vents have been reported (Rougeaux *et al.*, 1999a, b; Cambon-Bonavita *et al.*, 2002; Raguene *et al.*, 2003), there have been few reports on their ecological roles. The genome sequence of the deep-sea gammaproteobacterium *Idiomarina loihiensis* was reported and it was suggested that this species relies primarily on amino acids, rather than on carbohydrates, for carbon and energy (Hou *et al.*, 2004). The versatile signal transduction system of *I. loihiensis* apparently aids its survival and growth, and initiates the synthesis and secretion of highly viscous EPS(s) for attachment to proteinaceous particles. It would appear that deep-sea EPSs could serve as enhancers for the survival of deep-sea bacteria in a fluctuating environment

Abbreviations: COSY, correlation spectroscopy; EPS, exopolysaccharide; HRMS, high-resolution mass spectrometry; HSQC, heteronuclear single quantum correlation.

Supplementary data are available with the online version of this paper.

(Hou *et al.*, 2004). However, this hypothesis has not yet been confirmed by any experimental results.

The deep-sea hydrothermal vents only represent a small and particular portion of the whole deep-sea environment. Most deep-sea environments are influenced by high pressure, low temperature and low nutrient concentration. Many kinds of deep-sea psychrotolerant bacteria live in the abyssal ecological community. Survival in these conditions requires a complex suite of morphological, physiological and metabolic adaptations. Therefore, the investigation and study of deep-sea psychrotolerant bacteria may reveal important ecological characteristics enabling the adaptation of bacteria to the constantly changing deep-sea ecosystem (Rademacher *et al.*, 1988).

Psychrotolerant bacterium *Pseudoalteromonas* sp. SM9913 (its detailed characteristics are shown in Part I of the supplementary data available with the online version of this paper; the GenBank accession number of the 16S rDNA of this strain is AY305857), a gammaproteobacterium, was screened from 1855 m deep-sea sediment. It secreted at least three different extracellular proteases, MCP-01, MCP-02 (Chen *et al.*, 2003a; GenBank accession no. ABD14413.2 for MCP-01, EF029091 for MCP-02) and MCP-03 (unpublished results, GenBank accession no. ABD92880). The main extracellular protease MCP-01 was a cold-adapted protease which was thermolabile and susceptible to autolysis (Chen *et al.*, 2002, 2003a, b). Moreover, strain SM9913 secreted large quantities of EPSs. In this study, the EPS secreted by strain SM9913 was purified and its structure was characterized. On the basis of the results, some ecological roles provided by the EPS are suggested that are relevant for adaptation to the deep-sea ecosystem.

METHODS

EPS production of strain SM9913 at different temperatures.

In order to study the effect of temperature on EPS production, *Pseudoalteromonas* sp. strain SM9913 was cultured at 10 °C, 20 °C and 30 °C using the method previously described (Chen *et al.*, 2003a). The content of the EPSs in the culture supernatant was measured every 12 h by the phenol/H₂SO₄ method (Dubois *et al.*, 1956) after the culture was centrifuged at 10 000 g for 12 min at 25 °C (Eppendorf centrifuge 5804R). T-dextran of different molecular masses (T-10, T-40, T-70, T-500 and T-2000, from Amersham Pharmacia Biotech) were used as standards.

Isolation and purification of the EPS. The exopolymer was precipitated from the supernatant of the culture by addition of cold absolute ethanol (1:1). Then, it was centrifuged at 10 000 g for 10 min at 25 °C. The precipitate was dissolved in distilled water and decoloured with 5 % (w/v) activated charcoal. After removal of proteins from the exopolymer solution by the Sevag method (Staub, 1965), the crude EPS was obtained. The crude EPS was purified through gel-filtration chromatography, which was eluted with 0.1 M NaCl aqueous solution through a column (100 × 1.6 cm) of Sephadex G-100 at a flow rate of 12 ml h⁻¹. The EPS fraction was collected and further purified through anion-exchange chromatography on a DEAE-Sepharose Fast Flow column, which was eluted with a linear gradient of 0 to 1 M NaCl aqueous solution at a flow rate of 60 ml h⁻¹. The EPS fraction was collected. During these processes,

the polysaccharide content was assayed by the phenol/H₂SO₄ method and protein content was monitored by ultraviolet absorption spectroscopic analysis (Jasco). After dialysis against distilled water, concentration and lyophilization, the EPS was obtained.

The EPS obtained from chromatographic purification was further analysed on a Shimadzu analytical HPLC system with a Shimadzu autoinjector using a Waters Ultrahydrogel Linear column (7.8 × 300 mm). All samples were prepared as 1 % (w/v) aqueous solutions and 20 µl of sample solution was injected in each run process for 40 min. Analytical liquid chromatography was performed in redistilled water at a flow rate of 0.4 ml min⁻¹ at 35 °C. The sample was detected with a Shimadzu refractive index detector. Preliminary calibration of the column was conducted with different T-dextran standards. The molecular mass of the EPS was analysed by the same HPLC system with T-dextran of different molecular masses as standards. Chromatographic data were generated and processed with Shimadzu Class-VP software.

Structural characterization of the EPS

Constituent and methylation analysis of the EPS. For glycosyl linkage analysis, the EPS sample was permethylated, depolymerized, reduced and acetylated. The resultant partially methylated alditol acetates (PMAAs) were analysed by gas chromatography-mass spectrometry (GC-MS) as described by York *et al.* (1985). Initially, an aliquot of EPS sample was permethylated by the method of Ciucanu & Derek (1984), treating with sodium hydroxide and methyl iodide in dry DMSO. The permethylation was repeated twice to ensure the complete methylation of the exopolymer. Following sample workup, the permethylated material was hydrolysed using 2.0 M trifluoroacetic acid (2 h in a sealed tube at 121 °C), reduced with NaBD₄, and acetylated using acetic anhydride/trifluoroacetic acid. The resulting PMAAs were analysed on a Hewlett Packard 5890 GC interfaced to a 5970 mass selective detector (MSD, electron impact ionization mode), and separation was performed on a 30 m Supelco 2330 bonded-phase fused-silica capillary column.

NMR spectral studies of the EPS. The EPS sample was treated with D₂O to ensure the completion of the deuterium exchange before NMR analysis, dissolving the EPS solid (approx. 15 mg) in D₂O (99.9 %, 1.0 ml) at room temperature. After vortexing for 5 min at room temperature, the solution was frozen at -70 °C and lyophilized to dry. This procedure was performed three times, and the EPS solid obtained was dissolved in D₂O (99.9 %, 0.5 ml), then transferred into an NMR tube. The solution obtained was cloudy. However, the precipitate could be removed by centrifuging at 6000 g. The ¹H NMR spectrum of the cloudy solution was identical to that of the clear solution after centrifugation. The NMR spectra [¹H NMR, ¹³C NMR, ¹H-¹H correlation spectroscopy (H-¹H COSY), heteronuclear single quantum correlation (HSQC)] were recorded on a 500 MHz (DRX-500) Bruker NMR spectrometer.

Analysis of the ecological role of the EPS

Effect of the EPS on the thermostability and autolysis (self-digestion) of the cold-adapted protease MCP-01. Purification and activity assay of cold-adapted protease MCP-01 from strain SM9913 were all done using the method previously described (Chen *et al.*, 2003a). To study the effect of the EPS on MCP-01 thermostability, the protease solution with and without 1 % (w/v) the EPS was incubated at 40 °C for 150 min, and the residual protease activity was determined at intervals. The determination of MCP-01 autolysis at 4 °C and 40 °C was performed using capillary electrophoresis according to previously published procedures (Chen *et al.*, 2003b). To study the effect of the EPS on MCP-01 autolysis, the protease

solution with and without 1% (w/v) EPS was incubated at 40 °C and the autolysis process of the samples was monitored by capillary electrophoresis.

Analysis of metal-binding property of EPS. Metal adsorption of the EPS from strain SM9913 was carried out by Salehizadeh's method (Salehizadeh & Shojaosadati, 2003). In this experiment, 0.02% (w/v) EPS and 2.0 mM solution of each metallic ion (copper, magnesium, cobalt, manganese, iron, zinc and chromium) were used.

Flocculation analysis of the EPS. Kaolin was used to evaluate the flocculating ability of the EPS from strain SM9913 by the method of Anuradha & Bajpai (2005). The mixture of 0.5% (w/v) kaolin, 0.05% (w/v) CaCl_2 and 0.001% (w/v) EPS was vibrated in a table rotary shaker at 200 r.p.m. for 5 min and then equilibrated for 10 min at room temperature. The same sample without the EPS was used as a standard.

RESULTS

Effect of temperature on the production of the EPS secreted by strain SM9913

When strain SM9913 was cultured at 10 °C, 20 °C or 30 °C, after a short period of decrease, the sugar amount in the culture media continuously increased to a peak at 40–60 h and then declined. This indicated that the carbohydrate in the culture medium was utilized by strain SM9913 for growth at the beginning, and then strain SM9913 started to continuously secrete EPS. The yield of EPS increased as the temperature decreased in the tested range, indicating that the EPS production of strain SM9913 had cold-adaptation (see supplementary data, Part II, Fig. S1, available with the online version of this paper). Under optimal growth conditions (15 °C, 52 h), the yield of EPS reached 5.25 g l⁻¹ (dry weight), which was higher than that reported for the EPSs produced by other psychrotolerant microorganisms (Mancuso Nichols *et al.*, 2005a).

Isolation and purification of the EPS

Two EPS peaks were eluted from the gel-filtration chromatographic column of Sephadex G-100. The first fraction was collected and further purified by anion-exchange chromatography on a DEAE-Sepharose Fast Flow column (the latter was not collected because of limited contents). Homogeneity of the EPS purified by chromatography was analysed on the Shimadzu analytical HPLC system. Only one symmetrical acute peak was detected (see supplementary data, Part II, Fig. S2), which indicated that this EPS consisted of a single homogeneous component and could be used for structure analysis. The molecular mass of the EPS analysed by the same HPLC system was 4×10^4 Da.

Structural characterization of the EPS

Methylation analysis and glycosyl linkage analysis of the EPS. Glycosyl composition analysis of the EPS was initially performed and the results showed that it consisted mainly of glucose, with arabinose, xylose and a

minor peak for mannose. It did not contain any amino-residues. Linkage analysis of the EPS sample showed that it contained mainly 6-linked glucopyranosyl (6-Glc). Other linkages were terminal glucose, terminal arabinose and some minor peaks for terminal galactopyranosyl (t-Gal), 4-linked glucopyranosyl (4-Glc), 4-linked xylofuranosyl (4-Xyl-f) and 3,6-linked glucopyranosyl (3,6-Glc). These results showed that the main composition of the EPS was 6-Glc (61.8%). Other components included terminal arabinofuranosyl (t-Ara f, 11.0%) and terminal glucopyranosyl (t-Glc, 11.2%) together with a small amount of t-Gal (3.1%), 4-Xyl (f, 3.9%), 4-Glc (5.0%) and 3,6-Glc (4.0%). These results confirmed the results from the latter NMR analysis, and also provided the linkage between the repeating sugar units as an α -(1→6) linkage.

NMR analysis of the EPS. The ¹H NMR spectrum was first recorded at room temperature in D₂O, and only one anomeric proton signal was observed at 4.94 p.p.m. ($J=3.4$ Hz) (see supplementary data, Part II, Fig. S3). In order to avoid the overlap with H₂O peak at 4.70 p.p.m., the ¹H NMR spectrum was also collected at different temperatures (40 °C and 60 °C). The results indicated that the polysaccharide only presents one anomeric proton signal. From the coupling constant, the configuration of the sugar unit is α -anomer ($J_{\text{Heq-Hax}}=3.4$ Hz). The strong singlet at 2.19 p.p.m. implied the presence of an acetyl group (-COCH₃). The triplet at 1.14 p.p.m. ($J=7.1$ Hz) belongs to the CH₃ in an ethoxyl group (-OCH₂CH₃). The integration of the ¹H NMR spectrum indicated that the ratio between the sugar units, the acetyl group and the ethoxyl group is close to 4:5:1. The rest of the signals at 4.0 p.p.m. to 3.4 p.p.m. belong to the protons on the sugar skeleton. The coupling patterns between these signals are hard to identify by 1D NMR. ¹H-¹H COSY (phase sensitive) was employed to resolve the coupling pattern and the coupling constants (results summarized in supplementary data, Part II, Table S1). The coupling pattern and the coupling constant obtained from the phase-sensitive ¹H-¹H COSY, together with the chemical shift from the ¹H NMR spectra, showed that no amino group was present on the sugar skeleton (see supplementary data, Part II, Fig. S5). The coupling pattern and coupling constants strongly suggested that the repeating sugar unit has an α -glucose skeleton. The ¹³C NMR spectrum was recorded at room temperature in D₂O (see supplementary data, Part II, Fig. S4). The signal at 215.45 p.p.m. indicated the carbonyl group of the acetyl functional group. The most intensive peak at 30.27 p.p.m. was assigned to the methyl group in the acetyl group. The peaks at 66.01 and 14.12 p.p.m. confirmed the ethoxyl group. Only one peak was observed in the anomeric region (97.75 p.p.m.), which also confirmed one set of repeating sugar units. The peaks at 73.43, 71.43, 70.21, 69.57, 65.58 p.p.m. were assigned to the carbons on the sugar skeleton. The assignment of these carbon signals was facilitated by the HSQC spectrum (see supplementary data, Part II, Fig. S6; the final assignment of the ¹³C

signals is listed in supplementary data, Part II, Table S1). The down-field chemical shift of C3 indicated the attachment of the acetyl group at this position.

HRMS analysis of the EPS. The final assignment of the HRMS analysis is listed in supplementary data, Part II, Table S2. The observed $[M + Na^+]$ is 927.2395 (100.00 %); $[M + 1 + Na^+]$ is 928.2424 (36.44 %); $[M + 2 + Na^+]$ is 929.2421 (12.45 %) (supplementary data, Part II, Fig. S7). This result associated with the molecular formula $C_{36}H_{56}O_{26}Na^+$, which was consistent with the result obtained from the NMR analysis. The EPS contained four α -glucose units, five acetyl groups and one ethoxyl group.

Based on the data obtained and the analysis above, this EPS was structurally characterized as a linear arrangement of α -(1,6) $\rightarrow$ Glc and a high degree of acetylation. The repeating unit of the EPS was established as $\rightarrow$ 6)-[3,6-*O*-acetyl]- α -D-Glcp-(1 $\rightarrow$ 6)-[3-*O*-acetyl]- α -D-Glcp-(1 $\rightarrow$ 6)-[3-*O*-acetyl]- α -D-Glcp-(1 $\rightarrow$ 6)-[3-*O*-acetyl]- α -D-Glcp-(1 $\rightarrow$, and the structure of the EPS is shown in Fig. 1.

Ecological role of the EPS from strain SM9913

Effect of the EPS on the thermostability and autolysis of the cold-adapted protease MCP-01. Cold-adapted protease MCP-01, the main protease secreted by strain SM9913, was thermolabile and susceptible to autolysis (Chen *et al.*, 2002, 2003b). Our previous study showed that autolysis of the purified protease MCP-01 was the main reason for its thermolability (Chen *et al.*, 2003b). Here, protease MCP-01 was purified and the effect of the EPS secreted by strain SM9913 on the thermostability and autolysis of the protease was studied. The results showed that the EPS obviously enhanced the thermostability of protease MCP-01 at 40 °C. In the presence of EPS (1%, w/v), the protease activity of MCP-01 showed no evident change after 150 min incubation. In contrast, the protease activity in the absence of EPS was quickly lost, with 90 % of the activity lost after 135 min incubation at 40 °C (Fig. 2). The autolysis process of protease MCP-01 at 4 °C and 40 °C was monitored by capillary electrophoresis. Protease MCP-01 autolysed slowly at 4 °C; 57.3 % of the

Fig. 2. Effect of the EPS from strain SM9913 on the activity of the cold-adapted protease MCP-01 secreted by the same strain. ■, MCP-01 with the EPS (1%, w/v); ◆, MCP-01 without the EPS. The experiment was repeated three times; means of the three replicates are plotted.

protease had autolysed after incubation for 55 h. At 40 °C, protease MCP-01 autolysed rapidly and the peak of MCP-01 disappeared in 30 min. But when 1% (w/v) EPS was added into the MCP-01 solution, the peak of MCP-01 showed no evident change during 50 min incubation at 40 °C (see supplementary data, Part II, Fig. S8), indicating that the EPS prevented the autolysis of protease MCP-01 at 40 °C; this could be the reason why the EPS enhanced the thermostability of protease MCP-01.

Metal-binding property of the EPS. The metal-binding property of the EPS was studied and the adsorption percentage values (*Q*) of each metal tested were calculated. The results showed that Fe^{2+} (85.00 %), Zn^{2+} (58.15 %), Cu^{2+} (52.77 %) and Co^{2+} (48.88 %) were easily adsorbed by the EPS, while Mg^{2+} (30.69 %) and Mn^{2+} (25.67 %) were less adsorbed by the EPS. Of the metals tested, Cr^{6+} showed the lowest adsorption to the EPS (5.15 %). These results indicated that the EPS might be helpful for strain SM9913 to enrich metal ions in the nutrition-scant deep-sea environment.

Flocculation analysis of the EPS. The flocculation experiment showed that the EPS could make colloidal and suspended particles in solution conglomerate (see supplementary data, Part II, Fig. S9), suggesting that the EPS was a very good flocculating agent and had a good adsorptive effect. Therefore, it might play an important role for strain SM9913 in enriching nutrient particles in the deep-sea environment.

DISCUSSION

Many EPSs secreted by marine bacteria have been reported. Although EPSs from different strains may be different in

Fig. 1. Structure of the preponderant repeating units of the EPS from the deep-sea psychrotolerant strain SM9913.

yield, chemical composition, structure and physical properties, they have some common characteristics. Most EPSs produced by marine bacteria are heteropolysaccharides consisting of three or four different monosaccharides arranged in groups of ten or fewer to form repeating units (Decho, 1990). The monosaccharides may be pentoses, hexoses, amino sugars or uronic acids. EPSs from marine bacteria usually possess hydroxyl and carboxyl groups, which confer a net negative charge and acidic properties to the EPSs. Up to 20–50 % of these EPSs may be uronic acids. Most EPSs are linear overall and of varying lengths, with a mean molecular mass of 1×10^5 to 3×10^5 Da. Branches of one or more monosaccharides are often attached at regular intervals. Organic or inorganic (sulfate, phosphate) substituents may also be present (Mancuso Nichols *et al.*, 2005b). In recent years, some EPSs secreted by marine bacteria from deep-sea hydrothermal vents have been reported. Most of them are acidic polysaccharides with uronic acid content from 10 % to 40 % and high molecular mass up to 10^6 g mol⁻¹ (Guezennec, 2002).

In this study, the EPS secreted by strain SM9913, a deep-sea psychrotolerant bacterium isolated from sediment at 1855 m depth, was purified and its structure was elucidated. To our knowledge this is the first report on the EPS from a deep-sea psychrotolerant bacterium. Its structure was quite different from that of the EPSs secreted by marine bacteria from hydrothermal vents and other areas, including Antarctic *Pseudoalteromonas* strains (Mancuso Nichols *et al.*, 2005a; Guezennec, 2002). The EPS from strain SM9913 was an acetyl-rich EPS with a molecular mass of 4×10^4 Da, which was smaller than most of the EPSs secreted by the bacteria from deep-sea hydrothermal vents. Unlike most of the EPSs secreted by other marine bacteria, The EPS from strain SM9913 did not possess uronic acid. Its structure was a linear arrangement of α -(1,6)→Glc with a high degree of acetylation. Acetyl groups have been found in other marine EPSs such as the EPS produced by psychrotolerant strains CAM025 and CAM036 from Antarctic water and sea ice (Mancuso Nichols *et al.*, 2004, 2005). But an EPS with so high a degree of acetylation from a marine bacterium is an unusual finding. In contrast, the EPSs from deep-sea hydrothermal vent bacteria have no or few acetyl groups (Guezennec, 2002).

The ecological roles of EPSs from marine bacteria have been reviewed (Mancuso Nichols *et al.*, 2005b). In the marine environment, bacterial EPSs are essential in the production of aggregates, adhesion to surfaces and other organisms, biofilm formation and sequestering of nutrients, and thus provide protection and ecosystem stability (Mancuso Nichols *et al.*, 2005b). Although the structures of the EPSs from several deep-sea meso- or thermophilic bacteria have been elucidated (Raguenes *et al.*, 1996, 1997, 2003; Rougeaux *et al.*, 1999; Bowman, 1998; Muldoon *et al.*, 2003), there is little information on the ecological function of the EPSs from the deep-sea bacteria. In this study, besides the structural elucidation of the EPS from strain SM9913,

several experiments were done to investigate its ecological role in the cold deep-sea environment.

Most of the EPSs from marine bacteria are acidic extrapolsaccharides with net negative charge, which gives the molecule a 'sticky' quality (Mancuso Nichols *et al.*, 2005b). Some of them have been proved to have metal-binding capacity (Loaec *et al.*, 1997). The metal-binding properties of the EPSs produced by the Antarctic marine isolates were suggested by the authors as one potential ecological role for these polymers (Mancuso Nichols *et al.*, 2005a). The EPS from strain SM9913 included high levels of acetyl groups, which were polyanionic and therefore sticky for cations. Our data showed that this EPS was able to bind a range of metal cations, such as Fe²⁺, Zn²⁺, Cu²⁺ and Co²⁺, indicating that it might function in concentrating helpful metal ions to the surface of the strain in the microenvironment around the cell.

Recently, the genome sequence of the deep-sea gammaproteobacterium *I. loihiensis* was reported and it was suggested that this species relies primarily on amino acids, rather than on carbohydrates, for carbon and energy (Hou *et al.*, 2004). EPSs secreted by this species may function in colonizing proteinaceous particles in the surrounding environment (Hou *et al.*, 2004). The flocculation experiment on the EPS secreted by strain SM9913 showed that it was a very good flocculating agent and had a high ability to adhere to suspended particles in the surrounding environment, indicating that it could adhere to proteinaceous particles available in the deep-sea environment when it was secreted by strain SM9913.

Cold-adapted protease MCP-01, which was thermolabile and susceptible to autolysis, was the main protease secreted by strain SM9913. So it is very important for the strain's ability to utilize proteins in the surrounding environment. Our results showed that the EPS secreted by strain SM9913 could stabilize protease MCP-01 and effectively prevent its autolysis *in vitro*. This is believed to be the first report that EPS secreted by a marine bacterium has a stabilizing function on the proteases secreted by the same strain. Therefore, in the cold deep-sea environment, the EPS from strain SM9913 might play a role in stabilizing the proteases from the same strain *in situ*. It could also concentrate the proteases secreted by this strain by preventing protease diffusion. So the proteinaceous particles concentrated by the EPS could be effectively digested by the proteases into amino acids and oligopeptides and they, together with the concentrated metal ions, could be adsorbed by the strain as a source for growth and energy. Therefore, the EPS secreted by strain SM9913 may play important roles in the survival of this strain in the deep-sea nutrient-scarce environment.

Based on these experimental results and other studies, a schematic diagram of the lifestyle of deep-sea psychrotolerant strain SM9913 is proposed in Fig. 3, revealing an integrated mechanism of metabolic adaptation of this strain

Fig. 3. Schematic diagram of lifestyle of the deep-sea psychrotolerant strain SM9913 inferred from experimental analysis.

to the low temperature, organic and inorganic nutrient-scarce, constantly changing deep-sea environment.

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