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ORIGINAL ARTICLE

Comparative genomics reveals a deep-sea sediment-adapted life style of *Pseudoalteromonas* sp. SM9913

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Deep-sea sediment is one of the most important microbial-driven ecosystems, yet it is not well characterized. Genome sequence analyses of deep-sea sedimentary bacteria would shed light on the understanding of this ecosystem. In this study, the complete genome of deep-sea sedimentary bacterium *Pseudoalteromonas* sp. SM9913 (SM9913) is described and compared with that of the closely related Antarctic surface sea-water ecotype *Pseudoalteromonas haloplanktis* TAC125 (TAC125). SM9913 has fewer dioxygenase genes than TAC125, indicating a possible sensitivity to reactive oxygen species. Accordingly, experimental results showed that SM9913 was less tolerant of H_2O_2 than TAC125. SM9913 has gene clusters related to both polar and lateral flagella biosynthesis. Lateral flagella, which are usually present in deep-sea bacteria and absent in the related surface bacteria, are important for the survival of SM9913 in deep-sea environments. With these two flagellar systems, SM9913 can swim in sea water and swarm on the sediment particle surface, favoring the acquisition of nutrients from particulate organic matter and reflecting the particle-associated alternative lifestyle of SM9913 in the deep sea. A total of 12 genomic islands were identified in the genome of SM9913 that may confer specific features unique to SM9913 and absent from TAC125, such as drug and heavy metal resistance. Many signal transduction genes and a glycogen production operon were also present in the SM9913 genome, which may help SM9913 respond to food pulses and store carbon and energy in a deep-sea environment.

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Introduction

The deep-sea floor accounts for almost 60% of the Earth's surface (Brunnegarda *et al.*, 2004), most of which is covered with fine-grained sediments (Jørgensen and Boetius, 2007). Deep-sea sediment is a dynamic geo- and biosphere that hosts rich microbial communities (Whitman *et al.*, 1998; Jørgensen and Boetius, 2007). It is estimated that around 13% of total global bacteria live in the upper 10 cm of deep-sea sediments (Turley, 2000; Schippers *et al.*, 2005). In most deep-sea sediments, no light is present, temperatures are close to freezing (–1 °C to 4 °C), the pressure is very high and oxygen concentrations are usually very low compared with surface seawater (Jørgensen and Boetius, 2007). These extreme conditions have affected the sedimentary bacteria at various aspects, forcing bacteria to evolve special features to adapt for the deep-sea environment.

Genomic analysis provides an ideal technique for characterizing special features of deep-sea bacteria. The genomic analyses of deep-sea bacteria, such as *Photobacterium profundum* SS9 (Vezzi *et al.*, 2005), *Shewanella piezotolerans* WP3 (Wang *et al.*, 2008) and *Alteromonas macleodii* 'deep ecotype' (Ivars-Martinez *et al.*, 2008), have revealed some features common to many deep-sea bacteria. In general, the genomes of cultured deep-sea bacteria contain more transposable and phage related elements and larger intergenic spacers than that of surface bacteria (Ivars-Martinez *et al.*, 2008). Light-related genes, such as photoreactivation genes, are absent in deep-sea bacteria. Conversely, some genes are better represented in the deep-sea bacteria, such as those that are important for both cold and pressure adaptation, such as membrane unsaturation genes (Lauro *et al.*, 2008). Currently, only a few species of deep-sea bacteria have been subjected to sequence

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analyses. Considering the high diversity of deep-sea bacteria (Toffin *et al*., 2004; Sogin *et al*., 2006; Brown *et al*., 2009), more genomic sequences of representative bacterial strains must be analyzed to fully characterize the special features required for adaptation to deep-sea sediments.

Pseudoalteromonas is a genus of gamma-proteobacteria that is widespread in the world's oceans, from surface water to deep-sea sediments. *Pseudoalteromonas* species are usually associated with eukaryotic organisms and produce a large amount of biologically active agents, such as extracellular antibiotics, toxins and polysaccarides (Holmstrom and kjelleberg, 1999). *Pseudoalteromonas* species are cultivable bacteria found consistently in deep-sea sediments and produce large quantities of extracellular enzymes (Chen *et al*., 2003; Cui *et al*., 2008; Zhou *et al*., 2009), suggesting that they have an important role in the decomposition of particulate organic matter (POM) in deep-sea sediments. Although many *Pseudoalteromonas* strains have been recovered from sediments, the special features and adaptation mechanisms that allow them to thrive in extreme deep-sea conditions have barely been explored at the genomic level. *Pseudoalteromonas* sp. SM9913, isolated from deep-sea sediment at a water depth of 1855 meters near the Okinawa Trough, is a psychrophilic strain that produces a large quantity of proteases and exopolysaccharides (EPSs), indicating its function in the degradation of sedimentary particulate organic nitrogen and its potential uses in biotechnology applications (Chen *et al*., 2003; Qin *et al*., 2007a). Aside from its adaptability to cold temperatures, the deep-sea-specific features of SM9913 remain largely unknown. *Pseudoalteromonas haloplanktis* TAC125 which was isolated from the Antarctic coastal sea water and has already been sequenced by the whole genome shotgun method (Médigue *et al*., 2005) is closely related to SM9913. SM9913 and TAC125 are both psychrophilic strains with similar optimal growth temperatures, and both were isolated from permanently cold environments. Therefore, SM9913 and TAC125 represent different ecotypes of *Pseudoalteromonas* from cold deep-sea sediment and cold surface water, respectively, and are good models for the comparison of properties unique to bacteria in deep-sea sediment or surface seawater.

Genome sequence can provide a path toward understanding of how bacteria adapt to their environments. For example, the complete genome sequence analysis of *P. haloplanktis* TAC125 reflects its ability to cope with cold in Antarctic sea water (Médigue *et al*., 2005). Genome analysis of *Pseudoalteromonas tunicata* isolated from the surface of marine alga reflects its surface-associated marine lifestyle (Thomas *et al*., 2008). Comparative genomics is a powerful tool for understanding the ecological specialization of different ecotypes, which may shed light on the specific adaptations that bacteria make in different microenvironments (Ting *et al*., 2002; Kettler *et al*., 2007; Ivars-Martinez *et al*., 2008).

In this study, the complete genome sequence of *P.* sp. SM9913 is analyzed, and compared with the genome of *P. haloplanktis* TAC125. The genome sequencing and related functional analyses of SM9913 reveal some special features of *Pseudoalteromonas* that have allowed it to adapt to deep-sea sediment. This represents the first detailed comparative genomic analysis of *Pseudoalteromonas* species inhabiting dramatically different environments (deep-sea sediment versus surface seawater).

Materials and methods

Bacterial strains

P. sp. SM9913 (hereafter called SM9913) isolated from deep-sea sediment was cultured at 15 °C, the optimal temperature for SM9913 growth (Chen *et al*., 2003), using marine Luria–Bertani broth: 10 g peptone, 5 g yeast extract, 1 l artificial seawater, pH 7.5. The cells were harvested by centrifugation at 12 000 *g* at 4 °C for 10 min. DNA was extracted using a DNA extraction kit (Qiagen, Hilden, Germany). *P. haloplanktis* TAC125 (hereafter called TAC125) was obtained from the Institute Pasteur Collection (collection number CIP108707).

Genome sequencing

The genome sequence of strain SM9913 was determined using a combination of Sanger sequencing and 454 pyrosequencing. About 100 Mb data were obtained from one 454 GS FLX (Genome Sequencer FLX) sequencing run. The resulting sequences were assembled into 110 large contigs that were oriented by Sanger sequencing reads from paired ends of plasmid and fosmid libraries with average insert sizes of 3–5 and 40 kb, respectively. A total of 11 100 Sanger sequencing reads were used in the genome assembly. Gaps were closed by primer walking and PCR segment sequencing. The phred-phrap-consed package (Gordon *et al*., 1998) was used for assembly and finishing, and the finished genome was further validated by long PCR of 10 kB genome fragments.

Genome annotation and analysis

The tRNA genes were predicted by tRNAscan-SE (Lowe and Eddy, 1997). The rRNA genes were identified by BLAST search against Rfam (Griffiths-Jones *et al*., 2003) and rRNA gene sequences from TAC125. The open reading frames were found by GLIMMER 3.0 (Delcher *et al*., 1999). The predicted open reading frames were annotated by similarity searches against databases of nonredundant protein sequences from the National Center for Biotechnology Information (NCBI), clusters of orthologous groups of proteins (COGs) (Tatusova *et al*., 2001) and InterPro (Apweiler *et al*., 2001). The annotation of open reading frames was manually curated with

Artemis (Rutherford *et al.*, 2000). Signal peptide prediction was performed by SignalP 3.0 (Bendtsen *et al.*, 2004). Clustered regularly interspaced short palindromic repeats (CRISPRs) were found with CRISPR-finder (http://crispr.u-psud.fr/Server/CRISPRfinder.php). The codon adaptation index was calculated by the EMBOSS cai program using all ribosomal protein genes as highly expressed reference genes (Hjerde *et al.*, 2008).

Comparative genomics
The genome sequence of TAC125 was downloaded from NCBI. An all-versus-all search was performed using all proteins of SM9913 and TAC125 by BLASTP with an E-value cutoff of 1e-5. Orthologous proteins are defined as reciprocal best hit proteins with a minimum 50% identity and 70% of the length of the query protein, calculated by the BLAST algorithm. Proteins without orthologs are considered to be specific proteins. The COG function category was analyzed by searching all predicted proteins against the COG database on the basis of the BLASTP; the final results were put together by custom-made Perl scripts (available from the authors upon request). Average nucleotide identity was calculated according to the method of Konstantinidis and Tiedje (Konstantinidis and Tiedje, 2005) using SM9913 as the query genome. Genomic islands (GIs) were identified by G+C content variation across the genome and dinucleotide bias according to the methods of Karlin (Karlin, 2001), as well as the presence of transposable elements and the genes specific for SM9913. Therefore, the GIs identified contain mainly SM9913-specific genes.

Phenotypic characteristics and comparison
H_2O_2 resistance was tested in marine Luria–Bertani broth at 15 °C. Cultures of SM9913 and TAC125 were grown to an OD_{600} of 0.6, and then divided equally into four parts. H_2O_2 was added to a final concentration of 0, 5, 10 and 15 mM, respectively. The cultures were further incubated at 15 °C, and the OD_{600} of the cultures was measured at 1 h interval. The experiments were repeated twice. Swimming motility was determined using the hanging drop method (Qin *et al.*, 2007b). Sensitivity to antibiotics was tested using the disc-diffusion method, as described previously (Qin *et al.*, 2007b). The antibiotics contained within one disc were: kanamycin, 30 μg; tetracycline, 30 μg; penicillin, 1 μg; ampicillin, 10 μg; amoxicillin, 10 μg; and chloromycetin, 30 μg. Chitin degradation ability was tested according to Cottrell *et al.* (2000), that is, by observing cleared zones. Other physiological and biochemical properties were tested using the commercial systems API 20E, API ZYM (both from bioMerieux), following the manufacturer's instructions with some modifications (Qin *et al.*, 2007b). Flagella of cells grown in marine Luria–Bertani broth or on 0.3% agar were observed by transmission electron microscopy (JEM-100CXII). The cells were negatively stained with 2% phosphotungstic acid before observation.

Database accession numbers
The complete genome sequence of strain SM9913 was deposited in GenBank under accession nos. CP001796 and CP001797.

Results and discussion

General features
The general features of the SM9913 genome are summarized in Table 1, Figure 1 and Supplementary Table S1. Similar to TAC125, the genome of SM9913 is composed of two chromosomes (chrs), which are 3.3 Mb and 700 kb in size, respectively. The genomes of the two strains are quite similar in structure and size (Supplementary Figure S1). Similar to TAC125, chr II of SM9913 does not show a standard GC skew. This is consistent with the conclusion that these two strains are highly similar (16S rRNA gene identity of both strains is more than 99%, Figure 2). The average nucleotide identity between SM9913 and TAC125 is 85%, which indicates that the two strains are not the same species, but have a high identity, considering their widely divergent dwelling environments. The two chrs of SM9913 contain a total of 3711 predicted coding sequences, 66.9% of which can be annotated with known or predicted functions. The 62 tRNA genes, 8 rRNA operons and 1 extra 5S rRNA gene are all located in chr I. Elongated helices that are found in the 16S rRNA genes of some piezophiles (Lauro *et al.*, 2007) were not detected in SM9913.

SM9913 appears to possess all the genes of the glycolysis, tri-carboxylic acid cycle and pentose phosphate pathways. This indicates that SM9913 can metabolize glucose to generate energy, nicotinamide adenine dinucleotide and nicotinamide

Table 1 General features of the genome of *Pseudoalteromonas* sp. SM9913

	Chromosome I	*Chromosome II*
Size (bp)	3 332 787	704 884
G+C percentage	40.4	39.8
Number of ORFs	3077	634
Average size of ORFs (bp)	946	976
Percentage coding	87.8	88.6
Number of rRNA operons (16S-23S-5S)	8	0
5S rRNA (extra copies)	1	0
Number of tRNAs	62	0
Average CAI	0.54	0.52
ORFs with predicted function	2063	422
Hypothetical proteins	1014	212

Abbreviations: CAI, codon adaptation index; ORF, open reading frame.

adenine dinucleotide phosphate. The SM9913 genome also contains the key genes of the Entner–Doudoroff pathway, 6-phosphogluconate dehydratase (*PSM_A1394*) and 2-keto-3-deoxy-6-phosphogluconate aldolase (*PSM_A1395*). It harbors two sucrose phosphorylase genes (*PSM_B0264* and *PSM_B0464*) and does not contain the genes required to use mannose or galactose. This is consistent with experimental results that SM9913 can use sucrose, but not mannose (Table 2). SM9913 has one glutamine synthetase gene (*PSM_A0181*), which is related to ammonium assimilation (Brunnegarda *et al*., 2004). The strain cannot use nitrite or nitrate, consistent with the finding that it does not possess nitrite reductase or nitrate reductase genes.

SM9913 and TAC125 share 2698 orthologous genes (Figure 3a), accounting for 72.7% and 77.4% of all the genes of SM9913 and TAC125, respectively. There is no significant difference in amino-acid composition between the two strains (data not shown). As shown in Figure 3b, a larger proportion of SM9913-specific genes belonging to COGs representing the N (cell motility) and T (signal

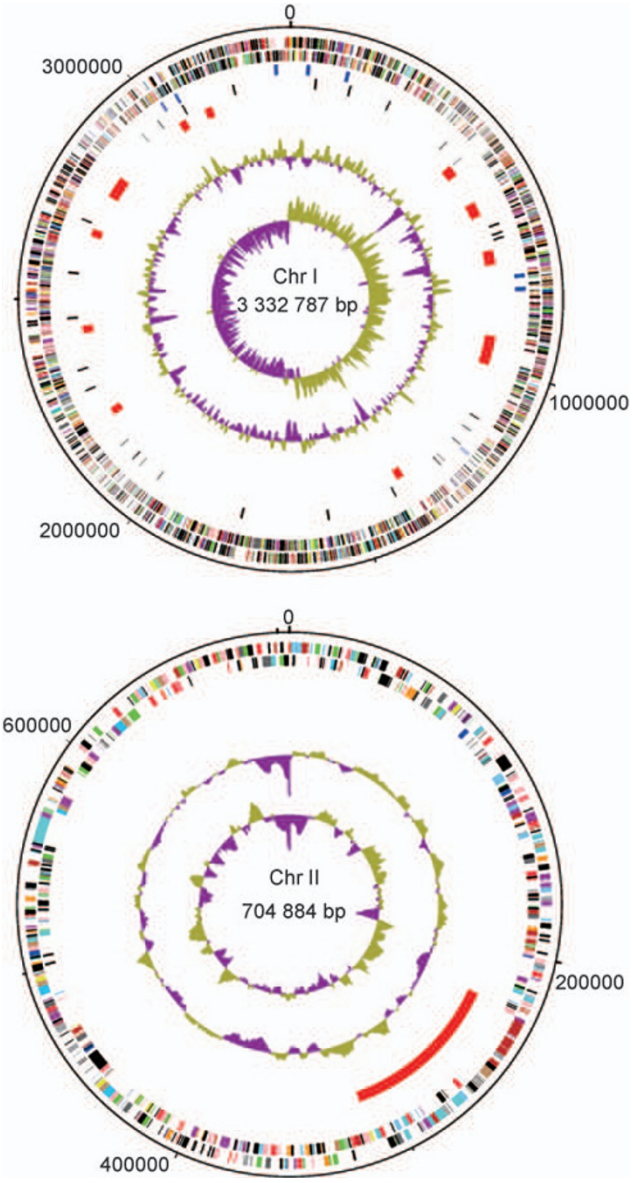

Figure 1 Circular representation of the SM9913 genome. From the outside inward: The first and second circles show predicted coding regions transcribed in the clockwise and counterclockwise directions, respectively. Colors indicate COG functional categories: dark grey indicates energy production and conversion (C); red indicates cell-cycle control, cell division and chromosome partitioning (D); green indicates amino-acid transport and metabolism (E); blue indicates nucleotide transport and metabolism (F); cyan indicates carbohydrate transport and metabolism (G); magenta indicates coenzyme transport and metabolism (H); yellow indicates lipid transport and metabolism (I); pale green indicates translation, ribosomal structure and biogenesis (J); light blue indicates transcription (K); orange indicates replication, recombination and repair (L); brown indicates cell wall/membrane/envelope biogenesis (M); pale pink indicates cell motility (N); light grey indicates post-translational modification, protein turnover or chaperones (O); mid red indicates inorganic ion transport and metabolism (P); light red indicates secondary metabolite (Q); pink indicates general function prediction only (R); dark red indicates unknown function (S); purple indicates signal transduction mechanisms (T); gold indicates intracellular trafficking, secretion and vesicular transport (U); navy blue indicates defense mechanisms (V); and black indicates hypothetical proteins. The third and fourth circles show the rRNA and tRNA, respectively. The fifth circle shows GIs. The sixth and seventh circles show percent G+C content and GC skew plot, respectively.

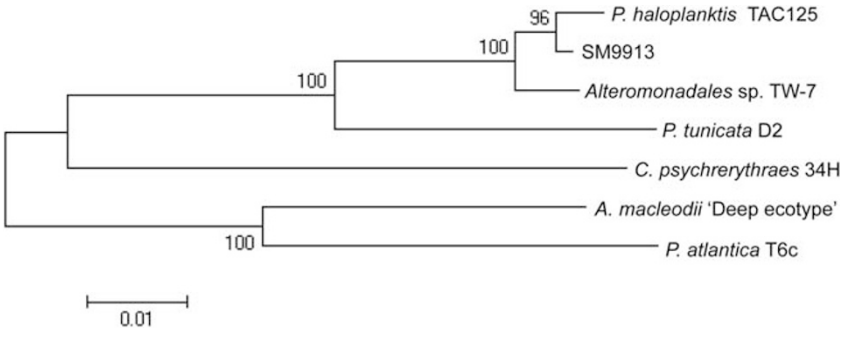

Figure 2 Phylogenetic tree based on the 16S rRNA gene sequences of SM9913 and related strains with completely sequenced genomes. The tree was generated by the neighbor-joining method using MEGA 3.1.

transduction mechanisms) groups, and fewer, unique genes in the I (lipid transport and metabolism) and Q (secondary metabolites biosynthesis, transport and catabolism) groups, compared with those of TAC125.

A total of 12 GIs larger than 15 kb can be identified by the methods described in the materials and methods section. Eleven GIs are located in chr 1 and one GI is located in chr 2 (Figure 1). General information about these GIs is summarized in Table 3. The average codon adaptation index of the GIs is similar to the average genome composition.

Table 2 Phenotypic characteristics of SM9913 and TAC125

	SM9913	*TAC125*
Gelatinase	+	+
Trypsin	+	+
Alpha-glucosidase	+	+
Beta-galactosidase	–	–
Beta-glucosidase	–	–
Arginine dihydrolase	+	+
Susceptible to		
Kanamycin	+	+
Tetracycline	–	–
Penicillin	–	+
Ampicillin	–	+
Amoxcillin	–	+
Chloromycetin	+	+
Acid form		
Mannose	–	+
Sucrose	+	–
Arabinose	–	–
Degradation of Chitin	–	–

Most genes in the GIs of SM9913 are specific genes that do not have orthologs in TAC125. These specific genes may confer some features of SM9913 that differentiate it from TAC125, which will be discussed in detail later.

Signal transduction genes

Histidine kinase and GGDEF domain-containing proteins are more enriched in deep sea water microbes than in surface sea water microbes (Konstantinidis *et al*., 2009). Histidine kinase is the sensing component of the two-component signal transduction system, and the GGDEF domain-contained protein is involved in cyclic diguanosine monophosphate synthesis (Römling *et al*., 2005). Cyclic diguanosine monophosphate is a novel global second messenger in bacteria, affecting multicellular behaviors, such as motility, phage and heavy metal resistance, EPS production and biofilm formation (Römling *et al*., 2005; Römling, 2009). The SM9913 genome contains 27 genes encoding GGDEF domain-containing protein and 5 genes encoding diguanylate cyclase that catalyzes the formation of cyclic diguanosine monophosphate. Meanwhile, TAC125 has only 15 genes encoding GGDEF domain-containing protein and no diguanylate cyclase genes. In addition, SM9913 harbors 33 histidine kinase genes in its genome, whereas TAC125 has only 20. The difference in the sizes of these families is primarily responsible for the fact that SM9913 has more specific genes belonging to signal transduction systems than does TAC125 (Figure 3b).

In the deep-sea bacterium *Ph. profundum* SS9 more signal transduction genes are overexpressed at 28

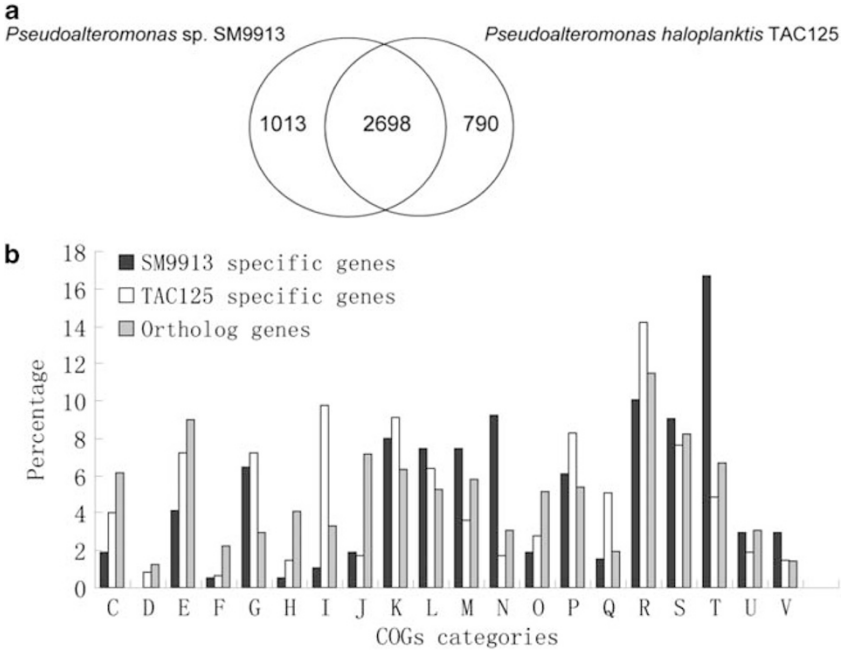

Figure 3 Comparison of the gene content of SM9913 and TAC125. (**a**) Venn diagram of the orthologous and specific genes in each strain. (**b**) The COG categories of the orthologous and specific genes in each strain. COG functional categories are described in Figure 1.

Table 3 Summary of genomic islands >15 kbp in SM9913

GI	*Size (kbp)*	*GC content (%)*[a]	*Number of genes*	*Hypothetical proteins*	*Number of transposase and integrase*	*Predicted function*	*Average CAI*
1	30.6	36.2 (4.2)	32	12	0	Exopolysaccharide biosynthesis	0.52
2	34.7	37.9 (2.5)	29	12	0	Durg resistance	0.50
3	32.5	36.5 (3.9)	28	17	0	Sugar transport and hydrolysis	0.51
4	72.2	41.3 (−0.9)	75	21	0	Lateral flagella biosynthesis	0.51
5	17.4	36.4 (4.0)	17	5	0	Exopolysaccharide biosynthesis	0.54
6	20.2	36.8 (3.6)	19	10	2	Unknown	0.52
7	17.4	35.7 (4.7)	20	6	0	Exopolysaccharide biosynthesis	0.54
8	17.5	40.3 (0.1)	17	6	3	Heavy metal resistance	0.52
9	52.1	39.0 (1.4)	56	27	3	Heavy metal resistance	0.51
10	15.5	39.6 (0.8)	16	10	0	Restriction modification system	0.52
11	22.8	36.8 (3.6)	21	9	6	Phage related	0.52
12	88.6	41.3 (−1.5)	74	35	0	Chitinase	0.54

[a]Number in parenthesis is the value of the average genomic GC content minus GC content of GIs.

mega Pascal than at 0.1 mega Pascal (Vezzi *et al.*, 2005). This shows that signal transduction genes have an important role in the adaptation of bacteria to deep-sea conditions. POM that have reached the sea bed from surface primary production is the major nutrients resource for deep-sea bacteria (Pfannkuche, 1992; Boetius *et al.*, 2000; Smith *et al.*, 2008). Every year, most nutrients arrive at the deep sea in pulse (Smith *et al.*, 2008; Witte *et al.*, 2003a, b). Deep-sea bacteria must respond to this nutrient pulse quickly as well. With its additional signal transduction genes, SM9913 may sense seasonal influx of POM keenly in the deep-sea environment.

Transposable elements

Transposases and integrases can mediate the movement of DNA sequences to new locations within or between genomes (Rice and Baker, 2001). SM9913 contains 28 transposase genes and 12 integrase genes, whereas TAC125 contains only 13 and 7, respectively. Konstantinidis *et al.* also reported that deep-sea bacteria usually have more transposable elements than bacteria in surface sea water (Konstantinidis *et al.*, 2009). This implies that there are more gene transfer incidents in deep-sea bacteria than in surface-sea bacteria. About one third of the transposase and integrase genes of SM9913 are located in GIs 6, 8, 9 and 11; GI-8 and GI-9 are related to heavy metal resistance (Table 3). The transposases and integrases may have function in the transfer of these resistance-related genes into SM9913. Under the strong selective pressure of the deep-sea environment, some adaptive mutations will be advantageous for bacteria to survive. The large number of transposases and integrases will promote the movement of these adaptive genes between different species, which would contribute to the diversity of deep-sea bacteria.

Sensitivity to reactive oxygen species (ROS)

When comparing the proteins involved in cold/salt adaptation between SM9913 and TAC125 (reported for TAC125 in Médigue *et al.*, 2005), we found that the number of these proteins in the two strains is almost the same, indicating that SM9913 and TAC125 have the similar cold/salt adaptation strategies. However, the number of dioxygenase genes in the two strains is quite different. TAC125 has twelve dioxygenase genes, whereas SM9913 has just four (*PSM_A0972*, *PSM_A1718*, *PSM_A1738* and *PSM_B0404*). The large number of dioxygenase genes in the genome is a strategy that TAC125 adopts against the ROS production that occurs due to the increased solubility of oxygen at low temperature. Indeed, TAC125 is remarkably resistant to H_2O_2 (Médigue *et al.*, 2005). SM9913 also lacks the fatty acid metabolism gene cluster of *PSHAa0894* to *PSHAa0910* that is related to ROS resistance in TAC125 (Médigue *et al.*, 2005). The smaller number of dioxygenase genes and lack of a ROS resistance-related gene cluster in the genome indicate that SM9913 may have lower H_2O_2 resistance than does TAC125. This was confirmed experimentally (Figure 4). SM9913 was only able to grow at concentrations of up to 5 mM H_2O_2, whereas TAC125 grew well even at 10–15 mM H_2O_2. Genome analysis also shows that SM9913 lacks the molybdopterin metabolism that results in ROS production, implying that SM9913 typically faces low ROS concentrations. Consistent with this finding, the oxygen concentration in sea water at a depth of 1800 m is about one third of that in surface sea water (Brown *et al.*, 2009). Furthermore, the oxygen concentration in deep sea sediment is even lower than that in the surrounding deep-sea water (Glud, 2008). The small number of dioxygenase genes in SM9913 and its sensitivity to ROS reflect its long-term life history in deep-sea sediment with low oxygen concentrations.

Exopolysaccharide biosynthesis

GI-1, -5 and -7 of SM9913 are related to EPS biosynthesis (Table 3). The genes in these GIs form three EPS biosynthesis clusters. Our previous study

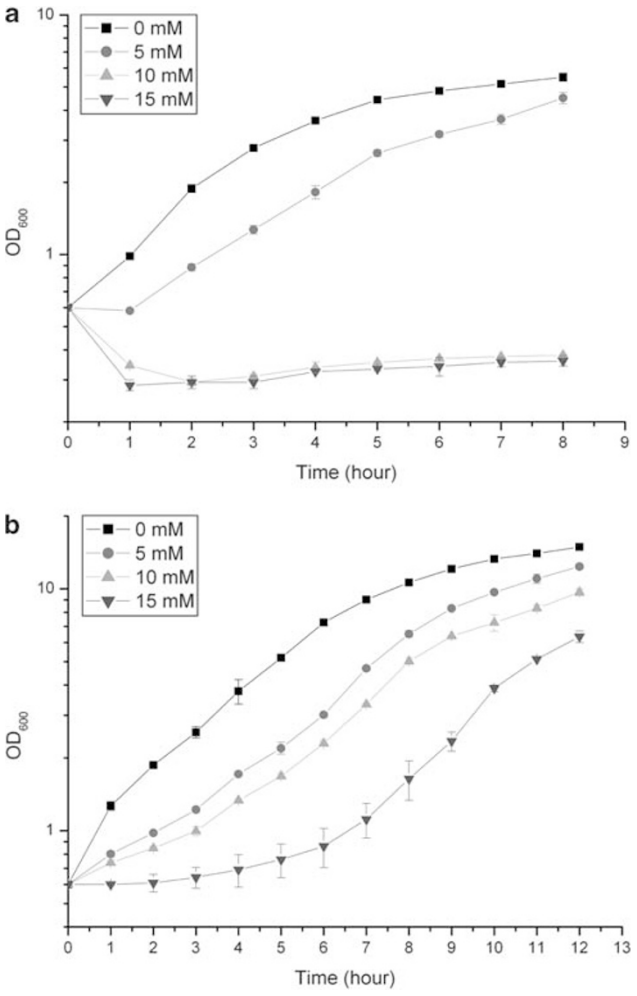

Figure 4 Growth curves of SM9913 and TAC125 at different concentrations of H_2O_2. (**a**) SM9913. (**b**) TAC125. The Y axis is logarithmic scale.

showed that SM9913 can produce large amounts of highly acetylated EPS (Qin *et al.*, 2007a). These EPS can protect the cold-adapted protease MCP-01 produced by SM9913 from autolysis and can bind many metal ions because of their net negative charge and acidic properties (Qin *et al.*, 2007a). Some deep-sea bacteria with sequenced genomes, such as *Idiomarina loihiensis* (Hou *et al.*, 2004) and *A. macleodii* 'deep ecotype' (Ivars-Martinez *et al.*, 2008), have EPS biosynthesis genes, showing that the production of EPS may be a common strategy that deep-sea bacteria adopt to endure extreme conditions. EPS can endow bacteria with other advantages as well, such as the ability to adhere to and colonize surfaces, and can speed biochemical interactions, protect the cell and concentrate dissolved organic matter in the marine environment (Nichols *et al.*, 2005; Qin *et al.*, 2007a).

Drug and heavy metal resistance

GI-2 of SM9913 contains a gene encoding an AcrA/E family efflux transporter (*PSM_A0563*) and a gene encoding an AcrB/AcrD/AcrF family protein (*PSM_A0564*), both of which are related to drug resistance (Nishino and Yamaguchi, 2001). SM9913 also has 12 multidrug-resistance genes and 4 β-lactamase genes. The percentage of SM9913-specific genes defined as 'defense mechanisms' by COG is 3.0%, but these genes are only 1.5% of the genes unique to TAC125. The Okinawa Trough is at the edge of the continental shelf of the East China Sea. Compared with Antarctic sea water, the water and sediments in the Okinawa Trough are likely to contain more materials from the continent, including antibiotics (Dang *et al.*, 2009). As a result of adaptation to their respective environments, SM9913 can resist some antibiotics, such as ampicillin, penicillin and amoxicillin, whereas TAC125 is susceptible to them (Table 2).

There are some heavy metal resistance and efflux genes in GI-8 and GI-9 of SM9913. On the basis of the genome survey, SM9913 is likely to be resistant to cadmium, cobalt, copper, magnesium, mercury and zinc. SM9913 also contains a Hg(II)-responsive transcriptional regulator gene (*PSM_A2609*) and a MerR family transcriptional regulator gene (*PSM_A2628*) in GI-9, which may be involved in the regulation of metal resistance. These genes may make SM9913 more resistant to some heavy metals than TAC125. Our experimental results showed that SM9913 is more resistant to zinc than TAC125. With 1 mM zinc acetate in the culture, SM9913 grew much faster than TAC125 (Supplementary Figure S2). Most deep-sea isolates are more resistant to heavy metals than surface bacteria. For example, the *A. macleodii* 'deep ecotype' strain is reported to be significantly more resistant to mercury and zinc than the *A. macleodii* 'surface' ecotype strain ATCC 27126 (Ivars-Martinez *et al.*, 2008). It is still unclear why deep-sea bacteria are more resistant to heavy metals than surface sea bacteria. However, SM9913 may be adapted to high metal concentrations because the negatively charged EPS that is secreted by deep-sea bacteria sometimes can adsorb more cations around the cell than are needed (Nichols *et al.*, 2005).

Flagella and motility

Flagellar motility is very important to allow bacteria to move toward favorable conditions, form biofilms and acquire nutrients. Deep-sea bacteria usually have both polar and lateral flagella for swimming and swarming, respectively (Eloe *et al.*, 2008; Wang *et al.*, 2008). Generally, two independent gene clusters are responsible for the synthesis of the two types of flagellar equipment. However, SM9913 seems to have three gene clusters for flagellum biosynthesis (Supplementary Figure S3). Cluster I, which is responsible for the synthesis of the lateral flagellum (LF), spans 32 883 bp from *PSM_A0884* to *PSM_A0920*. The LF gene cluster is located in GI-4 of SM9913, and is absent in TAC125. In the deep-sea

piezophilic bacterium *Ph. profundum* SS9, the LF gene cluster may be acquired by horizontal transfer, is only expressed under high pressure conditions and is absent in its shallow-water, pressure-sensitive relative *Ph. profundum* 3TCK (Eloe *et al.*, 2008). The deep-sea sedimentary bacterium *S. piezotolerans* WP3 contains a LF gene cluster that is upregulated at low temperatures (Wang *et al.*, 2008), whereas the surface-sea bacteria *Pseudoalteromonas tunicata* D2 and *Alteromonadales* sp. TW-7 both lack the LF gene cluster (Thomas *et al.*, 2008). Generally, it seems that deep-sea sedimentary bacteria usually have the LF gene cluster, whereas related surface bacteria lack it. Because LF is responsible for bacterial swarming, swarming should be an important movement for bacterial life in deep-sea sediments, but not necessary for surface bacteria. Although SM9913 has the LF gene cluster, expression of the LF could not be observed when the strain grew on 0.3% marine LB agar at atmospheric pressure, suggesting that the LF of SM9913 may be expressed only under high pressure conditions.

Cluster II and cluster III are responsible for synthesis of the polar flagellum (PF) in SM9913. Cluster II spans 32 907 bp from *PSM_A2229* to *PSM_A2262*, and cluster III contains 21 756 bp from *PSM_A2278* to *PSM_A2299*. The PF of SM9913 is visible under transmission electron microscopy (Supplementary Figure S4), which endows SM9913 with the ability to swim in sea water. It is surprising that there are two large gene clusters for the synthesis of PF in SM9913, as TAC125 and other PF-containing bacteria have only one PF gene cluster. However, analysis of the GIs shows that the two PF clusters in SM9913 are separated by GI-7. This indicates that the two PF gene clusters were actually a single PF gene cluster that has been interrupted by the insertion of other genes, which may have been horizontally transferred to SM9913. The PF observed indicates that the PF gene cluster is still active in SM9913 and that its function is not significantly affected by the insertion. The PF and LF motors are usually driven by different ion-motive forces in marine bacteria (Atsumi *et al.*, 1992). In SM9913, the putative proton-driven component LafTU (*PSM_A0904* and *PSM_A0905*), responsible for the power of LF, resides in the LF gene cluster. Four genes, *motA* (*PSM_A0714*), *motB* (*PSM_A0714*), *motX* (*PSM_A2815*) and *motY* (*PSM_A2160*), which are related to sodium-driven complexes and are associated with PF rotation (Eloe *et al.*, 2008), are also present in the genome. This confirms the presence of two types of flagellar equipment in SM9913.

With its two flagellar systems, SM9913 can swim in the sea water and swarm on the sediment particle surface, which is advantageous in the acquisition of nutrients. Only particulate materials can reach the deep-sea bed, so a surface-adapted motility system could allow SM9913 to attach to and move on particulate materials. SM9913 can form biofilms on water–solid interfaces (data not shown), which indicates its surface-attachment abilities. Attached bacteria tend to have larger genomes than do free-living bacteria have (Turley, 2000). This is the case for SM9913, as its genome is slightly larger than that of TAC125. SM9913 can produce an efficient protease to degrade particulate organic nitrogen (Chen *et al.*, 2003; Zhao *et al.*, 2008), reflecting its POM degradation ability in the deep sea. The surface-attached lifestyle would facilitate SM9913 to degrade POM. When nutrients are depleted, the strain can then swim through sediment pore fluids or seawater to newly arrived POM by PF.

Other GIs and related features

GI-12 contains three adjacent chitinase genes (*PSM_B0252*, *PSM_B0253* and *PSM_B0254*) with predicted signal peptides, implying that SM9913 can degrade chitin. However, our experimental results show that this is not the case. SM9913 can grow on enriched sea water agar containing chitin, but does not form clearing zones and can not grow on unenriched sea water agar after 7 days. This may be due to its lack of other essential chitin degradation elements, such as chitoporin. The SM9913 genome contains no annotated genes for chitoporin, chitodextrinase, N-aceytl-glucosaminidase or N-acetyl-hexosaminidase, all of which are important for the degradation of chitin (Hjerde *et al.*, 2008). We used the chitoporin protein sequence from *Vibrio furnissii* (AAF97616) to search the genome using BLASTP program with the E-value of 1e-2 and found no hits. These chitinase genes located in the GI implies that these genes may be laterally transferred, and so the genome lacks other chitin-degraded genes. This may explain why the strain cannot degrade chitin despite the presence of chitinase genes.

The SM9913 genome has 27 genes encoding nuclease excluding ribonucleases. There are three restriction endonuclease genes in the genome, two of which are located in GI-10. A DNA methylase gene (*PSM_A2853*) is also present in GI-10, next to a restriction endonuclease gene (*PSM_A2854*), which may form a restriction modification system to degrade foreign DNA elements (Ivars-Martinez *et al.*, 2008). Phages are abundant in deep sea environments and are much more abundant than bacteria (Suttle, 2005, 2007; Sorek *et al.*, 2008). Many bacteria contain CRISPR sequences that are thought to function as an anti-phage defense system, using an RNA-silencing-like mechanism to prevent phage infection (Barrangou *et al.*, 2007; Sorek *et al.*, 2008). Though there is a CRISPR-associated protein (*PSM_A2058*) in GI-6, we could not detect a CRISPR feature in SM9913 genome using the CRISPR-finder. Rather, a restriction modification system may be responsible for degrading alien DNA and protecting the strain from viral infection.

GI-2 contains one TonB-dependent siderophore receptor gene (*PSM_A0556*) that may be responsible for adsorbing iron ions from the environment (Moeck and Coulton, 1998; Ghysels *et al.*, 2005). There are a total of three TonB-dependent siderophore receptor genes in the SM9913 genome. Other genes of the siderophore uptake system, such as TonB2 and the TonB system transport proteins, *ExbB2* and *ExbD2* (Ghysels *et al.*, 2005), are all present in SM9913. Iron is always a growth-limiting factor for marine bacteria (Qin *et al.*, 2007a). Thus, it is reasonable to assume that the siderophore uptake system helps SM9913 adsorb iron in the marine environment.

Glycogen production

A glycogen production operon, including glucose-1-phosphate adenylyltransferase, glycogen synthase and glycogen branching enzyme, is present in chr II from *PSM_B0508* to *PSM_B0514*. This operon is absent in TAC125 and conserved in *Alteromonadales* sp. TW-7 and *P. tunicata* D2 (Thomas *et al.*, 2008). Bacterial glycogen is considered to be a storage product that provides both energy and carbon during starvation periods (Strange, 1968; Preiss, 1984). Nutrients arrive at the deep-sea in pulse seasonally every year (Pfannkuche, 1992; Witte *et al.*, 2003a, b). Therefore, SM9913 must accumulate glycogen with this operon when nutrients are widely available and use the stored glycogen when nutrients are absent from the environment, which would improve its ability to survive in the deep-sea environment.

Conclusion

Genomic and comparative genomic studies reveal some specific features of deep-sea sedimentary bacterium SM9913, such as drug and heavy metal resistance, sensitivity to H_2O_2 and many signal transduction genes. The predicted particle-associated lifestyle would facilitate SM9913 to use POM and thrive in deep-sea sediment.

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