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Genome analysis of *Rubritalea profundi* SAORIC-165^{T}, the first deep-sea verrucomicrobial isolate, from the northwestern Pacific Ocean[§]

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Although culture-independent studies have shown the presence of *Verrucomicrobia* in the deep sea, verrucomicrobial strains from deep-sea environments have been rarely cultured and characterized. Recently, *Rubritalea profundi* SAORIC-165^{T}, a psychrophilic bacterium of the phylum *Verrucomicrobia*, was isolated from a depth of 2,000 m in the northwestern Pacific Ocean. In this study, the genome sequence of *R. profundi* SAORIC-165^{T}, the first deep-sea verrucomicrobial isolate, is reported with description of the genome properties and comparison to surface-borne *Rubritalea* genomes. The draft genome consisted of four contigs with an entire size of 4,167,407 bp and G+C content of 47.5%. The SAORIC-165^{T} genome was predicted to have 3,844 protein-coding genes and 45 non-coding RNA genes. The genome contained a repertoire of metabolic pathways, including the Embden-Meyerhof-Parnas pathway, pentose phosphate pathway, tricarboxylic acid cycle, assimilatory sulfate reduction, and biosynthesis of nicotinate/nicotinamide, pantothenate/coenzyme A, folate, and lycopene. The comparative genomic analyses with two surface-derived *Rubritalea* genomes showed that the SAORIC-165^{T} genome was enriched in genes involved in transposition of mobile elements, signal transduction, and carbohydrate metabolism, some of which might be related to bacterial enhancement of ecological fitness in the deep-sea environment. Amplicon sequencing of 16S rRNA genes from the water column revealed that *R. profundi*-related phylotypes were relatively abundant at 2,000 m and preferred a particle-associated life style in the deep sea. These findings suggest that *R. profundi* represents a genetically unique and ecologically relevant verrucomicrobial group well adapted to the deep-sea environment.

Keywords: *Rubritalea*, *Verrucomicrobia*, genome, deep sea, 16S rRNA, particle-associated

Introduction

The phylum *Verrucomicrobia* is one of the major bacterial phyla that has a wide range of habitats including soil, freshwater, ocean, and plant- or animal-associated environments (Haukka *et al*., 2006; Freitas *et al*., 2012; He *et al*., 2017; Sun *et al*., 2018). In particular, members of the phylum are ubiquitous and abundant in soil ecosystems, accounting for up to 14–23% of soil bacterial communities (Bruce *et al*., 2010; Bergmann *et al*., 2011; Lei *et al*., 2017). Cells of the *Verrucomicrobia* are morphologically characterized by exhibiting an intracellular compartment, which is one of the shared phenotypic characteristics among the PVC (*Planctomycetes*, *Verrucomicrobia*, *Chlamydiae*) superphylum (Hedlund *et al*., 1997; Lee *et al*., 2009). Species of the phylum also possess a broad range of metabolic capabilities, including nitrogen-fixation, methane oxidation; and degradation of glycopolymers, polysaccharides, and human mucin (Cardman *et al*., 2014; Navarrete *et al*., 2015; Spring *et al*., 2016; He *et al*., 2017).

Among the diverse microbial habitats, the deep sea, referring to the part of the sea below a depth of 1,000 m (Jannasch and Taylor, 1984), is the largest habitat on earth in terms of volume. Specific prokaryotic groups have been identified to adapt to deep-sea environment with tolerance or preference to high pressure, low temperature, and oligotrophic conditions (Eloe *et al*., 2011). Since only a small portion of deep-sea microorganisms has been recovered using the conventional plating method equipped with a high-pressure chamber, physiological studies on deep-sea prokaryotes have been mostly performed only with a few cultured proteobacterial groups, such as the orders *Alteromonadales*, *Vibrionales*, and *Oceanospirillales* in the class *Gammaproteobacteria* and the order *Rhodobacterales* in the class *Alphaproterobacteria* (Yayanos *et al*., 1979; Jannasch and Wirsen, 1984; DeLong and Yayanos, 1985; Kato *et al*., 1995; Nogi *et al*., 2004; Eloe *et al*., 2011; Cao *et al*., 2014).

In this study, we focused on the genome analysis of *Rubri-*

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talea profundi SAORIC-165^T of the family *Rubritaleaceae* in the phylum *Verrucomicrobia*, which was isolated from a depth of 2,000 m in the northwestern Pacific Ocean (Song *et al.*, 2018). The genus *Rubritalea* currently consists of seven species that have been isolated from seawater, sediment, and marine animals such as sea squirts, sponges, and sea hares. Among the *Rubritalea* species, *R. profundi* is the only species that was retrieved from deep-sea water and the species, to the best of our knowledge, is the first deep-sea isolate belonging to the phylum *Verrucomicrobia*. *R. profundi* SAORIC-165^T showed good psychrophilic growth and contained a high proportion of anteiso-$C_{15:0}$ that is known to play an important role at low temperature (Annous *et al.*, 1997), indicating that strain SAORIC-165^T had some phenotypic properties, which assists this strain to adapt to the deep-sea environment.

Recent genomic approaches have gained a great deal of attention in elucidating bacterial adaptation to the deep-sea environment and revealed that an enrichment of transposable elements and genes involved in cold shock and protein folding might be related to adaptation of bacteria to deep-sea conditions (Hou *et al.*, 2004; Wang *et al.*, 2008; Konstantinidis *et al.*, 2009; Qin *et al.*, 2010). Therefore, through the genome analysis, it is worthwhile to investigate whether the first deep-sea verrucomicrobial isolate, strain SAORIC-165^T, has genomic potential to adapt to deep-sea environments. In this study, the whole genome sequence of strain SAORIC-165^T was obtained by next-generation sequencing technologies and compared to the genomes of two *Rubritalea* species that were isolated from marine sponges inhabiting littoral-sea environments. Furthermore, in order to find whether *Rubritalea*-related bacteria are present in deep-seawater, the vertical community structure of the domain *Bacteria* was determined by amplicon sequencing of 16S rRNA genes retrieved from the same sampling station as that of SAORIC-165^T.

Materials and Methods

Seawater sampling

Seawater samples were obtained from the station K2 (47° N, 160° E) in the subarctic gyre at five depths (0, 300, 1,000, 2,000, and 5,000 m) during the research cruise (MR-11-02) of RV 'Mirai' [Japan Agency for Marine-Earth Science and Technology (JAMSTEC)], in March 2011. Two to four liters of seawater samples collected from the five depths were filtered through a 3.0 μm pore-size Nuclepore polycarbonate membrane filter (Whatman) and the bacterial cells retained onto the filter were regarded as the particle-associated fraction. The filtrates were subsequently filtered through a 0.22-μm Sterivex filter (Millipore), and the bacterial cells collected onto the filter were considered as the free-living fraction. The filters were frozen on the ship and stored at -80°C until further analyses.

454 pyrosequencing of 16S rRNA gene

Genomic DNA extraction from the filters, PCR, and quality filtering and processing of 454 pyrosequencing sequences were performed as described previously (Kaneko *et al.*, 2017), except for PCR amplification of the V1–V3 hypervariable regions of bacterial 16S rRNA gene using the primers 27F (5′-AGRGTTTGATYMTGGCTCAG-3′) and 519R (5′-GWATTACCGCGGCKGCTG-3′). The quality-filtered sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity level. Representative sequences from each OTU were assigned to taxonomic hierarchy based on the SILVA database (v.119) (Pruesse *et al.*, 2007) implemented in the Mothur bioinformatics tool (Schloss *et al.*, 2001). The proportion of the sequences assigned to the phylum *Verrucomicrobia* was calculated based on the above taxonomic assignment. The proportion of *R. profundi*-related sequences was determined using BLAST+ software ver. 2.2.23 on a local computer, employing 454 pyrosequences as a database and the 16S rRNA gene sequence of SAORIC-165^T as a query. Pyrosequences showing > 97% similarity to SAORIC-165^T, longer than 150 bp, and belonging to the genus *Rubritalea* based on SILVA database were determined to be *R. profundi*-related bacteria.

Genome sequencing, annotation, and genome comparison

R. profundi SAORIC-165^T was isolated from a deep-seawater sample collected at a depth of 2,000 m in the station K2 (Song *et al.*, 2018). The genomic DNA of strain SAORIC-165^T was extracted from colonies grown on marine R2A agar using phenol-chloroform and ethanol precipitation (Johnson and Whitman, 2007) and used for library generation. Subsequent whole-genome sequencing was performed using both an Ion Torrent PGM system with single-end sequencing (Life Technologies) and 454 GS FLX Titanium with paired-end sequencing (Roche). The libraries were prepared using the Ion Xpress Plus Fragment Library Kit and the GS FLX Titanium Rapid Library MID Adaptors Kit for Ion Torrent PGM and 454 GS FLX Titanium, respectively. Before assembly, redundant reads were removed using a custom-built script, if any of the sequences had ≥ 95% identity to other sequences with exactly the same starting point, resulting in a total of 520,661 (Ion Torrent PGM) and 154,969 (454) reads. A hybrid assembly of the Ion PGM and 454 non-redundant reads was performed using the Newbler v2.8 (Roche).

Genome annotation was performed using the Rapid Annotation using Subsystem Technology (RAST) server (Aziz *et al.*, 2008) and the Integrated Microbial Genomes Expert Review (IMG-ER) (Markowitz *et al.*, 2009). Reconstruction of metabolic pathways were carried out using BlastKOALA based on KEGG pathway database (Kanehisa *et al.*, 2016). Functional analyses of protein-encoding genes were performed by BLASTp searches of the nr database of GenBank. The CGView program was used to generate a circular genome map after merging scaffolds of the SAORIC-165^T genome manually (Stothard and Wishart, 2005).

For genomic comparison among *Rubritalea* species, diverse functions of bioinformatics implemented in the IMG-ER were employed. Genomes of *R. marina* DSM 17716^T (2518645606, IMG genome ID) and *R. squalenifaciens* DSM 18772^T (2582581871), all the *Rubritalea* genomes available in the IMG-ER database, were compared to the SAORIC-165^T genome. Average nucleotide identity (ANI) and the functional profiles of the proteins based on Clusters of Orthologous Groups (COG) (Galperin *et al.*, 2015) and Pfam domains (Finn

et al., 2014) were evaluated using the 'Compare Genomes' function of the IMG-ER.

Nucleotide sequence accession number

The genome sequence of strain SAORIC-165^T has been deposited in GenBank under the accession number GCA_002954445.

Results and Discussion

General features of strain SAORIC-165^T and its genome

Morphological, physiological, biochemical, and phylogenetic characteristics of *R. profundi* SAORIC-165^T have been described previously (Song *et al*., 2018). In brief, the strain was a short-rod, facultatively anaerobic, non-motile, and red-pigmented bacterium that grew optimally at 10°C, utilizing a variety of carbon substrates, including glucose, galactose, lactose, and gluconate. Strain SAORIC-165^T was most closely related to *R. marina* Pol012^T, showing 95.7% 16S RNA gene sequence similarity and represented the first validly published verrucomicrobial species retrieved from the deep-sea environment.

After removing redundant reads from raw sequences of Ion Torrent PGM and 454 pyrosequencing, a total of 675,630 reads were assembled into 4 scaffolds, the sizes of which were 4,159,517, 3,284, 2,509, and 2,097 bp. The resulting draft genome sequence was 4,167,407 bp with 38.2 × coverage and its DNA G+C content was 47.5% (Fig. 1). Protein-coding genes connected to KEGG pathways were 754 genes. According to genome statistics based on IMG-ER (Table 1), the genome contained 3,892 genes, including 3,844 protein-coding genes, two rRNA genes, and 43 tRNA genes. Of the 3,844 genes, 1,681 and 2,558 genes in the SAORIC-165^T genome were assigned to the COG and Pfam databases, respectively.

The SAORIC-165^T genome contained a number of annotated genes for many metabolic pathways. The Embden-Meyerhof-Parnas pathway, the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle, and genes related to assimilatory sulfate reduction were conserved in the genome. The Entner-Doudoroff pathway, dissimilatory nitrate reduction, and seleno-compound metabolism were found to be incomplete. Genes related to the metabolism of fructose, galactose, propanoate, maltose, and sucrose were encoded in the genome. Metabolisms of thiamine, riboflavin, biotin, and vitamin B6 were impaired, while nicotinate/nicotinamide, pantothenate/coenzyme A, and folate biosynthesis were found to be functioning properly for strain SAORIC-165^T. The SAORIC-165^T genome contained a variety of genes for terpenoid and lycopene biosynthesis. Other metabolic pathways of strain SAORIC-165^T are summarized in Fig. 2. The major COG categories were translation, ribosomal structure, and bioge-

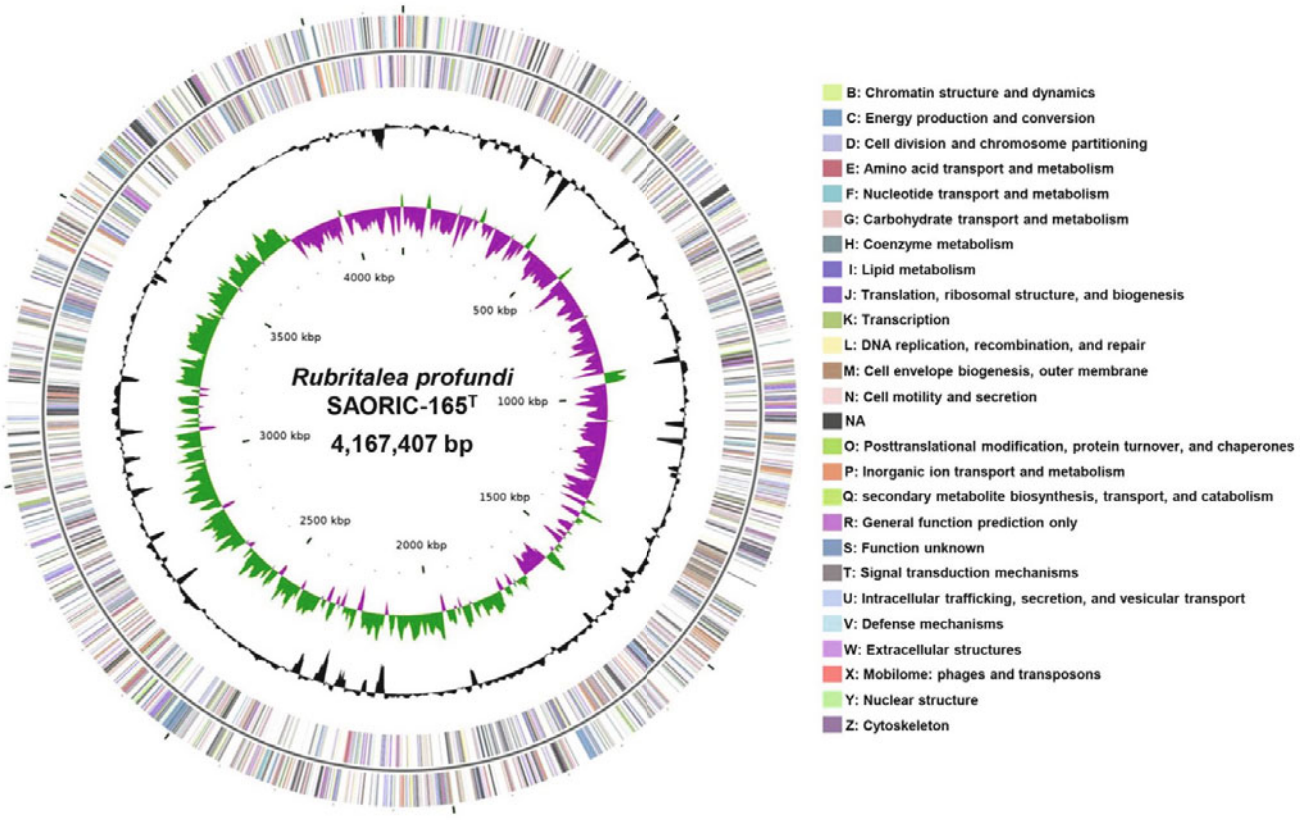

Fig. 1. Circular representation of the genome of strain *Rubritalea profundi* SAORIC-165^T. From outside to the center: forward strand (colored by COG categories), reverse strand (colored by COG categories), tRNA (blue), rRNA (red), GC ratio (black), GC skew (green and purple).

Table 1. Comparison of major genomic features of *Rubritalea profundi* SAORIC-165^T and two other *Rubritalea* species

Attribute	Strains		
	R. profundi SAORIC-165^T	*R. marina* DSM 17716^T	*R. squalenifaciens* DSM 18772^T
IMG genome ID	2716884913	2518645606	2582581871
Genome size (bp)	4,167,407	3,015,101	4,322,488
Scaffolds	4	31	17
G+C content (%)	49.5	51.6	52.0
Total genes	3,892	2,733	3,884
Protein coding genes	3,844	2,679	3,838
rRNA genes	2	8	4
tRNA genes	43	44	41
Protein coding genes			
with KEGG pathways	754	734	780
with COGs	1,681	1,503	1,788
with Pfam	2,558	1,982	2,539
ANI[a] with SAORIC-165^T (%)	-	73.6	72.2

All genomes were analyzed at JGI Integrated Microbial Genomes (http://img.jgi.doe.gov/).
[a] Average nucleotide identity.

nesis (J, 172 genes, 9.3%), amino acid transport and metabolism (E, 163 genes, 8.9%), general function prediction only (R, 160 genes, 8.7%), inorganic ion transport and metabolism (P, 141genes, 7.7%), cell wall/membrane biogenesis (M, 133 genes, 7.2%), energy production and conversion (C, 125 genes, 6.8%), coenzyme transport and metabolism (H, 116 genes, 6.3%), and carbohydrate transport and metabolism (G, 107 genes, 5.8%).

Comparison of gene contents: Unique genes in the SAORIC-165^T genome

Among seven *Rubritalea* species, two currently available whole genomes of *R. marina* DSM 17716^T and *R. squalenifaciens* DSM 18772^T that have been isolated from marine sponges (Scheuermayer *et al.*, 2006; Kasai *et al.*, 2007) were analyzed together with the SAORIC-165^T genome. Overall genome characteristics of the three *Rubritalea* species are summarized in Table 1. The 16S rRNA gene sequence similarities and average nucleotide identity (ANI) values between SAORIC-165^T and the two *Rubritalea* strains (DSM 17716^T-DSM 18772^T) were 95.7–93.2% and 73.6–72.2%, respectively. Strain SAORIC-165^T contained ~1.15 Mbp larger genome than strain DSM 17716^T, but ~0.16 Mbp smaller genome than strain DSM 18772^T. In the COG categories, the SAORIC-165^T genome showed a higher proportion of energy production and conversion (C), cell motility (N), and defense mechanisms (V) than two other *Rubritalea* genomes (Fig. 3).

Comparison of the annotation results based on Pfam data-

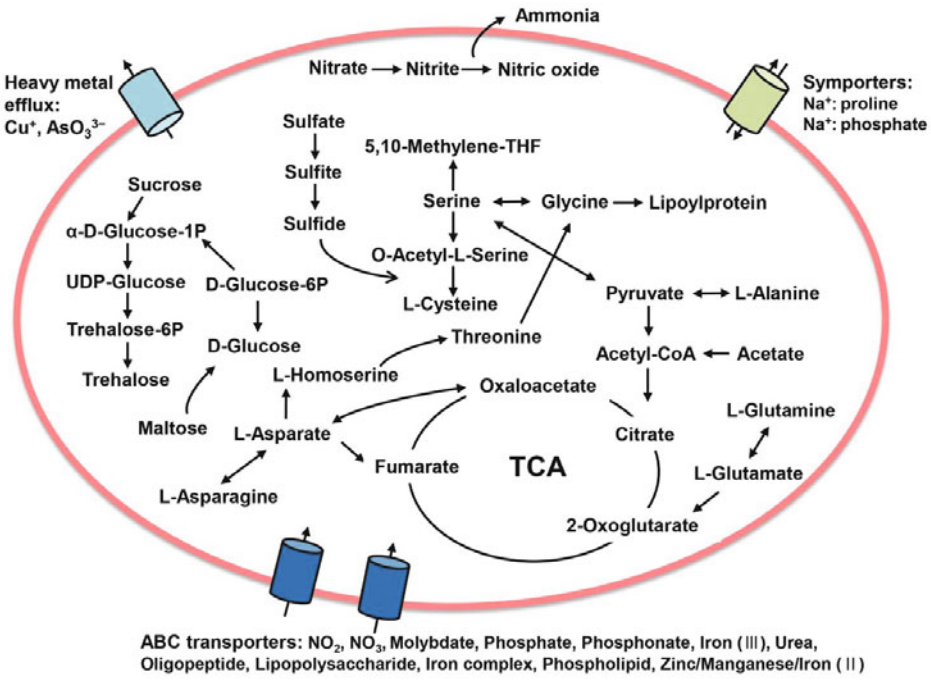

Fig. 2. Schematic overview of metabolic pathways predicted in the genome of *Rubritalea profundi* SAORIC-165^T. The presence and absence of genes were predicted within the Integrated Microbial Genomes system based on KEGG annotations.

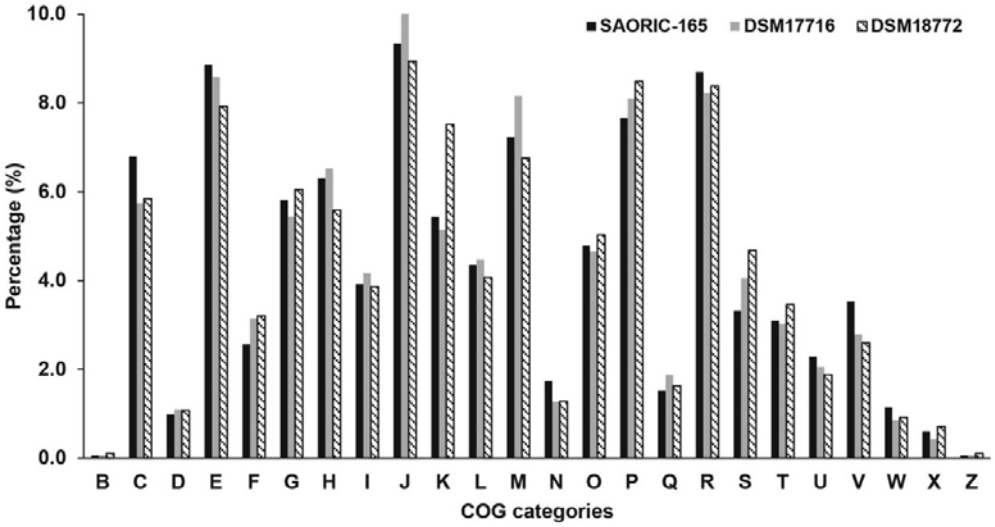

Fig. 3. **Comparison of the gene contents of strains *R. profundi* SAORIC-165^T, *R. marina* DSM 17716^T, and *R. squalenifaciens* DSM 18772^T, based on the COG categories.**

base indicated that many protein families (Pfam entries; hereafter designated as proteins) are encoded only in the SAORIC-165^T genome. Among the proteins unique to the SAORIC-165^T genome, those proteins that were present with more than two copies are summarized in Table 2. These unique and also abundant proteins were distributed among functional categories such as mobile elements, signal transduction, carbohydrate metabolism, replication, translation, transcription, defense mechanisms, and unknown functions. Other unique proteins found in the genome of SAORIC-165^T are summarized in Supplementary data Table S1.

Diverse transposases (PF01609, PF13751, PF04986, and PF05598) were uniquely found in the SAORIC-165^T genome. This transposase abundance is consistent with previous findings, revealing the enrichment of transposases in deep-sea bacterial genomes and metagenomes (Delong *et al*., 2006; Martín-Cuadrado *et al*., 2007; Konstantinidis *et al*., 2009; Lauro *et al*., 2014). Transposases were suggested to have a role in improving genetic plasticity of the microbial community inhabiting stressful or extreme environments by inducing transposons that facilitate genomic rearrangements and gene duplications (Leduc and Ferroni, 1994; Nelson *et al*., 2011; Vigil-Stenman *et al*., 2017).

Sel1 repeat family protein (PF08238), PDZ domain (PF00595), and PIN domain (PF01850) were found only in the SAORIC-165^T genome. These proteins are known to be involved in signal transduction mechanisms, facilitating potent abilities to sense environment signals (Walsh *et al*., 2003; Mittl and Schneider-Brachert, 2007; Matelska *et al*., 2017). Particularly, the signal transduction system of deep-sea bac-

Table 2. **Proteins present only in the genome of *Rubritalea profundi* SAORIC-165^T but absent in the genomes of *R. marina* DSM 17716^T and *R. squalenifaciens* DSM 18772^T**

Gene categories	Pfam ID	Gene	No. of genes
Mobile elements	PF01609	Transposase DDE domain	10
	PF13751	Transposase DDE domain	3
	PF04986	Putative transposase	2
	PF05598	Transposase domain (DUF772)	2
Signal transduction	PF08238	Sel1 repeat	3
	PF00595	PDZ domain	3
	PF01850	PIN domain	3
Carbohydrate metabolism	PF02449	β-Galactosidase	2
	PF07944	β-L-Arabinofuranosidase, GH127	2
Replication	PF08713	DNA alkylation repair enzyme	2
Translation	PF01253	Translation initiation factor SUI1	2
Transcription	PF01402	Ribbon-helix-helix protein, copG family	2
	PF13784	Fic/DOC family N-terminal	3
Defense mechanisms	PF09907	RelE-like toxin	3
	PF02452	MazF-like toxin	2
	PF13738	Pyridine nucleotide-disulphide oxidoreductase	2
Function unknown	PF09997	Predicted membrane protein (DUF2238)	2
	PF08332	Ca^{2+}/calmodulin-dependent protein kinase	2
	PF10707	PhoP regulatory network protein	2

teria has been reported to sense dynamic changes in dissolved oxygen, temperature, and pressure in deep-sea environments (Siebenaller and Garrett, 2002; Hou *et al.*, 2004). As for carbohydrate metabolism, the unique presence of β-galactosidase (PF02449) and β-L-arabinofuranosidase (PF07944) may be related to effective utilization of organic substrates present in such environments.

Several proteins found only in the SAORIC-165^T genome were related to DNA metabolism. Specifically, DNA alkylation repair enzyme (PF08713), ribbon-helix-helix protein, CopG family (PF01402), and fic/DOC family N-terminal (PF13784) were present in the genome. Since DNA replication is known to be impaired under high pressure and low temperature (Campanaro *et al.*, 2005; Lauro *et al.*, 2008), the psychrophilic bacterium, SAORIC-165^T, retrieved from the deep-sea ecosystem is likely to contain DNA repair proteins that are more than those in shallow-surface isolates. Ribbon-helix-helix protein, CopG family, one of transcriptional regulators, has been reported to be abundantly present in the deep-sea metagenome (Konstantinidis *et al.*, 2009). Likewise, other transcriptional regulators such as *acrR*, *csgD*, and *lysR* were also found to be abundant in deep-sea metagenomic clone libraries (Martín-Cuadrado *et al.*, 2007; Tseng *et al.*, 2015). Presence of fic family protein is also consistent with its abundance in the deep-sea metagenome (Konstantinidis *et al.*, 2009). In bacteria, the activity of some fic family proteins resembles classical toxin–antitoxin (TA) systems, which can enable bacteria to survive stress conditions by slowing metabolic processes and promoting dormancy (Sprenger *et al.*, 2017).

Among the proteins involved in defense mechanism, relE-like toxic component (PF09907), mazF-toxin (PF02452), and pyridine nucleotide-disulphide oxidoreductase (PF13738) were uniquely found in the SAORIC-165^T genome. RelE-like and mazF are known to be activated in response to nutritional stress (Christensen *et al.*, 2001; Gerdes *et al.*, 2005), which may be related to oligotrophic condition of the deep-sea environment. One of PF13738 protein families was affiliated with thioredoxin reductase that can cause bacterial cells to neutralize reactive oxygen species (ROS). Production of ROS is derived from pollutants, drugs, and xenobiotics; and induced by cold temperature, resulting in significant damage to cell structures (Mustacich and Powis, 2000; Awad *et al*, 2013; Ji *et al.*, 2013; Jung *et al.*, 2017). Considering that thioredoxin was up-regulated at low temperature (Ting *et al.*, 2011), presence of the thioredoxin reductase may support

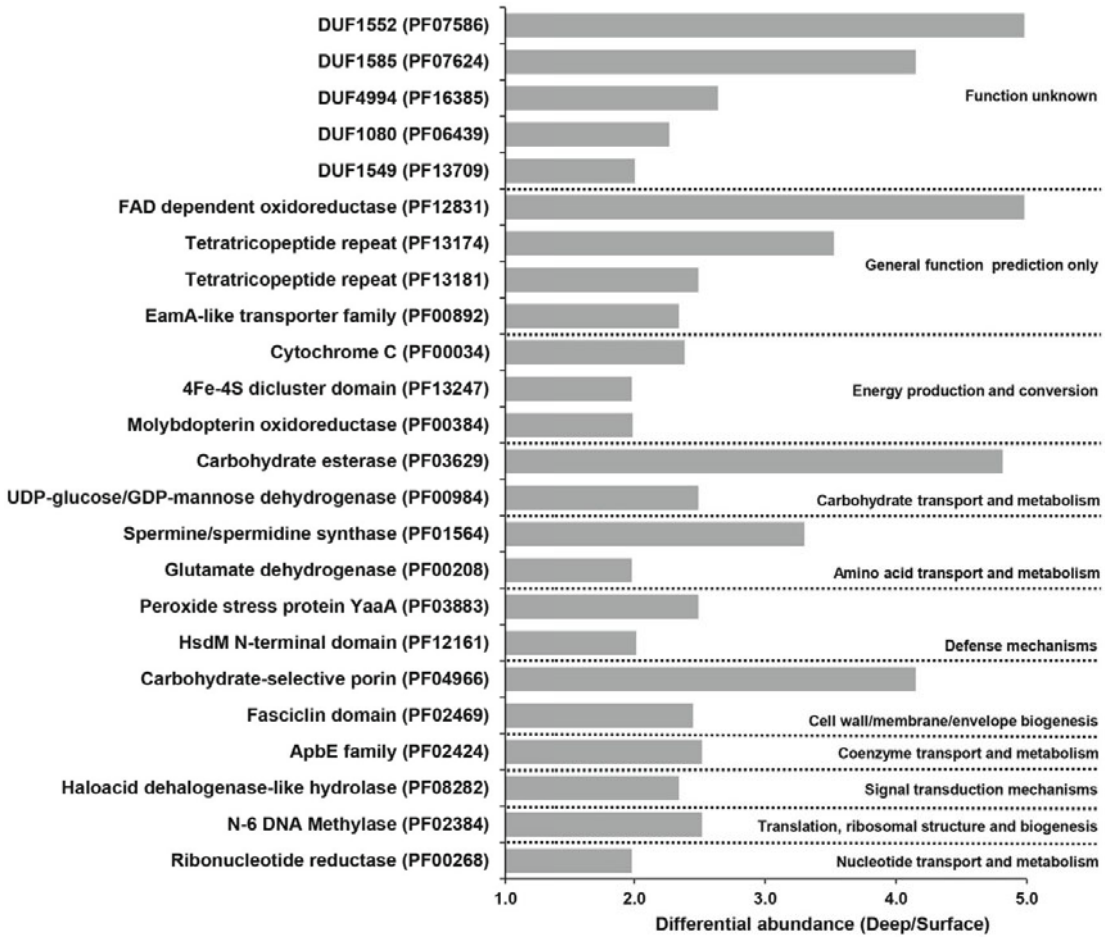

Fig. 4. Differential abundance of pfam domains in the genome of *Rubritalea profundi* SAORIC-165^T versus those on two *Rubritalea* genomes. Differential abundance was calculated using the equation: 2A/(B + C), where A, B, and C are the numbers of each pfam domain in the genomes of SAORIC-165^T, DSM 17716^T, and DSM 18772^T divided by total number of CDS in the corresponding genomes, respectively.

reduction of ROS in cold deep-sea environment. Additionally, proteins affiliated with DUF2238 (PF09997), Ca^{2+}/calmodulin-dependent protein kinase (PF08332), and *phoP* regulatory network protein (PF10707), were uniquely found in the genome.

Enriched genes in the SAORIC-165 genome: Genes for carbohydrate utilization

Compared to two other *Rubritalea* genomes, specific proteins were abundantly present in the SAORIC-165^T genome (Fig. 4). Differential abundance of pfam domains in the SAORIC-165^T genome versus those in the two *Rubritalea* genomes was calculated using the following equation: 2A/(B + C), where A, B, and C are numbers of each pfam domain in the SAORIC-165^T, DSM 17716^T, and DSM 18772^T genomes divided by total number of CDS in the corresponding genomes, respectively. Abundant proteins in the SAORIC-165^T genome were involved in the following functions: unknown functions, general function prediction only, energy production and conversion, carbohydrate metabolism, amino acid transport and metabolism, defense mechanisms, cell wall/membrane/envelope biogenesis, coenzyme transport and metabolism, signal transduction mechanisms, nucleotide transport and metabolism; and translation, ribosomal structure and biogenesis (Fig. 4).

FAD dependent oxidoreductases (PF12831) was abundantly found in the SAORIC-165^T genome. According to BLASTp results, of five PF12831 protein families, three genes were shown to encode xylan lyases. Xylan is a polysaccharide made from units of xylose (Huizing and Rietema, 1975) and is found in macro- and micro-algae in the marine environment. Differential abundance of xylan lyases in the SAORIC-165^T genome versus two *Rubritalea* genomes suggests that SAORIC-165^T may degrade algae-derived xylan transported to the deep-sea. In the physiological test, strain SAORIC-165^T utilized xylan at 10°C.

Carbohydrate esterases (PF03629) and UDP-glucose/GDP-mannose dehydrogenase (PF00984) were differentially abundant in the SAORIC-165^T genome. Of the 28 PF03629 protein families, 18 proteins were related to polysaccharide degradation; 6 proteins to xylan esterase and 12 proteins to sialate O-acetylesterase. Presence of these proteins involved in polysaccharide degradation is consistent in that many verrucomicrobial species have been considered to be active polysaccharide degraders in diverse environments (Cardman *et al.*, 2014). Studies indicate that biomass of algal cells produced by photosynthesis in the surface layer migrates from the upper water column to the deep-sea environment as sinking particles (Volk and Hoffert, 1985). Therefore, enrichment of these genes involved in carbohydrate metabolisms of the deep-sea isolate suggests that the deep-sea psychrophilic bacterium effectively utilize organic compounds in the deep-sea, which is characterized by oligotrophic condition and low temperature.

Enriched genes related to adaptation to the deep-sea environment

Many genes related to cold adaption, cell adhesion, and particle-associated life style were found abundantly in the SAORIC-165^T genome. The SAORIC-165^T genome contained differentially abundant cytochrome C (PF00034) compared to the two surface-derived *Rubritalea* genomes. High copy number of cytochrome C was also found in a deep-sea piezotolerant bacterium, *Shewanella piezotolerans* WP3 (Wang *et al.*, 2008). Since cytochrome C in the respiratory system is known to respond to high pressure and low temperature (Qureshi *et al.*, 1998; Yamada *et al.*, 2000; Ting *et al.*, 2010), enrichment of this gene in the deep-sea bacterial genome may enable the bacterium to respire stably under high pressure and low temperature deep-sea environments. Tetratricopeptide repeat (PF13174 and PF13181) and EamA-like transporter family (PF00892) were also abundantly found. Tetratricopeptide repeat is known to be up-regulated under conditions of low temperature (Ting *et al.*, 2010), but little is known about its function to date. The PF00892 protein family is known to be related to cold sensitivity of a deep-sea bacterium, *Photobacterium profundum* SS9 (Lauro *et al.*, 2008).

Spermine/spermidine synthase domain (PF01564), glutamate/leucine/phenylalanine/valine dehydrogenases (PF00208), carbohydrate-selective porin (PF04966), and Fasciclin domain (PF02469) were abundantly found in the genome. Spermidine is the most common polyamine in *Cyanobacteria* and it is known to promote survival of *Synechocystis* under long-term chill-light stress (Zhu *et al.*, 2015). Enrichment of glutamate dehydrogenases may be related to high concentra-

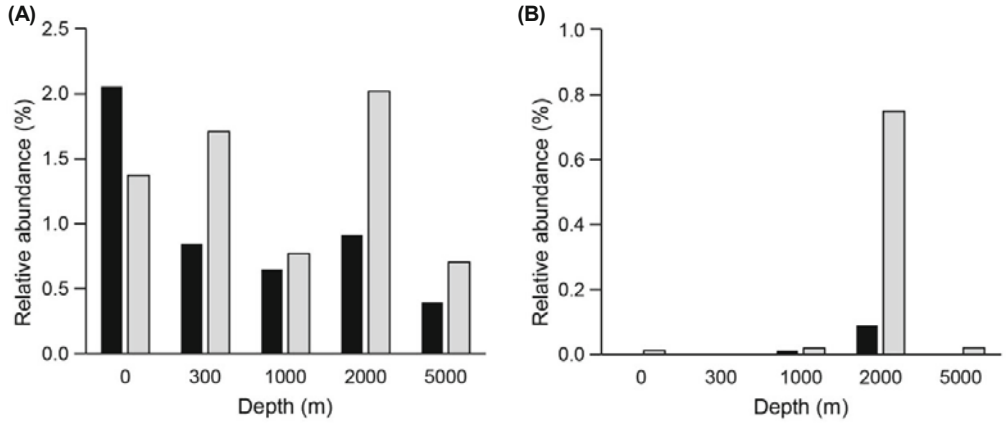

Fig. 5. Relative abundance of 16S rRNA gene sequences of the phylum *Verrucomicrobia* (A) and *Rubritalea*-related phylotypes (B) at station K2. Black bar, free-living fraction; gray bar, particle-associated fraction.

tions of glutamate in the deep sea (Takasu and Nagata, 2015). The carbohydrate-selective porin plays a central role in carbohydrate uptake (Wylie and Worobec, 1995). Fasciclin has been reported to have important roles in cell adhesion (Moody and Williamson, 2013).

Vertical distribution of *R. profundi*-related bacteria

Enrichment of the genes involved in carbohydrate utilization and cell adhesion in the SAORIC-165^T genome suggests that *R. profundi* may prefer a particle-attached life style. Results from the 454-amplicon pyrosequencing of 16S rRNA genes revealed that both the phylum *Verrucomicrobia* and *R. profundi*-related bacteria, albeit present in minority, were more abundantly present in the particle-associated fraction than in the free-living faction in the deep-ocean (Fig. 5). The verrucomicrobial sequences from the surface layer (0 m) were enriched in the free-living fraction whereas those from the deeper layers (300, 1,000, 2,000, and 5,000 m) were more abundant in the particle-associated fractions. Interestingly, *R. profundi*-related phylotypes were prominently found only at a depth of 2,000 m, from which strain SAORIC-165^T was isolated, and their abundance was 5 times higher in the particle-attached fraction. These results suggest that verrucomicrobial members related to *R. profundi* represent a deep-sea group of the phylum *Verrucomicrobia* and they prefer a particle-associated existence in the deep-sea.

In conclusion, comparative genomic analysis of *R. profundi* SAORIC-165^T, a deep-sea psychrophilic bacterium, revealed that the genome contained unique or differentially abundant genes involved in various functions including transposition of mobile elements, signal transduction, and carbohydrate metabolism. Many of these genes were related to microbial survival strategies that enhance their ecological fitness in the deep-sea, suggesting that strain SAORIC-165^T is an authentic bacterium adapted to the deep-sea environment. To the best of our knowledge, the SAORIC-165^T genome represents the first deep-sea genome to be reported for the phylum *Verrucomicrobia*.

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