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1 **Genome analysis of *Tepidibacter* sp. SWIR-1, an anaerobic**  
2 **endospore-forming bacterium isolated from a deep-sea**  
3 **hydrothermal vent**

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17 Running title: Genome analysis of *Tepidibacter* sp. SWIR-1

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20

21 **Abbreviations:** CDS, coding sequence; COG, clusters of orthologous groups; KEGG,  
22 Kyoto Encyclopedia of Genes and Genomes; CRISPR, Clustered regularly  
23 interspaced short palindromic repeat; *rrn*, ribosomal RNA operons; Ger, germination  
24 receptor protein; MCP, methyl-accepting chemotaxis protein.  
25

26 **Abstract**

27 *Tepidibacter* sp. SWIR-1, a putative new species isolated from deep-sea hydrothermal  
28 vent chimney on the Southwest Indian Ridge (SWIR), is an anaerobic, mesophilic and  
29 endospore-forming bacterium belonging to the family *Peptostreptococcaceae*. In this  
30 study, we present the complete genome sequence of strain SWIR-1, consists of a  
31 single circular chromosome comprising 4,122,966 nucleotides with 29.25 % G+C  
32 content and a circular plasmid comprising 38,843 nucleotides with 29.46 % G+C  
33 content. In total, 3,861 protein coding genes, 104 tRNA genes and 46 rRNA genes  
34 were obtained. SWIR-1 genome contains numerous genes related to sporulation and  
35 germination. Compared with the other three *Tepidibacter* species, SWIR-1 contained  
36 more spore germination receptor proteins. In addition, SWIR-1 contained more genes  
37 involved in chemotaxis and two-component systems than other *Tepidibacter* species.  
38 These results indicated that SWIR-1 has developed versatile adaptability to the  
39 Southwest Indian Ridge hydrothermal vent environment. The genome of strain  
40 SWIR-1 will be helpful for further understanding adaptive strategies used by bacteria  
41 dwelling in the deep-sea hydrothermal vent environments of different oceans.

42 **Keywords:** *Tepidibacter*, Complete genome, Deep-sea hydrothermal vent,  
43 Environmental adaptation.

44

## 45 **1. Introduction**

46 Deep-sea hydrothermal vents represent a typical chemotrophic extreme  
47 environment, possessing steep gradients in terms of temperature, redox potential, pH  
48 and substrate concentration [1, 2]. Due to the vigorous mixing of high-temperature  
49 reducing hydrothermal fluids and the surrounding cold oxidizing seawater, the  
50 environmental parameters of hydrothermal vents are in frequent fluctuations [3]. Vent  
51 bacteria employ a variety of strategies to adapt to such extreme environment. In turn,  
52 microbial community composition might shape animal populations dwelling in vent  
53 fields of different oceans. For example, vestimentiferan tube worms are the hallmark  
54 of Pacific hydrothermal vent fields, whereas swarming shrimps are a representative  
55 feature of Atlantic and Indian Ocean hydrothermal vents [4].

56 Genus of *Tepidibacter* taxonomically is affiliated to domain *Bacteria*, phylum  
57 *Bacillota*, class *Clostridia*, order *Eubacteriales*, and family *Peptostreptococcaceae*,  
58 and was first proposed by Slobodkin *et al.* with the description of *Tepidibacter*  
59 *thalassicus* [5]. At the time of writing, this genus includes only three valid published  
60 species (<https://lpsn.dsmz.de/genus/tepidibacter>). *Tepidibacter thalassicus* [5] and  
61 *Tepidibacter formicigenes* [6] were isolated from the deep-sea hydrothermal vent  
62 chimney of the East-Pacific Rise at a depth of 2,650 m and the Mid-Atlantic Ridge at  
63 a depth range of 800 - 1,000 m, respectively. The third, *Tepidibacter mesophilus*, was  
64 isolated from soil polluted by crude oil in the Karamay Oil Field [7]. The genomes of  
65 these three strains have been sequenced and draft genomes have been obtained.  
66 Recently, we have isolated a potential new species of genus *Tepidibacter*, strain

67 SWIR-1, from a deep-sea hydrothermal vent on the Southwest Indian Ridge. In this  
68 study, we report the complete genome sequence of strain SWIR-1, which is the first  
69 complete genome of genus *Tepidibacter*. Also, we compared the genome of SWIR-1  
70 with that of other *Tepidibacter*. These results will contribute to a better understanding  
71 of microbial adaptation to deep-sea hydrothermal vent habitats.

## 72 **2. Data description**

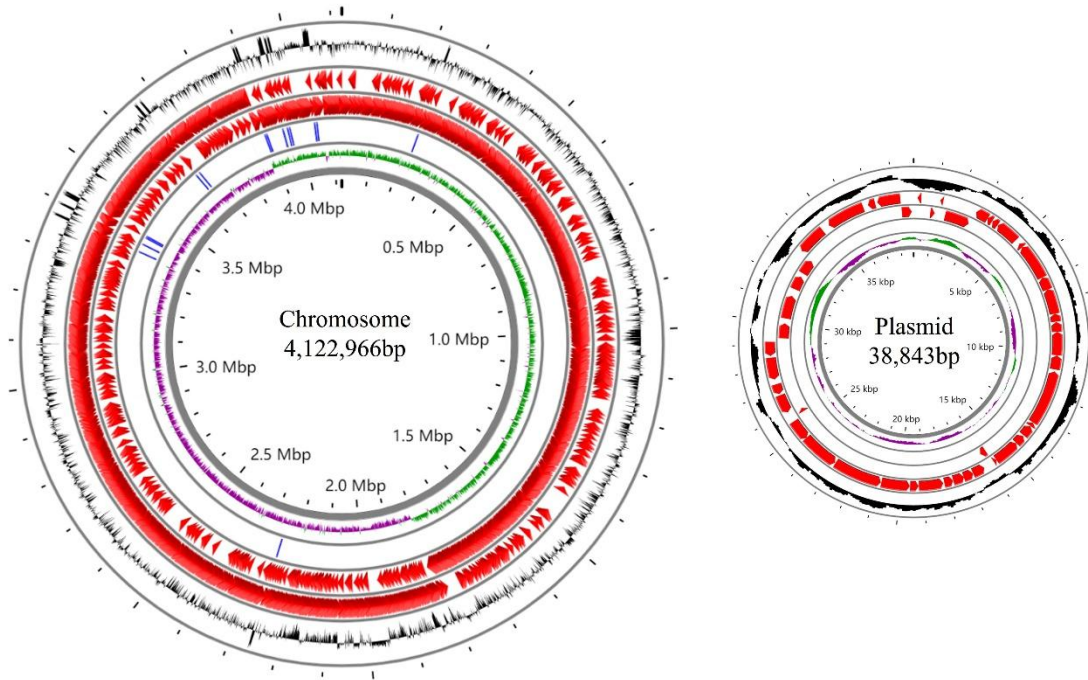
73 Strain SWIR-1 grown on DSMZ 904a medium (<https://www.dsmz.de/>) at 30 °C  
74 was harvested in late exponential-phase by centrifugation (4,500 g, 20 min). Genomic  
75 DNA was extracted with the SDS method. The harvested DNA was detected by the  
76 agarose gel electrophoresis and quantified by Qubit® 2.0 Fluorometer (Thermo  
77 Scientific). The whole genome of SWIR-1 was sequenced using Nanopore  
78 PromethION platform and Illumina NovaSeq PE150 at the Beijing Novogene  
79 Bioinformatics Technology Co., Ltd. About 6.1 Gb clean data were generated to reach  
80 approximately 1,246-fold depth of coverage. Clean reads were assembled using the  
81 Unicycler version 0.5.0 [8] and polished using Pilon v1.2 [9]. Gene predictions were  
82 made using GeneMarkS version 4.28 [10]. The rRNA and tRNA genes were  
83 identified using RNAmmer version 1.2 [11] and tRNAscan-SE version 2.0 [12],  
84 respectively. Clustered regularly interspaced short palindromic repeat (CRISPR)  
85 arrays were analyzed using the CRISPRFinder [13]. Gene annotation was performed  
86 by a sequence similarity search against the Clusters of Orthologous Groups (COG)  
87 database [14], and metabolic pathways were predicted by using KEGG Automatic  
88 Annotation Server (KAAS) [15]. The graphic circular map of the genome was

89 generated using MicroScope platform [16].

90 The resulting complete genome of *Tepidibacter* sp. SWIR-1 consists of a single  
91 circular chromosome comprising 4,122,966 nucleotides with 29.25 % G+C content  
92 and a circular plasmid comprising 38,843 nucleotides with 29.46 % G+C content (Fig.  
93 1; Table 1). It encodes 3,861 coding DNA sequences (CDSs) and 104 tRNA genes.  
94 The genome encodes 15 copies of ribosomal RNA operons (*rrn*) including 1 copy of  
95 16S-23S-5S-5S rRNA and 14 copy of 16S-23S-5S rRNA. Among them, 8 copies *rrn*  
96 located on forward strand and 7 copies *rrn* located on reverse strand. As reported,  
97 possessing multiple copies of *rrn* confer microorganisms with the ability to quickly  
98 respond to changes in external substrates [17]. Therefore, we believe that SWIR-1  
99 containing multiple *rrn* can quickly respond to environmental changes in  
100 hydrothermal vents.

101 Upon COG classification, 2,558 genes were assigned to 21 functional categories.  
102 The five most abundant COG categories are: amino acid transport and metabolism (E),  
103 transcription (K), energy production and conversion (C), signal transduction  
104 mechanisms (T), and inorganic ion transport and metabolism (P). Comparing to other  
105 *Tepidibacter* strains, SWIR-1 encode more proteins involved in the process of amino  
106 acid transport and metabolism (E), transcription (K), signal transduction mechanisms  
107 (T), defense mechanisms (V), and secondary metabolites biosynthesis, transport and  
108 catabolism (Q). While less proportion of CDSs affiliated to the process of replication,  
109 recombination and repair (L), translation, ribosomal structure and biogenesis (J), cell  
110 wall/membrane/envelope biogenesis (M), coenzyme transport and metabolism (H),

111 posttranslational modification, protein turnover, chaperones (O), Nucleotide transport  
 112 and metabolism (F), Cell motility (N), and cell cycle control, cell division,  
 113 chromosome partitioning (D). These differences may be related to the adaptation of  
 114 the strains to their local habitat.



115  
 116 Fig. 1. Schematic representation of the *Tepidibacter* sp. SWIR-1 genome. Labeling  
 117 from the outside to the center is as follows: circle 1, GC content; circle 2, genes on the  
 118 forward strand; circle 3, genes on reverse strand; circle 4, RNA genes (tRNAs green,  
 119 rRNAs blue); and circle 5, GC skew.

120

121 Table 1. General features and genome sequencing project information for  
 122 *Tepidibacter* sp. SWIR-1 according to MIGS recommendations

Item	Description
MIGS data	
Project_name	<i>Tepidibacter</i> sp. SWIR-1 Genome sequencing and assembly
NCBI BioProject	Accession: PRJNA947128
NCBI BioSample	Accession: SAMN 33842187
Geographic location	Indian Ocean



Latitude and longitude	37.78° S, 49.65° E	
Water depth	2,770 m	
Collection date	12–2018	
Environment (biome)	Ocean biome ENVO:01000048	
Environment (feature)	Marine hydrothermal vent ENVO:01000122	
Environment (material)	Marine hydrothermal vent chimney ENVO:01000129	
Observed biotic relationship	Free living	
Trophic_level	Chemoheterotroph	
Sequencing method	Oxford Nanopore PromethION and Illumina MiSeq	
Assembly method	Unicycler version 0.4.8	
Coverage	1246.58x	
Finishing_strategy	Complete	
General feature		
	Domain <i>Bacteria</i>	
	Phylum <i>Bacillota</i>	
Classification	Class <i>Clostridia</i>	
	Order <i>Eubacteriales</i>	
	Family <i>Peptostreptococcaceae</i>	
	Genus <i>Tepidibacter</i>	
Gram stain	Positive	
Cell shape	Rod	
Motility	Motile	
Relationship to oxygen	Obligate anaerobe	
Optimal temperature	30 °C	
Genomic features	Chromosome	Plasmid
Size (bp)	4,122,966	38,843
G+C content (%)	29.25	29.46
Number of predicted CDSs	3,814	47
Genes assigned to COG	2,558	8
Genes assigned to KEGG	1,637	2
Number of rRNA genes	16, 15, 15 (5S, 16S, 23S)	0
Number of tRNAs	104	0
Number of CRISPR Arrays	2	0

123

### 124 **3. Adaptations to deep-sea hydrothermal vent**

125 Sporulation is one of the common strategies utilized by bacteria to deal with  
126 unfavorable conditions in various habitats. Recently, many spore-forming bacteria  
127 have been isolated from hydrothermal vent samples, such as *Alkalicella caledoniensis*  
128 [18], *Longirhabdus pacifica* [19] and species from genus of *Crassaminicella* [20-22].

129 Despite being dormant, spores can still monitor changes in surrounding environmental  
130 conditions. When environmental conditions are again suitable for growth, these spores  
131 begin to germinate [23, 24]. Spores have strong resistance to extreme temperature, pH,  
132 oxidation and other unfavorable conditions, so spore-forming microorganisms can  
133 survive for a long time in these unfavorable environments [25]. Genomic analysis  
134 showed that strain SWIR-1, as well as other three *Tepidibacter* species, possesses  
135 over 70 sporulation related genes. These genes are involved in various stages of spore  
136 formation, such as sporulation initiation, forespore formation, cortex synthesis, coat  
137 deposition, spore maturation and mother cell lysis [26]. Dormant spore can germinate  
138 and convert back into a fully active vegetative cell once they encounter appropriate  
139 environmental conditions [27]. The first step in germination is to sense the signal of  
140 the surrounding environment through the Ger family receptor protein, and then  
141 triggers a series of germination events. Strain SWIR-1 contained 19 genes that  
142 encoded spore germination receptor proteins, including three copies of spore  
143 germination receptor protein KA (GerKA), fourteen GerKB, and two GerKC. While *T.*  
144 *formicigenes* (2 GerKA, 5 GerKB-2 and 1 GerKC), *T. thalassicus* (1 GerKA, 5GerKB  
145 and 1 GerKC) and *T. mesophilus* (1 GerKA, 5GerKB and 1 GerKC) contained no  
146 more than 10 such genes. GerKA is the dominant receptor and GerKC and GerKB are  
147 also required for efficient germination. We speculate that SWIR-1 contains more Ger  
148 receptor proteins, which may make it easier to sense changes in external signals,  
149 thereby controlling spore germination more efficiently.

150 Possessing a diversity of habitats with gradients of temperature and chemistry is

151 one of the hallmarks of deep-sea hydrothermal vent. Rapid search for substrates and a  
152 favorite environment is crucial for microorganisms to adapt to such extreme  
153 environments. In the genome of the strain SWIR-1, 48 genes encoding  
154 methyl-accepting chemotaxis proteins (MCPs) were observed. While only 10, 12 and  
155 18 MCP encoding genes were detected in the genome of *T. formicigenes*, *T.*  
156 *thalassicus* and *T. mesophilus*, respectively. MCPs are the predominant  
157 chemoreceptors in bacteria, they are believed to be involved in the chemotaxis and  
158 play an important role in bacterial adaptation to diverse environmental conditions [28].  
159 In addition, strain SWIR-1 contained 42 genes that encoded two-component system,  
160 while other three *Tepidibacter* genomes only contain no more than 10 such encoding  
161 genes. Two-component systems are crucial for strains to sense changes of surrounding  
162 environment [29], so we believe that the strain SWIR-1 has a more flexible  
163 adaptability to the deep-sea hydrothermal vent environment.

164 In conclusion, genome analysis revealed common genomic characteristics of  
165 genus *Tepidibacter* and identified more abundant *rrn*, *Ger* and MCP genes in SWIR-1  
166 compared to other *Tepidibacter*. Therefore, hydrothermal vents in Longqi field in the  
167 Southwest Indian Ridge might undergo higher biogeochemical variation than the vent  
168 fields in the East-Pacific Rise and the Mid-Atlantic Ridge.

#### 169 **4. Genome sequence accession numbers**

170 The complete genome sequence of *Tepidibacter* sp. SWIR-1 was deposited in the  
171 GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) under the accession  
172 number CP120733 (Chromosome) and CP120734 (Plasmid). The strain has been

173 submitted to Marine Culture Collection of China, MCCC (<http://www.mccc.org.cn/>)  
174 with accession number MCCC 1K07078.

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## 181 **Declaration of Competing Interest**

182 The authors declare that they have no known competing financial interests or  
183 personal relationships that could have appeared to influence the work reported in this  
184 paper.

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