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Data

Complete genome of *Mycetocola spongiae* MSC19^T isolated from deep-sea sponge *Cacospongia mycofijiensis* indicates the adaptation to deep-sea environment and sponge-microbe symbioses

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ABSTRACT

Genome of *Mycetocola spongiae* MSC19^T, a novel marine sponge-associated Actinobacteria isolated from the Mariana Trench sponge *Cacospongia mycofijiensis*, was sequenced. The genome has one circular chromosome of 3,196,754 bp, with an average GC content of 66.43 mol%, and 2887 coding sequences. Gene annotation shows that *M. spongiae* MSC19^T possesses series of genes related to adaptation to deep-sea environmental stresses including cold shock, heat shock, osmotic stress and oxidative stress. Genes encoding for heavy metal resistance, multidrug resistance and multiple natural product biosynthesis which are crucial for survival in the extreme environment are also detected in the genome. The potentials to synthesize kinds of vitamins and eukaryotic-like proteins indicates the possible nutrient exchange and mutual recognization between *M. spongiae* MSC19^T and its sponge host. The genome provides insights into the stress resistance and ecological fitness of bacterial symbionts in the deep-sea sponge holobionts.

1. Introduction

Sponges are ancient sessile filter-feeding metazoans that harbor complex microbial community (Thomas et al., 2010). Interests in marine sponges and their associated microorganisms have increased both for ecological functions and biotechnological potentials (Graca et al., 2015). Compared to shallow-water sponge, deep-sea sponge symbiotic microorganisms may more likely to adapt to extreme environment because deep-sea has its unique characteristics which are different from that of shallow water (Li et al., 2016). Culture-independent methods have demonstrated that deep-sea ecosystems contain a wide range of unique Actinobacteria that are not present in the terrestrial environment (Jensen and Lauro, 2008; Yi et al., 2021).

The genus *Mycetocola* was first identified to the family Microbacteriaceae, order Micrococcales, class Actinobacteria within the phylum Actinobacteria in 2001 (Tsukamoto et al., 2001). To date, 8 species in genus *Mycetocola* have been reported (https://lpsn.dsmz.de/genus/mycetocola). Members of this genus are described as short rod-shaped, Gram-positive, obligately aerobic and non-sporulating bacteria. They have been isolated from diverse ecosystems including mushroom, cheese, desert sand, snow, glacier and faeces of Tibetan antelopes (Bora et al., 2008; Luo et al., 2012; Zhu et al., 2013; Shen et al., 2013; Li et al., 2019). We recently identified a strain, *Mycetocola spongiae* MSC19^T, which was isolated from the deep-sea sponge *Cacospongia mycofijiensis* in the Mariana Trench (Chen et al., 2022). Here, we report the complete genome sequence of *M. spongiae* MSC19^T, and suggest how *M. spongiae* MSC19^T adapt to the extreme environment and its interaction with its sponge host.

2. Data description

The deep-sea sponge *Cacospongia mycofijiensi* which was collected from the junction of the Mariana Trench and the Yap Trench (11.439670°N, 139.406620°E), 2681 m in depth and was identified by 18S rRNA gene (GenBank Accession Number: OK135747). *Mycetocola spongiae* MSC19^T was isolated using the standard agar plate dilution method from grounded sponge samples, and purified on ISP2 agar plates (4.0 g yeast extract,10.0 g malt extract, 4.0 g glucose, 20.0 g agar, distilled water 1000 mL adjusted to pH 7.2). The *M. spongiae* MSC19^T grew optimally at 28–30 °C with 4% NaCl, but also could grow at temperatures 4–10 °C and NaCl concentrations up to 12%, corresponding to its ability to survive in deep-sea with high salinity in brine

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system.

General features of *M. spongiae* MSC19^T were shown in Table 1. *M. spongiae* MSC19^T was cultivated aerobically on ISP2 agar for 24 h at 28 °C for genomic DNA extraction using the SDS method (Lim et al., 2016). The extracted DNA was detected by the agarose gel electrophoresis and quantified by NanoDrop One spectrophotometer (NanoDrop Technologies) and Qubit® 3.0 Fluorometer (Thermo Scientific). The whole genome of *M. spongiae* MSC19^T was sequenced using Illumina NovaSeq 6000 PE150 and Oxford Nanopore PromethION 48 platform, Guangdong Magigene Biotechnology Co., Ltd. After quality control, reads were assembled using Unicycler v0.4.8 (Wick et al., 2017). Encoding gene prediction was performed using Glimmer version 3.02 (Delcher et al., 2007). Non-coding RNAs genes were predicted and analyzed using tRNA scan-SE v1.3.1 (Lowe and Eddy, 1997), rRNAmmer v1.2 (Lagesen et al., 2016) and the Rfam database (Griffiths-Jones et al., 2003).

The complete genome of *M. spongiae* MSC19^T consists of a single circular chromosome with a total length of 3,196,754 bp and an average G + C content of 66.43 mol% (Fig. 1). A total of 2887 coding sequences (CDS) were predicted in the genome. Among the CDS, 1514 (52.4%), 2339 (81.0%), 2567 (88.9%), 2614(90.5%), 2336(80.9%) and 1949 (67.5%) genes were categorized against the GO (Gene Ontology, http://www.geneontology.org/), COG (Clusters of Orthologous Groups, http://www.ncbi.nlm.nih.gov/COG), KEGG (Kyoto Encyclopedia of Genes and Genomes, https://www.genome.jp/kegg/), NR (Non-Redundant Protein Database databases, https://www.ncbi.nlm.nih.gov/), Pfamscan (ftp://ftp.ebi.ac.uk/pub/databases/Pfam/Tools/PfamScan.tar.gz) and Swiss-Prot (http://uniprot.org) databases, respectively. 2339 genes were assigned to 24 functional categories. The major five categories are transcription (12.2%), carbohydrate transport and metabolism (11.9%), amino acid transport and metabolism (11.3%), translation and ribosomal structure and biogenesis (8.1%) and inorganic ion transport and metabolism (8.1%), except for function unknown. Moreover, 5 Genomics islands, 1 prophage and 5 CRISPR were predicted by IslandPath-DIOMB (Hsiao et al., 2003), PHAST (Zhou et al., 2011) and CRISPRdigger (Ge et al., 2016), respectively.

Table 1
General features of *Mycetocola spongiae* MSC19^T.

Items	Description
General features	
Classification	Domain: Bacteria Phylum: Actinobacteria Class: Actinobacteria Order: Micrococcales Family: Microbacteriaceae Genus: *Mycetocola*
Gram stain	Positive
Cell shape	Rod
Colony color	Yellow
Temperature range (optimum)	4–32 °C (28–30 °C)
pH range (optimum)	5.5–12 (7.0–8.5)
NaCl% range (optimum)	0–12% (4%)
Oxygen requirements	Aerobic
Geographic location	The junction of the Mariana Trench and the Yap Trench
Sample type	Sponge *Cacospongia mycofijiensis*
Latitude and longitude	11.439670°N, 139.406620°E
Depth	2681 m
Collection date	October 2019
Biotic relationship	Free-living
Environment (biome)	Deep-sea biological sample
Environment (feature)	Sponge holobiont
Environment (material)	Sponge *Cacospongia mycofijiensis*
Genome characteristics	
Sequencing platform	Illumina NovaSeq 6000 PE150, Oxford Nanopore PromethION 48
Assembly method	Unicycle
Genome coverage	800×
Finishing quality	Complete genome
NCBI accession number	CP080203 (GenBank)
BioProject	PRJNA746413
BioSample	SAMN20206181
Size (bp)	3,196,754
DNA G + C content (%)	66.43
CDSs	2887
tRNAs	57
rRNA genes (5S, 16S, 23S)	13(5,4,4)
Gene islands	5

M. spongiae MSC19^T contains a great number of genes involved in adaptation to the challenging deep-sea habitat according to the annotation of COG. For example, 3 cold shock proteins (CSPs) and 8 heat shock proteins (HSPs) (Table S1) may function as chaperones to improve protein folding and synthesis in low- or elevated-temperature environments, respectively (Collins and Margesin, 2019; Maleki et al., 2016). Multiple genes participated in biosynthesis and transport of compatible solute, such as glycine betaine and choline, were identified in the genome. The important functions of these compatible solutes are to protect microbial cells against osmotic stress and temperature stress (Zou et al., 2016). Moreover, *M. spongiae* MSC19^T possesses dozens of genes involved in Na^+ pump-based transport systems (Table S2), including multi-subunit Na^+/H^+ antiporter, Na^+/H^+ antiporter such as *MnhABCDEF* as well as *NhaA*, *NhaP* and Na^+/proline symporter, etc. (Zhu et al., 2018). All these results suggest that *M. spongiae* MSC19^T is cold-adapted and halotolerant.

A variety of genes encoding enzymes for oxidative stress such as superoxide dismutase, catalase, peroxiredoxin, peroxidase, dioxygenase and peroxiredoxin were detected in the genome (Table S3) (Lim et al., 2019). Other bio-toxic stresses, like heavy metals and antibiotics, may be pump out of cells by a series of specific transport systems. *M. spongiae* MSC19^T is equipped with genes for resistance against heavy metals such as zinc, manganese, magnesium, nickel, copper, cobalt, cadmium and arsenic (Table S4). It also possesses ABC-type multidrug transport system, Na^+-driven multidrug efflux pump and multidrug exporter proteins encoded by *emrE* and *acrAB* (Table S5) to enhance antibiotic resistance (Baker-Austin et al., 2006).

As predicted by antiSMASH 6.0 (Blin et al., 2021), the genome of *M. spongiae* MSC19^T harbors four secondary metabolite biosynthetic gene clusters (Table S6). They are one T3PKS, one betalactone, one terpene and one ladderane gene cluster with low similarity ($\leq$ 50% of genes show similarity) to reported salinichelins, microansamycin and carotenoid. Among them, salinichelins are a new and unrelated group of peptidic siderophores (Bruns et al., 2018), microansamycins are a series of novel pentaketide ansamycins (Wang et al., 2018), carotenoids are widespread natural pigments acting as ROS and belongs to non-enzymatic systems for oxidative stress defense (Tao et al., 2007; Lim et al., 2019). According to the CAZy analysis (Cantarel et al., 2009), *M. spongiae* MSC19^T contains 80 CAZymes (Table S7) such as 24 carbohydrate esterases (CEs), 24 glycoside hydrolases (GHs) and 23 glycosyl transferases (GTs), suggesting its adaptability to utilize different classes of carbohydrates.

Sponge associated bacteria own many functions such as metabolic interactions with host by vitamin production and protein-protein interactions mediated through eukaryotic-like proteins (ELPs) (Kiran et al., 2018). Nearly complete sets of genes required for the synthesis of cobalamin (vitamin B12), pyridoxine (vitamin B6), thiamine (vitamin B1), riboflavin (vitamin B2) and biotin (vitamin B7) were identified in the genome of *M. spongiae* MSC19^T according to the KEGG database. Specially, the ability to de novo synthesize cobalamin is restricted to prokaryotes and only prokaryotes or plants can de novo synthesize pyridoxine (Bondarev et al., 2013), sponge host may need these vitamins from its symbiotic bacteria like *M. spongiae* MSC19^T. Sponge microbial metagenomes show abundant proteins comprising eukaryotic-type domains, such as ankyrin repeats (ANKs) and tetratricopeptide repeats (TPRs) which are involved in protein-protein interactions in eukaryotes, and leucine rich repeats (LRRs) domains (Reynolds and Thomas, 2016;

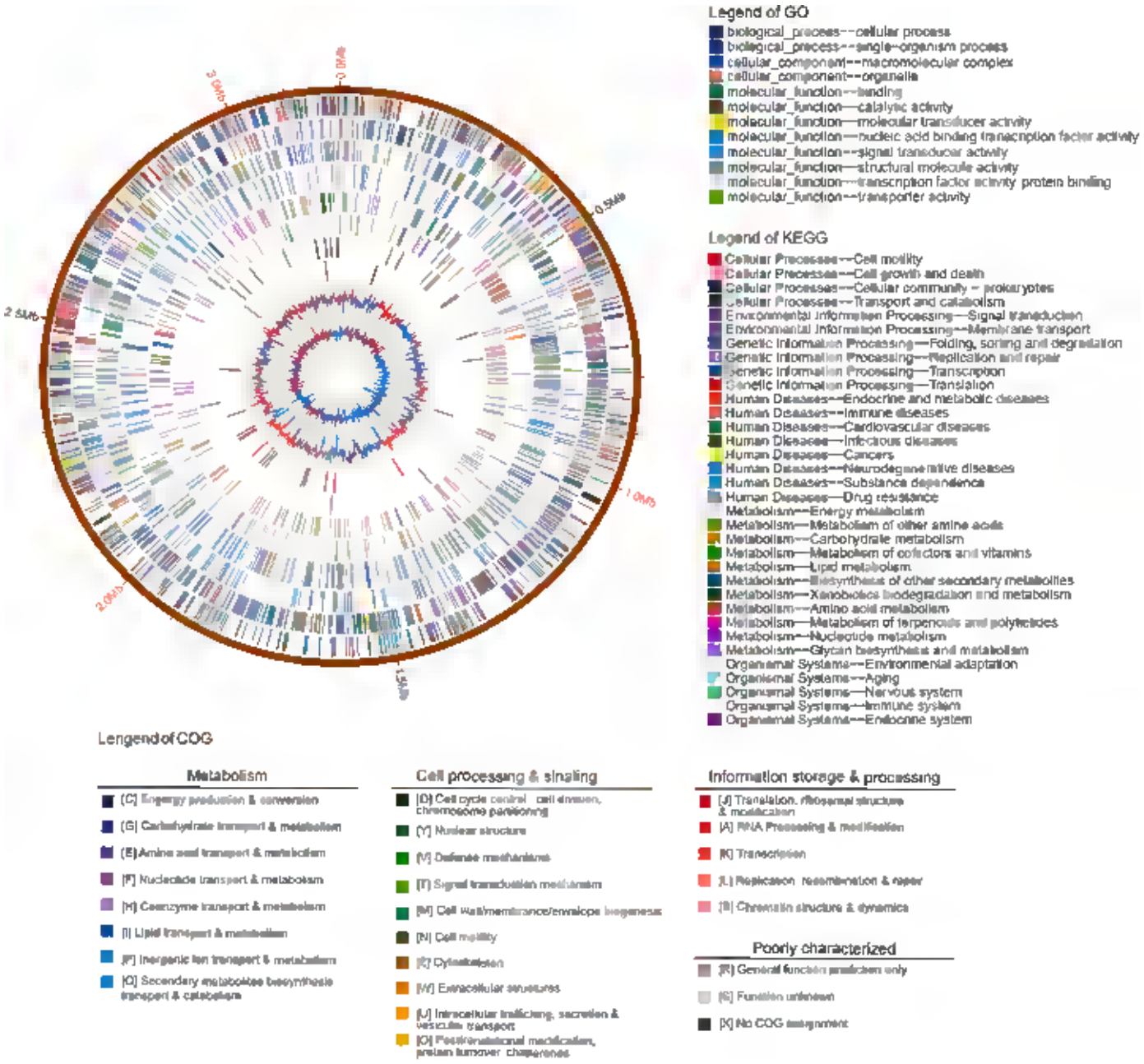

Fig. 1. Circular map of the genome of *Mycetocola spongiae* MSC19^T made by software Circos (Krzywinski et al., 2009). From the outside to the center: scale marks of the genome, forward COG-assigned CDSs, reverse COG-assigned CDSs, forward GO-assigned CDSs, reverse GO-assigned CDSs, forward KEGG-assigned CDSs, reverse KEGG-assigned CDSs, tRNA (black) and rRNA (red) genes on the forward strand, tRNA (black) and rRNA (red) genes on the reverse strand, GC content and GC skew. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Germer et al., 2017). *M. spongiae* MSC19^T contains two genes coding ANKs (COG0666, PF12796), four TPRs encoding genes (COG0457, PF14561) and six genes coding LRRs (COG4886, PF12799), indicating that *M. spongiae* MSC19^T may use these eukaryotic-type proteins (ELPs) to escape phagocytosis and/or control their symbiotic relationship with its sponge host (Liu et al., 2012).

In summary, the genome of *Mycetocola spongiae* MSC19^T which is a novel species of deep-sea sponge symbiotic bacterium was sequenced, and we revealed the genetic basis of *M. spongiae* MSC19^T to adapt to the deep-sea environment and the interactions with its sponge host.

3. Culture deposition and nucleotide sequence accession number

Mycetocola spongiae MSC19^T was deposited in the Marine Culture Collection of China (MCCC) and the Korean Collection for Type Cultures (KCTC) with accession numbers of MCCC 1K06265 and KCTC 49701, respectively. The complete genome sequence of *Mycetocola spongiae* MSC19^T is available in GenBank under accession number CP080203.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2022.100955.

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