Genome sequences of two cold-adapted *Cryobacterium* spp. SO1 and SO2 from Fildes Peninsula, Antarctica

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Psychrophilic and psychrotrophic bacteria play important roles in nutrient cycling in cold environments. These bacteria are suitable as model organisms for studying cold-adaptation, and sources of cold-active enzymes and metabolites for industrial applications. Here, we report the genome sequences of two *Cryobacterium* sp. strains SO1 and SO2. Genes coding major proteins related to cold- or thermal-stress adaptations and those with industrial applications found in their genomes are described.

Keywords: Fluctuation, genomes, King George Island, 16S rDNA, temperature.

IN general, *Cryobacterium* is Gram-positive aerobe with a pleomorphic rod shape[1]. Currently, the genus comprises only eight other known species: *C. psychrophilum* isolated from a region near Showa station, Antarctica[1,2]; *C. psychrotolerans*[3], *C. flavum* and *C. luteum*[4] and *C. levicorallinum*[5] isolated from China No. 1 glacier; *C. mesophilum* isolated from Bigeum Island[6]; *C. roopkundense* isolated from glacial lake Roopkund, Himalayan mountain range, India[7], and *C. arcticum* isolated from northeast Greenland[8]. All known *Cryobacterium* are cold-adapted bacteria except *C. mesophilum* which is a mesophile. Despite the numbers of *Cryobacterium* species reported from different geographical locations, not much is known about their genes and genomes. Our interest is to analyse genomes of *Cryobacterium* to determine the genes that they harbour. In this study, genomes of two cold-adapted *Cryobacterium* sp. SO1 and SO2 isolated from snow sample of the Fildes Peninsula were sequenced and analysed.

Two bacterial strains, SO1 and SO2, which grew well at 20°C were isolated from snow samples collected from the Fildes Peninsula, King George Island, Antarctica. They were routinely grown in Trypticase-soy medium. High-quality genomic DNAs were extracted using DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instruction. Strains SO1 and SO2 were identified based on their 16S rDNA sequences. PCR amplification of 16S rDNA was performed using primers, BSF-8/20 5′-AGAGTTTGATCCTGGCTCAG-3′ and BSR-1541/20 5′-AAGGAGGTGATCCAGCCGCA-3′. Amplified DNA fragments were sequenced using the Applied BioSystem sequencer, and aligned against sequences in NCBI GenBank using Basic Local Alignment Search Tool (BLAST). A phylogenetic tree was constructed based on the 16S rDNA sequences of bacteria using the MEGA6 software. The tree was inferred using the Tamura-Nei (TN93 + G + I) model based on 1000 resamples dataset[9,10]. Both strains, SO1 and SO2 formed distinctive clusters with *C. arcticum* SK1, *C. psychrotolerans* 0549, *C. roopkundense* RuGI7 and *C. psychrophilum* (Figure 1). Strains SO1 and SO2 were closest to *C. arcticum* SK1 with 99% similarity based on the BLAST results. The other species close to strains SO1 and SO2 were *C. psychrotolerans* 0549 with 97% and 98% similarity respectively, and *C. roopkundense* RuGl7 with 96% similarity. Based on the findings above, strains SO1 and SO2 are referred to as *Cryobacterium* sp. SO1 and *Cryobacterium* sp. SO2. Their 16S rDNA sequences were deposited together with their whole genome sequences in the DDBJ/ENA/GenBank.

Genome libraries of strains SO1 and SO2 were prepared using 300 cycles Miseq Reagent kit and sequenced using Illumina Mi-Seq next-generation sequencer. A total of 3,633,608 and 3,117,314 reads (101x and 85x coverage) were generated from the whole genome sequencing of strains SO1 and SO2 respectively. Sequences of strains SO1 and SO2 were assembled into 207 and 74 contigs respectively. The estimated genome sizes of both

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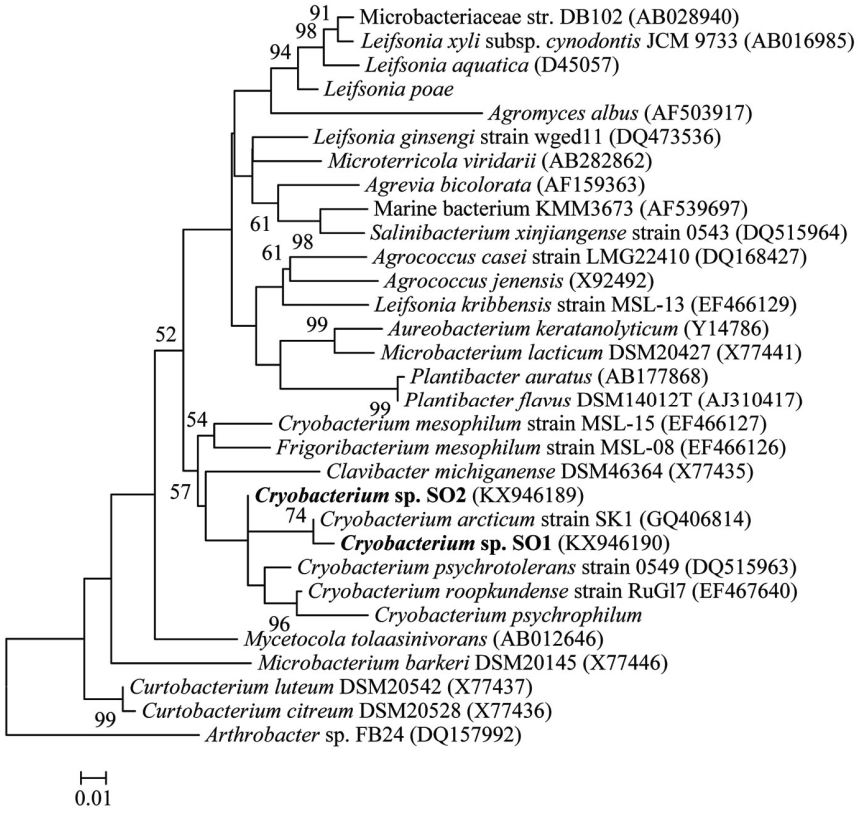

Figure 1. Neighbour-joining phylogenetic tree based on 16s rDNA sequence similarity among SO1, SO2, and species of Microbacteriaceae. *Arthrobacter* sp. FB24 (DQ157992) served as the outgroup. Numbers on the nodes (only those above 50% are shown) represent the bootstrap percentages based on 1000 resampled dataset using neighbour-joining analysis. Accession numbers in parentheses. Bar, 0.01 substitutions per nucleotide position.

strains were approximately 4.0 and 4.2 Mb with G + C contents of 67.5% and 68.5% respectively. The percentage of genome G + C contents are consistent to *C. psychrophilum*, *C. psychrotolerans*, *C. roopkundense* and *C. arcticum* which were 65%, 67%, 64.7% and 67.8% respectively[1–3,7,8]. Assembled genomes were annotated using rapid annotation subsystem technology (RAST)[11]. Genomes of strains SO1 and SO2 were found to harbour 3682 and 3891 putative protein coding sequences respectively. The whole genome sequences of *Cryobacterium* spp. SO1 and SO2 have been deposited in DDBJ/ENA/GenBank under the accession numbers MQTR01000000 and MQTP01000000.

Common genes in cold-adapted bacteria such as molecular chaperones, heat-shock proteins (HSPs), cold-shock proteins (CSPs), low-temperature requirement protein A and antifreeze proteins (AFPs) were found in the genomes of strains SO1 and SO2 (Table 1). These genes are essential for bacteria to survive in cold environment with fluctuating temperatures[12,13]. An increase and decrease in ambient temperature alters the expression of molecular chaperones, HSP and CSP genes to ensure transcription, translation and post-translation process of the cells continue and the proteins or enzymes are functional[12,13].

Several thermal stress-related genes of *Cryobacterium* spp. SO1 and SO2, *C. arcticum* PAMC 27867[14], *C. psychrotolerans*[15] and *C. roopkundense* RUGI7[16] are listed in Table 1. Some of the genes that are present in *Cryobacterium* sp. strains SO1 and SO2 were different. For instance, the *Hsp*20 and low-temperature requirement *A* genes are present in strain SO2 but not in strain SO1. It was found that strains SO1 and SO2, and *C. psychrotolerans*[15], each harboured an antifreeze glycopeptide polyprotein (AFGP) gene, which was absent in the closely related *C. arcticum* PAMC 27867 (ref. 14) and *C. roopkundense* RUGI7 (ref. 16) (Figure 1). However, strains SO1 and SO2, and *C. arcticum* PAMC 27867 (ref. 14) harboured a common antifreeze protein type I gene (Table 1) that was absent in *C. psychrotolerans* and *C. roopkundense* RUGI7. Interestingly, *C. arcticum*[14] and *C. roopkundense* RUGI7 (ref. 16) each have an antifreeze protein gene that was different from the antifreeze protein type I gene. AFGP, antifreeze protein type I and antifreeze protein probably have similar functions to ensure that the host carrying one of them can withstand cold shocks and survive.

Apart from thermal stress genes, *Cryobacterium* spp. SO1 and SO2 also encode enzymes with wide

Table 1. Presence and absence of heat-stress, cold-stress, and antifreeze related genes in the genomes of strains (1) SO1, (2) SO2, (3) *Cryobacterium arcticum* PAMC 27867, (4) *C. psychrotolerans* and (5) *C. roopkundense* RuGI7. Present, √; absent, –.

	Strains				
Thermal stress related genes	1	2	3	4	5
Chaperone protein *Dna*J	√	√	√	√	√
Chaperone protein *Dna*K	√	√	√	√	√
Heat shock protein 60 family chaperone *Gro*EL	√	√	√	√	√
Heat shock protein 60 family co–chaperone *Gro*ES	√	√	√	√	√
Heat shock protein *Grp*E	√	√	√	√	√
Heat–inducible transcription repressor *Hrc*A	√	√	√	√	√
Heat shock protein *Hsp*20	–	√	√	√	–
Heat shock protein *Htp*X	√	√	√	√	√
Cold shock protein *Csp*A	√	√	√	√	√
Cold shock protein *Csp*C	√	√	√	√	√
Low temperature requirement A	–	√	–	–	–
Antifreeze glycopeptide AFGP polyprotein	√	√	–	√	–
Type I antifreeze protein	√	√	√	–	–
Antifreeze protein	–	–	–	√	√

biotechnological and industrial applications. Among them were: alkaline phosphatase, Uracil-DNA glycosylase, RNase polymerases and ligases for molecular work, as well as cold-active enzymes such as protease, lipase, amylase, chitinase, superoxide dismutase, β-galactosidase and neopullulanase. Enzymes coded by those genes are valuable especially if they are active at low temperatures.

Overall, the genome data of these two *Cryobacterium* spp. SO1 and SO2 provided knowledge about genes they harboured. These genome data form the platform for future analysis of thermal-stress adaptation by cold-active bacteria, through gene expression study. Additionally, genes encoding proteins and enzymes with wide range of applications can be harnessed for use in the bioindustry sectors.

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ACKNOWLEDGEMENTS. This work was funded by the Ministry of Science Technology and Innovation (MOSTI), Malaysia (Flagship Grant no. FP1213E036). We thank personnel at the INACH especially Drs José Retamales and Marcelo Leppe for their advice and logistic support.

doi: 10.18520/cs/v115/i9/1706-1708