Sains Malaysiana 52(7)(2023): 1955-1966
http://doi.org/10.17576/jsm-2023-5207-05

Genomic Analysis of a Novel Antarctic Bacterium, *Cryobacterium* sp. SO2 Provides Insights into its Genomic Potential for Production of Antimicrobial Compounds

(Analisis Genom Bakteria Antartika Baharu, *Cryobacterium* sp. SO2 Memberi Cerapan tentang Potensi Genomnya untuk Pengeluaran Sebatian Antimikrob)

TEOH, C.P.[1], LAVIN, P.[2], GONZÁLEZ-ARAVENA, M.[3] & WONG, C.M.V.L.[1,*]

[1]*Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia*
[2]*Departamento de Biotecnologia, Facultad de Ciencias del Mar y Recursos Biologicos, Universidad de Antofagasta, 601 Avenida Angamos, Antofagasta 1270300, Chile*
[3]*Instituto Antártico Chileno, Plaza Muñoz Gamero 1055, Punta Arenas, Chile*

Received: 17 February 2023/Accepted: 19 June 2023

ABSTRACT

A novel strain of *Cryobacterium* designated as SO2, was isolated from the Antarctic. Hence, this study was undertaken to gain further insight into the antimicrobial compounds and secondary metabolites produced by *Cryobacterium* sp. SO2. It was found that strain SO2 is a Gram-positive that exhibits an irregular rod shape, which formed yellow to orange pigmented colonies on semi-solid media. Strain SO2 grows at temperatures ranging from 4 to 25 ºC. It has a complete genomic size of 4.097 Mb. SO2 has a DNA G+C content of 68.43%, and genomic annotation showed that the genome contained 3,862 CDS, 10 rRNA, 55 tRNA and 1 tm-RNA. Phylogenetic and OrthoANI analysis suggested *Cryobacterium* sp. strains SO1, N22, TMB1-8, LW097, TMN39-1, *C. zongtaii* TMN-42, *C. arcticum* PAMC27867 and *C. soli* GCJ02 as its closest phylogenetic neighbour. Genome annotation shows that strain SO2 confers β-lactamase class A, cephalosporin-C deacetylases, and 27 drug-resistance or efflux coding genes, and allows resistance to ceftazidime. Functional annotation identifies 28.74% of predicted genes are of unknown functions. Genome mining indicates that there are six putative secondary metabolite gene clusters in strain SO2. They are made up of RRE-containing, terpene, beta-lactone, T3PKS, NAPAA, and 2dos. This finding shows strain SO2 harbours genes that may be involved in the production of compounds with antibacterial and antioxidant activities.

Keywords: Complete genome; *Cryobacterium* sp.; drug-resistant; psychrotolerant; secondary metabolite gene cluster

ABSTRAK

Strain baharu *Cryobacterium* yang ditetapkan sebagai SO2 telah dipencilkan dari Antartika. Oleh itu, kajian ini dijalankan untuk mendapatkan pemahaman yang lebih mendalam mengenai sebatian antimikrob dan penghasilan metabolit sekunder oleh *Cryobacterium* sp. SO2. Didapati bahawa strain SO2 adalah Gram-positif yang mempamerkan bentuk rod yang tidak teratur, yang membentuk koloni berpigmen kuning hingga oren pada media separa pepejal. Strain SO2 tumbuh pada suhu antara 4 hingga 25 ºC. Saiz genomnya yang lengkap adalah 4.097 Mb. Strain SO2 mempunyai kandungan G+C DNA sebanyak 68.43% dan anotasi genom menunjukkan terdapatnya 3,862 CDS, 10 rRNA, 55 tRNA dan 1 tm-RNA. Analisis filogenetik dan OrthoANI mencadangkan *Cryobacterium* sp strain SO1, N22, TMB1-8, LW097, TMN39-1, *C. zongtaii* TMN-42, *C. arcticum* PAMC27867 dan *C. soli* GCJ02 merupakan jiran filogenetik terdekat. Anotasi genom menunjukkan bahawa strain SO2 mengandungi β-laktamase kelas A, *cephalosporin*-C *deacetylases* dan 27 gen kerintangan atau efluks dadah dan menyebabkan kerintangan strain SO2 terhadap ceftazidime. Anotasi kefungsian mengenal pasti 28.74% gen yang diramalkan mempunyai fungsi yang tidak diketahui. Perlombongan genom strain SO2 menunjukkan terdapatnya enam kluster gen metabolit sekunder. Mereka terdiri daripada RRE, terpena, beta-lakton, T3PKS, NAPAA dan 2dos. Penemuan ini menunjukkan strain SO2 mengandungi gen yang mungkin terlibat dalam aktiviti antibakteria dan antioksidan.

Kata kunci: *Cryobacterium* sp.; genom lengkap; kerintangan dadah; kluster gen metabolit sekunder; psikrotoleran

Introduction

The rapid emergence of antibiotic-resistant pathogens, compared to the discovery of new antibiotics, is endangering the effectiveness of available antibiotics. In 1940, penicillin-resistant *Staphylococcus* was reported (Centers for Disease Control and Prevention [CDC] 2013; Spellberg & Gilbert 2014). Several new beta-lactam were discovered by the 1950s, and the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was reported by 1960s (CDC 2013; Sengupta, Chattopadhyay & Grossart 2013; Spellberg & Gilbert 2014). By 1972, vancomycin was introduced for the treatment of MRSA and coagulase-negative staphylococci (CDC 2013; Sengupta, Chattopadhyay & Grossart 2013). Nevertheless, vancomycin resistance in coagulase-negative staphylococci was first reported in 1979 (Sengupta, Chattopadhyay & Grossart 2013). The pharmaceutical industry has introduced new antibiotics throughout the years, but the discovery of new antibiotics has been relatively fewer (Spellberg & Gilbert 2014). Therefore, it is important for the discovery of novel lead compounds to keep pace with emerging multi-drug-resistant pathogens (Ventola 2015).

Microorganisms are the source of a wide diversity of natural products, especially members of the phylum Actinobacteria (Bérdy 2005). Actinobacteria have been reported as a great source of new biological active agents, including antibacterial, antifungal, antitumor, anticancer, anti-inflammatory, antiviral, cytotoxic, and immunosuppressive activities (Dharmaraj 2010; Hassan & Shaikh 2017; Manivasagan et al. 2014; Silva et al. 2020). The genus *Cryobacterium* is classified under the Actinobacteria phylum and the Microbacteriaceae family (Suzuki et al. 1997). Members of the *Cryobacterium* genus are Gram-positive, aerobic and exhibit irregular rod-shaped morphology (Bajerski et al. 2011; Reddy et al. 2010; Suzuki et al. 1997; Zhang et al. 2007). They have been isolated from different geographical locations, such as Antarctica, the Arctic, China, South Korea, and India. At the time of writing, fifteen *Cryobacterium* species have been identified, but potential valuable novel bioactive molecules of *Cryobacterium* species have yet to be reported.

Despite the substantial number of *Cryobacterium* spp. that have been identified and the fact that they belong to Actinobacteria, little is known about their phenotypic traits, genomes, genes they encode, or antimicrobial compounds they produce. Hence, phenotypic and genomic analyses were carried out in this study to understand *Cryobacterium* sp. SO2 better. The PacBio RSII sequencer was used to sequence its entire genome to obtain a single contig. Bioinformatics analysis was then used to mine the strain SO2 complete genome data for potential novel biological active agents, such as peptides and antimicrobial compounds.

Materials and Methods

Sources of Bacterial Strain

Strain SO2 was isolated from the snow sample near the Estrellas lake (S 62°12’15” W 58°57’43”), Fildes Peninsula, King George Island, Antarctica (Teoh et al. 2018) and was used in this study.

Morphology and Physiology

Cell shapes were determined using a compound light microscope and scanning electron microscope (SEM). A modified hexamethyldizilazane (HMDS) drying method from Murtey and Ramasamy (2016) was used to prepare cells before examination under SEM. The sample was centrifuged at 4,500 rpm for 1 min. The supernatant was discarded and 1 mL of 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) was added to the sample, followed by resuspension and incubation at 4 °C for 6 h for fixation. After fixation, the sample was centrifuged at 4,500 rpm for 1 min. The supernatant was discarded, and the pellet was washed twice with 0.05 M phosphate buffer. The specimen was dehydrated once in each graded series of 35, 50, 80, 95, and twice for 100% (v/v) ethanol. The sample was centrifuged at 4,500 rpm for 1 min between each dehydration step after 5 min of incubation. The supernatant was discarded, and the pellet was suspended in 1 mL of HMDS for 10 min. HMDS was decanted after centrifugation and the pellet was dried overnight. The dried specimen was mounted onto an SEM specimen stub using double-sided sticky tape, coated with gold, and viewed under SEM.

The growth performance of strain SO2 on different agar media was determined. Agar media, namely Luria-Bertani (LB), R2A, Nutrient (NA) and Tryticase Soy (TS) media from Difco were used. Ten-fold serial dilutions of culture were performed, and 1 uL of cell suspension from each dilution was transferred onto the agar media. Culture agar plates were incubated at 4, 10, 15, 20, and 28 °C for two weeks. Quantitative growth measurements were performed by growing strain SO2 in Trypticase Soy broth media. Cultures were incubated at 10, 15, 20, and 25 °C. Optical densities for triplicate cultures at 600_{nm} were measured at 24-hour intervals using a spectrophotometer and SpectraMax M2 microplate readers. The average and standard deviation of optical densities for cultures were calculated and a graph was constructed.

GENOMIC DNA EXTRACTION, SEQUENCING, ASSEMBLY AND ANNOTATION

Cryobacterium sp. SO2 was cultivated in TS broth media at 20 °C with shaking at 180 rpm for 2 days. Cells were harvested when the culture reached the mid-logarithm phase (OD_{600}=0.4). Cells were pelleted by centrifuging at 10,000 rpm for 5 min. The supernatant was discarded and the genomic DNA was then extracted using the DNeasy Blood and Tissue kit (Qiagen) according to the manufacturer's instructions. Extracted genomic DNA was suspended in sterilized distilled water. The concentration of genomic DNA was examined using the Invitrogen Qubit 2.0 fluorometer and the Invitrogen Qubit® dsDNA HS assay. As for the purity of genomic DNA, a Thermo Scientific NanoDrop 2000 UV/Vis spectrophotometer was used for examination.

The genomic DNA was then sequenced by Macrogen (Seoul, South Korea) for Single-Molecule Real-Time (SMRT) sequencing on a PacBio RSII system. A 20 kb SMRT bell template DNA library was constructed and loaded into 1 SMRT cell 8Pac V3. Sequencing was then performed using the DNA Polymerase Binding Kit P6V2 Reagent. The sequencing reads were then subjected to Canu v2.1 for correction, trimming and assembly at default settings (Koren et al. 2017). The assembled genome was then circularized using Circlator v1.5.5 at default settings (Hunt et al. 2015). The genome completeness of the assembled genome was determined with actinobacteria_odb9 lineage using Benchmarking Universal Single-Copy Ortholog (BUSCO) (Simão et al. 2015). The assembled genome sequence was then annotated using Prokka (Seemann 2014). Previously, the MiSeq-generated genome sequence had been deposited at DDBJ/ENA/GenBank under the accession MQTP00000000. The PacBio-generated Whole Genome Shotgun project described in this paper was deposited at DDBJ/ENA/GenBank under the accession CP117849. In addition, for ease of comparison, re-annotation on other genomes was carried out using Prokka and used throughout this study.

PHYLOGENETIC ANALYSIS

The phylogenetic tree was constructed based on whole-genome sequences by using the PhyloSift pipeline (Darling et al. 2014) with all the settings left by default. PhyloSift recognized, concatenated and aligned numbers of elite genes that are known to be conserved among prokaryotes. The phylogenetic tree was then inferred by using FastTree (Price, Dehal & Arkin 2009).

ANTIBIOTICS ASSAY

The log-phase culture of strain SO2 was cultivated in the Trypticase Soy broth medium. The cultures were subsequently transferred onto Trypticase Soy agar medium and evenly swabbed using a cotton swab. Antibiotic discs were then transferred onto the agar media and incubated at 20 °C for 5 days. The following antibiotics were tested: Clarithromycin 15 ug (CLR15), Lincomycin 10 ug (MY10), Cephalothin 30 ug (KF30), Rifampicin 5 ug (RD5), Erythromycin 15 ug (E15), Ceftazidime 30 ug (CAZ30), Clindamycin 2 ug (DA2), Cefixime 5 ug (CFM5), Nitrofurantoin 100 ug (F100) and Gentamicin 10 ug (CN10). Three blank discs, each containing 10 uL of sterile broth culture, were included as negative controls, while CN10 was used as the positive control due to its broad-spectrum antibacterial activity. The average diameter of the formed halo zones was recorded.

BIOINFORMATIC ANALYSES

To determine genomic similarity of strain SO2 and neighbouring *Cryobacterium* species resulting from PhyloSift, the OrthoANI was measured by using the Orthologous Average Nucleotide Identity Tool (OAT) (Lee et al. 2016). All the closely phylogenetic-related genomes were re-annotated by using Prokka (Seemann 2014) to ease downstream analysis. The resulting general feature format (.gff) files were inputted into Roary pangenome pipeline v1.006924 for pan-genome analysis (Page et al. 2015). The predicted proteins of strain SO2 and *Cryobacterium* sp. were inputted into eggNOG-mapper v0.90.0 and eggnog database 4.5.1 (Huerta-Cepas et al. 2016, 2017) for functional annotation at the default setting. BlastKOALA was implemented for the KEGG orthology (KO) prediction (Kanehisa, Sato & Morishima 2016). Lastly, the secondary metabolite of strain SO2 was predicted by using anti-SMASH 7.0 beta (Medema et al. 2011).

RESULTS AND DISCUSSION

Cryobacterium sp. SO2 is a Gram-positive bacterium. When viewed under an SEM, it exhibited an irregular rod-shaped (Figure 1). Most of them had an a verage length of about 0.6 µm, with a small number of cells that formed longer rods. As depicted in Figure 2, *Cryobacterium* sp. SO2 formed yellow to yellowish orange pigmented colonies on agar media, which were circular, entire and pulvinate. The qualitative growth test showed *Cryobacterium* sp. SO2 grew best on the TSA medium, followed by NA, LB, and R2A. Strain SO2 grew at temperatures ranging from 4 to 25 °C but not at 28 °C. Quantitative growth measurements showed that the culture of strain SO2 reached the early log phase on day 1. Strain SO2 grown at 20°C had the highest growth rate, followed by 25, 15, and 10 °C at the log phase (between 1 and 5 days) (Figure 3). Psychrotolerant have broad ranges of growth temperatures, capable of growing at 0 °C and below, while their optimal growth temperatures are usually above 15 °C and their maximum growth temperature is 30 °C (Bakermans 2012). This suggested that *Cryobacterium* SO2 is psychrotolerant.

GENOME SEQUENCING

The concentration of extracted gDNA is 170.4 ng/uL, with absorbance ratio of A260/280 = 1.940 and A260/230 = 1.940. The raw data from SMRT sequencing resulted in a total of 1,087,890,530 bases of polymerase reads, consisting of 73,024 reads with an average length of 14,896 nucleotide bases and a polymerase read quality of 0.861. The polymerase reads were pre-processed in the SMRT portal to remove adapters sequences, resulting in a total of 1,086,626,470 subread bases, which made up of 100,797 subreads with an average length of 10,780 nucleotide bases. After correction and trimming, 7,954 reads with a mean length of 23,715 bases were recovered, providing a genome coverage of 46 times.

GENOME PROPERTIES

The genome size of strain SO2 is 4,097,436 bp (Table 1). Computational analysis showed that strain SO2 had a high DNA G+C content of 68.43%. This is a common characteristic of bacteria from the genus *Cryobacterium*. The N50 of the genome was 4,097,436 bp. As annotated, 3,862 genes were identified, and they were made up of 3,796 CDSs, 10 rRNA, 55 tRNA and 1 tm-RNA. There were 1,406 (36.4%) genes identified as hypothetical proteins. The completeness of the genome for strain SO2 was quantitatively assessed using BUSCO and predicted as 94.9% complete, 2.3% fragmented and 2.8% missing.

Orthology prediction was carried out for functional annotation based on Clusters of Orthologous Groups (COGs). As predicted, there were 3,440 (89.07%) genes classified as COGs and the remaining 422 (10.93%) genes were not recognized. Functional annotation showed that the majority of the ortholog genes of strain SO2 are assigned as category ‘S’, whereby the function of 1002 (28.74%) genes was unknown. There were 320 (9.18%) genes assigned to categories G (Carbohydrate transport

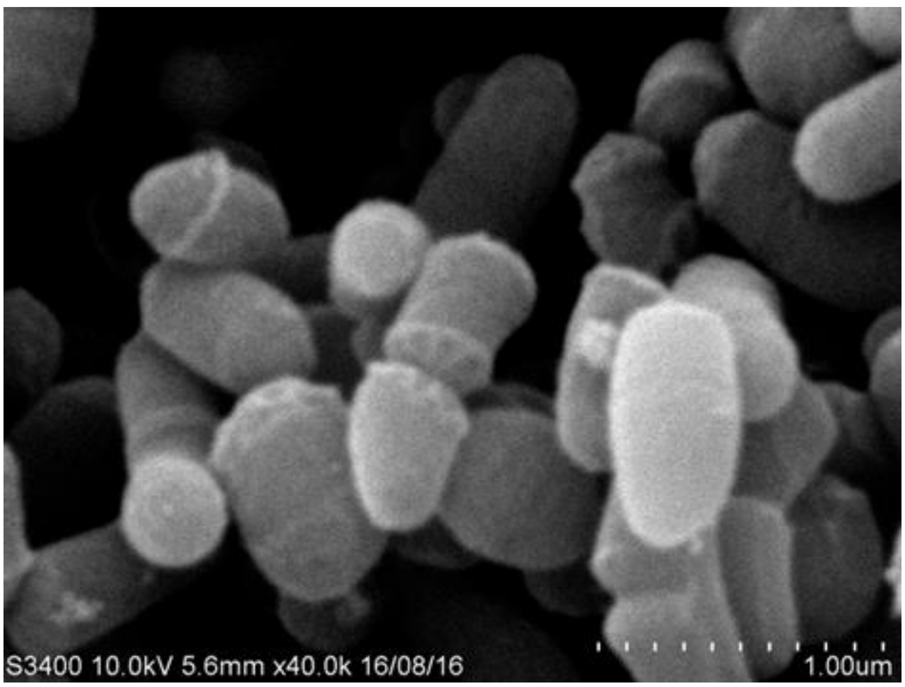

FIGURE 1. SO2 observed under SEM at 40,000× magnification

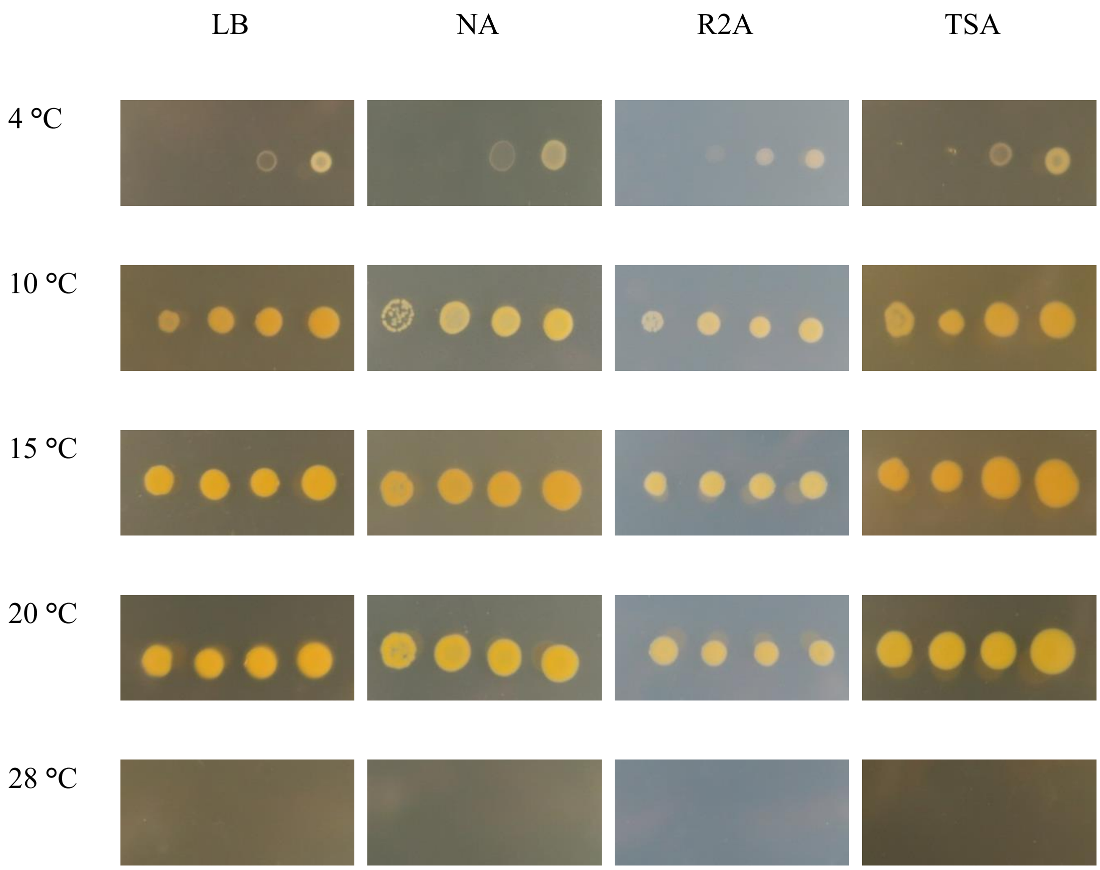

FIGURE 2. *Cryobacterium* sp. SO2 cultivated on LB, NA, R2A, and TSA media and incubated 4, 10, 15, 20 and 28 °C

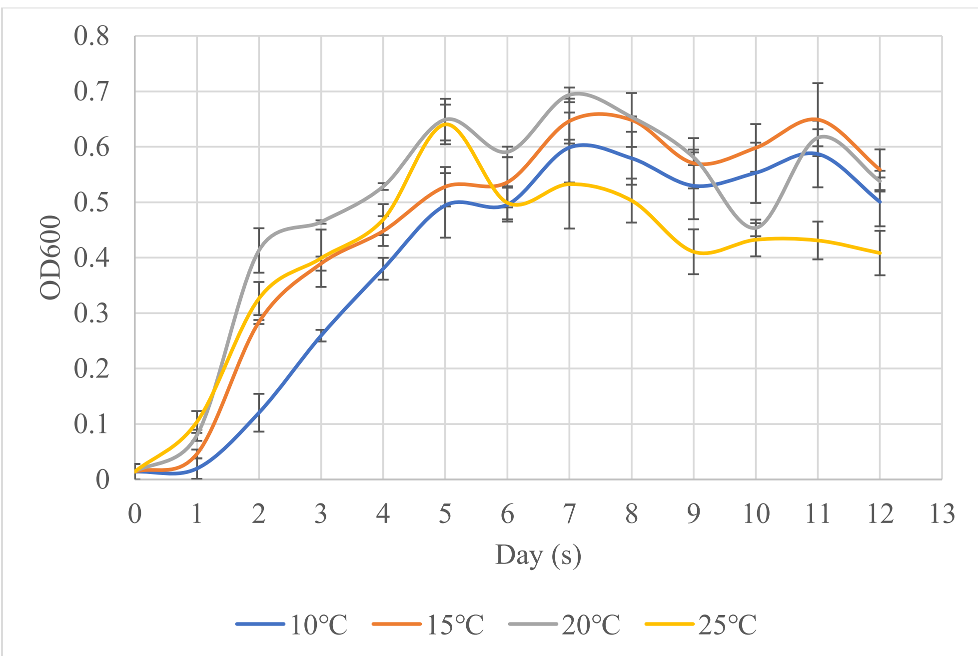

FIGURE 3. SO2 cultivated in TS broth media, incubated at 10, 15, 20 and 25 °C

TABLE 1. Genome properties of strain SO2

Feature	Value	(%)
Genome size (bp)	4,097,436	100
Contigs No.	1	
GC content (%)	2,803,875	68.43
N50	4,097,436	100
L50	1	
Total number of genes	3,862	100
Protein coding sequences (CDS)	3,796	98.29
CDS with predicted function	2,390	61.89
Hypothetical genes	1,406	36.41
Mean gene length	963.38	
rRNA	10	0.26
tRNA	55	1.42
tmRNA	1	0.03

and metabolism), followed by 292 (8.39%) genes assigned to categories K (Transcription), 257 (7.37%) genes assigned to categories E (Amino acid transport and metabolism), 254 (7.29%) genes assigned to categories P (Inorganic ion transport and metabolism) and 156 (4.48%) genes assigned to categories M (Cell wall/membrane/envelope biogenesis) (Figure 4).

The Basic Local Alignment Search Tool (BLASTn) result indicated that the 16S rDNA sequence of strain SO2 shared 99.93% identity with *C. soli* GCJ02 (CP030033.1), 99.93% identity with *Cryobacterium* sp. Asd M3-6 (FM955863.1) and 99.87% identity with *C. arcticum* PAMC27867 (CP016282.1). To better understand the relatedness of *Cryobacterium* species and strain SO2, the phylogenetic relationship was examined using PhyloSift software. *Cryobacterium* sp. SO2 forms a distinctive cluster with strains SO1, N22, TMB1-8, LW097, TMN39-1 and *C. zongtaii* TMN-42 (Figure 5). Further comparison was then carried out using OAT (Orthologous Average Nucleotide Identity Tool) (Lee et al. 2016), whereby OrthoANI was implemented for the measurement of genome similarity among strain SO2 and relevant *Cryobacterium* species. According to Lee et al. (2016), the proposed cut-off value for species demarcation is 95~96% for OrthoANI. Low identity values resulted between strain SO2 and other species (Figure 6).

In this study, the relatedness of *Cryobacterium* species was further measured by using a pangenome analysis. *Cryobacterium* sp. showed huge variability among the neighbouring species. There are 544 genes that were identified as the core genome, 4,832 genes as the shell genome and 12,590 genes as the cloud genome. According to Guimarães et al. (2015), the core genome can be used as the indicator to examine the diversity of the studied genomes. When the diversity of organisms used for pangenome study increases, the size of the core genome will be reduced. According to Page et al. (2015), the core for the highly related bacteria is about 1,000 genes for every million bases in pangenome analysis. The analysis had shown that the core genome was way too small, and this is most probably due to the species used in this analysis being way too diverse. These resulting data have shown that *Cryobacterium* sp. SO2 is a novel, newly isolated cold-adapted *Cryobacterium* species.

Antibiotic profiles give an overview of what types of antibiotics that strain SO2 is susceptible to or resistant to. The average size of halo zones was measured and recorded. Strain SO2 was resistant to CAZ30 but susceptible to all other antibiotics tested (Table 2). The smaller the size of the halo zones, the less susceptible they are to the tested antibiotics. The following shows the susceptibility of strain SO2 to the antibiotics: F100< CFM5< CN10< MY10< DA2< E15< KF30 <RD5 < CLR15.

KEGG analysis shows that strain SO2 possesses a gene (BJQ94_00098) that was identified as β-lactamase class A (EC:3.5.2.6). β-lactamase class A is a group of key antibiotic resistance enzymes to β-lactam compounds (Philippon et al. 2016). β- lactamase class A involved in the hydrolyzation of penicillin to penicilloic acid. In addition, there were three genes, BJQ94_01585, BJQ94_02275 and BJQ94_03673, that were identified as cephalosporin-C deacetylase (EC:3.1.1.41). BlastP analysis shows that these genes share low similarity (<61%). Cephalosporin-C deacetylases (CAH) are involved in the hydrolysis of the acetyl ester bond of cephalosporin C and may also play a role in the biosynthesis of cephamycin C. In addition, the annotation for the genome of strain SO2 indicated the presence of 27 multidrug resistance or efflux coding genes.

These genes most probably confer resistance to a narrow range of cephalosporin, such as CAZ30. As reported by Poirel et al. (2007), *Acinetobacter* spp. shows resistance to kanamycin, tobramycin, amikacin, gentamicin, rifampin and sulfonamides but is susceptible to chloramphenicol, tetracycline, fosfomycin, nalidixic acid and fluoroquinolones. This is because the gene from isolates *Acinetobacter* species encodes a narrow-spectrum β-lactamase (Poirel et al. 2007). *Cryobacterium* sp. SO2 may exhibit the same pattern of resistance. Strain SO2 resisted ceftazidime but was susceptible to cephalothin and cefixime. Nevertheless, strain SO2 may have other mechanisms of resistance towards ceftazidime that do not rely on spectrum β-lactamase.

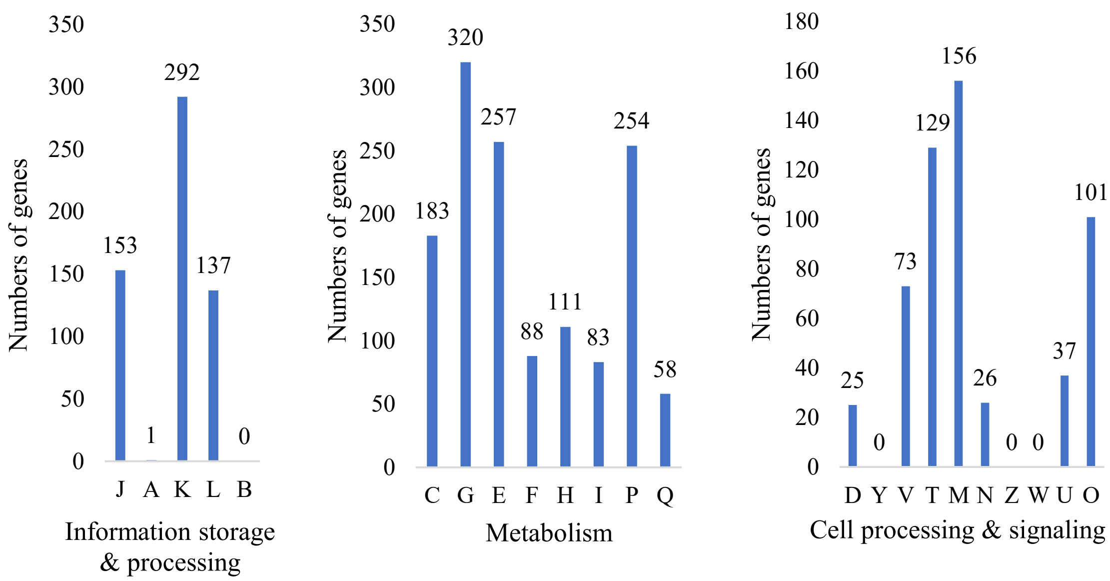

FIGURE 4. COG functional categories of *Cryobacterium* sp. SO2. Information storage and processing: [J] Translation, ribosomal structure, and biogenesis, [A] RNA processing and modification, [K] Transcription, [L] Replication, recombination and repair, [B] Chromatin structure and dynamics. Metabolism: [C] Energy production and conversion, [G] Carbohydrate transport and metabolism, [E] Amino acid transport and metabolism, [F] Nucleotide transport and metabolism, [H] Coenzyme transport and metabolism, [I] Lipid transport and metabolism, [P] Inorganic ion transport and metabolism, [Q] Secondary metabolites biosynthesis, transport, and catabolism. Cell processing and signalling: [D] Cell cycle control, cell division, chromosome partitioning, [Y] Nuclear structure, [V] Defense mechanisms, [T] Signal transduction mechanisms, [M] Cell wall/membrane/envelope biogenesis, [N] Cell motility, [Z]Cytoskeleton, [W] Extracellular structures, [U] Intracellular trafficking, secretion, and vesicular transport, [O] Posttranslational modification, protein turnover, chaperones. The y-axis represents the numbers of genes

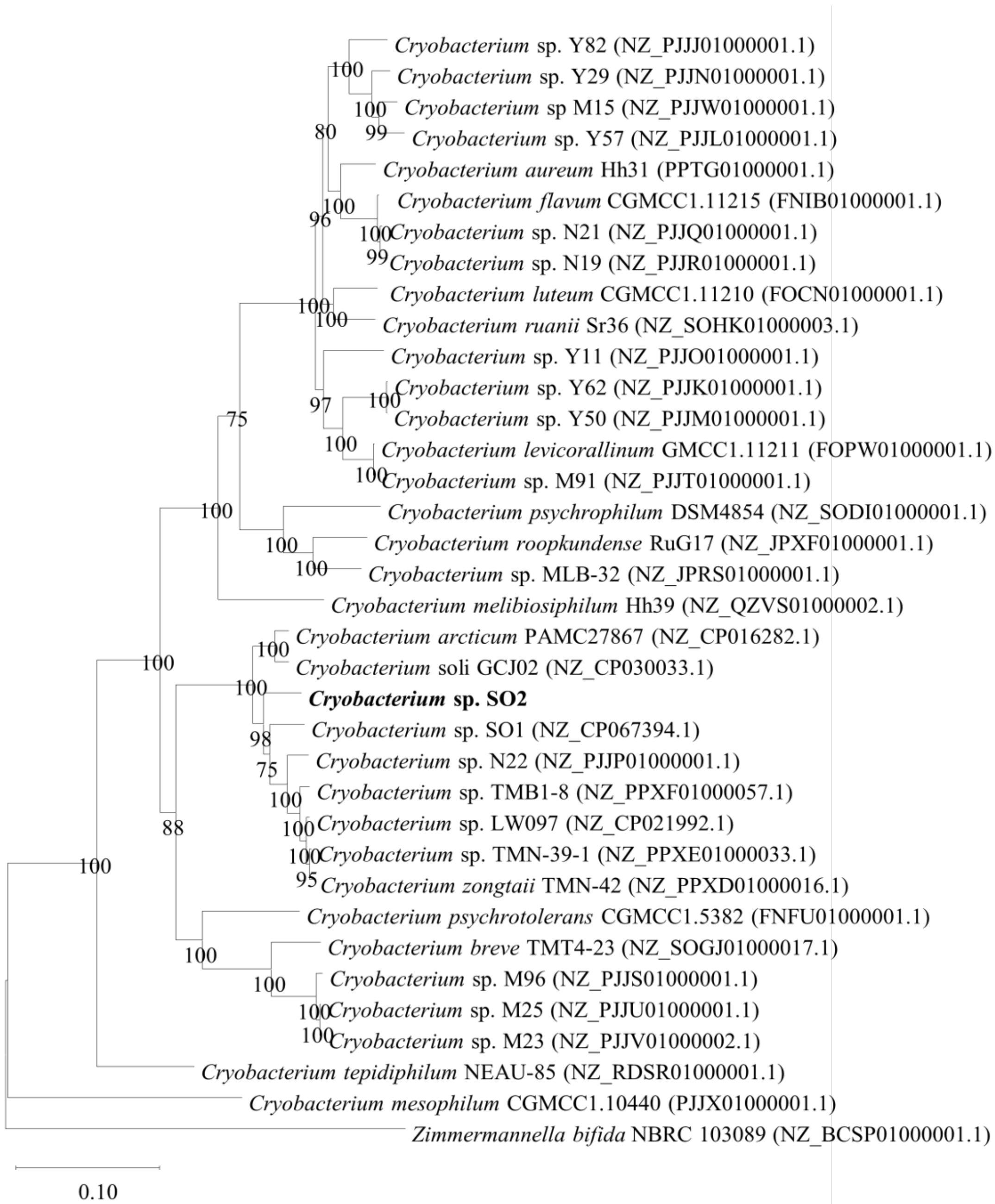

FIGURE 5. Phylogenetic tree of *Cryobacterium* sp. SO2 and neighbouring species were constructed using the Phylosift pipeline. *Z. bifida* NBRC 103089 served as the outgroup

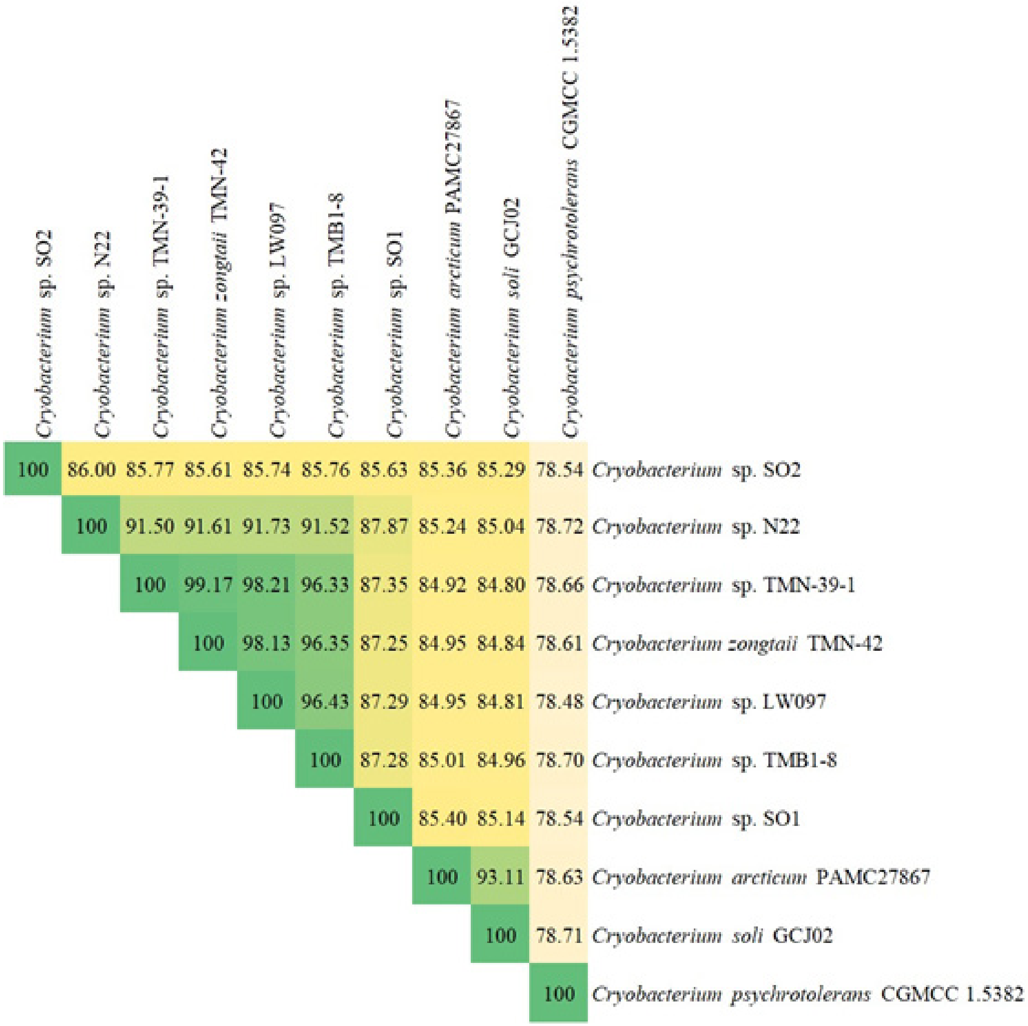

FIGURE 6. Orthologous Average Nucleotide Identity (Lee et al. 2016) for *Cryobacterium* sp. SO2 and neighbouring species

TABLE 2. Antibiotics assay on *Cryobacterium* sp. SO2

Antibiotics disc	The average size of halo zones (cm)	Class
Clarithromycin 15 ug (CLR15)	4.27	Macrolide
Lincomycin 10 ug (MY10)	3.30	Lincosamide
Cephalothin 30 ug (KF30)	3.53	Beta-lactam
Rifampicin 5 ug (RD5)	3.70	Antimycobacterials
Erythromycin 15 ug (E15)	3.50	Macrolide
Ceftazidime 30 ug (CAZ30)	Resistant	Cephalosporin
Clindamycin 2 ug (DA2)	3.43	Lincomycin
Cefixime 5 ug (CFM5)	1.20	Cephalosporin
Nitrofurantoin 100 ug (F100)	0.93	Nitrofuran
Gentamicin 10 ug (CN10)*	2.23	Aminoglycoside
Negative control	-	-

*: refers to the positive control

The secondary metabolites produced by strain SO2 were predicted using the antiSMASH software. It was found that strain SO2 conferred only six gene clusters of known and unknown secondary metabolites. In contrast, other Actinobacteria usually have large numbers of gene clusters. For instance, *Streptomyces coelicolor* A3(2) and *S. avermitilis* confer more than 29 and 37 putative gene clusters, respectively (Bentley et al. 2002; Ōmura et al. 2001). Regions 1 and 2 are RRE-element-containing clusters, and Region 2 displays low similarity (18%) to actinokineosin. Gene clusters with low similarities (40%) are most probably species-specific and might encode metabolites with novel chemical structures and biological activities (Zhong et al. 2013). For Regions 1, 4 (Type III Polyketide Synthase) and 6 (2-deoxy-streptamine aminoglycoside), as predicted, there are no known homologous sequences in the public database. Whereby, their products could be predicted partially from the gene organization. These clusters probably encode new biologically active compounds (Paulus et al. 2017).

Region 3 is identified as a terpene and beta-lactone containing a protease inhibitor, displaying 50% similarity to carotenoid. Up until 2018, there are about 850 carotenoids reported. Carotenoids appear in various colors, most noticeably yellow, orange, red, and purple. Carotenoids are valuable components in various industries due to their antioxidant, anti-inflammatory, and anti-cancer properties (Kaulmann & Bohn 2014; Maoka 2020; Mata-Gómez et al. 2014). Strain SO2 appeared as yellow to orange colored colonies, indicating possible production of carotenoids.

Region 5 is identified as non-alpha poly-amino acids like e-Polylysin, displaying 100% similarity to ε-Poly-L-lysine. ε-Poly-L-lysine is an antibacterial agent that is widely used due to its broad antimicrobial spectrum (Ye et al. 2013). The above genome analysis showed that strain SO2 likely encodes several bioactive substances with antimicrobial properties, and this can be explored further in the future.

Conclusion

Cryobacterium sp. SO2 is a psychrotolerant organism that is capable of growing at temperatures as low as 4 °C. The information from this work will facilitate our understanding of bacteria from this genus better because there is very little genomic research on *Cryobacterium* species. *In sillico* bioinformatics analysis of the genome of *Cryobacterium* sp. SO2 shows that this strain is most probably a novel species as low core genome size and ANIb values were identified. In addition, strain SO2 was predicted to produce natural products such as antimicrobial compounds and enzymes. The antiSMASH analysis results showed that strain SO1 encodes several bioactive substances, some of which have distinctive properties and could be novel antimicrobial compounds. Future works can focus on the characterization of bioactive compounds produced by strain SO2 and elucidation of their antimicrobial properties.

TABLE 3. Putative secondary metabolite gene clusters of strain SO2

Region	Type	From	To	Most similar known cluster	Similarity
Region 1	RRE-containing	661,616	678,879		
Region 2	RRE-containing	1,486,434	1,506,694	actinokineosin	18%
Region 3	terpene, betalactone	1,775,350	1,817,376	carotenoid	50%
Region 4	T3PKS	3,011,743	3,053,005		
Region 5	NAPAA	3,080,829	3,114,875	ε-Poly-L-lysine	100%
Region 6	2dos	3,930,879	3,952,018		

ACKNOWLEDGEMENTS

The funding support from the Ministry of Science, Technology, and Innovation (MOSTI), Malaysia, under the Antarctica Flagship Programme (Sub-Project 1: FP1213E036) is gratefully acknowledged.

REFERENCES

Bajerski, F., Ganzert, L., Mangelsdorf, K., Lipski, A. & Wagner, D. 2011. *Cryobacterium arcticum* sp. nov., a psychrotolerant bacterium from an Arctic soil. *International Journal of Systematic and Evolutionary Microbiology* 61(8): 1849-1853.

Bakermans, C. 2012. Psychrophiles: Life in the cold. In *Extremophiles: Microbiology and Biotechnology,* edited by Anitori, R.P. Poole: Caister Academic Press. pp. 53-60.

Bentley, S.D., Chater, K.F., Cerdeño-Tárraga, A.M., Challis, G.L., Thomson, N.R., James, K.D., Harris, D.E., Quail, M.A., Kieser, H., Harper, D. & Bateman, A. 2002. Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3 (2). *Nature* 417(6885): 141-147.

Bérdy, J. 2005. Bioactive microbial metabolites. *The Journal of Antibiotics* 58(1): 1-26.

Centers for Disease Control and Prevention (CDC). 2013. *Antibiotic Resistance Threats in the United States, 2013*. Centers for Disease Control and Prevention, Office of Infectious Disease Antibiotic Resistance Threats in the United States, 2013, accessed 25th May 2023 https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf

Darling, A.E., Jospin, G., Lowe, E., Matsen, F.A., Bik, H.M. & Eisen, J.A. 2014. PhyloSift: phylogenetic analysis of genomes and metagenomes. *PeerJ* 2: e243. https://doi.org/10.7717/peerj.243

Dharmaraj, S. 2010. Marine *Streptomyces* as a novel source of bioactive substances. *World Journal of Microbiology and Biotechnology* 26(12): 2123-2139.

Guimarães, L.C., de Jesus, L.B., Viana, M.V.C., Silva, A., Ramos, R.T.J., Soares, S.C. & Azevedo, V. 2015. Inside the pan-genome-methods and software overview. *Current Genomics* 16: 245-252.

Hassan, S.S. & Shaikh, A.L. 2017. Marine actinobacteria as a drug treasure house. *Biomedicine & Pharmacotherapy* 87: 46-57.

Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering, C., & Bork, P. 2017. Fast genome-wide functional annotation through orthology assignment by eggNOG-Mapper. *Molecular Biology and Evolution* 34(8): 2115-2122.

Huerta-Cepas, J., Szklarczyk, D., Forslund, K., Cook, H., Heller, D., Walter, M.C., Rattei, T., Mende, D.R., Sunagawa, S., Kuhn, M., Jensen, L.J., von Mering, C. & Bork, P. 2016. eggNOG 4.5: A hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. *Nucleic Acids Research* 44(D1): D286-D293.

Hunt, M., de Silva, N., Otto, T.D., Parkhill, J., Keane, J.A. & Harris, S.R. 2015. Circlator: Automated circularization of genome assemblies using long sequencing reads. *Genome Biology* 16(1): 294.

Kanehisa, M., Sato, Y. & Morishima, K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. *Journal of Molecular Biology* 428(4): 726-731.

Kaulmann, A. & Bohn, T. 2014. Carotenoids, inflammation, and oxidative stress -implications of cellular signaling pathways and relation to chronic disease prevention. *Nutrition Research* 34(11): 907-929.

Koren, S., Walenz, B.P., Berlin, K., Miller, J.R., Bergman, N.H. & Phillippy, A.M. 2017. Canu: Scalable and accurate long-read assembly via adaptive *k* -mer weighting and repeat separation. *Genome Research* 27(5): 722-736.

Lee, I., Ouk Kim, Y., Park, S.C. & Chun, J. 2016. OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *International Journal of Systematic and Evolutionary Microbiology* 66(2): 1100-1103.

Manivasagan, P., Kang, K.H., Sivakumar, K., Li-Chan, E.C.Y., Oh, H.M. & Kim, S.K. 2014. Marine actinobacteria: An important source of bioactive natural products. *Environmental Toxicology and Pharmacology* 38(1): 172-188.

Maoka, T. 2020. Carotenoids as natural functional pigments. *Journal of Natural Medicines* 74(1): 1-16.

Mata-Gómez, L.C., Montañez, J.C., Méndez-Zavala, A. & Aguilar, C.N. 2014. Biotechnological production of carotenoids by yeasts: An overview. *Microbial Cell Factories* 13(1): 12. https://doi.org/10.1186/1475-2859-13-12

Medema, M.H., Blin, K., Cimermancic, P., de Jager, V., Zakrzewski, P., Fischbach, M.A., Weber, T., Takano, E. & Breitling, R. 2011. AntiSMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Research* 39(2): W339-W346.

Murtey, M. & Ramasamy, P. 2016. Sample preparations for scanning electron microscopy - Life sciences. In *Physics, Optics and Lasers: Modern Electron Microscopy in Physical and Life Sciences,* edited by Janecek, M. & Kral, R. InTech. pp. 163-185.

Ōmura, S., Ikeda, H., Ishikawa, J., Hanamoto, A., Takahashi, C., Shinose, M., Takahashi, Y., Horikawa, H., Nakazawa, H., Osonoe, T., Kikuchi, H., Shiba, T., Sakaki, Y. & Hattori, M. 2001. Genome sequence of an industrial microorganism *Streptomyces avermitilis*: Deducing the ability of producing secondary metabolites. *Proceedings of the National Academy of Sciences* 98(21): 12215-12220.

Page, A.J., Cummins, C.A., Hunt, M., Wong, V.K., Reuter, S., Holden, M.T.G., Fookes, M., Falush, D., Keane, J.A. & Parkhill, J. 2015. Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31(22): 3691-3693.

Paulus, C., Rebets, Y., Tokovenko, B., Nadmid, S., Terekhova, L.P., Myronovskyi, M., Zotchev, S.B., Rückert, C., Braig, S., Zahler, S., Kalinowski, J. & Luzhetskyy, A. 2017. New natural products identified by combined genomics-metabolomics profiling of marine *Streptomyces* sp. MP131-18. *Scientific Reports* 7(1): 42382. https://doi.org/10.1038/srep42382

Philippon, A., Slama, P., Dény, P. & Labia, R. 2016. A structure-based classification of class A β-Lactamases, a broadly diverse family of enzymes. *Clinical Microbiology Reviews* 29(1): 29-57.

Poirel, L., Corvec, S., Rapoport, M., Mugnier, P., Petroni, A., Pasteran, F., Faccone, D., Galas, M., Drugeon, H., Cattoir, V. & Nordmann, P. 2007. Identification of the novel narrow-spectrum β-Lactamase SCO-1 in *Acinetobacter* spp. from Argentina. *Antimicrobial Agents and Chemotherapy* 51(6): 2179-2184.

Price, M.N., Dehal, P.S. & Arkin, A.P. 2009. FastTree: Computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution* 26(7): 1641-1650.

Reddy, G.S.N., Pradhan, S., Manorama, R. & Shivaji, S. 2010. *Cryobacterium roopkundense* sp. nov., a psychrophilic bacterium isolated from glacial soil. *International Journal of Systematic and Evolutionary Microbiology* 60(4): 866-870.

Seemann, T. 2014. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 30(14): 2068-2069.

Sengupta, S., Chattopadhyay, M.K. & Grossart, H.P. 2013. The multifaceted roles of antibiotics and antibiotic resistance in nature. *Frontiers in Microbiology* 47(4). https://doi.org/10.3389/fmicb.2013.00047

Silva, L.J., Crevelin, E.J., Souza, D.T., Lacerda-Júnior, G.V., de Oliveira, V.M., Ruiz, A.L.T.G., Rosa, L.H., Moraes, L.A.B. & Melo, I.S. 2020. Actinobacteria from Antarctica as a source for anticancer discovery. *Scientific Reports* 10(1): 13870.

Simão, F.A., Waterhouse, R.M., Ioannidis, P., Kriventseva, E.V. & Zdobnov, E.M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31(19): 3210-3212.

Spellberg, B. & Gilbert, D.N. 2014. The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases* 59(2): S71-75.

Suzuki, K.I., Sasaki, J., Uramoto, M., Nakase, T. & Komagata, K. 1997. *Cryobacterium psychrophilum* gen. nov., sp. nov., nom. rev., comb. nov., an obligately psychrophilic Actinomycete to accommodate "*Curtobacterium psychrophilum*" Inoue and Komagata 1976. *International Journal of Systematic Bacteriology* 47(2): 474-478.

Teoh, C.P., Wong, C.M.V.L., Lee, D.J.H., González, M.A., Najimudin, N., Lee, P.C. & Cheah, Y.K. 2018. Genome sequences of two cold-adapted *Cryobacterium* spp. SO1 and SO2 from Fildes Peninsula, Antarctica. *Current Science* 115(9): 1706-1708.

Ventola, C.L. 2015. The antibiotic resistance crisis: part 1: Causes and threats. *P&T: A Peer-Reviewed Journal for Formulary Management* 40(4): 277-283.

Ye, R., Xu, H., Wan, C., Peng, S., Wang, L., Xu, H., Aguilar, Z.P., Xiong, Y., Zeng, Z. & Wei, H. 2013. Antibacterial activity and mechanism of action of ε-poly-l-lysine. *Biochemical and Biophysical Research Communications* 439(1): 148-153.

Zhang, D.C., Wang, H.X., Cui, H.L., Yang, Y., Liu, H.C., Dong, X.Z. & Zhou, P.J. 2007. *Cryobacterium psychrotolerans* sp. nov., a novel psychrotolerant bacterium isolated from the China No. 1 glacier. *International Journal of Systematic and Evolutionary Microbiology* 57(4): 866-869.

Zhong, X., Tian, Y., Niu, G. & Tan, H. 2013. Assembly and features of secondary metabolite biosynthetic gene clusters in *Streptomyces ansochromogenes*. *Science China Life Sciences* 56(7): 609-618.

*Corresponding author; email: michaelw@ums.edu.my