Complete genome sequence of *Microbulbifer* sp. CCB-MM1, a halophile isolated from Matang Mangrove Forest, Malaysia

Tsu Horng Moh¹, Nyok-Sean Lau¹, Go Furusawa¹ and Al-Ashraf Abdullah Amirul¹,²*

Abstract: *Microbulbifer* sp. CCB-MM1 is a halophile isolated from estuarine sediment of Matang Mangrove Forest, Malaysia. Based on 16S rRNA gene sequence analysis, strain CCB-MM1 is a potentially new species of genus *Microbulbifer*. Here we describe its features and present its complete genome sequence with annotation. The genome sequence is 3.86 Mb in size with GC content of 58.85%, harbouring 3313 protein coding genes and 92 RNA genes. A total of 71 genes associated with carbohydrate active enzymes were found using dbCAN. Ectoine biosynthetic genes, *ectABC* operon and *ask_ect* were detected using antiSMASH 3.0. Cell shape determination genes, *mreBCD* operon, *rodA* and *rodZ* were annotated, congruent with the rod-coccus cell cycle of the strain CCB-MM1. In addition, putative *mreBCD* operon regulatory gene, *bolA* was detected, which might be associated with the regulation of rod-coccus cell cycle observed from the strain.

Keywords: Complete genome sequence, *Microbulbifer*, Halophile, Mangrove, Estuarine sediment

Introduction

*Microbulbifer* sp. CCB-MM1 is a halophile isolated from an estuarine sediment sample taken from Matang Mangrove Forest, Malaysia. The genus *Microbulbifer* was proposed by González [1] with the description of *Microbulbifer hydrolyticus* which was isolated from marine pulp mill effluent. *Microbulbifer* are typically found in high-salinity environments including marine sediment [2], salt marsh [3], costal soil [4] as well as mangrove soil [5]. They were known for their capability to degrade a great variety of polysaccharides including cellulose [1, 5], xylan [1, 5, 6], chitin [1, 5, 6], agar [3, 6] and alginate [7]. *Microbulbifer* strains are potential sources of carbohydrate active enzymes with biotechnological interest. One of the species, *Microbulbifer mangrovi* had been reported with the ability to degrade more than 10 different polysaccharides [7].

Polysaccharides have a broad range of industrial applications. The most common storage polysaccharide, starch, can be used as food additives [8], excipients [9] and substrates in fermentation process to produce bioethanol [10]. Structural polysaccharides such as cellulose, chitosan and chitin, on the other hand, can be used to develop high-performance materials due to their renewability, biodegradability, biological inertness and low cost [11–13]. However, polysaccharides from natural sources are often not suitable for direct application. Chemical modifications involving the reactive groups (carboxyl, hydroxyl, amido, and acetamido groups) on the backbone of polysaccharide are required to alter their chemical and physical properties to suit the application purposes [14]. In the past years, explorations and researches are in favor of enzymatic method using carbohydrate active enzymes [15]. This alternative method offers the advantages of substrate specificity, stereospecificity, and environment friendly [16]. Hence, the discovery of novel carbohydrate active enzymes has great biotechnological interest and *Microbulbifer* strains are potential sources of these enzymes.

Therefore, we sequenced the genome of *Microbulbifer* sp. CCB-MM1 with primary objective to identify potential carbohydrate active enzyme coding genes. The genome
insights will serve as baseline for downstream analyses including enzyme activity assays and functional elucidation of these genes. To date, there are seven genomes of *Microbulbifer* publicly available from GenBank, namely *Microbulbifer agarilyticus* S89 (NZ_AFP00000000.1) [17], *Microbulbifer variabilis* ATCC 700307T (NZ_AQYJ0000000.1), *Microbulbifer elongatus* HZ11 (NZ_JELR00000000.1) [18], *Microbulbifer* sp. ZGT114 (LQBR00000000.1), *Microbulbifer thermotolerans* DAU221 (CP014864.4) [19], *Microbulbifer* sp. Q7 (LROY00000000.1) and *Microbulbifer* sp. WRN-8 (LRFG00000000.1). All of the *Microbulbifer* genomes are assembled to draft assembly only except the *Microbulbifer thermotolerans* DAU221 genome. Here we present the complete genome of *Microbulbifer* sp. CCB-MM1 and some insights from comparative analysis with seven other *Microbulbifer* genomes.

**Organism information**

**Classification and general features**

*Microbulbifer* sp. strain CCB-MM1 was isolated from mangrove sediment obtained from Matang Mangrove Forest. The isolation was done using the method previously described [20] with the use of H-ASWM (2.4% artificial sea water, 0.5% tryptone, 10 mM HEPES, pH 7.6) [21]. CCB-MM1 is a Gram-negative, aerobic, non-spore-forming and halophilic bacterium (Table 1). Its shape appears to be associated with its growth phases where it is rod-shaped at exponential phase (Fig. 1a) and cocci-shaped at stationary phase (Fig. 1b). The rod-shaped cell size ranges from approximately 1.3 to 2.5 µm in length and 0.3 µm in width while the diameter of coccus cells is approximately 0.6 µm. The colonies observed on agar plate are white in colour, circular, and raised with entire edge.

The 16S rRNA gene sequence of CCB-MM1 was amplified and sequenced using the universal primer pair 27F and 1492R [22]. The 16S rRNA gene sequence analysis was performed by using BLASTN [23] against NCBI 16S ribosomal RNA (Bacteria and Archaea) database. BLAST report revealed that the closely related strains include *Microbulbifer rhiZhospaeae* Cs16bT (98.1%), *Microbulbifer taiwanensis* CC-LN1-12T (97.3%), *Microbulbifer maritimus* TF-17T (97.4%), *Microbulbifer pacificus* SPO729T (97.3%), and *Microbulbifer gwangyangensis* GY2T (97.3%). Based on the threshold of Proteinobacteria-specific 16S rRNA gene sequence similarity at 98.7% [24], the analysis suggests that CCB-MM1 is a new species belonging to the genus *Microbulbifer*. To reconstruct a phylogenetic tree of *Microbulbifer*, the 16S rRNA sequences of other *Microbulbifer* type strains were downloaded from GenBank. Then, these sequences were aligned using MUSCLE [25, 26] and MEGA6 [27] was used to reconstruct a neighbour-joining tree [28] with 1000 replications of bootstrap method test [29].

![Table 1](image)

| MIGS ID | Property | Term | Evidence code* |
|---------|----------|------|----------------|
|         | Classification | Domain Bacteria | TAS [70] |
|         | Phylum | Proteobacteria | TAS [71] |
|         | Class | Gammaproteobacteria | TAS [72] |
|         | Order | Cellvibionales | TAS [73, 74] |
|         | Family | Microbulbiferaceae | TAS [73, 74] |
|         | Genus | Microbulbifer | TAS [1] |
|         | Species Unknown | IDA |
|         | Strain CCB-MM1 | IDA |
|         | Gram stain | Negative | IDA |
|         | Cell shape | Rod-coccus | IDA |
|         | Motility | Non-motile | IDA |
|         | Sporulation | Non-sporulating | NAS |
|         | Temperature range | Mesophile | NAS |
|         | Optimum temperature | 30 °C | NAS |
|         | pH range; Optimum | 6.0–9.0; 7.0 | IDA |
|         | Carbon source | Not reported | |
|         | Habitat | Estuarine sediment | IDA |
|         | Salinity | Halophile | NAS |
|         | Oxygen | Aerobic | IDA |
|         | Biotic relationship | Free-living | NAS |
|         | Pathogenicity | Non-pathogenic | NAS |
|         | Geographic location | Malaysia: Matang Mangrove Forest | IDA |
|         | Sample collection time | October 1, 2014 | IDA |
|         | Latitude | 4.8528 N | IDA |
|         | Longitude | 100.5577 E | IDA |
|         | Depth | 10 cm | IDA |
|         | Altitude | Not reported | |

*Evidence codes - IDA inferred from direct assay, TAS traceable author statement (i.e., a direct report exists in the literature), NAS non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from http://www.geneontology.org/G0.evidence.shtml of the Gene Ontology project [75].

shown in Fig. 2, CCB-MM1 formed a cluster with *M. rhizospaeae* Cs16bT in the phylogenetic tree.

**Genome sequencing information**

**Genome project history**

Genome of CCB-MM1 was sequenced in October 2015. The whole genome sequencing and annotation were done by Centre for Chemical Biology (Universiti Sains Malaysia). The complete genome sequence is
available in GenBank under the accession number CP014143. The project information is summarized in Table 2.

**Growth conditions and genomic DNA preparation**

CCB-MM1 was cultured aerobically in 100 mL of H-ASWM for overnight (16 h) at 30 °C with shaking. The genomic DNA was extracted using modified phenol-chloroform method [30]. The integrity of extracted genomic DNA was assessed by gel electrophoresis using 0.7% agarose gel and the quantification was done using NanoDrop 2000 Spectrophotometer (Thermo Scientific, USA).

**Genome sequencing and assembly**

The whole genome of CCB-MM1 was sequenced using PacBio RS II platform with P6-C4 chemistry (Pacific Biosciences, USA). Two SMRT Cells were used and 2,674,097,380 pre-filter polymerase read bases were obtained, which was approximately 692X coverage of the genome. The reads were assembled using HGAP3 protocol [31] on SMRT Portal v2.3.0 with reads more than 25,000 bp in length being used as seed bases. The assembly result was a circular chromosome with the size of 3,864,326 bp, average base coverage of 431X and 100% base calling. The assembled sequence was polished twice using the resequencing protocol until the consensus concordance reached 100%.

**Genome annotation**

The genome was annotated using Prokka 1.11 pipeline [32]. The pipeline uses Prodigal [33], Rfam [34], Aragorn [35], SignalP [36] and Infernal [37] to predict the coding sequences (CDS), ribosomal RNA genes, transfer RNA genes, signal leader peptides and non-coding RNAs, respectively. In addition, the translated CDS output by Prokka were used to BLAST against protein databases including non-redundant protein database (nr) from GenBank, Swiss-Prot and TrEMBL from UniProt [38], and KEGG database [39]. COG functional categories assignment was done using RPS-BLAST [40].

**Table 2** Project information

| MIGS ID | Property                  | Term                              |
|---------|---------------------------|-----------------------------------|
| MIGS-31 | Finishing quality         | Complete                          |
| MIGS-28 | Libraries used            | PacBio P6-C4 chemistry, size selected 10 kb library, two SMRT Cells |
| MIGS-29 | Sequencing platform       | PacBio RS II                      |
| MIGS-31.2| Fold coverage            | 431x                              |
| MIGS-30 | Assemblers                | HGAP 3, PacBio SMRT Analysis v2.3 |
| MIGS-32 | Gene calling method       | Prodigal                          |
|         | Locus tag                 | AUP74                             |
|         | Genbank ID                | CP014143                          |
|         | GenBank date of release   | September 30, 2016                |
|         | GOLD ID                   | Gp0156207                         |
|         | BIOPROJECT                | PRJNA305828                       |
| MIGS-13 | Source material identifier | SAMN04334609                      |
|         | Project relevance         | Environmental                     |
search against the COG database [41]. In addition, antiSMASH 3.0 [42] was used to identify biosynthetic gene clusters and dbCAN [43] was used to identify carbohydrate active enzymes.

**Genome properties**

CCB-MM1 only contains one circular chromosome and no plasmid. The size of the chromosome is 3,864,326 bp with an overall of 58.85% G + C content (Table 3). The complete genome consists of 3313 ORFs, 79 tRNA, 12 rRNA and 1 tmRNA genes. Of all the 3313 predicted ORFs, 2030 of them can be assigned with functional prediction and 2563 of them can be assigned to COG functional categories (Table 4). The circular map of the genome generated using CGView Comparison Tool [44] is depicted in Fig. 3.

**Insights from the genome sequence**

**Comparative genomics**

There are seven genomes of *Microbulbifer* strains publicly available in GenBank to date. To assess the relatedness between CCB-MM1 and publicly available *Microbulbifer* genomes, ANI values between the genotypes were calculated using method based on MUMmer alignment [45]. Based on the results (Table 5), the ANI values ranged from 85.58% (*Microbulbifer* sp. ZGT114 and *Microbulbifer* sp. WRN-8) to 83.45% (*Microbulbifer thermotolerans* DAU221). These ANI values fall below 95% [46], suggesting that CCB-MM1 represents a different species from the other seven sequenced species. Interestingly, the ANI value between genomes of *Microbulbifer* sp. ZGT114 and *Microbulbifer* sp. WRN-8 is 99.99%, which suggests that these two strains belong to the same species. The circular map comparing CCB-MM1 genome and seven other *Microbulbifer* genomes is shown in Fig. 4.

**Carbohydrate active enzymes**

dbCAN [43] was used to predict carbohydrate-active enzyme coding genes present in CCB-MM1 genome, particularly genes belonging to glycoside hydrolase and polysaccharide lyase families that could provide us the insights on carbohydrate degrading capability of CCB-MM1. The analysis was done by running HMMER3 [47]

### Table 3 Genome statistics

| Attribute                  | Value     | % of Total |
|----------------------------|-----------|------------|
| Genome size                | 3,864,326 | 100.00     |
| DNA coding (bp)            | 3,487,727 | 90.25      |
| DNA G + C (bp)             | 2,274,198 | 58.85      |
| DNA scaffolds              | 1         | -          |
| Total genes                | 3406      | 100.00     |
| Protein coding genes       | 3313      | 97.27      |
| RNA genes                  | 92        | 2.70       |
| Pseudo genes               | 1         | 0.03       |
| Genes in internal clusters | -         | -          |
| Genes with function prediction | 2030   | 59.62      |
| Genes assigned to COGs     | 2563      | 75.27      |
| Genes with Pfam domains    | 2856      | 83.88      |
| Genes with signal peptides | 403       | 11.84      |
| Genes with transmembrane helices | 851  | 24.99      |
| CRISPR repeats             | 0         | 0          |

*The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

### Table 4 Number of genes associated with general COG functional categories

| Code | Value | % age | Description                        |
|------|-------|-------|------------------------------------|
| J    | 229   | 6.9   | Translation, ribosomal structure and biogenesis |
| A    | 2     | 0.1   | RNA processing and modification   |
| K    | 127   | 3.8   | Transcription                     |
| L    | 111   | 3.3   | Replication, recombination and repair |
| B    | 0     | 0.0   | Chromatin structure and dynamics  |
| D    | 41    | 1.2   | Cell cycle control, cell division, chromosome partitioning |
| Y    | 0     | 0.0   | Nuclear structure                 |
| V    | 64    | 1.9   | Defense mechanisms                |
| T    | 109   | 3.3   | Signal transduction mechanisms    |
| M    | 218   | 6.6   | Cell wall/membrane/envelope biogenesis |
| N    | 8     | 0.2   | Cell motility                     |
| Z    | 2     | 0.1   | Cytoskeleton                      |
| W    | 3     | 0.1   | Extracellular structures          |
| U    | 48    | 1.4   | Intracellular trafficking, secretion, and vesicular transport |
| O    | 173   | 5.2   | Posttranslational modification, protein turnover, chaperones |
| X    | 3     | 0.1   | Mobilome: prophages, transposons  |
| C    | 180   | 5.4   | Energy production and conversion  |
| G    | 131   | 4.0   | Carbohydrate transport and metabolism |
| E    | 212   | 6.4   | Amino acid transport and metabolism |
| F    | 53    | 1.6   | Nucleotide transport and metabolism |
| H    | 113   | 3.4   | Coenzyme transport and metabolism |
| I    | 133   | 4.0   | Lipid transport and metabolism   |
| P    | 167   | 5.0   | Inorganic ion transport and metabolism |
| Q    | 55    | 1.7   | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 226   | 6.8   | General function prediction only  |
| S    | 224   | 6.8   | Function unknown                  |
| -    | 751   | 22.7  | Not in COGs                       |

*The total is based on the total number of protein coding genes in the annotated genome.*
scan using HMMs profile downloaded from dbCAN (version: dbCAN-fam-HMMs.txt.v4) with an e-value cut off of $1 \times 10^{-18}$ and coverage cut off of 0.35. A total of 71 carbohydrate-active genes were detected and further analysis of these genes using SignalP predicted that 25 of them contain signal peptides. As shown in Table 6, we had found 29 genes associated with GH families including GH3, GH5, GH13, GH16, GH20, GH23, GH31, GH38, GH103 and GH130, however, we found no genes associated with PL families in the genome. Annotation of the GH genes revealed that CCB-MM1 genome possesses genes encoding cellulase (GH5), alpha-amylase, pullulanase (GH13) and beta-glucanase (GH16) with potential interest for biotechnological applications. While gene coding for beta-hexosaminidase, one of the chitinolytic enzymes [48], is present in the genome of CCB-MM1, gene that codes for chitinase was not detected. This suggests that CCB-MM1 lacks the ability to degrade chitin, although further assays are required to confirm the phenotype.

**Rod-coccus cell cycle**

*Microbulbifer* were found to demonstrate rod-coccus cell cycle, in association with different growth phases [49].

![Fig. 3 Circular map of the genome of Microbulbifer sp. CCB-MM1 generated using CGView Comparison Tool [44]. Circles (from outside) representing the following: 1. COG functional categories for forward coding sequence; 2. Forward sequence features; 3. Reverse sequence features; 4. COG functional categories for reverse coding sequence; 5. GC content; 6. GC skew](image)

**Table 5** ANI value(%) between *Microbulbifer* sp. CCB-MM1 genome and seven other *Microbulbifer* genomes calculated using ANIm [45]

|            | CCB-MM1  | ZGT114  | WRN-8  | HZ11  | S89  | Q7    | ATCC 700307T | DAU221 |
|------------|----------|---------|--------|-------|------|-------|--------------|--------|
| CCB-MM1    | 100.00   | 85.58   | 85.58  | 84.75 | 84.65| 84.61 | 84.37        | 83.45  |
| ZGT114     | 85.58    | 100.00  | 99.99  | 84.65 | 84.64| 84.70 | 84.29        | 83.85  |
| WRN-8      | 85.58    | 99.99   | 100.00 | 84.65 | 84.65| 84.70 | 84.29        | 83.87  |
| HZ11       | 84.75    | 84.65   | 84.65  | 100.00| 85.23| 85.58 | 84.68        | 83.71  |
| S89        | 84.65    | 84.64   | 84.70  | 85.23 | 100.00| 85.03 | 84.77        | 83.66  |
| Q7         | 84.61    | 84.70   | 84.67  | 85.58 | 85.03| 100.00| 84.75        | 83.77  |
| ATCC 700307| 84.37    | 84.29   | 84.29  | 84.68 | 84.77| 84.75 | 100.00       | 83.59  |
| DAU221     | 83.45    | 83.85   | 83.87  | 83.71 | 83.66| 83.77 | 83.59        | 100.00 |

CCB-MM1 = *Microbulbifer* sp. CCB-MM1; ZGT114 = *Microbulbifer* sp. ZGT114; WRN-8 = *Microbulbifer* sp. WRN-8; HZ11 = *Microbulbifer* elongatus HZ11; S89 = *Microbulbifer* agarilyticus S89; Q7 = *Microbulbifer* sp. Q7; ATCC 700307T = *Microbulbifer* variabilis ATCC 700307T; DAU221 = *Microbulbifer* thermotolerans DAU221
This cell cycle was also observed in CCB-MM1. In CCB-MM1 genome, we found genes which are known to be involved in determining and maintaining the rod shape of bacteria, including mreBCD [50] (AUP74_00016, AUP74_00017 and AUP74_00018), rodA [51] (AUP74_01706) and rodZ [52] (AUP74_01850). BLAST analysis showed that these genes are present in all other Microbulbifer genomes. In addition, we detected the presence of general stress response gene, bolA, in all Microbulbifer genomes. It has been demonstrated that the overexpression of bolA in E.coli inhibited cell elongation and reduced the transcription of mreBCD operon [53]. The gene, mreB, and its product, actin homolog have been studied for their functions in several species of bacteria. This protein lies beneath the cell surface, forming actin-like cables which function as guidance for the synthesis of longitudinal cell wall [54]. While MreB is not essential in E. coli [55], it is found to be essential for Streptomyces coelicolor [56], Rhodobacter sphaeroides [57] and Bacillus subtilis [58]. In E. coli, depletion of MreB caused cells to change from rod-like to spherical shape but these cells were able to survive [59]. In contrast, the spherical-shaped B. subtilis cells eventually lyse. For CCB-MM1, the spherical-shaped cells do not lyse but grow into rod-shaped again after being transferred into fresh medium. We infer that mreB gene may have important functions in determining Microbulbifer cell shape and the rod-coccus cycle of Microbulbifer is likely regulated by BolA through inhibition of mreB transcription when triggered by stress.

**Secondary metabolites, ectoine**

Ectoine and hydroxyectoine are compatible solutes found primarily in halophilic bacteria. When triggered by osmotic stress, bacteria produce and accumulate them intracellularly to balance the osmotic pressure [60]. Apart from osmotic stress, they were also protectants against temperature stress [61]. A cluster of genes responsible for the biosynthesis of ectoine [62] has been identified in CCB-MM1 genome using antiSMASH 3.0 [42]. These genes encode for aspartate kinase (Ask_Ect) (AUP74_00280), L-ectoine synthase (EctC) (AUP74_00281), diaminobutyrate-2-oxoglutarate transaminase (EctB) (AUP74_00282), L-2,4-diaminobutyric acid acetyltransferase (EctA) (AUP74_00283) and HTH transcriptional regulator (AUP74_00284). The lack of the gene ectD, ectoine hydroxylase, in CCB-
Table 6 GH enzyme coding genes found in CCB-MM1 genome

| GH Family | Annotation                                    | Signal peptide | Locus tag     |
|-----------|-----------------------------------------------|----------------|---------------|
| 3         | Periplasmic beta-glucosidase precursor        | Yes            | AUP74_01723   |
|           | Periplasmic beta-glucosidase precursor        | No             | AUP74_01724   |
|           | Beta-hexosaminidase                           | No             | AUP74_02396   |
|           | Beta-hexosaminidase A precursor               | Yes            | AUP74_02833   |
| 5         | Cellulase (glycosyl hydrolase family S)       | No             | AUP74_03275   |
|           | hypothetical protein                          | No             | AUP74_03276   |
| 13        | Pullulanase precursor                         | Yes            | AUP74_00304   |
|           | Oligo-1,6-glucosidase                         | No             | AUP74_00394   |
|           | Cyclomaltoextrinase                           | Yes            | AUP74_00399   |
|           | 4-alpha-glucanotransferase                    | No             | AUP74_00401   |
|           | Alpha-amylose precursor                       | Yes            | AUP74_00413   |
|           | Sucrose phosphorylase                         | No             | AUP74_03226   |
| 16        | Glucan endo-1,3-beta-glucosidase A1 precursor | No             | AUP74_01725   |
|           | Beta-glucanase                                | Yes            | AUP74_01727   |
| 20        | NN\(^{4}\)-diacetylchitobiose precursor       | No             | AUP74_01890   |
| 23        | Membrane-bound lytic murein transglycosylase F precursor | Yes | AUP74_00546   |
|           | Membrane-bound lytic murein transglycosylase F precursor | No | AUP74_01553   |
|           | Membrane-bound lytic murein transglycosylase F precursor | Yes | AUP74_01554   |
|           | murein transglycosylase C                     | Yes            | AUP74_01596   |
|           | Membrane-bound lytic murein transglycosylase D precursor | Yes | AUP74_02266   |
|           | Soluble lytic murein transglycosylase precursor | Yes | AUP74_02385   |
|           | Membrane-bound lytic murein transglycosylase F precursor | No | AUP74_03185   |
|           | Membrane-bound lytic murein transglycosylase F precursor | No | AUP74_03186   |
|           | Membrane-bound lytic murein transglycosylase F precursor | Yes | AUP74_03326   |
| 31        | Alpha-xylosidase                              | Yes            | AUP74_00400   |
| 38        | Mannosylglycerate hydrolase                   | No             | AUP74_01043   |
| 103       | Membrane-bound lytic murein transglycosylase B precursor | Yes | AUP74_01186   |
|           | Membrane-bound lytic murein transglycosylase B precursor | Yes | AUP74_01707   |
| 130       | 4-O-beta-D-mannosyl-D-glucose phosphorylase   | No             | AUP74_03278   |

MM1 genome suggests that it only has the ability to synthesize ectoine but not hydroxyectoine. By using BLASTP, we searched and found similar gene cluster in other Microbulbifer genomes except Microbulbifer variabilis ATCC 700307\(^{\dagger}\). While the reason for the absence of these genes in Microbulbifer variabilis ATCC 700307\(^{\dagger}\) is unknown, our findings suggest that Microbulbifer utilized only ectoine instead of ectoine/hydroxyectoine mixture. The transcriptional regulator of ectoine operon, EctR, found in Methylophaga thalassica belongs to MarR family [63]. HTH transcriptional regulator (AUP74_00284) in CCB-MM1 also contains the conserved domain of MarR family. This implies that the HTH transcriptional regulator is likely the putative transcriptional regulator of ectoine operon in Microbulbifer. Ectoine has attracted considerable biotechnological interest due to its stabilizing effects that extend from proteins [64], nucleic acids [65] to whole cells [66]. Such properties allow it to be used in skin care product as cell protectants [66], protein stabilizers [67] and medical application as cryoprotectants in cryopreservation of human cells [68].

**Conclusion**

In this study we presented the complete genome sequence of Microbulbifer sp. CCB-MM1 with genome size of 3.86 Mb and G + C content of 58.85%. We discussed some insights on its phenotypic characteristics from the genomic perspective, covering carbohydrate active enzymes, rod-coccus cell cycle and secondary metabolite, ectoine. The genome sequence provides valuable information for functional elucidations of novel enzymes for both biotechnological application and fundamental research purposes.
Abbreviations

ANL: Average nucleotide identity; antiSMASH: Antibiotics & Secondary Metabolite Analysis Shell; CCB: Centre for Chemical Biology; dbCAN: Database for automated carbohydrate-active enzyme annotation; GH: Glycoside hydrolase; H-ASWM: High nutrient artificial seawater media; MM: Matang Mangrove; PL: Polysaccharide lyase

Acknowledgements

We would like to thank Balachandra Dinesh for isolating Microbulbifer sp. CCB-MM1 and Ka Kei Sam for extracting the genomic DNA. N.-S. Lau and G. Furusawa gratefully acknowledge the post-doctoral fellowships granted by Universiti Sains Malaysia. T.H. Moh also acknowledges the financial support provided by Ministry of Higher Education Malaysia (MOHE) through MyBrain15 MyMaster scholarship.

Funding

This work was conducted as part of the mangrove project supported by Research University (RU) mangrove project grant (1001/PCCB/8/70009) to Centre for Chemical Biology, Universiti Sains Malaysia.

Authors’ contributions

TH performed the genome assembly, annotation, bioinformatics analyses and wrote the manuscript. NS and GF designed the experiments and revised the manuscript. AAA coordinated the project and determined the project direction. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 4 October 2016 Accepted: 29 June 2017
Published online: 06 July 2017

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