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1 ***Robertkochia solimangrovi* sp. nov., isolated from**  
2 **mangrove soil, and emended description of the genus**  
3 ***Robertkochia***

4  
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19 **Keywords:** *Robertkochia solimangrovi*; polyphasic taxonomy; *Flavobacteriaceae*; mangrove

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21 The full length 16S rRNA gene of strain CL23<sup>T</sup> has been deposited at  
22 EMBL/DDBJ/GenBank with accession number [MK258111](#).

23 The whole genome shotgun project of strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> are  
24 available at EMBL/DDBJ/GenBank under accession [QKWN00000000](#) and [QXMP00000000](#)  
25 respectively.

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## 30 ABSTRACT

31 To date, there is sparse information for the genus *Robertkochia* with *Robertkochia marina*  
32 CC-AMO-30D<sup>T</sup> as the only described member. We report here a new species isolated from  
33 mangrove soil of Malaysia Tanjung Piai National Park and perform polyphasic  
34 characterization to determine its taxonomy position. Strain CL23<sup>T</sup> is a Gram-negative,  
35 yellow-pigmented, strictly aerobic, catalase-positive and oxidase-positive bacterium. The  
36 optimal growth conditions were determined to be at pH 7.0, 30–37°C and 1–2% (w/v) NaCl.  
37 The major respiratory quinone was menaquinone-6 (MK-6) and the highly abundant polar  
38 lipids were four unidentified lipids, a phosphatidylethanolamine and two unidentified  
39 aminolipids. The 16S rRNA similarity between CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> is  
40 96.67%. Strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> are clustered together and were  
41 distinguished from taxa of closely related genera in 16S rRNA phylogenetic analysis.  
42 Genome sequencing revealed the strain CL23<sup>T</sup> has a genome size of 4.4 Mbp and a G+C  
43 content of 40.72 mol%. Overall genome indexes (OGRIs) including digital DNA-DNA  
44 hybridization (dDDH) value and average nucleotide identity (ANI) are 17.70% and  
45 approximately 70%, below the cut-off 70% and 95% respectively, indicated that strain CL23<sup>T</sup>  
46 is a distinct species to that of *R. marina* CC-AMO-30D<sup>T</sup>. Collectively, based on phenotypic,  
47 chemotaxonomic, phylogenetic and genomic evidence presented, strain CL23<sup>T</sup> is proposed as  
48 a new species with the name *Robertkochia solimangrovi* sp. nov. (=KCTC 72252<sup>T</sup> =LMG  
49 31418<sup>T</sup>). An emended description of the genus *Robertkochia* is also proposed.

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*Flavobacteriaceae* is one of the widely spread bacterial families composed of 158 genera at the time of writing [1]. The genus *Robertkochia* was introduced by Hameed et al. in 2014 [2] as one of the new genera in the family *Flavobacteriaceae*. Until now, the genus consisted of a single species *Robertkochia marina* CC-AMO-30D<sup>T</sup>, which was isolated from surface seawater at Taichung harbour, Taiwan [2]. The species was described as Gram negative, strictly aerobic, orange-pigmented and with iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> G and iso-C<sub>17:0</sub> 3-OH as predominant fatty acids. The report for *Robertkochia* is scarce as the previous study only focused on taxonomic assignment with one species reported so far [2]. Furthermore, the genome of this genus and prospective application have not been studied or reported.

*Robertkochia* and many other members of the *Flavobacteriaceae* are halophilic or halotolerant bacteria that reside in diverse saline environments such as seawater, mangrove forest and marine sediment [3-5]. Mangroves are inter-tidal wetlands that connect terrestrial and marine ecosystems [6]. Due to periodic tidal flats, drastic changes in salinity and nutrient availability of the mangrove environment make it a unique ecosystem [7]. Free living and symbiotic bacteria in such environment were found to play essential roles in maintaining mangrove ecosystem such as recycling of organic matter and biotransformation of minerals [8-10]. It was estimated that less than 5% of species in mangrove environment have been described so far [11]. Therefore, it could be considered as one of the interesting areas to be explored. In the present study, strain CL23<sup>T</sup> was isolated from soil obtained from mangrove forest located at Tanjung Piai National Park, Johor, Malaysia. This strain was characterized using polyphasic approach (phenotypic, chemotaxonomic and genomic aspects) following the recommended guidelines [12, 13] and new criteria for classification [14] to elucidate its taxonomy position. The results indicated that strain CL23<sup>T</sup> represents a new species within *Robertkochia* genus, with the name *Robertkochia solimangrovi* sp. nov. is proposed.

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## 99 ISOLATION AND HOME HABITAT

100 Soil from the mangrove forest was sampled at Tanjung Piai National Park (GPS location:  
101 1°16'06.0" N, 103°30'31.2" E) in September 2017 with permit (CJB F No. 734342) granted  
102 by Johor National Parks Corporation. The soil samples were serially diluted with sterile  
103 distilled water ( $10^{-1}$  to  $10^{-8}$ ). A 0.1 ml of diluted sample was spread onto marine agar 2216  
104 (MA; BD Difco) and incubated at 30–35°C for 1 to 14 days. A yellow-pigmented strain  
105 designated as CL23<sup>T</sup> was isolated from MA and re-streaked twice to obtain a pure culture.  
106 The strain was maintained in marine broth 2216 (MB; BD Difco) with 20 % (v/v) glycerol at  
107 –80°C. Strain CL23<sup>T</sup> was deposited at Korean Collection for Type Cultures (KCTC) and  
108 Belgian Co-ordinated Collections of Micro-organisms (BCCM) under accession of KCTC  
109 72252<sup>T</sup> and LMG 31418<sup>T</sup>, respectively. For comparative polyphasic taxonomy  
110 characterization, *R. marina* CC-AMO-30D<sup>T</sup> (=JCM 18552<sup>T</sup>) was obtained from Japan  
111 Collection of Microorganisms (JCM). Both strains were routinely cultured on MA and in MB  
112 at 30°C for 48 h, unless specified otherwise.

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## 116 16S rRNA PHYLOGENY

117 Genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen) and was purified  
118 by DNA Clean and Concentrator<sup>TM</sup>-25 (Zymo Research) following manual instructions. The  
119 16S rRNA gene of strain CL23<sup>T</sup> was amplified by PCR using universal primers: 27F (5'-  
120 AGAGTTTGATCMTGGCTCAG-3') and 1525R (5'-AAGGAGGTGWTCARCC-3') [15].  
121 The 16S rRNA gene was sequenced at Apical Scientific Pte. Ltd., Seri Kembangan, Malaysia.  
122 After the sequencing, the raw sequences were trimmed, and the sequences were aligned using  
123 ClustalW. The nearly full-length 16S rRNA gene was searched against EzBioCloud database  
124 for identification. The amplified 16S rRNA gene of strain CL23<sup>T</sup> was also cross-checked  
125 with the genome data to ensure the acquisition of full-length gene (1522 bp). The 16S rRNA  
126 gene of strain CL23<sup>T</sup> (MK258111) shared highest similarity (96.67%) with *R. marina* CC-  
127 AMO-30D<sup>T</sup> (JX235674), which is below the accepted threshold of 98.7% for species  
128 delineation [14]. The 16S rRNA gene similarity was less than 94% between strain CL23<sup>T</sup> and

129 other members of closely related genera: *Joostella marina* En5<sup>T</sup> (93.82%), *Joostella*  
130 *atrarenae* M1-2<sup>T</sup> (93.82%), *Zhouia spongiae* HN-Y44<sup>T</sup> (93.75%) and *Pustulibacterium*  
131 *marinum* E403<sup>T</sup> (93.35%).

132  
133 Phylogenetic trees of 16S rRNA were built following the Neighbor-joining (NJ) [16] and  
134 Maximum Likelihood (ML) [17] algorithms using MEGA 7.0 software [18] based on 1000  
135 bootstrap replications [19] and Kimura-2 parameter. Following the 16S rRNA phylogenetic  
136 analysis (Fig. 1), strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> formed a clade in NJ and ML  
137 trees, confirming the placement of strain CL23<sup>T</sup> within *Robertkochia* genus. The high  
138 bootstrap value at the node separating the branch of strain CL23<sup>T</sup> and *R. marina* CC-AMO-  
139 30D<sup>T</sup> in 16S rRNA phylogenetic tree supported that these two strains are distinct between  
140 each other.

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## 144 PHENOTYPIC AND CHEMOTAXONOMIC CHARACTERIZATION

145 Colony morphology was observed on MA at 30°C after 48 h of incubation. The Gram  
146 staining was performed according to the protocol as described previously [20]. The malachite  
147 green staining was used to assess the presence of endospore in 7-day old cultures [21]. The  
148 Gram stain reaction and endospore formation were examined under light microscope (Nikon  
149 ECLIPSE E200). Cell morphology was examined under scanning electron microscope (SEM;  
150 JEOL JSM-IT300LV). The bacterial motility was investigated by hanging-drop approach  
151 [22]. The presence of flexirubin-type pigment was determined by flooding the cells with 20 %  
152 (w/v) KOH [12].

153

154 Catalase activity was detected by effervescence using 3 % (v/v) H<sub>2</sub>O<sub>2</sub> while oxidase activity  
155 was determined by oxidation of tetramethyl-*p*-phenylenediamine. Hydrolysis of starch, casein,  
156 L-tyrosine, hypoxanthine, xanthine, Tween 20, Tween 40, Tween 60, Tween 80,  
157 carboxymethyl-cellulose (CMC) and xylan were tested according to Smibert and Krieg [21].  
158 Bile esculin hydrolysis was investigated using the method of Facklam and Moody [23]. Other  
159 biochemical characteristics were revealed by API 20 E and API 20 NE kits (BioMérieux,  
160 France). Carbohydrate utilization and enzyme activity profile of both strains were

161 investigated by API 50 CHB and API ZYM kits (BioMérieux, France), respectively. All API  
162 kits were carried out by following the manufacturer's instructions with slight modification in  
163 which inoculation was supplemented up to 2 % (w/v) NaCl.

164

165 Growth under anaerobic condition was tested by incubating the bacteria on MA for 14 days at  
166 30°C using AnaeroGen (Oxoid) in an anaerobic jar (Mitsubishi Gas Chemical). Growth was  
167 tested on the following media: Reasoner's 2A agar (R2A; HiMedia), Nutrient agar (NA;  
168 Merck), Tryptic soy agar (TSA; Merck), Luria-Bertani agar (LBA; Conda) and Muller Hinton  
169 agar (MHA; Sigma) supplemented with 2 % (w/v) NaCl at 30°C for 7 days. The temperature  
170 range (4, 9, 15, 20, 25, 30, 37, 40, 42, 45 and 50°C) and the optimum temperature for growth  
171 were determined using MB at pH 7. The pH range (in intervals of 1.0 pH unit) and optimum  
172 pH for growth were investigated using MB at 30°C. The pH was adjusted with the following  
173 buffer systems: 50 mM citrate phosphate (pH 4–5), 50 mM sodium phosphate (pH 6–8) and  
174 50 mM glycine–NaOH (pH 9–10) [24]. The pH was verified after autoclaving. To test NaCl  
175 tolerance and optimal concentration, the bacteria were grown in a medium containing yeast  
176 extract (1.0 g l<sup>-1</sup>), peptone (5.0 g l<sup>-1</sup>), MgCl<sub>2</sub> (5.0 g l<sup>-1</sup>), MgSO<sub>4</sub>·7H<sub>2</sub>O (2.0 g l<sup>-1</sup>), CaCl<sub>2</sub> (0.5 g  
177 l<sup>-1</sup>), KCl (1.0 g l<sup>-1</sup>) and NaCl (0, 0.5, 1–11 %, w/v) [10].

178

179 Antibiotic susceptibility of bacteria against 21 antibiotics was tested using the disk diffusion  
180 method on MA at 30°C for 48 h [25]. The antibiotics disks (Oxoid) used were: ampicillin (10  
181 µg), bacitracin (10 IU), carbenicillin (100 µg), chloramphenicol (100 µg), clindamycin (2 µg),  
182 doxycycline (30 µg), erythromycin (60 µg), gentamicin (10 µg), kanamycin (50 µg),  
183 lincomycin (2 µg), minocycline (30 µg), neomycin (30 µg), novobiocin (5 µg), oleandomycin  
184 (15 µg), oxacillin (1 µg), penicillin G (10 IU), piperacillin (100 µg), polymyxin B (300 IU),  
185 rifampicin (5 µg), streptomycin (10 µg) and tetracycline (30 µg).

186

187 Strain CL23<sup>T</sup> was determined as a Gram negative, rod-shaped, non-spore forming, oxidase  
188 positive and catalase positive bacterium with motile ability by gliding. The colony was in a  
189 circular form with 0.5–1.0 mm diameter, smooth surface, convex elevation, entire margin and  
190 has translucent property on MA after 48 h incubation. Under SEM, cells of strain CL23<sup>T</sup> were  
191 0.2–0.4 µm in width and 2.3–3.2 µm in length. The notable distinctive features to  
192 differentiate strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> are shown in Table 1. In terms of  
193 morphology, strain CL23<sup>T</sup> is yellow pigmented while *R. marina* CC-AMO-30D<sup>T</sup> was found  
194 to be orange pigmented. Strain CL23<sup>T</sup> grew well in 15–42°C, pH 5–9 and 0–9 % (w/v) NaCl,

195 and in general strain CL23<sup>T</sup> demonstrated a broader growth range compared to *R. marina*  
196 CC-AMO-30D<sup>T</sup> (Table 1). The optimal growth conditions of strain CL23<sup>T</sup> were observed at  
197 30–37°C, pH 7 and 1–2 % (w/v) NaCl. Strain CL23<sup>T</sup> was also able to produce acetoin, β-  
198 galactosidase and weakly positive toward amygdaline according to API 20 E but not for *R.*  
199 *marina* CC-AMO-30D<sup>T</sup>. Based on API ZYM, strain CL23<sup>T</sup> was able to produce α-  
200 galactosidase, β-galactosidase and α-mannosidase, which were absent in *R. marina* CC-  
201 AMO-30D<sup>T</sup>. Both strains were further distinguished by the hydrolysis capability of gelatin,  
202 Tween 20, Tween 40, Tween 60, and exhibiting resistance towards ampicillin, penicillin G,  
203 piperacillin and bacitracin (Table 1).

204

205 For the chemotaxonomic analysis, cellular fatty acids were extracted following the protocol  
206 of Microbial Identification System (MIDI, version 6.1) [26]. Biomass of strain CL23<sup>T</sup> and its  
207 reference strain *R. marina* CC-AMO-30D<sup>T</sup> were harvested from MA after 48 h of incubation  
208 at 30°C. The cells were saponified with methanolic base, then the resulting sodium salts of  
209 fatty acids were methylated. In the final step, methyl esters were transferred to the organic  
210 phase and washed. Fatty acid methyl esters were analyzed on an Agilent 6890 equipped with  
211 Ultra-2 capillary column and subsequently identified in the RTSBA6 library. As exhibited in  
212 Table 2, the predominant cellular fatty acid of strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup>  
213 were found to be iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> G and iso-C<sub>17:0</sub> 3-OH (> 10%). Nonetheless, some fatty  
214 acid patterns and abundance of strain CL23<sup>T</sup> varied when compared to *R. marina* CC-AMO-  
215 30D<sup>T</sup>, such as summed features 3 (3.64%) and 9 (5.24%) were constituted in strain CL23<sup>T</sup> but  
216 none for *R. marina* CC-AMO-30D<sup>T</sup>. On top of that, the amount of iso-C<sub>16:0</sub>, anteiso-C<sub>15:0</sub> and  
217 iso-C<sub>16:0</sub> 3-OH of strain CL23<sup>T</sup> are remarkably lower than *R. marina* CC-AMO-30D<sup>T</sup> (Table  
218 2).

219

220 The polar lipids and respiratory quinone analyses of strain CL23<sup>T</sup> were performed by Dr.  
221 Brian Tindall at the Identification Service, DSMZ, Braunschweig, Germany. In brief, the  
222 respiratory quinones were extracted by solvent methanol: hexane (2:1 v/v), separated by TLC  
223 and High Performance Liquid Chromatography (HPLC) following the standard method by  
224 Tindall [27]. The polar lipids were extracted using chloroform: methanol solvent and  
225 separated by two-dimensional silica gel thin layer chromatography (TLC) [28]. Total lipid  
226 material was identified using molybdotophosphoric acid and specific functional groups were  
227 determined using spray reagents specific for defined functional groups.

228



229 The major respiratory quinone of strain CL23<sup>T</sup> was identified to be menaquinone-6 (MK-6),  
230 which matched to *R. marina* [2] and other members in *Flavobacteriaceae* family [12]. In  
231 terms of polar lipids, strain CL23<sup>T</sup> has four unidentified lipids (L1, L2, L3 and L4), a  
232 phosphatidylethanolamine (PE) and two unidentified aminolipids (AL1 and AL2) as major  
233 polar lipids (Fig. S1). Additionally, three unidentified glycolipids (GL1, GL2 and GL3) and  
234 an unknown lipid (L5) were observed in minor amounts. The unidentified lipids (L1–L3) and  
235 glycolipids (GL1–GL3) were not detected in *R. marina* CC-AMO-30D<sup>T</sup> [2]. Moreover, an  
236 unidentified phospholipid (PL) was contained in *R. marina* CC-AMO-30D<sup>T</sup> in which this  
237 lipid was not found in strain CL23<sup>T</sup> [2].

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## 241 GENOMIC CHARACTERIZATION

242 The genome of reference strain *R. marina* CC-AMO-30D<sup>T</sup> was not available at the time of  
243 study, therefore, both the genomes of strain CL23<sup>T</sup> (NCBI accession: QKWN000000000) and  
244 *R. marina* CC-AMO-30D<sup>T</sup> (NCBI accession: QXMP000000000) were sequenced in this study.  
245 Whole genome sequencing of strain CL23<sup>T</sup> was accomplished on an Illumina HiSeq 2500  
246 platform (2 × 150 bp). The raw reads were filtered, and the quality data was *de novo*  
247 assembled using SOAPdenovo 2.04 [29]. The resulting genome was annotated using NCBI  
248 Prokaryotic Genome Annotation Pipeline (PGAP) [30].

249

250 The assembled genome of strain CL23<sup>T</sup> consists of 23 contigs with 322× depth of sequencing  
251 coverage (average), made up the size of genome with 4,407,290 bp in length and a GC  
252 content of 40.72 mol%. The genome size of strain CL23<sup>T</sup> is significantly larger than *R.*  
253 *marina* CC-AMO-30D<sup>T</sup> (3,571,649 bp). The GC content of strain CL23<sup>T</sup> is slightly lower  
254 than *R. marina* CC-AMO-30D<sup>T</sup> (43.67 mol%). Based on PGAP annotation, a total of 3669  
255 protein coding genes was found in genome of strain CL23<sup>T</sup>. The genes responsible for  
256 phosphatase activity were found in the genome of strain CL23<sup>T</sup> and *R. marina* CC-AMO-  
257 30D<sup>T</sup> with a total of 12 and 7 phosphatases were encoded respectively (Table S1). This  
258 correlated to API ZYM results in which both strains were positive to acidic and alkali  
259 phosphatases. Notably, the number of phosphatases annotated is higher in strain CL23<sup>T</sup> as  
260 compared to *R. marina* CC-AMO-30D<sup>T</sup>. On the other hand, strain CL23<sup>T</sup> consists of a series

261 of genes for assimilatory sulfate reduction into sulfite (sulfate adenylyltransferase subunit  
262 CysN and CysD, adenylylsulfate kinase and phosphoadenylylsulfate reductase) and then  
263 sulfite reduction into sulfide (FAD-binding oxidoreductase and LLM class flavin-dependent  
264 oxidoreductase) (Table S1). Nevertheless, the genes responsible for reduction of sulfite to  
265 sulfide are absent in *R. marina* CC-AMO-30D<sup>T</sup> (Table S1). Furthermore, strain CL23<sup>T</sup> also  
266 encodes a set of genes for reduction of nitrate to ammonia (*NirBD* and *NrfAH*) in which  
267 *NirBD* genes were not found in genome of *R. marina* CC-AMO-30D<sup>T</sup> (Table S1). These  
268 genes suggest that strain CL23<sup>T</sup> participates in nutrient recycling in mangrove environments.

269

270 Multilocus sequence analysis (MLSA) was conducted on five housekeeping genes of strain  
271 CL23<sup>T</sup>, *R. marina* CC-AMO-30D<sup>T</sup> and related genera, which the sequences were retrieved  
272 from genome data. The sequences of housekeeping genes were aligned individually and then  
273 concatenated in the following order: *rpoB*–*gyrB*–*recA*–*mutL*–*atpD*. The phylogenetic tree of  
274 concatenated housekeeping genes was constructed using MEGA 7.0 similarly as described in  
275 section “16S rRNA phylogeny”. In this tree (Fig. 2), strain CL23<sup>T</sup> and *R. marina* CC-AMO-  
276 30D<sup>T</sup> are clustered together but well distinguished from each other with high level of support  
277 (>90% bootstrap value). Likewise, the phylogenetic tree based on whole genome sequences  
278 that built using REALPHY 1.12 [31] also supported that both strain CL23<sup>T</sup> and *R. marina*  
279 CC-AMO-30D<sup>T</sup> are grouped in the same clade (Fig. S2).

280

281 To further underpin the classification of strain CL23<sup>T</sup> as a new species, the overall genome  
282 related indexes (OGRIs) were determined. Average nucleotide identity based on BLAST  
283 (ANIb) was calculated using JSpeciesWS [32]. ANI based on USEARCH (OrthoANIu) was  
284 determined by ChunLab's online ANI calculator [33]. The digital DNA-DNA hybridization  
285 (dDDH) value was calculated by Genome-to-Genome Distance Calculator [34].

286

287 The ANIb and OrthoANIu values between strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup> are  
288 69.35% and 70.47% respectively. These ANI values are below the recommended 95–96% for  
289 species delineation [35]. Similarly, the dDDH value between two strains was found to be  
290 17.70%, lower than 70%, the cut off for species boundary [34]. Combining the interpretation  
291 of ANI and dDDH values, the result revealed the identity of strain CL23<sup>T</sup> as a distinct species  
292 within the same genus as *R. marina* CC-AMO-30D<sup>T</sup>.

293

294 Based on polyphasic taxonomy characterization including phenotypic, chemotaxonomic,  
295 phylogenetic and genomic aspects, the results clearly indicated that strain CL23<sup>T</sup> (=KCTC  
296 72252<sup>T</sup> =LMG 31418<sup>T</sup>) represents a new species within the genus *Robertkochia*, for which  
297 the name *Robertkochia solimangrovi* sp. nov. is proposed.

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### 301 **DESCRIPTION OF *ROBERTKOCHIA SOLIMANGROVI* SP. NOV.**

302

303 *Robertkochia solimangrovi* sp. nov. (so.li.man.gro'vi. L. neut. n. *solum* soil; N.L. neut. n.  
304 *mangrovum* a mangrove; N.L. gen. n. *solimangrovi* of soil of a mangrove, pertaining to  
305 where the type strain was isolated.)

306 The cells are Gram-negative, rod shape, approximate 0.2–0.4 µm in width and 2.3–3.2 µm in  
307 length with motile ability by gliding. Colony is yellow-pigmented, in circular form with 0.5–  
308 1.0 mm diameter, smooth surface, convex elevation, entire margin and has translucent  
309 property after 48 hours incubation at 30°C on MA. Flexirubin-type pigment is absent. Cells  
310 are positive for oxidase and catalase. Growth occurs at 15–42 °C (optimum, 30–37°C), pH  
311 5–9 (optimum, pH 7) and in the presence of 0–9 % (w/v) NaCl (optimum, 1–2 % (w/v) NaCl).  
312 Grows well on MA, however, no growth is observed on R2A, NA, LBA, TSA and MHA  
313 media. No growth is observed on MA in anaerobic condition. The predominant fatty acids  
314 are iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> G and iso-C<sub>17:0</sub> 3-OH. The major isoprenoid quinone is menaquinone-6  
315 (MK-6). The major polar lipids are four unidentified lipids, a phosphatidylethanolamine and  
316 two unidentified aminolipids. Xylan, esculin, Tween 20, 40 and 60 are hydrolyzed. L-  
317 Tyrosine is weakly hydrolyzed. Casein, starch, CMC, Tween 80, xanthine and hypoxanthine  
318 are not hydrolyzed. In the API 20 E strip, positive for ONP-β-D-galactopyranoside and  
319 acetoin production; weakly positive for fermentation/oxidation of amygdaline; negative for  
320 arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase,  
321 urease and gelatinase, production of H<sub>2</sub>S and indole, utilization of citrate,  
322 fermentation/oxidation of D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, D-  
323 saccharose, D-melibiose and L-arabinose. In the API 20 NE strip, positive for hydrolysis of  
324 pNP-β-D-galactopyranoside and esculin ferric citrate; negative for nitrate reduction, indole

325 production, arginine dihydrolase, gelatinase and urease, fermentation of D-glucose and  
326 assimilation of D-glucose, D-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-  
327 maltose, potassium gluconate, capric acid, adipic acid, malic acid and phenylacetic acid. In  
328 the API 50 CHB strip, acid is produced from D-galactose, D-glucose, D-mannose, esculin  
329 ferric citrate, D-cellobiose, D-maltose, D-lactose, D-melibiose, D-saccharose, D-trehalose, D-  
330 melezitose, D-raffinose, amidon, glycogen and gentibiose; acid is weakly produced from  
331 methyl- $\alpha$ -D-glucopyranoside, arbutin, salicin and D-turanose; acid is not produced from  
332 glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol,  
333 methyl- $\beta$ -D-xylopyranoside, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol,  
334 D-sorbitol, methyl- $\alpha$ -D-mannopyranoside, N-acetyl-glucosamine, amygdalin, inulin, xylitol,  
335 D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate,  
336 potassium 2-ketogluconate and potassium 5-ketogluconate. In the API ZYM strip, alkali  
337 phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase,  
338 cystine arylamidase, trypsin, chymotrypsin, acid phosphatase, naphthol-AS-BI-  
339 phosphohydrolase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\alpha$ -glucosidase,  $\beta$ -glucosidase, N-acetyl-  
340  $\beta$ -glucosaminidase and  $\alpha$ -mannosidase are present; weak positive reaction for  $\alpha$ -fucosidase  
341 and negative results for lipase (C14) and  $\alpha$ -fucosidase. Cells are susceptible to carbenicillin,  
342 clindamycin, doxycycline, lincomycin, minocycline, novobiocin, oleandomycin, rifampicin  
343 and tetracycline, but not to ampicillin, bacitracin, chloramphenicol, erythromycin, gentamicin,  
344 kanamycin, neomycin, oxacillin, penicillin G, piperacillin, polymyxin B and streptomycin.

345

346 The type strain is CL23<sup>T</sup> (=KCTC 72252<sup>T</sup> =LMG 31418<sup>T</sup>), isolated from soil of mangrove  
347 collected from Tanjung Piai National Park, Johor, Malaysia.

348

349 The EMBL/DDBJ/GenBank accession for 16S rRNA gene of strain CL23<sup>T</sup> is [MK258111](#).  
350 Genome metrics are as follows: genome size, 4,407,290 bp; number of contigs, 23; G+C  
351 content, 40.72 mol%. The Whole Genome Shotgun project of strain CL23<sup>T</sup> is available at  
352 EMBL/DDBJ/GenBank under accession [QKWN00000000](#). The version described in this  
353 paper is QKWN01000000.

354

355 **EMENDED DESCRIPTION OF GENUS *ROBERTKOCHIA***

356

357 The characteristics of the genus *Robertkochia* are described according to Hameed et al. 2014  
358 [2] with following amendments and additional information. Oxidase is either positive or  
359 negative and catalase is positive. The DNA G+C content of the type strain of type species is  
360 43.67 mol% based on genome data. The Whole Genome Shotgun project of type strain of  
361 type species is available at EMBL/DDBJ/GenBank under accession [QXMP00000000](#). The  
362 version described in this paper is QXMP01000000.

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## 365 **AUTHOR STATEMENTS**

366

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386

### 387 *Ethical statement*

388 No human and animal experiments are involved.

389

390 *Conflicts of interest*

391 All authors declared that there are no conflicts of interest.

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393

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## 394 **ABBREVIATIONS**

395 ANI, average nucleotide identity, dDDH, digital DNA-DNA hybridization; MA, marine agar;  
396 MB, marine broth; MK, menaquinone; ML, maximum likelihood; NJ, neighbor-joining;  
397 OGRI, overall genome related index; PE, phosphatidylethanolamine.

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522 **FIGURES AND TABLES**

523

524 **Table 1.** Differential phenotypic characteristics of strain CL23<sup>T</sup> and *Robertkochia marina*  
 525 CC-AMO-30D<sup>T</sup>.

526 Strains: 1, strain CL23<sup>T</sup>; 2, *R. marina* CC-AMO-30D<sup>T</sup>. All data were obtained from this study.  
 527 +, Positive reaction; –, negative reaction; w, weakly positive reaction. All strains were  
 528 positive for catalase; hydrolysis of xylan and aesculin; production of acid from D-glucose,  
 529 esculin ferric citrate, D-cellobiose, D-maltose, D-saccharose, D-trehalose, D-melezitose,  
 530 amidon and glycogen in API 50 CHB strips; and activity of alkali phosphatase, esterase (C4),  
 531 esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin,  
 532 chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, β-  
 533 glucosidase and N-acetyl-β-glucosaminidase. Both strains were negative for flexirubin-type  
 534 pigment; growth under anaerobic condition; growth on R2A, NA, LBA, TSA and MHA  
 535 media; hydrolysis of casein, starch, CMC, Tween 80, xanthine and hypoxanthine; nitrate  
 536 reduction; indole and H<sub>2</sub>S production; urease; acid production from glycerol, erythritol, D-  
 537 arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside,  
 538 D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl-α-D-  
 539 mannopyranoside, N-acetyl-glucosamine, amygdalin, inulin, xylitol, D-lyxose, D-tagatose, D-  
 540 fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate and potassium 2-ketogluconate  
 541 and potassium 5-ketogluconate in API 50 CHB strips; and activity of lipase (C14) and β-  
 542 glucuronidase (API ZYM).

Characteristics	1	2
Colony pigmentation	Yellow	Orange
Oxidase	+	–
Growth parameters		
pH range	5–9	6–7
Temperature range (°C)	15–42	20–40
Temperature optimum (°C)	30–37	30
NaCl range (% w/v)	0–9	0.5–4
NaCl optimum (% w/v)	1–2	2
Hydrolysis of		
Tween 20	+	w
Tween 40	+	–
Tween 60	+	w
Tyrosine	w	–

Gelatin	–	+
Production of Acetoin	+	–
Oxidation of Amygdaline	w	–
Utilization of D-Galactose	+	–
D-Mannose	+	–
Arbutin	w	–
Salicin	w	–
D-Lactose	+	–
D-Melibiose	+	–
D-Raffinose	+	–
Gentiobiose	+	–
D-Turanose	w	–
Enzyme activity (API ZYM)		
α-Galactosidase	+	–
β-Galactosidase	+	–
α-Mannosidase	+	w
α-Fucosidase	w	–
Antibiotic susceptibility (per disc)		
Ampicillin (10 µg)	–	+
Penicillin G (10 IU)	–	+
Piperacillin (100 µg)	–	+
Bacitracin (45 µg)	–	+

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558 **Table 2.** Cellular fatty acid profiles (%) of strain CL23<sup>T</sup> and *Robertkochia marina* CC-AMO-  
559 30D<sup>T</sup>.

560 Strains: 1, strain CL23<sup>T</sup>; 2, *R. marina* CC-AMO-30D<sup>T</sup>. All data presented in the table are  
561 from this study. TR, trace ( $\leq 0.5\%$ ); –, not detected. Major components ( $> 10\%$ ) are  
562 highlighted in bold.

Fatty acid	1	2
<b>Branched saturated</b>		
iso-C <sub>13:0</sub>	TR	2.4
iso-C <sub>14:0</sub>	–	2.4
iso-C <sub>15:0</sub>	<b>21.8</b>	<b>19.9</b>
iso-C <sub>16:0</sub>	3.4	6.1
anteiso-C <sub>15:0</sub>	2.3	5.8
<b>Unsaturated</b>		
C <sub>15:1</sub> ω5c	0.7	–
C <sub>17:1</sub> ω6c	1.7	–
C <sub>17:1</sub> ω8c	0.8	–
<b>Branched unsaturated</b>		
iso-C <sub>15:1</sub> G	<b>10.8</b>	<b>23.3</b>
iso-C <sub>16:1</sub> G	–	1.6
iso-C <sub>16:1</sub> H	1.0	–
anteiso-C <sub>15:1</sub> A	TR	2.8
<b>Hydroxy</b>		
C <sub>15:0</sub> 2-OH	0.9	1.5
C <sub>15:0</sub> 3-OH	2.0	0.6
C <sub>16:0</sub> 3-OH	1.4	TR
C <sub>17:0</sub> 3-OH	1.1	TR
iso-C <sub>16:0</sub> 3-OH	2.6	6.5
iso-C <sub>17:0</sub> 3-OH	<b>29.5</b>	<b>15.5</b>
<b>Summed features *</b>		
3 †	3.6	–
9 #	5.2	–

563 \* Summed features are groups of two or three fatty acids that cannot be separated by GLC  
564 with the MIDI system.

565 † Summed feature 3 consisted of iso-C<sub>15:0</sub> 2-OH, C<sub>16:1</sub>ω6c and/or C<sub>16:1</sub>ω7c and annotated here as  
566 iso-C<sub>15:0</sub> 2-OH based on the equivalent chain length (ECL).

567 # Summed feature 9 consisted of iso-C<sub>17:1</sub>ω9c and/or C<sub>16:0</sub> 10-methyl.

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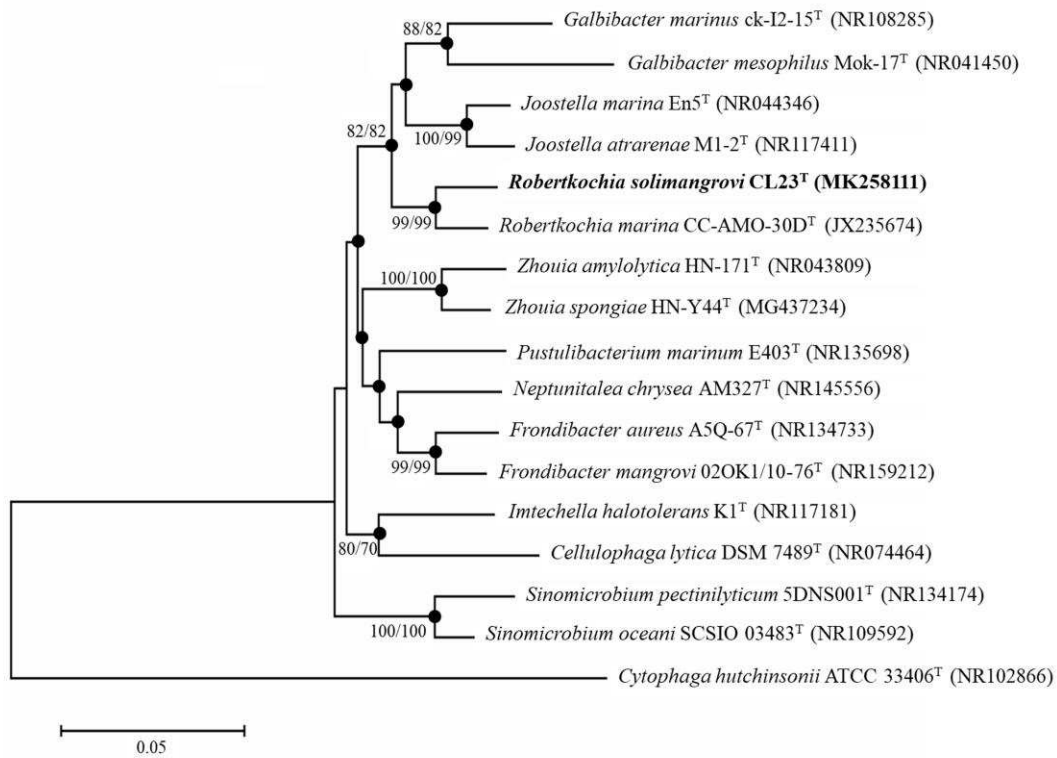
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572 **Figure legends**

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575 **Fig. 1.** Neighbor joining 16S rRNA phylogenetic tree manifesting the relationship of strain  
 576 CL23<sup>T</sup> with closely related taxa of family *Flavobacteriaceae*. Corresponding Genbank  
 577 accession numbers are indicated in parentheses. Bootstrap values  $\geq 70\%$  based on 1000  
 578 resampled datasets are depicted as percentages at nodes. Bootstrap value from left to right for  
 579 NJ and ML calculated with same sequence set. Filled circles indicate that corresponding  
 580 nodes were also recovered in dendrograms generated using ML algorithm. The sequence of  
 581 *Cytophaga hutchinsonii* ATCC 33406<sup>T</sup> was used as outgroup. Bar, 0.05 substitutions per  
 582 nucleotide position.

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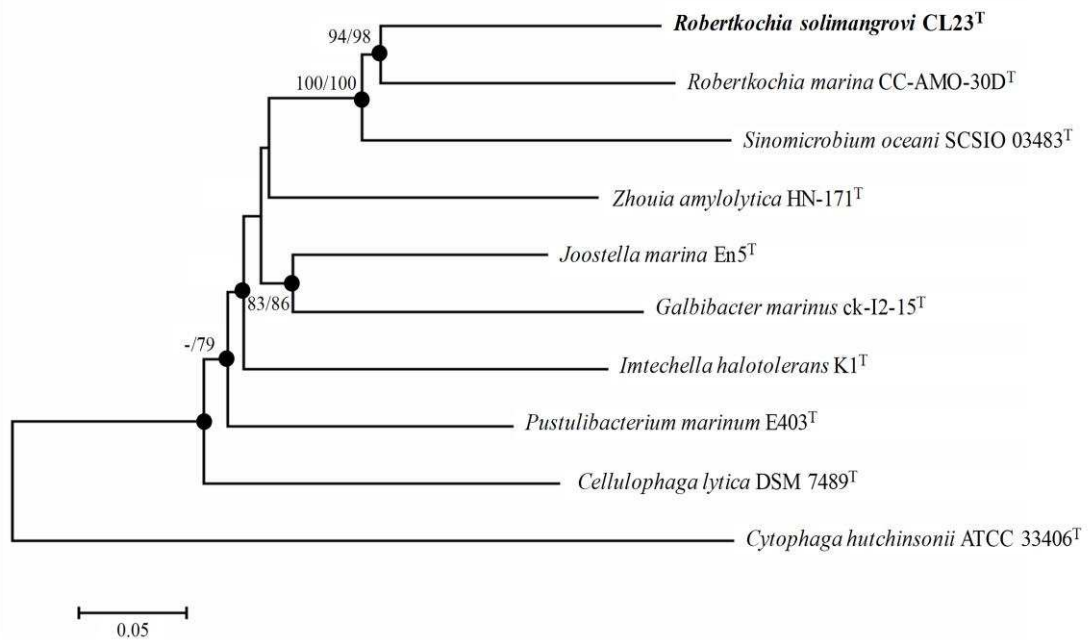
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 590 **Fig. 2.** Neighbor joining phylogenetic tree based on the concatenated sequences of five  
 591 housekeeping genes: *rpoB*–*gyrB*–*recA*–*mutL*–*atpD*, indicating the position of strain CL23<sup>T</sup>.  
 592 Bootstrap values  $\geq 70\%$  based on 1000 resampled datasets are depicted as percentages at  
 593 nodes; value  $< 70\%$  is indicated by a dash. Bootstrap value from left to right for NJ and ML  
 594 calculated with same sequence set. Filled circles indicate that corresponding nodes were also  
 595 recovered in dendrograms generated using ML algorithm. The sequence of *Cytophaga*  
 596 *hutchinsonii* ATCC 33406<sup>T</sup> was used as outgroup. Bar, 0.05 substitutions per nucleotide  
 597 position.

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## Supplementary Materials

### ***Robertkochia solimangrovi* sp. nov., isolated from mangrove soil, and emended description of the genus *Robertkochia***

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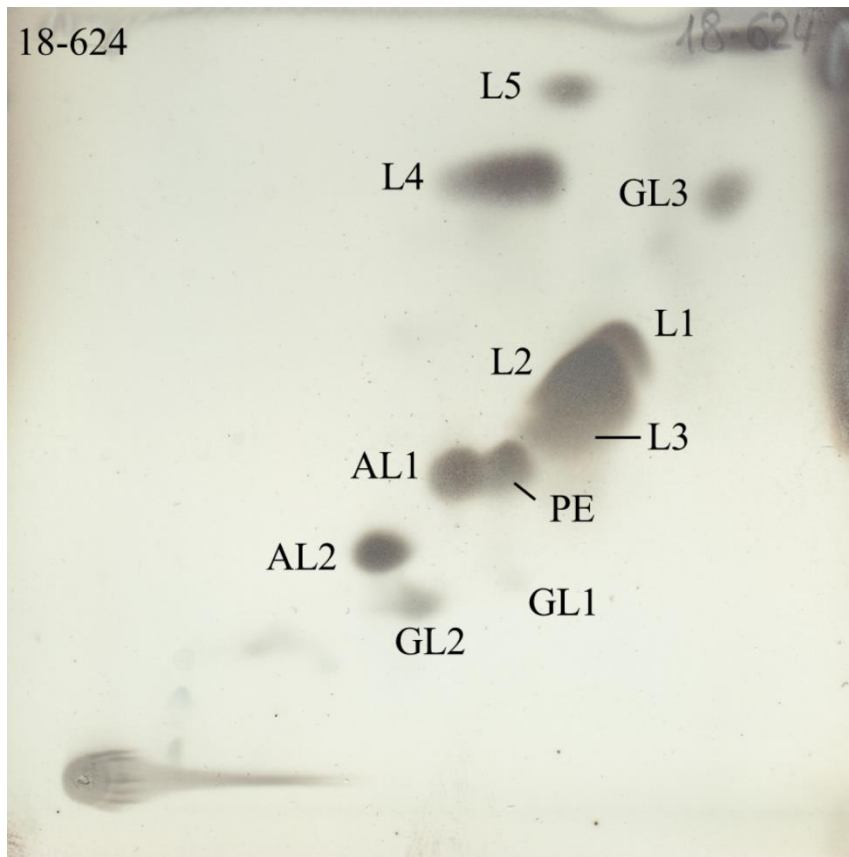
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<sup>3</sup> Johor National Parks Corporation, Kota Iskandar, 79575 Iskandar Puteri, Johor, Malaysia

<sup>4</sup> Centre for Novel Agricultural Products, Department of Biology, University of York, Wentworth Way, York, YO10 5DD, United Kingdom

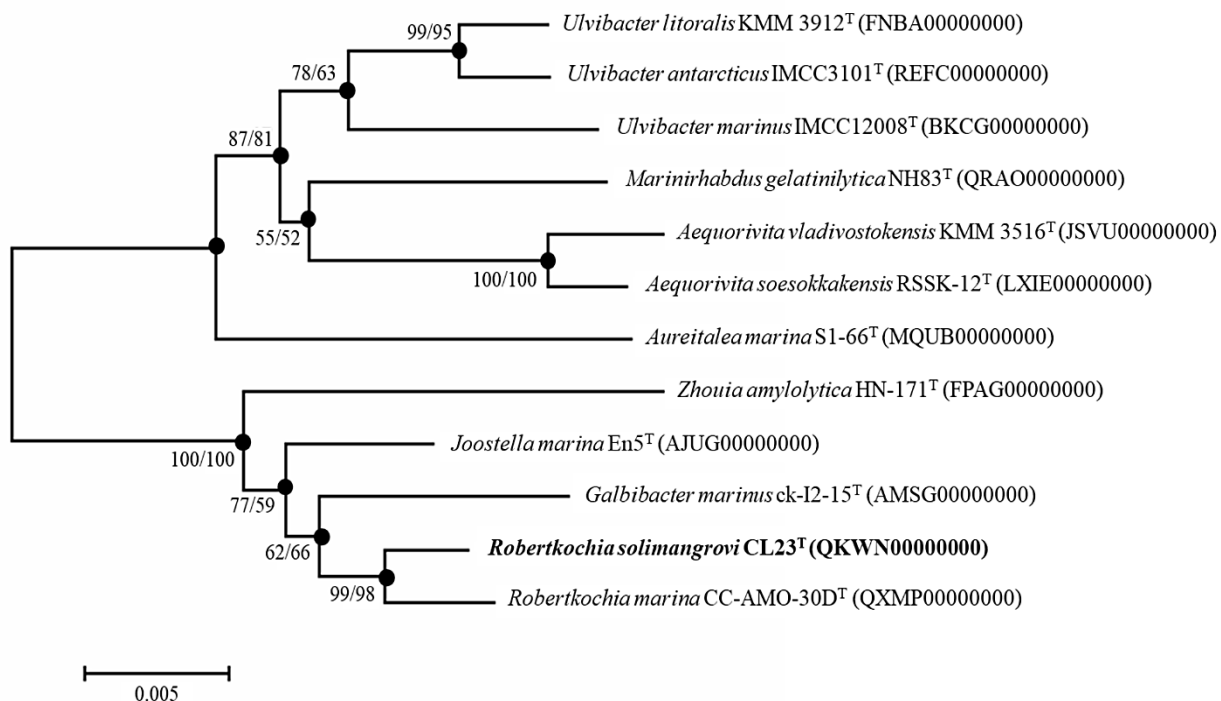
**\*Correspondence:** Chun Shiong Chong, [cschong@utm.my](mailto:cschong@utm.my); Neil C. Bruce, [neil.bruce@york.ac.uk](mailto:neil.bruce@york.ac.uk)

Supplementary figures



**Fig. S1.** Polar lipids profile of strain CL23<sup>T</sup>. Unidentified lipids; L1–L5, phosphatidylethanolamine; PE, unidentified aminolipids; AL1–AL2, unidentified glycolipids; GL1–GL3.





**Fig. S2.** Neighbor joining phylogenomic tree manifesting the relationship of strain CL23<sup>T</sup> with closely related taxa of family *Flavobacteriaceae*. Corresponding Genbank accession numbers are indicated in parentheses. Bootstrap values  $\geq 50\%$  based on 1000 resampled datasets are depicted as percentages at nodes. Bootstrap value from left to right for NJ and ML calculated with same sequence set. Filled circles indicate that corresponding nodes were also recovered in dendrograms generated using ML algorithm. Bar, 0.005 substitutions per nucleotide position.

## Supplementary tables

**Table S1.** List of potential genes for phosphatases, sulfur reduction and nitrate reduction encoded in the genome of strain CL23<sup>T</sup> and *R. marina* CC-AMO-30D<sup>T</sup>.

Category	Bacterial strain	NCBI Annotation	Accession		
Phosphatases	CL23 <sup>T</sup>	alkaline phosphatase family protein	TRZ44267		
		alkaline phosphatase	TRZ44500		
		alkaline phosphatase	TRZ44343		
		pyrophosphatase	TRZ44378		
		sodium-translocating pyrophosphatase	TRZ44400		
		alkaline phosphatase family protein	TRZ43533		
		alkaline phosphatase family protein	TRZ43596		
		alkaline phosphatase	TRZ42861		
		HAD family phosphatase	TRZ42760		
		alkaline phosphatase family protein	TRZ42969		
		alkaline phosphatase family protein	TRZ41972		
		HAD family phosphatase	TRZ41063		
		<i>R. marina</i> CC-AMO-30D <sup>T</sup>	alkaline phosphatase family protein	TRZ46762	
			alkaline phosphatase family protein	TRZ45488	
	alkaline phosphatase family protein		TRZ45685		
	sodium-translocating pyrophosphatase		TRZ44743		
	HAD family phosphatase		TRZ42656		
	pyrophosphatase		TRZ41149		
	Sulfur reduction	CL23 <sup>T</sup>	HAD family phosphatase	TRZ40862	
			sulfate adenylyltransferase subunit CysN	TRZ46029	
sulfate adenylyltransferase subunit CysD			TRZ46030		
adenylyl-sulfate kinase			TRZ46031		
phosphoadenylylsulfate reductase			TRZ44200		
phosphoadenylylsulfate reductase			TRZ42776		
FAD-binding oxidoreductase			TRZ41175		
LLM class flavin-dependent oxidoreductase			TRZ41182		
<i>R. marina</i> CC-AMO-30D <sup>T</sup>		sulfate adenylyltransferase subunit CysN	TRZ40960		
		sulfate adenylyltransferase subunit CysD	TRZ40970		
		adenylyl-sulfate kinase	TRZ40959		
		phosphoadenylylsulfate reductase	TRZ46694		
		Nitrate reduction	CL23 <sup>T</sup>	nitrite reductase ( <i>NirBD</i> )	TRZ44395
				nitrite reductase (NAD(P)H) ( <i>NirBD</i> )	TRZ42280

	nitrite reductase (NAD(P)H) small subunit ( <i>NirBD</i> )	TRZ42281
	NAD(P)H-nitrite reductase ( <i>NirBD</i> )	TRZ42287
	ammonia-forming cytochrome c nitrite reductase ( <i>NrfAH</i> )	TRZ42033
	cytochrome c nitrite reductase small subunit ( <i>NrfAH</i> )	TRZ42034
<i>R. marina</i> CC- AMO-30D <sup>T</sup>	ammonia-forming cytochrome c nitrite reductase ( <i>NrfAH</i> )	TRZ44150
	cytochrome c nitrite reductase small subunit ( <i>NrfAH</i> )	TRZ44178