

1 ***Marinobacter maroccanus* sp. nov., a moderately halophilic bacterium**  
2 **isolated from a saline soil**

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16  
17 **Keywords:** *Marinobacter maroccanus* sp. nov.; halophilic bacterium.

18  
19 The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *rpoD* gene  
20 sequences and the whole genome shotgun project of strain N4<sup>T</sup> are MG563241,  
21 MG551593, and PSSX01000000, respectively.

22

23 **Abstract**

24  
25 During the taxonomic investigation of exopolymer producing halophilic bacteria, a rod-  
26 shaped, motile, Gram-stain-negative, aerobic, halophilic bacterium, designated strain  
27 N4<sup>T</sup>, was isolated from a natural saline soil located in the northern Morocco. The optimal  
28 growth of the isolate was at 30–37 °C and at pH 6.0–9.0, in the presence of 5–7% (w/v)  
29 NaCl. Useful tests for the phenotypic differentiation of strain N4<sup>T</sup> from other *Marinobacter*  
30 species included α-chymotrypsin and α-glucosidase activities and the carbohydrate  
31 assimilation profile. The major fatty acids detected in strain N4<sup>T</sup> were C<sub>18:1</sub> ω9c, C<sub>16:0</sub>, and  
32 C<sub>16:1</sub> ω7c/C<sub>15:0</sub> iso 2-OH. Sequence analysis of the 16S rRNA indicated that strain N4<sup>T</sup>  
33 belonged to the genus *Marinobacter* and was closely related to *Marinobacter adhaerens*  
34 NC17506<sup>T</sup> (99.04%), *Marinobacter salsuginis* SD-14B<sup>T</sup> (98.97%), and *Marinobacter*  
35 *flavimaris* SW-145<sup>T</sup> (98.36%). Phylogenetic analysis of the *rpoD* gene sequence also  
36 showed that the nearest neighbours of strain N4<sup>T</sup> were *M. adhaerens* (90.63%) and *M.*  
37 *salsuginis* (91.13%). Strain N4<sup>T</sup> showed 87.98% similarity in the average nucleotide  
38 identity (ANI) with *M. flavimaris* and *M. salsuginis*, and 87.47% with *M. adhaerens*. In the  
39 in-silico genome-to-genome distance (GGD), strain N4<sup>T</sup> showed DNA-DNA hybridization  
40 (DDH) values of 33.30% with *M. adhaerens*, 34.60% with *M. flavimaris* and 34.70% with  
41 *M. salsuginis*. DNA G+C content of N4<sup>T</sup> was 57.3 mol%. Based on the results of  
42 phenotypic characterization, phylogenetic analysis and genome comparison, strain N4<sup>T</sup>  
43 represents a novel species of the genus *Marinobacter*, for which the name *Marinobacter*  
44 *maroccanus* sp. nov. is proposed. The type strain is N4<sup>T</sup> (=CECT 9525<sup>T</sup>=LMG 30466<sup>T</sup>).

45

46 The genus *Marinobacter* Gauthier *et al.* 1992 [1], with *Marinobacter*  
47 *hydrocarbonoclasticus* as its type species and 42 species with validly published names  
48 described until now, belongs to the family *Alteromonadaceae* within the  
49 *Gammaproteobacteria* [2]. Members of the genus *Marinobacter* are Gram negative, rod  
50 shaped, motile, mesophilic, halophilic, aerobic, oxidase- and catalase- positive, and can  
51 grow anaerobically by denitrification [3]. Most of them have been isolated from saline  
52 environments like seawater [4], sea sediments [5], marine aggregates [6], Antarctic  
53 environment [7], oil polluted saline soil [8] and saltern crystalizing pond [9]. This genus  
54 comprises many species that could be of great biotechnological interest since halotolerant  
55 and halophilic microorganisms are well known for their potential biotechnological  
56 applications [10].

57  
58 During a search of exopolymer producing halophilic bacteria, strain N4<sup>T</sup> was isolated from  
59 a natural saline soil of a wetland located in Douar Hjar Melaghi, in the Ouezzane province  
60 (34° 44' 33.006" N 5° 11' 19.803"W). The sample was diluted in 5% w/v sea salt solution,  
61 transferred to plates containing MY agar medium [11] supplemented with a 10% w/v of  
62 sea salt solution [12] and incubated at 37°C for 7 days. Isolated colony of strain N4<sup>T</sup> was  
63 selected and transferred to fresh plates of the same medium and maintained aerobically  
64 at 4°C and in MY broth containing 5% sea salt solution with 20% (v/v) glycerol at –80°C.

65  
66 Growth conditions, Gram staining, and motility were observed by light microscopy while  
67 morphology, cell size and shape of cells were determined by transmission electron  
68 microscopy. Growth at 0, 0.5, 1, 2, 3, 5, 7.5, 10, 15, 20, 25 and 30% (w/v) NaCl was  
69 assessed in MY agar at pH 7.0 and incubated at 30°C. Temperature and pH ranges were  
70 studied on solid MY supplemented with 5 % w/v sea salts solution. For temperature range,  
71 the plates were incubated at temperatures varying from 4 to 45°C. For pH range, the pH  
72 of the medium was adjusted to 5, 5.5, 6, 7, 8, 9 and 10. The plates were incubated for 7  
73 days for all the tests. Growth on MacConkey agar and Cetrimide agar both supplemented  
74 with 5% sea salt solution was tested. Oxidase and catalase activities and hydrolysis of  
75 starch, casein, gelatin, lecithin, DNA, tyrosine and Tween 20 and 80 were determined  
76 according to Barrow and Feltham (1993) [13]. API 20NE, API 50CH and API ZYM strips

77 (bioMérieux, France) were used to study the utilization of carbohydrates and enzymes  
78 activities. The inoculum was prepared by suspending colonies of strain N4<sup>T</sup> in a 5% (w/v)  
79 sea salt solution and transferred to the API strips following the recommendations of the  
80 manufacturer. In the case of API 50CH, the media was supplemented with a 5% (w/v) sea  
81 salt solution. The API 20NE, API CH50 and API ZYM tests were also done for reference  
82 strains *Marinobacter flavimaris* LMG 23834<sup>T</sup> and *Marinobacter guineae* M3B<sup>T</sup>.  
83 Susceptibility to antibiotics was tested according to the method described by Bauer *et al.*  
84 (1966) [14] using the following antibiotics: amoxycillin /clavulanic acid (30 µg), ampicillin  
85 (10 µg), ceftriaxone (30 µg), doxycycline hydrochloride (30 µg), nalidixic acid (30 µg),  
86 norfloxacin (10 µg), ofloxacin (5 µg), oxacillin (5 µg), penicillin G (6 µg), polymixin B (300  
87 µg), rifampicin (30 µg), spectinomycin (100 µg), sulphamethoxazole (25 µg) (Oxoid),  
88 cefuroxime (30 µg) (Bio-Rad), chloromphenicol (30 µg), ciprofloxacin (5 µg), gentamycin  
89 (30 µg), pristinomycin (15 µg) (Himedia) and vancomycin (30 µg) (Bioanalyse).

90  
91 For fatty acids analysis, strain N4<sup>T</sup> was cultivated on solid MY medium supplemented with  
92 5% sea salt solution for 2 days at 37°C. Fatty acids were extracted according to the  
93 protocol of the Microbial Identification System (Microbial ID; MIDI), and profiles were  
94 determined using the Sherlock Microbial Identification system (MIDI, database TSBA 40,  
95 version 4.10) [15].

96  
97 Genomic DNA was extracted using a genomic DNA extraction kit (REAL, Durviz S. L.,  
98 València, Spain). 16S rRNA gene was amplified by PCR using primers 16F27 [16] and  
99 16S15R [17]. Partial amplification of the gene *rpoD* with primers 70Fs and 70Rs [18] was  
100 also performed. The amplified products were purified using the ExoSAP-IT<sup>®</sup> (Affymetrix,  
101 Santa Clara, CA, USA). Purified PCR products were later sequenced by the Genomics  
102 Unit of Scientific and Technological Centers from University of Barcelona (CCiTUB) using  
103 the same primers as for the PCRs plus 16S11F and 16S5R primers [17] in the case of the  
104 gene 16S rRNA. Pairwise sequence similarity values between the obtained 16S rRNA  
105 sequences and reference sequences were calculated by the Identify tool included in the  
106 EzBioCloud portal (<http://www.ezbiocloud.net/>) [19]. Multiple sequence alignments and  
107 phylogenetic analysis of 16S rRNA and *rpoD* gene sequences from strain N4<sup>T</sup> (GenBank

108 accession nos. MG563241 and MG551593, respectively) and related species (taken from  
109 the GenBank database, [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) were performed using MEGA version 7  
110 [20]. Phylogenetic trees were constructed using the neighbour-joining method and their  
111 topological robustness was evaluated by bootstrap analysis based on 1,000 replicates.

112  
113 The whole genome of the strain N4<sup>T</sup> was sequenced by the Centre for Genomic  
114 Regulation (CRG, Barcelona) using Illumina Hi-seq platform (2 x 125 bp reads and  
115 501.49x coverage). Assembly of the contigs was performed with the program a5-  
116 assembler [21], Prokka [22] was used for the annotation of the genes and Mauve [23] for  
117 genome sequence alignment and reordering contigs according to the reference genome  
118 of *Marinobacter adhaerens* HP15<sup>T</sup> (NC\_017506). Genome annotation was also acquired  
119 from NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) [24]. The whole genome  
120 shotgun project of strain N4<sup>T</sup> was deposited in GenBank under the accession no.  
121 PSSX01000000. The in-silico genome-to-genome distance (GGD) between genomes  
122 was calculated using the genome-to-genome calculator 2.0 (GGDC), a digital DNA-DNA  
123 hybridization (dDDH) method provided by DSMZ (<http://ggdc.dsmz.de>) [25]. The average  
124 nucleotide identity (ANI) and the GC content were calculated using the software OAT  
125 hosted in the EzBioCloud portal [19, 26].

126  
127 Cells of strain N4<sup>T</sup> were rod-shaped, Gram-negative and motile. The strain grows  
128 optimally in the media that contains 5-7% NaCl (w/v) at pH 7-8 and 30-37°C. N4<sup>T</sup> was  
129 catalase- and oxidase-positive and able to hydrolyze lecithin and Tween 20 and 80. Many  
130 phenotypic characteristics differentiated strain N4<sup>T</sup> from other *Marinobacter* species,  
131 mainly for the  $\alpha$ -chymotrypsin and  $\alpha$ -glucosidase activities and the carbohydrate  
132 assimilation profile (Table 1). The API ZYM system revealed that the strain was positive  
133 for acid phosphatase, alkaline phosphatase, esterase (C4), esterase (C8), lipase (C14),  
134 cystine arylamidase, leucine arylamidase, valine arylamidase, naphthol-AS-BI-  
135 phosphohydrolase,  $\alpha$ -chymotrypsin,  $\alpha$ -glucosidase and N-acetyl- $\beta$ -glucosaminidase.  
136 The API 20NE system showed that the strain was positive for the reduction of nitrates to  
137 nitrites and the assimilation of D-maltose, malate and trisodium citrate. Using the API

138 50CH system, the strain was able to oxidize D-glucose, D-fructose, D-maltose, D-sucrose  
139 and glycerol.

140  
141 The fatty acid profile of strain N4<sup>T</sup> was similar to those of *Marinobacter* species previously  
142 described. The major fatty acids detected in strain N4<sup>T</sup> were C<sub>18:1</sub> ω9c (22.84%), C<sub>16:0</sub>  
143 (22.43%), C<sub>16:1</sub> ω7c/C<sub>15:0</sub> iso 2-OH (15.93 %), C<sub>12:0</sub> 3-OH (7.97%), C<sub>12:0</sub> (6.99%) and C<sub>16:1</sub>  
144 ω9c (4.86%) which are the most common fatty acids of the most phylogenetically closely  
145 related species (Table 2).

146  
147 Phylogenetic analysis based on 16S rRNA and *rpoD* gene sequences revealed that strain  
148 N4<sup>T</sup> belongs to the genus *Marinobacter* (Figs 1 and 2). Strain N4<sup>T</sup> showed the highest 16S  
149 rRNA gene sequence similarities with *Marinobacter adhaerens* HP15<sup>T</sup> (99.04%), *M.*  
150 *salsuginis* SD-14B<sup>T</sup> (98.97%), *M. flavimaris* SW-145<sup>T</sup> (98.36%), *M. similis*  
151 A3d10<sup>T</sup> (98.22%), *M. salinus* Hb8<sup>T</sup> (98.15%), *M. sediminum* R65<sup>T</sup> (98.15%) and *M.*  
152 *lipolyticus* SM19<sup>T</sup> (98.02%). Strain N4<sup>T</sup> could not be discriminated from *M. adhaerens* and  
153 *M. salsuginis* since 16S rRNA gene sequence similarities were >98.65%, the threshold  
154 for species delineation [27]. On the basis of *rpoD* gene sequences, the nearest neighbours  
155 of strain N4<sup>T</sup> were again the type strains of *M. adhaerens* and *M. salsuginis* (Fig. 2), with  
156 sequence similarities of 90.63 and 91.13%, respectively. These similarity values are below  
157 the species cut-off value (>97%) described for other genera as *Aeromonas* and  
158 *Pseudomonas* [28, 29], suggesting that this strain could represent a novel *Marinobacter*  
159 species.

160  
161 A total genome length of 4,340,695 bp was obtained from 61 contigs, with an *N*<sub>50</sub> of  
162 166,663 and a GC content of 57.3%. Genome annotation from PGAP revealed 4,007  
163 genes, with 3,899 coding genes, 3 complete rRNAs (5S, 16S, and 23S), 45 tRNAs, and 4  
164 ncRNAs. Based on 16S rRNA gene sequence similarities and phylogenetic positions,  
165 genomic sequences of the type strains of *M. adhaerens* (NC\_017506), *M. salsuginis*  
166 (PRJNA187995) and *M. flavimaris* (PSSW00000000) were selected for genome  
167 comparison. Strain N4<sup>T</sup> showed ANI values of 87.98% with *M. flavimaris* and *M.*  
168 *salsuginis*, and 87.47% with *M. adhaerens*, and DDH values of 34.70%, 34.60% and

169 33.30% with *M. salsuginis*, *M. flavimaris*, and *M. adhaerens*, respectively. As species  
170 boundary for ANI and DDH values are 95–96 and 70 %, respectively, genome comparison  
171 results confirmed that strain N4<sup>T</sup> constitutes a novel *Marinobacter* species [30].

172  
173 Based on the results of phenotypic characterization, phylogenetic analysis (16S rRNA and  
174 *rpoD* genes) and genome comparison (ANI and DDH), strain N4<sup>T</sup> represents a novel  
175 species of the genus *Marinobacter*, for which the name *Marinobacter maroccanus* sp. nov.  
176 is proposed.

177

### 178 **DESCRIPTION OF *MARINOBACTER MAROCCANUS* SP. NOV.**

179  
180 *Marinobacter maroccanus* (ma.roc.ca'nus. L. masc. adj. *maroccanus*, from the L.  
181 *Maroccanum Regnus*, Morocco, where the type strain was isolated).

182 Cells are rod-shaped (0.5×1.6–2 μm), Gram-stain-negative, aerobic, motile and non-  
183 spore-forming. After 2 days of incubation at 30°C, colonies on solid MY medium  
184 supplemented with 5% sea salt solution are circular (1.5–2 mm in diameter), smooth and  
185 brownish that darkens over incubation time. Temperature, salt and pH ranges for growth  
186 are 4–40°C, 2–15% NaCl and pH 6–9 respectively. Optimal growth occurs at 5–7% NaCl,  
187 30–37°C and pH 7–8. Not able to growth on MacConkey agar and Cetrimide agar. Cells  
188 are oxidase- and catalase-positive, reduce nitrate to nitrite. Indole, arginine dihydrolase  
189 and acid from glucose are not produced. Lecithin and Tween 20 and 80 are hydrolyzed  
190 but not casein, DNA, esculin, gelatin, starch, tyrosine or urea. Positive for the utilization  
191 of D-fructose, D-glucose, D-maltose, D-sucrose, glycerol, malate, trissodium citrate, but  
192 negative for adipic acid, amygdaline, arbutine, capric acid, D-adonitol, D- or L-arabinose,  
193 D- or L-arabitol, D-cellobiose, D-fucose, D-galactose, D-lactose, D-lyxose, D-mannitol, D-  
194 mannose, D-melezitose, D-melibiose, D-raffinose, D-ribose, D-sorbitol, D-tagatose, D-  
195 trehalose, D-turanose, D- or L-xylose, dulcitol, erythritol, esculine ferric citrate,  
196 gentiobiose, glycogen, inositol, inulin, L-rhamnose, L-sorbose, methyl αD-  
197 glucopyranoside, methyl αD-mannopyranoside, methyl-βD-xylopyranoside, N-  
198 acetylglucosamine, phenilacetic acid, potassium gluconate, potassium 2-ketogluconate,  
199 potassium 5-ketogluconate, salicin, starch or xylitol. Enzymatic activities are observed for

200 acid phosphatase, alkaline phosphatase,  $\alpha$ -chymotrypsin, cysteine arylamidase, esterase  
201 (C4), esterase (C8),  $\alpha$ -glucosidase, leucine arylamidase, lipase (C14), N-acethyl- $\beta$ -  
202 glucosaminidase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, but not for  $\alpha$ -  
203 fucosidase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase,  $\alpha$ -  
204 mannosidase or trypsin. Resistant to cefuroxime (30  $\mu$ g), ofloxacin (5  $\mu$ g), oxacillin (5  $\mu$ g),  
205 vancomycin (30  $\mu$ g), but susceptible to amoxicillin/clavulanic acid (30  $\mu$ g), ampicillin (10  
206  $\mu$ g), ceftriaxone (30  $\mu$ g), chloramphenicol (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), doxycycline  
207 hydrochloride (30  $\mu$ g), gentamycin (30  $\mu$ g), nalidixic acid (30  $\mu$ g), norfloxacin (10  $\mu$ g),  
208 penicillin G (6  $\mu$ g), polymixin B (300  $\mu$ g), pristinomycin (15  $\mu$ g), rifampicin (30  $\mu$ g),  
209 spectinomycin (100  $\mu$ g) and sulphamethoxazole (25  $\mu$ g). The predominant cellular fatty  
210 acids are C<sub>18:1</sub>  $\omega$ 9c (22.84%), C<sub>16:0</sub> (22.43%), C<sub>16:1</sub>  $\omega$ 7c/C<sub>15:0</sub> iso 2-OH (15.93 %), C<sub>12:0</sub> 3-  
211 OH (7.97%), C<sub>12:0</sub> (6.99%) and C<sub>16:1</sub>  $\omega$  9c (4.86%).

212  
213 The type strain, N4<sup>T</sup> (=CECT 9525<sup>T</sup>=LMG 30466<sup>T</sup>), was isolated from a hypersaline  
214 environment in Douar Hjar Melaghi, Ouezzane province, Morocco. The DNA G+C content  
215 of the type strain is 57.3 mol%.

216  
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222

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- 315

316 **Table 1.** Differential phenotypic characteristics of strain N4<sup>T</sup> and other species of the  
 317 genus *Marinobacter*

318 Strains: 1, N4<sup>T</sup>; 2, *M. adhaerens* HP15<sup>T</sup> [6]; 3, *M. flavimaris* SW-145<sup>T</sup> [31]; 4, *M. lipolyticus*  
 319 SM19<sup>T</sup> [32]; 5, *M. salinus* Hb8<sup>T</sup> [33]; 6, *M. salsuginis* SD-14B<sup>T</sup> [34]; 7, *M. sediminum* KMM  
 320 3657<sup>T</sup> [35]; 8, *M. similis* A3d10<sup>T</sup> [4]. +, Positive; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
NaCl range for growth (%)	2-15	0.5-20	1-20	1-15	0.5-15	1-20	0.5-18	0.5-20
Temperature range for growth (°C)	4-40	4-45	4-45	15-40	10-37	10-45	4-42	4-40
Nitrate reduction to nitrite	+	–	+	–	+	+	ND	+
Nitrite reduction to N <sub>2</sub>	–	–	–	–	–	+	–	–
Hydrolysis of:								
Casein	–	ND	–	ND	ND	+	–	ND
Gelatin	–	–	–	–	+	+	–	–
API ZYM test:								
α-Chymotrypsin	+	ND	–	ND	+	–	–	–
α-Glucosidase	+	ND	–	ND	–	–	–	–
Utilization of:								
D-Cellobiose	–	–	–	–	–	–	+	–
D-Fructose	+	–	+	+	–	–	–	–
D-Glucose	+	–	–	+	+	+	+	–
D-Maltose	+	–	–	+	+	–	–	–
D-Mannose	–	–	–	–	+	–	+	–
D-Sucrose	+	–	–	–	–	–	+	–
Citrate	+	–	–	–	+	–	–	–
Glycerol	+	–	–	–	ND	+	+	–
Malate	+	+	+	ND	+	+	–	ND
Mannitol	–	–	–	+	+	–	–	–
Phenylacetic acid	–	+	–	–	+	–	–	–
DNA G+C content (mol%)	57.3	56.90	58.00	57.00	54.51	55.90	56.50	57.60

321

322 **Table 2.** Fatty acid compositions (%) of strain N4<sup>T</sup> and other species of the genus  
 323 *Marinobacter*.

324 Strains: 1, N4<sup>T</sup>; 2, *M. adhaerens* HP15<sup>T</sup> [6]; 3, *M. flavimaris* SW-145<sup>T</sup> [31]; 4, *M. lipolyticus*  
 325 SM19<sup>T</sup> [32]; 5, *M. salinus* Hb8<sup>T</sup> [33]; 6, *M. salsuginis* SD-14B<sup>T</sup> [34]; 7, *M. sediminum* KMM  
 326 3657<sup>T</sup> [35]; 8, *M. similis* A3d10<sup>T</sup> [4]. –, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
C <sub>10:0</sub>	0.37	0.2	0.5	1.5	–	–	–	–
C <sub>12:0</sub>	6.99	6.00	9.1	8.3	6.6	7.3	4.15	0.60
C <sub>13:0</sub>	0.05	–	–	–	–	–	–	–
C <sub>14:0</sub>	1.29	1.2	1.1	–	0.9	1.1	0.92	1.00
C <sub>15:0</sub>	0.15	–	0.7	1.0	–	–	–	0.3
C <sub>16:0</sub>	22.43	21.7	26.7	28.5	19.8	22.9	21.78	15.20
C <sub>17:0</sub>	0.63	1.1	3.7	3.6	0.9	0.5	1.31	2.00
C <sub>18:0</sub>	2.03	3.4	3.3	2.7	1.1	2.90	2.20	4.90
C <sub>14:1</sub> ω7c	–	–	–	–	–	–	–	2.20
C <sub>16:1</sub> ω9c	4.86	9.0	10.2	10.5	12.8	10.5	13.28	8.10
C <sub>16:1</sub> ω7c	–	–	–	–	–	–	15.87	20.90
C <sub>16:1</sub> ω5c	0.17	–	–	–	–	–	–	–
C <sub>17:1</sub> ω8c	0.85	2.00	3.8	2.9	2.1	3.8	2.79	4.9
C <sub>18:1</sub> ω9c	22.84	21.6	17.4	13.9	11.7	17.2	16.12	21.20
C <sub>18:1</sub> ω7c	2.84	3.70	1.2	2.3	–	–	2.91	14.30
C <sub>18:3</sub> ω6c (6,9,12)	0.72	1.65	–	–	–	–	1.88	–
C <sub>19:0</sub> ω8c cyclo	–	–	1.7	–	–	–	–	–
C <sub>16:1</sub> ω6c/C <sub>16:1</sub> ω7c	–	–	–	–	13.6	13.10	–	–
C <sub>18:1</sub> ω6c/C <sub>18:1</sub> ω7c	–	–	–	–	2.1	3.00	–	–
C <sub>13:0</sub> iso	0.08	0.13	–	–	–	–	–	–
C <sub>15:0</sub> iso	0.06	–	–	–	–	–	–	–
C <sub>17:0</sub> iso	0.16	0.32	–	–	–	–	–	–
C <sub>10:0</sub> 3-OH	0.12	–	–	–	–	–	–	–
C <sub>11:0</sub> 3-OH	0.10	0.2	–	–	–	0.5	–	–
C <sub>12:0</sub> 2-OH	0.14	–	–	–	–	–	–	–
C <sub>12:0</sub> 3-OH	7.97	7.9	10.5	–	5.5	9.3	8.04	2.80
C <sub>12:1</sub> 3-OH	0.07	–	–	11.3	–	–	–	–

C <sub>15:0</sub> iso 3-OH	0.74	–	–	–	–	–	–	–
C <sub>16:1</sub> 2-OH	0.39	–	–	–	–	–	–	–
C <sub>18:0</sub> 3-OH	–	–	–	–	15.7	–	–	–
C <sub>14:0</sub> 3-OH/C <sub>16:1</sub> iso I	0.05	–	–	–	–	–	–	–
C <sub>16:1</sub> ω7c/C <sub>15:0</sub> iso 2-OH	15.93	14.6	6.8	–	–	–	–	–
C <sub>17:1</sub> iso ω9c/C <sub>16:0</sub> 10 methyl	–	–	–	–	4.5	–	–	–
C <sub>16:0</sub> N alcohol	2.40	3.19	–	–	–	–	–	–
C <sub>16:1</sub> ω7c alcohol	0.65	–	–	–	–	–	–	–
C <sub>16:0</sub> 10 methyl	3.56	0.6	0.2	4.0	–	2.6	–	–
C <sub>17:0</sub> 10 methyl	0.87	1.09	–	–	–	–	–	–

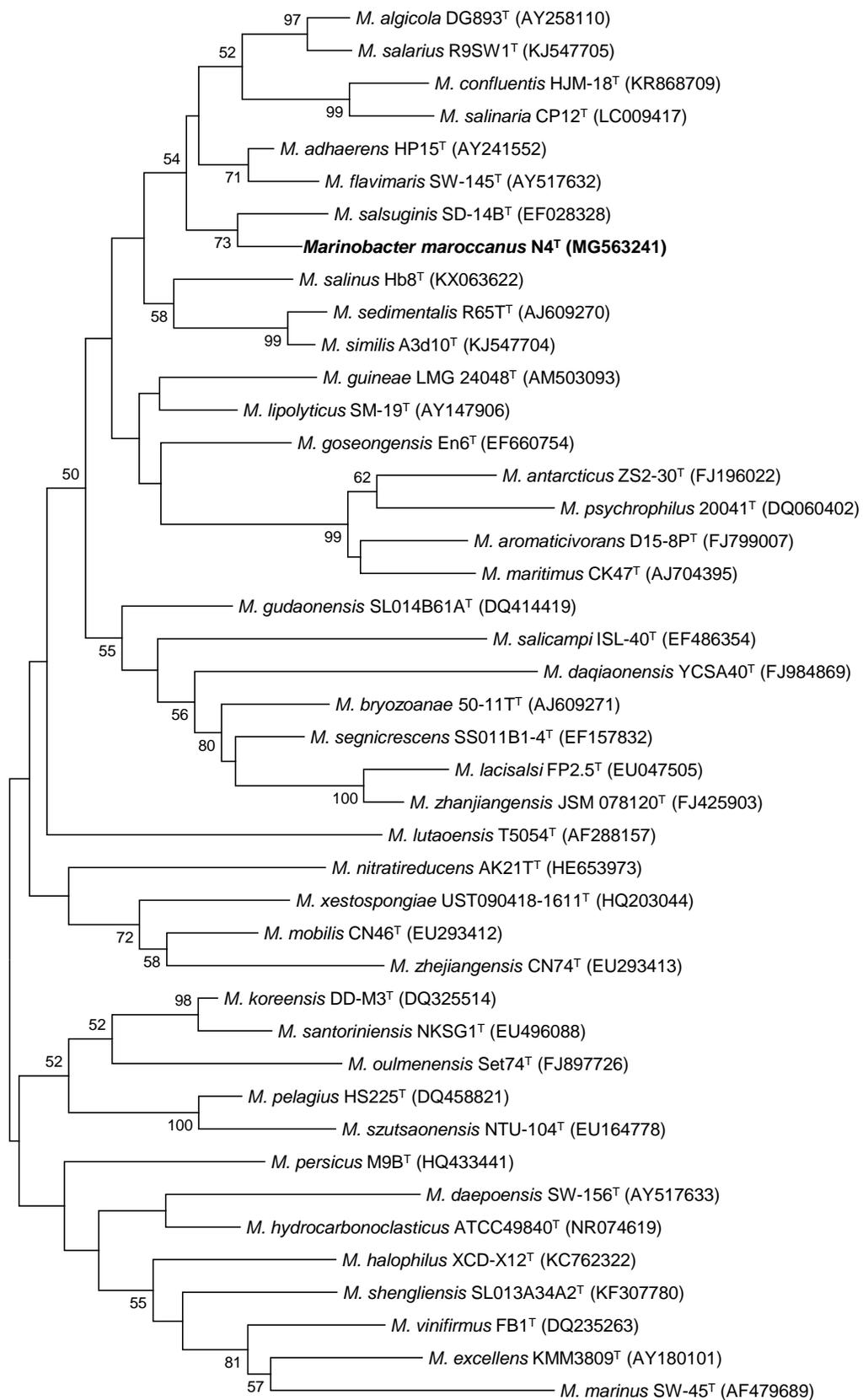
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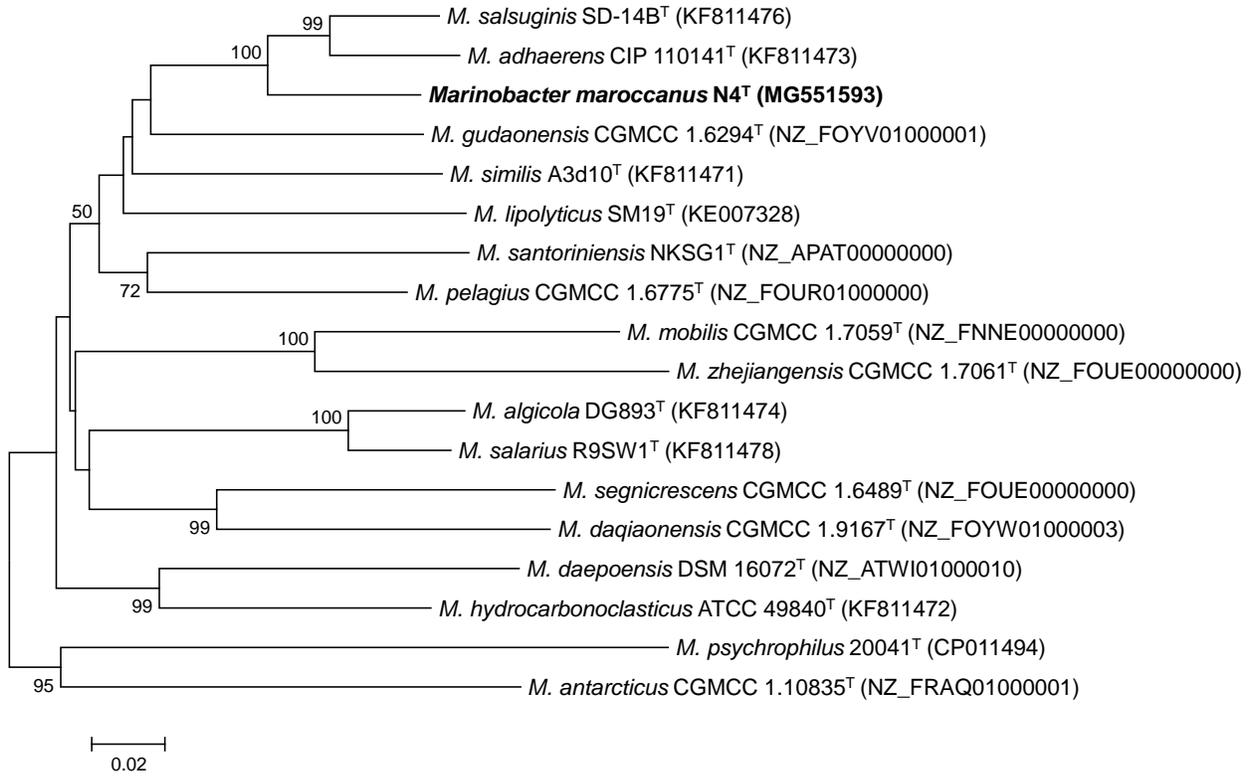
328 **FIGURE LEGENDS**

329  
330 **Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing  
331 the position of strain N4<sup>T</sup> with respect to other members of the genus *Marinobacter*.  
332 GenBank accession numbers are indicated in parentheses. Bar, distance of 0.005  
333 substitutions per nucleotide position as calculated by MEGA. Bootstrap values (>50 %)  
334 after 1,000 replicates are shown.

335  
336 **Fig 2.** Neighbour-joining phylogenetic tree based on *rpoD* gene sequences, showing the  
337 position of strain N4<sup>T</sup> with respect to other members of the genus *Marinobacter*. GenBank  
338 accession numbers are indicated in parentheses. Bar, distance of 0.02 substitutions per  
339 nucleotide position as calculated by MEGA. Bootstrap values (>50 %) after 1,000  
340 replicates are shown.

341





343