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ORIGINAL PAPER

Microbulbifer hainanensis sp. nov., a moderately halopilic bacterium isolated from mangrove sediment

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Abstract A new bacterium was successfully isolated from a mangrove sediment sample in Haikou City, Hainan Province, China. The organism is a Gram-negative, rod-shaped, non-motile and strictly aerobic bacterium, named NBU-8HK146^T. Strain NBU-8HK146^T was able to grow at temperatures of 10–40 °C, at salinities of 0–11% (w/v) and at pH 5.5–9.5. Voges–Proskauer, methyl red reaction and hydrolysis of Tween 20 were negative. Catalase and oxidase activities, H_2S production, hydrolysis of starch, casein, Tweens 40, 60 and 80 were positive. The major cellular fatty acids were $C_{16:0}$, iso-$C_{15:0}$ and summed feature 9. The major respiratory quinone was ubiquinone-8 (Q-8). The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and two unidentified glycolipids. According to 16S rRNA gene sequence similarities, strain NBU-8HK146^T shared 98.0%, 97.9%, 97.7%, 97.6% and 97.3% similarities to the species with validated name *Microbulbifer taiwanensis* CC-LN1-12^T, *Microbulbifer rhizosphaerae* Cs16b^T, *Microbulbifer marinus* Y215^T, *Microbulbifer donghaiensis* CN85^T and *Microbulbifer aggregans* CCB-MM1^T, respectively. Phylogenetic analyses indicated that strain NBU-8HK146^T formed a distinct lineage with strains *Microbulbifer taiwanensis* CC-LN1-12^T and *Microbulbifer marinus* Y215^T. Both digital DNA-DNA hybridization values (19.5–22.7%) and average nucleotide identity values (73.2–78.9%) between strain NBU-8HK146^T and related species of genus *Microbulbifer* were below the species delineation cutoffs. The DNA G+C content was 58.9 mol%. Many proteins involving in the adaption of osmotic stress in the salt environment of mangrove were predicted in genome of strain NBU-8HK146^T. From phenotypic, genotypic, phylogenetic and chemotaxonomic characteristics, strain NBU-8HK146^T can be regarded as a new *Microbulbifer* species for which the name *Microbulbifer hainanensis*. The type strain is NBU-8HK146^T (= KCTC 82226^T = MCCC 1K04737^T).

Keywords *Microbulbifer hainanensis* · Mangrove sediment · Polyphasic taxonomy · Moderately halopilic

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Introduction

Most of microorganisms (> 99%) do not grow on synthetic media in the laboratory and remain unexplored (Epstein 2013). In recent years, cultivation methodologies have been developed to obtain more challenging to cultivate more microbes. A novel method of in situ cultivation of environmentally relavant microorganisms was developed by Kaeberlein et al. (2002). This method places environmental cells into a membranous contraption and subsequently incubating this contraption in the natural habitat of the cells. The expectation is that naturally occurring nutrients and growth factors will diffuse in and enable growth of those cells that normally do not grow in the lab. In our recent survey of microbial diversity of mangrove sediments, a modified high-throughput ichip method (Nichols et al. 2010; Berdy et al. 2017) was used. In this way, a bacterial strain NBU-8HK146^T was isolated, and characterized by using a polyphasic taxonomic approach. Based on initially molecular characterization, it seemed to be a novel species of the genus *Microbulbifer*.

In 1997, the genus *Microbulbifer* was proposed to accommodate a Gram-negative, strictly aerobic gamma proteobacteria capable of utilizing a variety of hydrocarbons (González et al. 1997). At the time of writing, the genus *Microbulbifer* contained 25 validly published species (https://www.bacterio.net/genus/microbulbifer), which were isolated from diverse habitats such as pulp mill effluent (González et al. 1997), coastal soil (Kämpfer et al. 2012), marine sediment (Zhang et al. 2012; Wang et al. 2009; Xiong et al. 2019), deep-sea sediment (Miyazaki et al. 2008), estuarine sediment (Moh et al. 2017), marine solar saltern (Yoon et al. 2007), mangrove forests (Baba et al. 2011; Vashist et al. 2013), gastrointestinal tract of a purple sea urchin (Lee et al. 2017), marine algae (Nishijima et al. 2009), marine environments (Jeong et al. 2013), coastal sand (Huang et al. 2020), the rhizosphere of the halophyte (Camacho et al. 2016) and salt marshes (Yoon et al. 2003). Members of the genus *Microbulbifer* are Gram-staining negative, rod-shaped, strictly aerobic and oxidase-positive, with Q-8 as the predominant respiratory quinone. Following the polyphasic taxonomic approach, we propose that strain NBU-8HK146^T represent a novel species of the genus *Microbulbifer*.

Material and methods

Bacterial strains and culture condition

A mangrove sediment sample was collected from Haikou in Hainan Province, China (110°34′E, 19°57′N), in December 2017. Marine broth 2216 (MB, Difco) was used for a modified high throughput ichip in situ cultivation (Berdy et al. 2017). The medium was solidified with 2.0% agar (MA). About 3.0 g mangrove sediment sample was diluted, mixed with MA medium at 55 °C, and added to the holes of the modified ichips. Then, we returned the ichips to the mangrove sediment in Haikou (Zhang et al. 2018). After 2 weeks of in situ cultivation, many colonies appeared in the ichips, and a cream-colored colony was selected and purified by repeated restreaking on MB. The isolate was usually cultured in MB at 26 °C and stored at − 80 °C with 25% (v/v) glycerol. *Microbulbifer taiwanensis* LMG 26125^T, *Microbulbifer marinus* JCM 17211^T and *Microbulbifer hydrolyticus* DSM 11525^T were used as reference species for comparative studies.

Morphological, physiological and biochemical characterization

Cell morphology and motility were examined by means of optical microscope (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) after 2 days incubation at 26 °C on MA. The exponentially growing cells incubated on the MA plate were suspended and stained with uranyl acetate, then fixed on a copper mesh, and examined with a transmission electron microscope (Sun et al. 2018). Gram staining was performed according to Dong and Cai (2001). Motility was checked by microscopic observation and inoculation on semi-solid MB medium with 0.5% agar (w/v). Growth was determined in modified MB (with original Na^+ and Cl^- removed) containing various NaCl concentrations 0, 0.5 and 1–10.0% (at intervals of 1%, w/v) and the temperature range in MB at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 45, 50 and 55 °C. The pH range for growth was determined at pH 4.0–10.0 (at intervals of 0.5) in MB supplemented with the following buffers: ammonium acetate (pH 4.0–5.0), MES (pH 5.5–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) and CAPSO (pH 9.0–10.0) at a concentration of 30 mM. All growth

conditions were tested in quadruplicate, and OD_{600} was measured after incubation at 26 °C at 140 rpm for 24 h.

The following biochemical and physiological tests were carried out on strains NBU-8HK146^T, *M. taiwanensis* LMG 26125^T, *M. marinus* JCM 17211^T and *M. hydrolyticus* DSM 11525^T in MB unless otherwise indicated. Catalase activity was detected via bubble production in 3% (v/v) H_2O_2 solution. Oxidase activity was assessed by oxidation of 1% *p*-aminodimethylaniline oxalate. Indole, methyl red, Voges-Proskauer test, H_2S production, and hydrolysis of starch, casein, Tweens 20, 40, 60 and 80 were tested as described by Zhu (2011). Other enzyme activities, physiological and biochemical properties, and acid production tests were determined by using API ZYM, API 20NE and API 50 CH strips (bioMérieux) according to the manufacturer's instructions, except for using 2.0% (w/v) NaCl solution for preparing cell suspensions. For the API 50CH test, we used modified MB without yeast extract and peptone, but adding 0.02 g/L yeast extract and 0.01 g/L phenol red. Anaerobic growth was determined with an Anaero-Pack-MicroAero (2.5 l; MGC, Japan) system by using sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM) or sodium nitrate (20 mM) as electron acceptors, respectively. Same media under aerobic condition were used as control. Susceptibility to antibiotics was tested on MA using antibiotic discs and considered susceptible when the diameter of the inhibition zone was over 1.5 cm. The antibiotics tested were (μg per disc, unless indicated): amikacin (30), amoxicillin (20), ampicillin (10), bacitracin (0.04 IU), carbenicillin (100), cefamezin (30), cefoxitin (30), cefradine (30), cephalexin (30), chloramphenicol (30), ciprofloxacin (5), clindamycin (2), doxycycline (30), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (2), minocycline (30), nalidixic acid (30), neomycin (30), norfloxacin (10), novobiocin (30), nystatin (100), ofloxacin (5), oxacillin (1), penicillin G (10 IU), polymyxin B (300 IU), rifampicin (5), streptomycin (10), tetracycline (30) and vancomycin (30).

Chemotaxonomic characterization

Biomass for chemotaxonomic and molecular studies was obtained by cultivation in MB at 26 °C for 24 h, with shaking at 140 rpm. All the following tests for chemotaxonomic characterization were performed on strains NBU-8HK146^T, *M. taiwanensis* LMG 26125^T, *M. marinus* JCM 17211^T and *M. hydrolyticus* DSM 11525^T under the same conditions. For fatty acid methyl esters (FAMEs) analysis, late exponential-phase cells were harvested from MB. The identification and quantification of FAMEs were performed using the Sherlock Microbial Identification System (MIDI) with the standard MIS Library Generation Software version 6.1 according to manufacturer's instructions. Respiratory quinones were extracted and analyzed by using reversed-phase HPLC as described by Minnikin et al. (1984). Total lipids were extracted as described by Kates (1986) and detected by two-dimensional TLC silica-gel 60 F_{254} aluminium-backed thin-layer plates (10 × 10 cm, Merck 5554), and further analyzed as described by Minnikin et al. (1984). The TLC plates were sprayed with phosphomolybdic acid (with 5% phosphomolybdic acid in ethanol) to reveal total lipids, α-naphthol/H_2SO_4 to reveal glycolipids, molybdenum blue to reveal phospholipids and ninhydrin to reveal aminolipids (Zhang et al. 2015).

Phylogenetic analysis and genome analysis

The 16S rRNA gene was amplified by PCR using universal bacterial primers 27F and 1492R (Sun et al. 2017). Purified PCR products were cloned into the vector pMD19-T (TaKaRa). The recombinant plasmid transformed into *Escherichia coli* DH5α and then commercially sequenced. The almost-complete 16S rRNA gene sequence (1494 nt) was compared with those of closely related species by EzBioCloud's Identify Service (http://www.ezbiocloud.net/identify) (Yoon et al. 2017a, b) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequences alignments and phylogenetic tree reconstructions were performed by using MEGA 7 (Kumar et al. 2016). Phylogenetic trees were reconstructed by using three different methods: neighbour-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971) and maximum-likelihood (Felsenstein 1981) methods. Evolutionary distances were calculated using the Kimura 2-parameter model (Kimura, 1980) for the neighbour-joining method. The topology of the phylogenetic trees was evaluated by using the bootstrap values based on 1000 resamplings. Phylogenomic analysis

was performed online by Type (strain) Genome Server (TYGS) (Meier-Kolthoff et al. 2019).

The whole genomes of strain NBU-8HK146^T and *M. taiwanensis* LMG 26125^T were sequenced using an Illumina HiSeq 4000 system (Illumina) at the Beijing Genomics Institute (Shenzhen, China). The paired-end fragment libraries were sequenced according to the Illumina HiSeq 4000 system's protocol. Raw reads of low quality from paired-end sequencing (those with consecutive bases covered by fewer than five reads) were discarded. The sequenced reads were assembled using SOAPdenovo v1.05 software (Li et al. 2008). The coding sequences (CDSs) were annotated by using Rapid Annotation using Subsystem Technology (RAST) server online (Overbeek et al. 2014) [34], and analyzed subsequently against Clusters of Orthologous Groups of proteins (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases (Tatusov et al. 2003; Kanehisa et al. 2016). Genome data publicly available of related *Microbulbifer* species were retrieved from the NCBI Genome database. The average nucleotide identity (ANI) values between strain NBU-8HK146^T and related species were calculated using the ANI calculator online service (Yoon et al. 2017a, b). Digital DNA-DNA hybridization (dDDH) values were calculated by the genome-to-genome distance calculator (GGDC) server version 2.1 (Meier-Kolthoff et al. 2013). The genomic DNA G+C content of NBU-8HK146^T and *M. taiwanensis* LMG 26125^T was calculated from genome sequence.

Results and conclusion

Morphological, physiological and biochemical characteristics

Colonies of strain NBU-8HK146^T were 0.5 mm in diameter, circular, elevated and cream-colored after growing on MA at 26 °C for 24 h. Cells were Gram-negative, rod-shaped and non-motile (Fig. S1). Strain NBU-8HK146^T grew at 0–11% (w/v) NaCl (optimum 3%, w/v), 10–40 °C (optimum 32 °C) and pH 5.5–9.5 (optimum 7.0). No growth was detected under anaerobic conditions on MA even after two weeks with thiosulfate, sulifite, nitrite or nitrate as electron acceptors. Other physiological and biochemical characteristics of strain NBU-8HK146^T are given in the species description.

Chemotaxonomy results

The predominant fatty acids (> 10%) of strain NBU-8HK146^T were $C_{16:0}$ (10.2%), iso-$C_{15:0}$ (30.1%) and summed feature 9 (21.9%). The complete fatty acid profiles were summarized in Table 1. Ubiquinone-8 (Q-8) was the major respiratory quinone. The polar lipids included phosphatidylethanolamine (PE), phosphatidylglycerol (PG), three unidentified glycolipids (GL1, GL2 and GL4), two unidentified aminolipids (AL1 and AL2) and three unidentified lipids (L1, L2 and L5) (Fig. S2).

Phylogenetic analysis and genome characterization

Nearly full-length 16S rRNA gene sequence (1494 nt) of strain NBU-8HK146^T gained by PCR was completely identical with that obtained by genome sequencing. Result of 16S rRNA gene sequence alignment showed that strain NBU-8HK146^T belonged to the genus *Microbulbifer*. Strain NBU-8HK146^T shared 98.0%, 97.9%, 97.7%, 97.6% and 97.3% similarities to the species with validated name *Microbulbifer taiwanensis* CC-LN1-12^T, *Microbulbifer rhizosphaerae* Cs16b^T, *Microbulbifer marinus* Y215^T, *Microbulbifer donghaiensis* CN85^T and *Microbulbifer aggregans* CCB-MM1^T, respectively. Phylogenetic analysis revealed that strain NBU-8HK146^T was affiliated with species in the genus *Microbulbifer*, and closely related to *M. taiwanensis* CC-LN1-12^T and *M. marinus* Y215^T on the different phylogenetic trees (Fig. 1, Fig. S3 and Fig. S4). Result of phylogenomic analysis also supported that strain NBU-8HK146^T belonged to genus *Microbulbifer* (supplementary Fig. S5).

The draft genomes of strain NBU-8HK146^T and *M. taiwanensis* LMG 26125^T were composed of 111 contigs for 4,726,775 bp with 58.9% G+C content and 116 contigs for 4,821,019 bp with 60.2% G+C content, respectively. The genome of strain NBU-8HK146^T contained 4,094 protein-coding genes and 74 RNA genes including one operon of 16S-23S-5S rRNA genes and 45 tRNA genes, while *M. taiwanensis* LMG 26125^T contained 4136 protein-coding genes and 115 RNA genes including one operon of 16S-23S-

Table 1 Cellular fatty acids for strain NBU-8HK146^T and related type strains of the genus *Microbulbifer*

Fatty acid	1	2	3	4
Saturated				
$C_{10:0}$	0.5	0.8	0.6	0.8
$C_{14:0}$	1.5	1.4	1.6	1.6
$C_{16:0}$	10.2	11.5	12	11.6
$C_{17:0}$	1.3	2.5	1.4	2.9
$C_{18:0}$	tr	0.5	0.7	0.6
Branched				
iso-$C_{11:0}$	3.2	3.1	3	3.4
iso-$C_{15:0}$	30.1	16.8	19.9	22.1
iso-$C_{17:0}$	5.1	2.7	7.8	4.7
iso-$C_{15:1}$ F*	2.2	2.4	2.3	3.4
Hydroxy				
$C_{10:0}$ 3-OH	0.8	–	0.7	0.5
iso-$C_{11:0}$ 3-OH	4.4	5.2	4.9	3.2
Unsaturated				
$C_{17:1}$ $\omega 6c$	–	1.2	0.6	–
$C_{17:1}$ $\omega 8c$	0.8	1.8	0.9	1.6
$C_{17:0}$ cyclo	2	–	–	2.2
$C_{19:0}$ cyclo $\omega 8c$	1.4	–	–	–
$C_{18:1}$ $\omega 7c$ 11-methyl	–	–	–	1.5
Summed Features*				
3	2.9	9.1	4.3	4.6
8	6.7	19	10.9	11.5
9	21.9	19.2	26.1	21.4

Taxa: 1, strain NBU-8HK146^T; 2, *M. taiwanensis* LMG 26125^T; 3, *M. marinus* JCM 17211^T; 4, *M. hydrolyticus* DSM 11525^T. All data were taken from this study unless otherwise indicated. Values are percentages of the total fatty acid content. Fatty acids amounting to < 0.5% of the total fatty acids in all strains listed are omitted. tr, trace component (< 0.5%); -, not detected

*The double-bond position indicated by a capital letter is unknown. Summed feature 3 contained $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; Summed feature 8 contained $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; Summed feature 9 contained iso-$C_{17:1}$ $\omega 9c$ and/or $C_{16:0}$ 10-methyl

5S rRNA genes and 46 tRNA genes. Among the whole CDSs predicted by using RAST server online, 3348 CDSs (81.8%) of strain NBU-8HK146^T could be assigned to COG database, and the major COG categories were energy production and conversion (C, 8.36%), amino acid transport and metabolism (E, 7.53%), translation, ribosomal structure and biogenesis (J, 7.35%), transcription (K, 6.75%), and cell wall/membrane/envelope biogenesis (M, 6.51%), While 3261 CDSs (78.8%) of strain *M. taiwanensis* LMG 26125^T could be assigned to COG database, and the major COG categories were amino acid transport and metabolism (E, 8.25%), translation, ribosomal structure and biogenesis (J, 7.33%), transcription (K, 6.93%), cell wall/membrane/envelope biogenesis (M, 6.59%), and energy production and conversion (C, 6.35%). It showed strain NBU-8HK146^T contained more genes related to energy production and conversion comparing to *M. taiwanensis* LMG 26125^T. The percentage of CDSs assigned to KEGG database is 69.7% and 65.4% for strain NBU-8HK146^T and *M. taiwanensis* LMG 26125^T, respectively. The major pathways of both strains were metabolic pathways including carbohydrate metabolism, amino acid metabolism, metabolism of cofactors and vitamins, energy metabolism and lipid metabolism. The draft genome sequences of strain NBU-8HK146^T and *M. taiwanensis* LMG 26125^T were deposited in DDBJ/EMBL/GenBank under the accession number JACZCR000000000 and JACZFR000000000, respectively. The ANI values between strain NBU-8HK146^T and related species were 73.2–78.9%, which were lower than the threshold value of 95% ANI relatedness for species delineation (Richter and Rosselló-Móra 2009), and the dDDH values between strain NBU-8HK146^T and related species were 19.5–22.7%, which were far below the proposed cut-off borderline of 70% (Wayne et al. 1987) (Table S2).

Strain NBU-8HK146^T was isolated from mangrove sediment which containing about 2% salt concentration. The experiments showed the strain could grow at 0–11% NaCl concentration with 3% for optimal growth. The adaption mechanism to maintain osmotic balance was analyzed. According to the annotation of genome sequence, Trk system potassium uptake proteins were found, which were responsible for K^+ uptake and transport, including one *TrkH* gene and one *TrkA* gene. Two copies of potassium efflux system KefA proteins and two copies glutathione regulated potassium-efflux proteins KefB/C were found that were related to K^+ efflux. In addition, the genome contained 6 copies of Na^+/ H^+ antiporter proteins related to Na^+ efflux. The genome of strain NBU-8HK146^T also contained 12 genes related to the synthesis and transport of the compatible solutes glycine betaine and ectoine for resistance to osmotic stress: 9 proteins and 3 proteins involving in synthesis

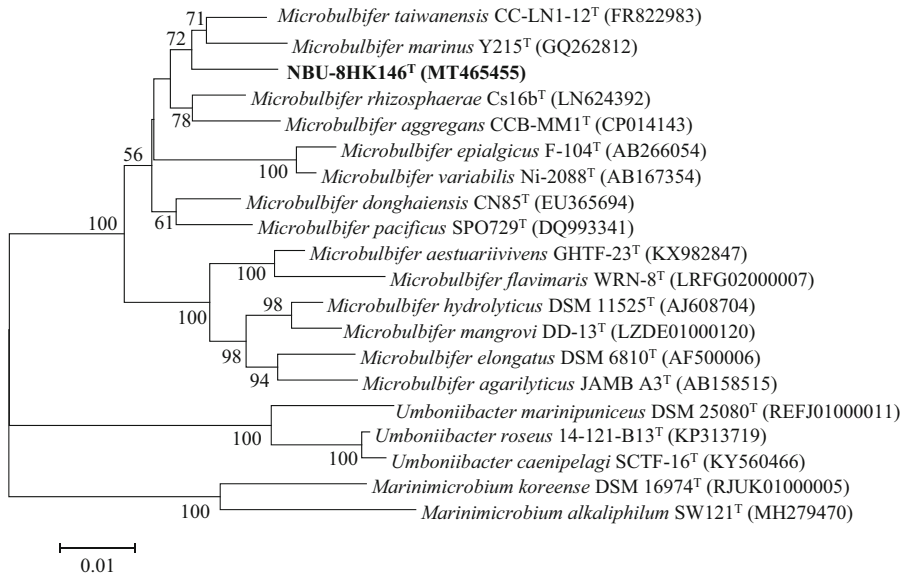

Fig.1 Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the phylogenetic relationships of strain NBU-8HK146^T and related taxa. Bootstrap values are based on 1000 resamplings. Bootstrap values higher than 50% are indicated at branch-points. Bar, 0.01 substitutions per nucleotide positions

and transport of glycine betaine and ectoine, respectively. All these proteins and systems mentioned played an important role in the adaption of osmotic stress in the salt environment of mangrove.

Taxonomic conclusion

The major cellular fatty acids ($C_{16:0}$, iso-$C_{15:0}$ and summed feature 9), predominant respiratory quinone (ubiquinone Q-8), major polar lipids (PE, PG, GL1 and GL2), phylogenetic and phylogenomic trees supported that strain NBU-8HK146^T should be classified into the genus *Microbulbifer*. However, there are some additional differences between strain NBU-8HK146^T and the related type strains of genus *Microbulbifer*. The detailed fatty acid profile showed that strain NBU-8HK146^T possessed a higher amount of iso-$C_{15:0}$ than three reference species, while possessing lower amount of summed feature 3, summed feature 8, $C_{16:0}$ and $C_{17:0}$ than three reference species (Table 1). The detailed polar lipid profile showed all these strains contained PE, PG, GL1, GL2, GL4, AL2, L1 and L2, while NBU-8HK146^T possessed AL1 and L5, *M. marinus* JCM 17211^T contained GL3, AL1, L3 and L4, *M. hydrolyticus* DSM 11525^T possessed L3 (Fig. S2). There were also several phenotypic differences between NBU-8HK146^T and related type strains (Table 2). Strain NBU-8HK146^T could tolerate NaCl concentration up to 11.0%, but other three strains could not grow at NaCl concentration higher than 7.0%. Hydrolysis of starch was negative for strain *M. taiwanensis* LMG 26125^T but positive for other three reference species. Meanwhile, the ANI values (73.2–78.9%) and dDDH values (19.5–22.7%) between strain NBU-8HK146^T and related species were below the thresholds recommended for species delineation 95% (ANI) and 70% (dDDH), respectively. All of the above confirmed that strain NBU-8HK146^T represented a novel species within the genus *Microbulbifer*.

Based on the phenotypic, chemotaxonomic and genotypic characteristics described above, we identified strain NBU-8HK146^T as a novel species of the genus *Microbulbifer*, for which the name *Microbulbifer hainanensis* sp. nov. is proposed.

Description of *Microbulbifer hainanensis* sp. nov.

Microbulbifer hainanensis (hai.nan.en'sis. N.L. fem. adj. hainanensis of or pertaining to Hainan, a tropical province of China, from which the mangrove sediment sample was collected).

Cells are Gram-negative, rod-shaped, aerobic, non-motile and moderately halophilic. The cell size is 0.3–0.5 × 2.6–5.0 μm. Colonies on Marine agar 2216 are 0.5 mm in diameter, circular, elevated and cream-colored after 24 h at 26 °C. The concentration of sodium chloride for cell growth is 0–11%, and the

Table 2 Differential characteristics of strain NBU-8HK146^T and related type strains of the genus *Microbulbifer*

Characteristic	1	2	3	4
Habitat	Mangrove sediment	Coastal soil[a]	Marine sediment[b]	Mill waste[c]
Cell morphology	Rods	Rod-shaped or coccoid[a]	Rod-shaped or ovoid[b]	Rod-shaped[c]
Cell size (μm)	0.3–0.5 × 2.6–5.0	0.6–0.9 × 3.0[a]	0.3–0.5 × 2.5–5.0[b]	1.1–1.7 × 0.3–0.5[c]
Pigmentation	Cream	Brown	Light yellow	Cream
Temperature range (optimum, °C)	10–40 (32)	ND (28)[a]	15–40 (25–30)[b]	10–41 (37)[c]
pH range (optimum)	5.5–9.5 (7)	ND[a]	4.5–10.0 (7.0–8.0)[b]	6.5–8.5 (7.5)[c]
NaCl range (optimum) (w/v)	0–11.0% (3.0%)	0–7.0% (3.0–4.0%)[a]	0–7.0% (2.0–3.0%)[b]	0.1–1 M (0.1–0.5 M)[c]
Veoges-Proskauer	−	−	−	+
Catalase activity	+	+	−	+
Hydrolysis of				
Starch	+	−	+	+
Casein	+	+	−	+
Tween 80	+	–	−	+
API 20NE test results:				
Nitrate reduction	+	−	+	+
Glucose, arginine dihydrolase	−	−	+	−
API ZYM test results:				
Esterase (C4)	+	−	+	+
Trypsin	−	−	+	−
α-Chymotrypsin	+	+	+	−
α-Galactosidase	−	+	−	−
α-Glucosidase	−	−	+	+
Vβ-Galactosidase, α-mannosidase, cystine arylamidase	+	−	−	−
API 50CH:				
D-Arabinose, D-ribose, L-xylose, L-sorbose	+	−	−	−
L-Arabinose, D-xylose, gentiobiose	+	−	−	+
D-Alactose	−	+	+	−
D-Fructose, L-rhamnose, L-fucose	−	−	+	−
D-Mannose, α-methyl-D-mannopyranoside	+	+	−	−
Salicin	+	+	+	−
Melibiose, sucrose	−	+	−	−
D-Turanose, D-fucose	+	+	−	+
D-Lyxose	+	−	+	−
5-Ketogluconate, cellobiose	+	−	+	+
Susceptibility to				
Amoxicillin	R	S	R	S
Ampicillin, carbenicillin, cefoxitin	S	R	R	S
Clindamycin, cephalexin, cefradine, lincomycin	R	R	S	R

Table 2 continued

Characteristic	1	2	3	4
Ciprofloxacin, streptomycin, kanamycin	S	S	S	R
Chloramphenicol, cefamezin, gentamicin, nalidixic acid, rifampicin	S	R	S	S
Doxycycline, vancomycin	S	R	S	R
Tetracycline, polymyxin B	S	S	R	S
Minocycline	S	S	R	R
Penicillin G	S	R	R	R
DNA G + C content (mol%)	58.9	60.2	54.0[b]	57.7[c]

Taxa: 1, strain NBU-8HK146^T; 2, *M. taiwanensis* LMG 26125^T; 3, *M. marinus* JCM 17211^T; 4, *M. hydrolyticus* DSM 11525^T. All data were taken from this study unless otherwise indicated. Data marked with [a, b, c] were taken from Kämpfer et al. (2012), Zhang et al. (2012) and González et al. (1997), respectively. −, negative; + , positive; ND, no data; R, resistant; S, susceptible. The same characteristics shared by these four strains were listed in Table S1.

optimal growth concentration is 3%. Growth occurs at 10–40 °C (optimum 32 °C) and pH 5.5–9.5 (optimum pH 7). Veoges-Proskauer, methyl red reaction and hydrolysis of Tween 20 were negative. Catalase and oxidase activities, hydrolysis of starch, casein, H_2S production, Tweens 40, 60 and 80 were positive. In the API ZYM kit, positive for activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucin arylamidase, valin arylamidase, cystin arylamidase, acid phosphohydrolase, α-chymotrypsin, naphtol-AS-BI-phosphohydrolase, β-galactosidase, α-mannosidase and *N*-acetyl-β-glucosaminidase. In the API 50CH kit, positive for acid production from *N*-acetyl-β-D-glucosamine, amygdalin, aesculin, D-arabinose, L-arabinose, cellobiose, D-fucose, glycogen, gentiobiose, D-glucose, 2-ketogluconate, 5-ketogluconate, D-lyxose, D-mannose, α-methyl-D-mannopyranoside, maltose, D-ribose, L-sorbose, salicin, starch, D-turanose, D-xylose and L-xylose. Sensitive to ampicillin, amikacin, chloramphenicol, cefoxitin, cefamezin, carbenicillin, ciprofloxacin, doxycycline, erythromycin, gentamicin, kanamycin, minocycline, neomycin, norfloxacin, nalidixic acid, novobiocin, ofloxacin, penicillin G, polymyxin B, rifampicin, streptomycin, tetracycline and vancomycin. The major cellular fatty acids were $C_{16:0}$, iso-$C_{15:0}$ and summed feature 9. The major respiratory quinone was ubiquinone-8. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, three unidentified glycolipids (GL1, GL2 and GL4), two unidentified aminolipids (AL1 and AL2), and three unidentified lipids (L1, L2 and L5). The DNA G+C content of the genomic DNA of the type strain is 58.9 mol% (Table 1).

The type strain NBU-8HK146^T (= KCTC 82226 ^{T} = MCCC 1K04737^T) was isolated from a mangrove sediment sample taken from Haikou, Hainan province, China. The GenBank accession numbers for the 16S rRNA gene and the draft genome data of strain NBU-8HK146^T are MT465455 and JACZCR000000000, respectively.

Authors' contributions W.Y.Z. and S.H. conceived the study. Y.P.C., S.T.Z., C.B.G. and F.L.X performed the research. D.J and S.Y.L. analyzed data. Y.P.C. and S.T.Z. wrote the paper. All authors read and approved the manuscript.

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Availability of data and material (data transparency) The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and draft genome sequence of strain NBU-8HK146^T are MT465455 and JACZCR000000000, respectively. A transmission electron micrograph of the cells, average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) values, polar lipids and phylogenetic trees (maximum-likelihood and maximum-parsimony trees) are available as supplementary materials. Supplementary data associated with this article can be found in the online version.

Declarations

Conflict of interest The authors declare there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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