Muricauda Chongwuensis Sp. Nov., Isolated From Coastal Seawater of China

Ming-Xia Chen (chenmx1257@163.com)
Huaqiao University https://orcid.org/0000-0001-9420-9339
Xiao-Yu He
Huaqiao University
He-Yang Li
Third Institute of Oceanography, Ministry of Natural Resources

Research Article

Keywords: Muricauda chongwuensis, nonobligate bacterial predator, polyphasic taxonomy

Posted Date: June 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-631001/v1

Version of Record: A version of this preprint was published at Archives of Microbiology on October 5th, 2021. See the published version at https://doi.org/10.1007/s00203-021-02591-1.

Abstract

In the course of screening for bacterial predator, a Gram-negative, non-flagellated, gliding, long rod-shaped and yellow-pigmented bacterium, designated strain HICW^T, was isolated from coastal seawater of China. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain HICW^T represented a member of the genus *Muricauda* and showed the highest sequence similarity to *M. aquimarina* JCM11811^T (98.8%) and *M. ruestringensis* DSM13258^T (98.1%). NaCl was required for growth. Optimum growth occurred at 25–30 ^oC, 2.0–3.0% (w/v) NaCl with pH 7.0. Strain HICW^T showed some similar characteristics to the nonobligate bactertal predators, the cells can attach the prey cells. Strain HICW^T contained MK-6 as the predominant respiratory quinone and had iso-$C_{15:0}$, iso-$C_{15:1}$ G and iso-$C_{17:0}$ 3-OH as the major cellular fatty acids. The polar lipids contained phosphatidylethanolamine (PE), three unknown phospholipids (PL1–PL3), one unknown aminolipids (AL) and three unknown polar lipids (L1–L3). The genome size of strain HICW^T was approximately 3.8 Mbp, with a G+C content of 41.4%. On the basis of the polyphasic evidence, strain HICW^T is proposed as representing a new species of the genus *Muricauda*, for which the name *Muricauda chongwuensis* sp. nov. is proposed. The type strain is HICW^T (=JCM 33643^T=MCCC 1K03769^T).

Introduction

According to the LPSN (www.bacterio.net/index.html), the family Flavobacteriaceae comprises 145 genera, including genus *Muricauda*, which was proposed by Bruns et al. (2001), and subsequently emended by Yoon et al. (2005) and Hwang et al. (2009). Members of genus *Muricauda* share the characteristics of being Gram-negative, non-motile, strictly or facultatively aerobic, yellow pigmented rod and having menaquinone-6 (MK-6) as the major isoprenoid quinone and DNA G+C contents of 41.0–55.0 mol%. Their fatty acid profiles are characterised by large amounts of branched- and straight-chain fatty acids. At the time of writing, there are 25 valid species in the genus *Muricauda* listed in the LPSN (https://lpsn.dsmz.de/genus/muricauda). These *Muricauda* species were isolated from various saline environments, such as intertidal or tidal sediment and sand (Bruns et al. 2001; Yoon et al. 2008; Lee et al. 2012; Kim et al. 2013; Li et al. 2019; Kim et al. 2020) , salt lake (Yoon et al. 2005), crude oil-contaminated seawater(Hwang et al. 2009), coastal hot spring (Arun et al. 2009), surface seawater (Wang et al. 2017; Chen et al. 2019; Zhang et al. 2015), surface marine snow (Su et al. 2017), deep sea water and sediment (Zhang et al. 2018; Zhang et al. 2020; Liu et al. 2018; Dong et al. 2020), mangrove sediment (Yang et al. 2013), Antarctic seawater (Wu et al. 2013), sponge (Park 2019; Yoon et al. 2012), shrimp gill (Liu et al. 2020), rhizosphere of a marine macroalga (Bae et al. 2007).

Predatory bacteria can be considered any bacteria that kill or destroy other microbes and consume them as a nutritional resource (Pérez et al. 2016). Most described predatory bacteria except members of *Bdellovibrio*-and-like organisms (BALOs) (Williams and Chen 2020) are nonobligate predators, such as *Ensifer adhaerens* (Germida and Casida 1983), *Agromyces ramosus* and *Lysobacter* (Jurkevitch and Davidov 2006; Svercel et al. 2011), *Pseudobacteriovorax antillogorgiicola* (Mccauley et al. 2015),

Bradymonas sediminis (wang et al. 2015; Mu et al. 2020), *Wenzhouxiangella* Strain AB-CW3 (Sorokin et al. 2020). In the course of screening for bacterial predator distributed in the coastal waters of China, a yellow-pigmented strain, designated HICW^T, was isolated. Strain HICW^T showed some similar characteristics to nonobligate bactertal predators, and the results of 16S rRNA gene sequence comparisons indicated that it was phylogenetically related to the genus *Muricauda* in the family Flavobacteriaceae. The present study determined the taxonomic status of strain HICW^T by using a polyphasic approach.

Materials And Methods

Strain and culture condition

The sample was collected from coastal waters, near the town Chongwu in Southeast China (118.545685 °E, 24.53178 °N). Sample was brought to the lab and stored at 4 ^{o}C (refrigerator) for up to 2 days before being processed. Strain HICW^T was isolated and purified over five times using the seawater double-layer agar plating method as previously described with *Vibrio alginolyticus* LF TCBS 15 (=MCCC 1K03520) as the prey bacterium (Schoeffield and Williams 1990; Ye et al. 2019). Cells from the plaque were examined by light microscopy (CX22RFS1, OLYMPUS) with 1% (w/v) crystal violet staining. A single-plaque (with slender rod cells of strain HICW^T and a few residual prey cells) on the double-layer plate was picked into a 20 ml tube-type bottle with 2 ml 1/40 (v/v) marine broth 2216E (MB, peptone 5 g, yeast extract 1 g, seawater 1 L, pH 7.2–7.6) and incubated at 28 °C with 200 r.p.m for 2–3 days. Small amounts of cells (each around $1\times10^{5-6}$ cell ml^{-1}) of strain HICW^T and prey strain LF TCBS 15 were detected in 1/40 MB culture. The axenic independent strain HICW^T was purified from the 1/40 MB co-culture by the standard dilution plating on marine agar 2216E (MA, pH 7.2–7.6), after incubation at 28 °C for 6-7 days. A yellow colony different from the prey strain was picked, checked by light microscopy (CX22RFS1, OLYMPUS) with 1% (w/v) crystal violet staining, and then purified by streaking three times on MA. The strain was maintained in MB at 28 °C for 24 h and preserved in MB supplemented with 20% (v/v) glycerol at -20 °C and -80 °C. For long-term storage, the cultures of strain HICW^T were lyophilized *in* 10% (w/v*) skim* milk, and then deposited at the Marine Culture Collection of China (MCCC) and Japan Collection of Microorganisms (JCM). *M. aquimarina* JCM11811^T and *M. ruestringensis* DSM13258^T were obtained from Marine Culture Collection of China and cultivated as reference strains under identical conditions.

Phenotypic and biochemical characterisation

Cell morphology were observed by light microscopy (CX22RFS1, OLYMPUS) and transmission electron microscopy (TEM, HT7800, Hitachi). For negative stains, cells from 24 h cultures on MA were resuspended with 0.1 mol/L phosphate buffer (pH7.4), then a 400-mesh grid was inverted over a drop of cell suspensions for 1 min. The grid was then washed on 2 drops of water and the cells were stained with 2.0% (w/v) uranyl acetate for 10 s.

For predatory characteristic detection, strain HICW^T was cultivated either in double-layer agar plate or in seawater with washed prey cells (around 1×10^9 cell ml^{-1}) (Ye et al. 2019). Light microscopy (CX22RFS1, OLYMPUS) and transmission electron microscopy (TEM, HT7800, Hitachi) were used to assess the cell-to-cell contact with attachment to the prey. For negative stains, a 400-mesh grid was inverted over a drop of 24 h co-cultures seawater, washed on 1 drop of water, and the cells were stained as mentioned above.

Gram staining was performed using a Gram Stain kit (QingDao Hopebio-Technology Co., Ltd) according to the instructions of the manufacturer. Growth temperature and pH values were assessed in MB at different temperatures (15, 20, 25, 28, 30, 35, 37, 40 and 45 ^oC) and pH values (3.0–10.0 at 1.0 unit intervals) for 42 h. MB with different pH values were prepared using the following biological buffers: citrate/phosphate (pH 3.0–7.0), Tris/HCl (pH 7.0–9.0) and sodium carbonate/sodium bicarbonate (pH 9.0–10.0). Growth at various NaCl concentrations (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0% (w/v)) was investigated using MB with different NaCl concentrations prepared in the laboratory according to the formula of the medium. Gliding motility was examined on MA with 1.0% agar (w/v) and using the hanging-drop method after growing cells in MB broth for 48 h at 28°C (Bernardet et al. 2002). The aerobic condition of the strain HICW^T was determined by a semi-solid (0.5%) stab culture (Dong and Cai 2001). The presence of flexirubin-type pigments were investigated as described by Reichenbach (1989) and Bernardet (2002). An acetone: methanol (7:2, v/v) mixture was used to extract bacterial carotenoids pigments, and the whole-cell spectrum for carotenoid pigments was detected using the UV–visible spectrophotometer (AOE Instruments (Shanghai) Co., Ltd.) according to Hameed et al. (2011). Oxidase reagent (Hangzhou Microbial Reagent Co., Ltd) was used for testing oxidase activity, and catalase activity was evaluated by the production of oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution according to the method of Dong & Cai (2001). Additional enzymic activities and biochemical features of strain HICW^T and the reference strains were determined by the API ZYM and API 20NE kits (bioMérieux) according to the manufacturer's instructions, except that inocula were prepared by suspending cells in artificial seawater (Yang et al. 2013). Antibiotic susceptibility tests were performed using 6 mm filter-paper discs (Hangzhou Microbial Reagent Co., Ltd) with antibiotics added at the following concentrations (μg per disc unless stated otherwise): penicillin (10 U), erythromycin (15), neomycin (30), gentamicin (10), tetracycline (30), doxycycline (30), minocycline (30), kanamycin (30), amikacin (30), oxacillin (1), ampicillin (10), carbenicillin (100), piperacillin (100), cefradine (30), cephalexin (30), cefazoline (30), cefuroxime (30), ceftazidime (30), ceftriaxone (30), cefoperazone (75).

Chemotaxonomic characterization

For analysis of the cell fatty acids, bacteria were cultured in MB at 28 ^oC for 24 h, the harvested cells were saponified, methylated and extracted using the standard MIDI (Sherlock Microbial Identification System, version 6.0B) protocol. The whole-cell fatty acid pattern were then analysed by gas chromatography (model 6850, Agilent Technologies) and identified using the TSBA6.0 database of the Microbial Identification System (Athalye et al. 1985; Sasser 1990). Polar lipids were extracted and examined using two-dimensional thin-layer chromatography according to Kates (1972). Isoprenoid quinones were

extracted from freeze-dried cells with chloroform/methanol (2:1, v/v) and analysed by reversed-phase HPLC (Collins et al. 1984).

Phylogenetic and genomic analyses

The genomic DNA of strain HICW^T was extracted using a Rapid Bacterial Genomic DNA Isolation Kit (B518225, Shanghai Sangon Biological Engineering Technology & Services Co., Ltd). The whole genome of strain HICW^T was sequenced by the Guangdong Magigene Biotechnology Co., Ltd., using Solexa paired-end (150 bp library) sequencing technology protocol. SPAdes software (http://cab.spbu.ru/software/spades/) was used to do genome assembly with multiple-Kmer parameters (Bankevich et al. 2012). The draft genome data of HICW^T has been deposited in GenBank with the accession number WYET00000000. The G+C contents of the genomic DNA were calculated from the sequenced genome (https://www.ezbiocloud.net/tools/ani). Open Reading Frames (ORFs) were predicted using Prodigal v2.6.3 (Hyatt et al. 2010) and the predicted protein coding sequences (CDS) were searched against the GenBank, Clusters of Orthologous Groups (COGs) and KEGG databases to analyse gene functions and metabolic pathways.

The partial 16S rRNA gene (around 1400 bp) was respectively amplified from the chromosomal DNA and plaques of the strain HICW^T on the double-layer agar plate with *V. alginolyticus* LF TCBS 15 as the prey cells. The universal bacterial primers 27F and 1492R (Delong 1992) and the purified PCR product was sequenced by Xiamen Bioray Biotechnology Co., Ltd. The complete 16S rRNA gene sequence of the strain HICW^T was obtained from its draft genome sequence. The 16S rRNA gene sequence analyses were carried out with the online tool EzBioCloud (http://eztaxon-e. ezbiocloud.net) (Kim et al. 2012). 16S rRNA gene sequences of related taxa were selected from the GenBank database. Phylogenetic trees were reconstructed using the MEGA software package version 7.0 (Kumar et al. 2016) with distance options according to the default parameter model and clustering with the neighbor-joining (NJ), maximum-likelihood (ML) and maximum-parsimony (MP) methods, supported using bootstrap values with 1000 replications.

The whole genome average nucleotide identity (gANI) was calculated using the algorithm as described by Yoon et al. (2017) with the web service of EzBioCloud (https://www.ezbiocloud.net/tools/ani) and the digital DNA-DNA hybridizations (dDDH) were determined online at http://ggdc.dsmz.de/ggdc.php# using the Genome-to-Genome Distance Calculation (GGDC) version 2.1 (Meier-Kolthoff et al. 2013). Genomic data of related species were downloaded from GenBank database.

Results And Discussion

Phenotypic and biochemical characteristics

Cells of strain HICW^T were Gram-negative, facultatively anaerobic, slender rods without any flagella, 1.8–3.7 μm in length, 0.3–0.4 μm in width after culturing on MA for 24 h at 28 ^oC (Table 1, Fig. S1).

Appendages were not found, and outer membrane vesicles (OMVs) detached from the bacterial cell surface were detected (Fig. 1). Colonies of strain HICW^T were yellow, smooth, convex, raised and circular, 0.5-1 mm in diameter and semitransparent after incubation on MA for 72 h at 28 ^oC (Fig. S2a). Colony spreading was observed on MA with 1.0% agar (w/v) (Fig. S2b) and cells glided slowly at the bottom surface of the cover slip. Flexirubin-type pigments were not detected, but a carotenoid pigment with maximal absorption at 454 nm was present (Fig. S3). Growth occured at 15–40 ^oC (optimum 25–30 ^oC), but not above 45 ^oC or lower than 10 ^oC (Fig. S4a). Growth occured at 0.5–8.0% (w/v, optimum 2.0–3.0%) NaCl (Fig. S4b), NaCl was necessary for growth, but no growth was observed at 9.0% and 10% (w/v) NaCl (data not shown). The pH range for growth was 6.0–8.0 with an optimal pH 7.0 (Fig. S4c).

Plaques were formed on lawns of *V. alginolyticus* LF TCBS 15 when strain HICW^T was isolated□purified and cultivated using double-layer agar plate in the first 6 months. Arc-shaped concaves on the plate became visible after a 24 h incubation at 28 °C, then they extended slowly and turned to be clear sunken plaques (Fig. S5). A multitude of slender rods of strain HICW^T and a small amount of big rods of *V. alginolyticus* LF TCBS 15 were checked from the sunken plaques. The results indicated that strain HICW^T has the abilities to hydrolyze agars and prey on other bacteria. The 16S rRNA gene sequence from the sunken plaques showed most closely related to the genus *Muricauda* (around 83% similarity). When the plaques were picked and incubated in seawater with prey cells (around 1×10^9 cell ml^{-1}) at 28 °C for 48-120 h, strain HICW^T showed a poor cell growth, the turbidity of seawater co-cultured system did not decreased obviously, and only a few cells of strain HICW^T were found, which can not be separated from prey cells using centrifugalization or membrane filtration. This situation bothered us for 6 months. During this period, double-layer agar plate method was mainly used to culture strain HICW^T. It was almost impossible to get enough pure cells (even >50% purity) of strain HICW^T from the agar or liquid co-cultured system by the ways we had used, which limited the further physiological, biochemical and genomic analyses. So, it is imperative to purify the axenic independent strain HICW^T. Then, a 1/40 MB, lack of nutrients, was used to incubate the plaques, in which both strain HICW^T and prey grew a little bit on the same order. The method of standard dilution plating on MA was tried to purify the axenic independent strain HICW^T from the 1/40 MB co-cultured system. Fortunately, yellow colonies of strain HICW^T different from the prey strain were detected on 10^{-3} diluted plates (10 µl 10^{-3} diluted co-cultures). Accordingly, the numbers of strain HICW^T in the 1/40 MB co-cultured system was estimated at 10^{-5} CFU ml^{-1}, which was consistent with the results of microscope counting (10^{5-6} cell ml^{-1}). These results also indicated that strain HICW^T did not belong to an obligate predator, and the axenic independent colones were not like the prey-independent mutant of *Bdellovibrio bacteriovorus* (Barel and Jurkevitch 2001) with a frequency of 10^{-6}–10^{-7}. However, after purification, the axenic independent strain HICW^T appeared to lose agar hydrolysis ability and plaque-forming activity against *V. alginolyticus* LF TCBS 15. Cells from early co-cultured system (preserved with 20% (v/v) glycerol at -20 °C or -80 °C) also showed the same phenomenon. The similar property was reported on *Pseudobacteriovorax antillogorgiicola* RKEM611 to lose predatory activity after subsequent transfers on solid media (Mccauley et al. 2015). Since then,

the predation activity was observed in seawater co-cultured system by Light microscopy and TEM. Strain HICW^T attached to the prey cells (Fig. 2, Fig. S6), and one cell attached to one or more prey (Fig. 2, Fig. S6 b-e). Empty prey cell adjacent to cell of strain HICW^T was detected (Fig. 2d). Although strain HICW^T showed some similar characteristics to the nonobligate bactertal predators, the predation activity was weak in the liquid co-culture system and the predatory mechanism of strain HICW^T is still confused. More conclusive evidences are needed to prove strain HICW^T as a bacterial predator. Here, we defined it as a potential predator or quasi-predator.

In the API 20NE strip, strains HICW^T and the reference strains were positive for D-glucose fermentation, β-glucosidase (aesculin hydrolysis) and β-galactosidase activities; and negative for denitrification, indole production, arginine dihydrolase activity, urease activity and assimilation of D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate and penylacetic acid. In the API ZYM strip, all strains were positive for alkaline phosphatase, acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphtol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and β-glucuronidase (weakly). Nitrate reduction, gelatin hydrolysis, assimilation profile of several substrates and enzymatic activities were the physiological properties differentiating among strain HICW^T and closely related species of the genus *Muricauda* (Table 1). Other physiological and biochemical characteristics of strain HICW^T are given in the species description.

Chemotaxonomic characteristics

The major fatty acids (>10%) of strain HICW^T were iso-$C_{15:0}$ (31.4%), iso-$C_{15:1}$ G (23.5%) and iso-$C_{17:0}$ 3-OH (22.5%). These are the three major fatty acids of species of the genus *Muricauda* (Bruns et al. 2001). The differences in fatty acid content between strain HICW^T and related species in the genus *Muricauda* are shown in Table S1.The predominant isoprenoid quinone of strain HICW^T was menaquinone-6 (MK-6), which was consistent with other *Muricauda* species for which quinones have been analysed. Strain HICW^T contained phosphatidylethanolamine (PE), three unknown phospholipids (PL1–PL3), one unknown aminolipids (AL) and three unknown polar lipids (L1–L3) (Fig. S7). All species with available data of the genus *Muricauda* contain PE as the major lipid.

Phylogenetic and genomic analyses

Genome features of strain HICW^T are summarized in Table 2. The draft genome size of strain HICW^T was determined to 3777431 bp. There were 13 contigs for strain HICW^T. The coverage values (sequencing depth) of the genome sequences of strain HICW^T was 428×, whereas the N50 and N90 values were 1121114 bp and 125639 bp, respectively. The G+C content of the genomic DNA of strain HICW^T was 41.4%, similar to *M. aquimarina* JCM11811^T (43.4%) and *M. ruestringensis* DSM 13258^T (41.4%) (Table 1). A total of 3569 CDSs with sequence length of 3434949 bp were predicted, which account for 90.9% of the genome, and 37 tRNA and 5 rRNA (one 23S rRNA, one 16S rRNA and three 5S rRNA) genes were

identified. In all, 2896 CDSs were assigned to COG families and 1714 CDSs were included in 202 pathways.

The complete 16S rRNA gene sequences of strain HICW^T was 1514 bp in length. Comparisons of the sequence with the corresponding 16S rRNA gene sequences in the EzBioCloud databases showed that strain HICW^T belonged to the genus *Muricauda* and shared the highest sequence similarity with 16S rRNA gene of *M. aquimarina* JCM11811^T (98.8%) and *M. ruestringensis* DSM13258^T (98.1%). In the neighbour-joining tree based on 16S rRNA gene sequences of strain HICW^T and related type strains, the new isolate belonged to the family Flavobacteriaceae, fell into the same cluster with the members of the genus *Muricauda* and was most closely related to *M. aquimarina* JCM11811^T (Fig. 3). The maximum-parsimony and maximum-likelihood trees showed essentially the same topology (Fig. S8, S9).

Additionally, The ANI value for comparisons between strain HICW^T and *M. aquimarina* JCM11811^T and *M. ruestringensis* DSM13258^T were79.2% and 80.6% (Table 1), respectively, which were clearly lower than the threshold of 94–96% for bacterial species delineation (Kim et al. 2014; Richter 2009). The dDDH relatedness for strain HICW^T with *M. aquimarina* JCM11811^T and *M. ruestringensis* DSM13258^T were 34.1% and 34.5% (Table 1), respectively, which were also clearly below the 70% threshold DDH value generally accepted for the delineation of species (Richter 2009).

Taxonomic Conclusion

Based on the results of phenotypic, biochemical, chemotaxonomic, phylogenetic and genomic analyses, it is clear that strain HICW^T is genetically distinct from other strains of the genus *Muricauda* and represents a new species of the genus *Muricauda*, for which the name *Muricauda chongwuensis* sp. nov. is proposed.

Description of *Muricauda chongwuensis* sp. nov.

Muricauda chongwuensis (chong.wu.en'sis. N.L. fem. adj. *chongwuensis* of chongwu, the city where the type strain was isolated)

Cells of strain HICW^T are Gram-negative, facultatively anaerobic, gliding rods (1.8–3.7 μm in length, 0.3–0.4 μm in width after culturing on MA for 24 h at 28 ^oC) without any flagella. Outer membrane vesicles (OMVs) detach from the bacterial cell surface. Colonies are yellow, smooth, convex, circular (1–2 mm in diameter) and semitransparent after growth on MA for 48 h at 28 ^oC. Flexirubin-type pigments are not formed, but a carotenoid pigment with maximal absorption at 454 nm and 480 nm is present. Growth occurs at 15–40 ^oC (optimum 25–30 ^oC), but not above 45 ^oC or lower than 10 ^oC. Growth occurs at 0.5–8.0% (w/v, optimum 2.0–3.0%) NaCl, NaCl is necessary for growth. The pH range for growth is 6.0–8.0 with an optimal pH 7.0. Cells show some similar characteristics to nonobligate bacterial predators, attach to the prey cells. Catalase is positive, and oxidase is negative.

In the API 20E strip, positive for reduction of nitrate to nitrite (weakly), D-glucose fermentation, β-glucosidase (Aesculin hydrolysis), beta-galactosidase activities and the utilization of D-glucose, L-arabinose, D-mannose, D-maltose (weakly) and potassium gluconate (weakly), but negative for denitrification, indole production, arginine dihydrolase activities, urease activities, gelatin hydrolysis and the utilization of D-mannitol, N-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. In API ZYM strip, activities of acid phosphatase, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase (weakly), α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are present. Sensitive to (μg per disc unless stated otherwise) erythromycin (15), minocycline (30), amikacin (30) and piperacillin (100), but resistant to penicillin (10 U), neomycin (30), gentamicin (10), tetracycline (30), doxycycline (30), kanamycin (30), oxacillin (1), ampicillin (10), carbenicillin (100), cefradine (30), cephalexin (30), cefazoline (30), cefuroxime (30), ceftazidime (30), ceftriaxone (30) and cefoperazone (75) (Table S2). The major respiratory quinine is MK-6. The polar lipids contain phosphatidylethanolamine (PE), three unknown phospholipids (PL1–PL3), one unknown aminolipids (AL) and three unknown polar lipids (L1–L3). The predominant fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH.

The type strain, HICW^T (=JCM 33643^T=MCCC 1K03769^T), was isolated from coastal waters of Chongwu in Southeast China (118.545685 °E, 24.53178 °N). The draft genome size of strain HICW^T is approximately 3.8 Mbp, with a G+C content of 41.4%. The GenBank/EMBL/DDBJ accession numbers for the draft genome sequence and the 16S rRNA gene sequence of *Muricauda chongwuensis* HICW^T are WYET00000000 and MK920190, respectively.

Abbreviations

BALOs, *Bdellovibrio*-and-like organisms; rRNA, ribosomal RNA; MB, marine broth 2216E; MA, marine agar 2216E; MCCC, Marine Culture Collection of China; JCM, Japan Collection of Microorganisms; TEM, transmission electron microscope; gANI, genome average nucleotide identity; dDDH, digital DNA-DNA hybridization; MP, maximum-parsimony; ML, maximum-likelihood; NJ, neighbor-joining; OMVs, outer membrane vesicles.

Declarations

Author contributions M-XC and H-YL conceived the project. M-XC and X-YH perfomed the experiment and analysed the data. M-XC wrote the manuscript. All authors have revised the manuscript.

Funding This work was supported by the National Natural Science Foundation of China (grants no. 41506179), National Programme on Global Change and Air-Sea Interaction (grant no. GASI-03-01-03-01), Natural Science Foundation of Fujian Province (grant no. 2015J01613) and Fujian Provincial Key Laboratory of Marine Ecological Conservation and Restoration (grant no. EPR2020002).

Data availability The GenBank/EMBL/DDBJ accession numbers for the draft genome sequence and the 16S rRNA gene sequence of Muricauda chongwuensis HICWT are WYET00000000 and MK920190, respectively.

Code availability Not applicable.

Conflict of interest The authors declare that they have no conflict of interest.

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

Supplementary Information Two supplementary table and nine supplementary figures are available with the online version of this paper.

References

1. Arun AB, Chen WM, Lai WA, Chao JH, Rekha PD, Shen FT, Singh S, Young CC (2009) *Muricauda lutaonensis* sp. nov., a moderate thermophile isolated from a coastal hot spring. Int J Syst Evol Microbiol 59:2738–2742
2. Athalye M, Noble WC, Minnikin DE (1985) Analysis of cellular fatty acids by gas chromatography as a tool in the identification of medically important coryneform bacteria. J Appl Bacteriol 58:507–512
3. Bae SS, Kwon KK, Yang SH, Lee HS, Kim SJ, Lee JH (2007) *Flagellimonas eckloniae* gen. nov. sp. nov. a mesophilic marine bacterium of the family Flavobacteriaceae, isolated from the rhizosphere of *Ecklonia kurome*. Int J Syst Evol Microbiol 57:1050–1054
4. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) SPAdes: a new genome assembly algorithm and its applications to single cell sequencing. J Comput Biol 19:455–477
5. Barel G, Jurkevitch E (2001) Analysis of phenotypic diversity among host-independent mutants of *Bdellovibrio bacteriovorus* 109J. Arch Microbiol 176:211-216
6. Bernardet JF, Nakagawa Y, Holmes B (2002) Proposed minimal standards for describing new taxa of the family Flavobacteriaceae and emended description of the family. Int J Syst Evol Microbiol 52:1049–1070
7. Bruns A, Rohde M, Berthe-Corti L (2001) *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. Int J Syst Evol Microbiol 51:1997–2006
8. Collins MD, Costas M, Owen RJ (1984) Isoprenoid quinone composition of representatives of the genus Campylobacter. Arch Microbiol 137:168–170
9. Dang YR, Sun YY, Sun LL, Yuan XX, Li Y, Qin QL, Chen XL, Zhang YZ, Shi M, Zhang XY (2019) *Muricauda nanhaiensis* sp. nov., isolated from seawater of the South China Sea. Int J Syst

Evol Microbiol 69:2089–2094

10. Delong EF (1992) Archaea in coastal marine environments. Proc Natl Acad Sci USA 89:5685–5689
11. Dong B, Zhu S, Chen T, Ren N, Chen X, Chen Y, Xue Z, Shen X, Huang Y, Yang J, Chen J (2020) *Muricauda oceani* sp. nov. isolated from the East Pacific Ocean. Int J Syst Evol Microbiol 70: 3839-3844
12. Dong XZ, Cai MY (2001) Determinative manual for routine bacteriology. Scientific Press, Beijing (in Chinese)
13. Germida JJ, Casida LE (1983) *Ensifer adhaerens* predatory activity against other bacteria in soil, as monitored by indirect phage analysis. Appl Environ Microbiol 45:1380–1388
14. Hameed A, Arun AB, Ho HP, Chang CMJ, Rekha PD, Lee MR, Singh S, Young CC (2011) Supercritical carbon dioxide micronization of zeaxanthin from moderately thermophilic bacteria *Muricauda lutaonensis* CC-HSB-11^T. J Agric Food Chem 59:4119–4124
15. Hwang CY, Kim MH, Bae GD, Zhang GI, Kim YH, Cho BC (2009) *Muricauda olearia* sp. nov., isolated from crude-oil-contaminated seawater, and emended description of the genus *Muricauda*. Int J Syst Evol Microbiol 59:1856–1861
16. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ (2010) Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119
17. Jurkevitch E, Davidov Y (2006) Phylogenetic diversity and evolution of predatory prokaryotes. In: Jurkevitch E (eds) Predatory prokaryotes. Microbiology Monographs, vol 4. Springer, Berlin, Heidelberg
18. Kates M (1972) Techniques of lipidology: isolation, analysis and identification of lipids. In: Work TS and Work E (eds) Laboratory Techniques in Biochemistry & Molecular Biology, vol. 3. Elsevier, Amsterdam, pp 0151–0155
19. Kim D, Yoo Y, Khim JS, Yang D, Pathiraja D, Choi IG, Kim JJ (2020) *Muricauda ochracea* sp. nov. isolated from a tidal flat in the Republic of Korea. Int J Syst Evol Microbiol 70:4555-4561
20. Kim JM, Jin HM, Jeon CO (2013) *Muricauda taeanensis* sp. nov., isolated from a marine tidal flat. Int J Syst Evol Microbiol 63:2672–2677
21. Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351
22. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, Na H, Park SC, Jeon YS, Lee JH, Yi H, Won S, Chun J (2012) Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. Int J Syst Evol Microbiol 62:716–721
23. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874
24. Lee SY, Park S, Oh TK, Yoon JH (2012) *Muricauda beolgyonensis* sp. nov., isolated from a tidal flat. Int J Syst Evol Microbiol 62:1134–1139

25. Li G, Lai Q, Yan P, Shao Z (2019) *Roseovarius amoyensis* sp. nov. and *Muricauda amoyensis* sp. nov., isolated from the Xiamen coast. Int J Syst Evol Microbiol 69:3100–3108
26. Liu L, Yu M, Zhou S, Fu T, Sun W, Wang L, Zhang XH (2020) *Muricauda alvinocaridis* sp. nov., isolated from shrimp gill from the Okinawa Trough. Int J Syst Evol Microbiol 70:1666–1671
27. Liu SQ, Sun QL, Sun YY, Yu C, Sun L (2018) *Muricauda iocasae* sp. nov., isolated from deep sea sediment of the South China Sea. Int J Syst Evol Microbiol 68:2538–2544
28. McCauley EP, Haltli B, Kerr RG (2015) Description of *Pseudobacteriovorax antillogorgiicola* gen. nov. sp. nov. a bacterium isolated from the gorgonian octocoral *Antillogorgia elisabethae*, belonging to the family Pseudobacteriovoracaceae fam. nov. within the order Bdellovibrionales. Int J Syst Evol Microbiol 65:522–530
29. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 14:60
30. Mu DS, Wang S, Liang QY, Du ZZ, Tian R, Ouyang Y, Wang XP, Zhou A, Gong Y, Chen GJ, Nostrand JV, Yang Y, Zhou J, Du ZJ (2020) Bradymonabacteria, a novel bacterial predator group with versatile survival strategies in saline environments. BMC Microb 8:126
31. Park JS (2019) *Muricauda hymeniacidonis* sp. nov., isolated from sponge of *Hymeniacidon sinapium*. Int J Syst Evol Microbiol 69:3800–3805
32. Pérez J, Moraleda-Muñoz A, Marcos-Torres FJ, Muñoz-Dorado J (2016) Bacterial predation: 75 years and counting!. Environ Microbiol 18:766–779
33. Reichenbach H (1989) Order I. *Cytophagales* Leadbetter 1974. In: Staley JT, Bryant MP, Pfennig N, Holt JG (eds) Bergey's Manual of Systematic Bacteriology, vol. 3. Williams & Wilkins, Baltimore, pp 2011–2013
34. Richter M, Rosselló-Móra R (2009) Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci USA 106:19126–19131
35. Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. MIDI Inc., Newark
36. Schoeffield AJ, Williams HN (1990) Efficiencies of recovery of bdellovibrios from brackish- water environments by using various bacterial species as prey. Appl Environ Microbiol 56:230–236
37. Sorokin DY, Mosier D, Zorz JK, Dong X, Strous M (2020) *Wenzhouxiangella* strain AB-CW3, a proteolytic bacterium from ypersaline Soda Lakes that preys on cells of Gram-positive bacteria. Front Microbiol 11:597686
38. Su Y, Yang X, Wang Y, Liu Y, Ren Q, Zhang XH (2017) *Muricauda marina* sp. nov., isolated from marine snow of Yellow Sea. Int J Syst Evol Microbiol 67:2446–2451
39. Svercel M, Saladin B, Moorsel SJ, Wolf S, Bagheri HC (2011) Antagonistic interactions between filamentous heterotrophs and the cyanobacterium *Nostoc muscorum*. BMC Res Notes 4:357
40. Wang Y, Yang X, Liu J, Wu Y, Zhang XH (2017) *Muricauda lutea* sp. nov., isolated from seawater. Int J Syst Evol Microbiol 67:1064–1069

41. Wang ZJ, Liu QQ, Zhao LH, Du ZJ, Chen GJ (2015) *Bradymonas sediminis* gen. nov. sp. nov. isolated from coastal sediment, and description of Bradymonadaceae fam. nov. and Bradymonadales ord. nov. Int J Syst Evol Microbiol 65:1542–1549
42. Williams HN, Chen H (2020) Environmental regulation of the distribution and ecology of *Bdellovibrio* and like organisms. Front Microbiol 11:545070
43. Wu YH, Yu PS, Zhou YD, Xu L, Wang CS, Wu M, Oren A, Xu XW (2013) *Muricauda antarctica* sp. nov., a marine member of the *Flavobacteriaceae* isolated from Antarctic seawater. Int J Syst Evol Microbiol 63:3451–3456
44. Yang C, Li Y, Guo Q, Lai Q, Wei J, Zheng T, Tian Y (2013) *Muricauda zhangzhouensis* sp. nov., isolated from mangrove sediment. Int J Syst Evol Microbiol 63:2320–2325
45. Ye XS, Chen MX, Li HY, He XY, Zhao Y (2019) *Halobacteriovorax vibrionivorans* sp. nov., a novel prokaryotic predator isolated from coastal seawater of China. Int J Syst Evol Microbiol 69:3917–3923
46. Yoon BJ, Oh DC (2012) *Spongiibacterium flavum* gen. nov. sp nov. a member of the family Flavobacteriaceae isolated from the marine sponge *Halichondria oshoro*, and emended descriptions of the genera *Croceitalea* and *Flagellimonas*. Int J Syst Evol Microbiol 62:1158–1164
47. Yoon JH, Kang SJ, Jung YT, Oh TK (2008) *Muricauda lutimaris* sp. nov., isolated from a tidal flat of the Yellow Sea. Int J Syst Evol Microbiol 58:1603–1607
48. Yoon JH, Lee MH, Oh TK, Park YH (2005) *Muricauda flavescens* sp. nov. and *Muricauda aquimarina* sp. nov., isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea, and emended description of the genus *Muricauda*. Int J Syst Evol Microbiol 55:1015–1019
49. Yoon SH, Ha SM, Lim J, Kwon S, Chun J (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. Antonie Van Leeuwenhoek 110:1281–1286
50. Zhang X, Liu X, Lai Q, Du Y, Sun F, Shao Z (2018) *Muricauda indica* sp. nov., isolated from deep sea water. Int J Syst Evol Microbiol 68:881–885
51. Zhang Y, Gao Y, Pei J, Cao J, Xie Z, Liu R, Wang L, Wei Y, Fang J (2020) *Muricauda hadalis* sp. nov., a novel piezophile isolated from hadopelagic water of the Mariana Trench and reclassification of *Muricauda antarctica* as a later heterotypic synonym of *Muricauda teanensis*. Int J Syst Evol Microbiol 70:4315-4320
52. Zhang Z, Gao X, Qiao Y, Wang Y, Zhang XH (2015) *Muricauda pacifica* sp. nov., isolated from seawater of the South Pacific Gyre. Int J Syst Evol Microbiol 65:4087–4092

Tables

Table 1. Different characteristics of strain HICW^T and related type strains of *Muricauda*

Characteristic	1	2	3
Pigmentation	yellow	orange	yellow
Cell size (μm)	0.3–0.4×1.8–3.7	0.2–0.5×2.5–6.0	0.3–0.6×1.1–1.7
Facultative anaerobe	+	–	+
Oxidase activity	–	+	+
Catalase activity	+	+	–
α-fucosidase	+	–	+
Ranges (optima) for growth			
Temperature (°C)	15–40 (25–30)	10–44 (30–37)	8–40 (20–30)
NaCl (%, w/v)	0.5–8.0 (2.0–3.0)	0.5–9.0 (2.0–3.0)	0.5–9.0 (2.0–3.0)
pH	6.0–8 .0(7.0)	6.0–9.0 (7.0)	6.0–9.0 (8.0)
Nitrate reduction	W	–	–
Gelatin hydrolysis	–	–	W
Utilization of:			
D-glucose	+	–	+
L-arabinose	+	–	+
D-mannose	+	–	+
N-acetyl-glucosamine	–	–	+
D-maltose	W	–	+
Potassium gluconate	W	–	W
Draft genome size (Mb)	3.8	3.4	3.8
DNA G+C content (mol%)	41.4	43.4	41.4
dDDH values to HICW^T (%)	100	34.1	34.5
ANI values to HICW^T (%)	100	79.2	80.6

Strains: 1, HICW^T; 2, *M. aquimarina* JCM 11811^T; 3, *M. ruestringensis* DSM 13258^T. The tests for oxidase activities, the API 20NE and API ZYM strip were performed on strain HICW^T and the reference strains in this study. Genome sequences of the reference strains were taken from GenBank and analysed in this study. Other data for the reference strains were taken from their original description (Bruns et al. 2001; Yoon et al. 2005). +, Positive; W, weakly positive; –, negative

Table 2. General features of the genome of strain HICW^T

Attribute	Characteristics
Genome size (bp)	3777431
Number of contigs	13
sequencing depth	428
N50 length (bp)	1121114
N90 length (bp)	125639
G+C content (%)	41.4
Number of coding genes	3569
rRNA	5
tRNA	37

Figures

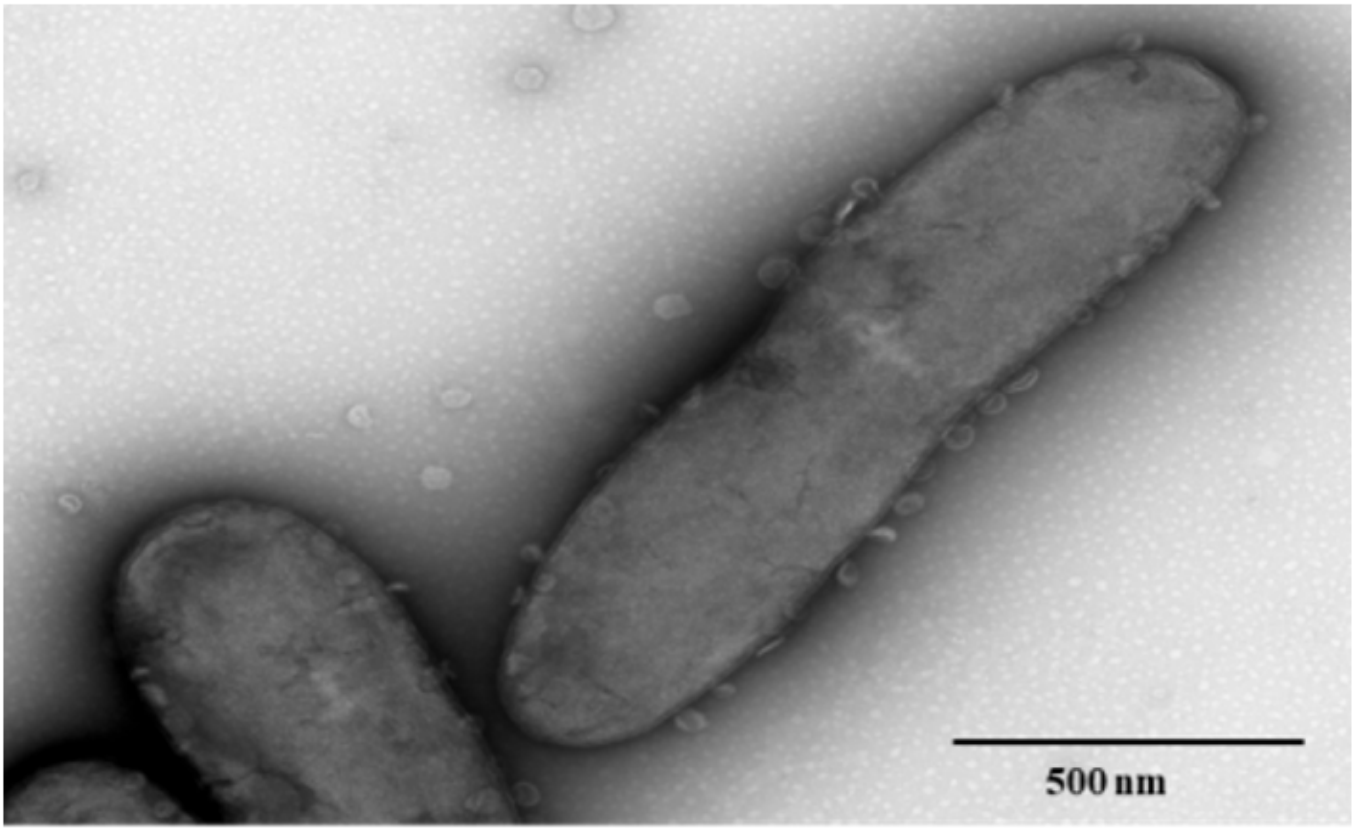

Figure 1

Transmission electron microscopy images of strain HICWT.

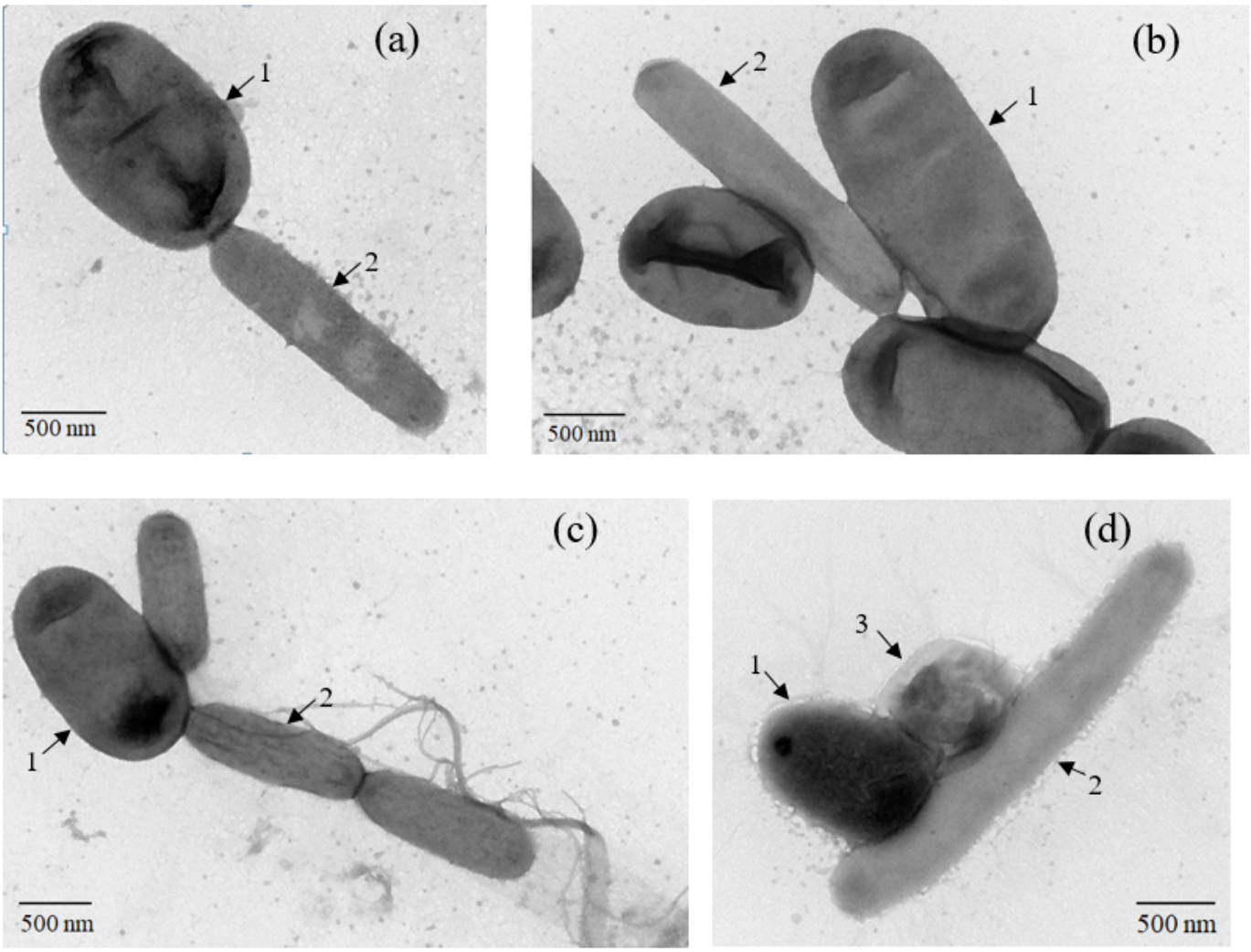

Figure 2

Transmission electron microscopy images of strain HICWT attachs to Vibrio alginolyticus LF TCBS15. arrow 1, Vibrio alginolyticus LF TCBS15; arrow 2, strain HICWT; arrow 3, empty prey cell.

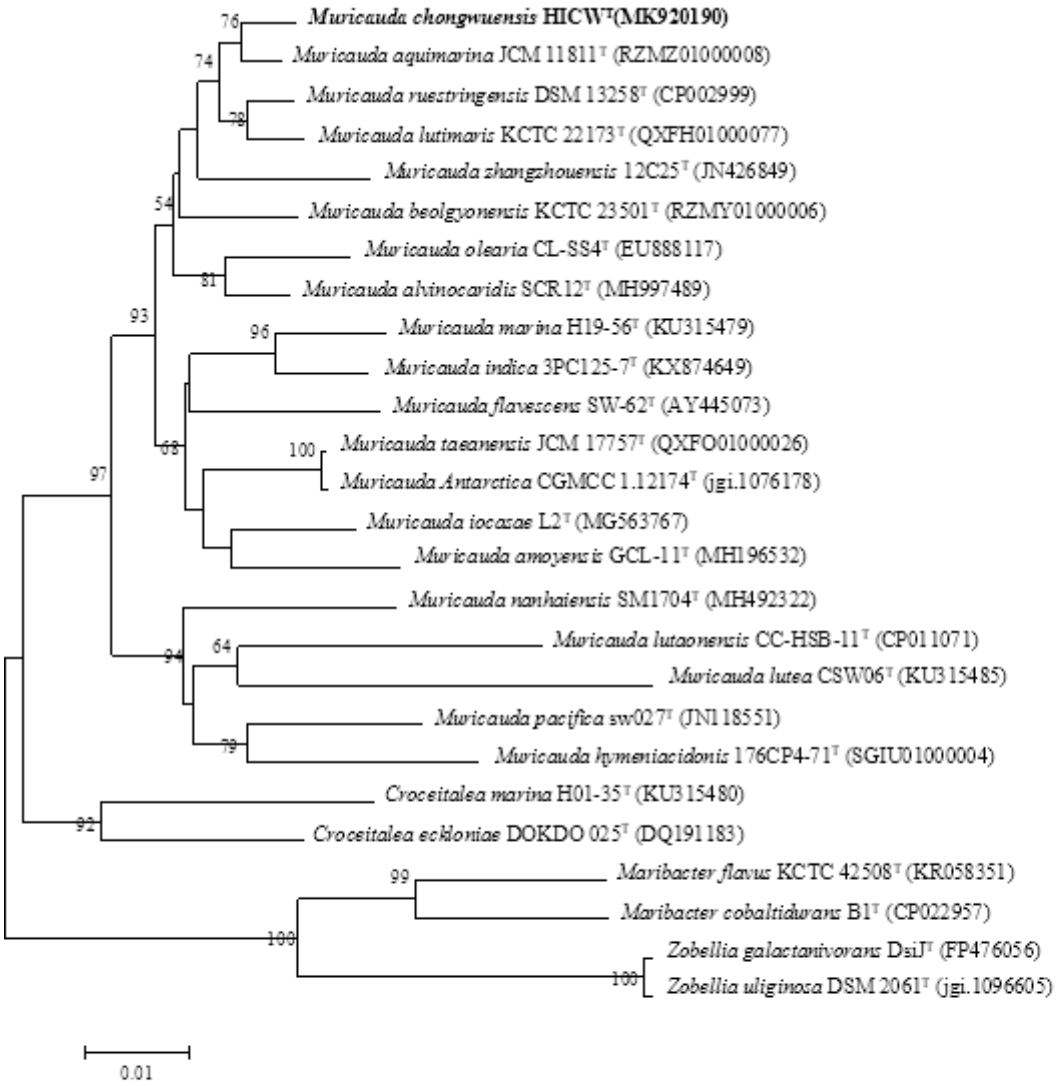

Figure 3

Neighbour-joining tree showing phylogenetic positions of Muricauda chongwuensis HICWT and its related species based on 16S rRNA gene sequences. Accession numbers for the type strains are shown in parenthesis. Only bootstrap values (expressed as percentages of 1000 replications) greater than 50 are shown at branching points. Bar, 0.01 substitutions per nucleotide position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 4.supplementsMuricaudachongwuensissp.nov..docx