



Anaerophilus nitritogenes gen. nov., sp. nov., isolated from salt lake sediment in Xinjiang Province, China

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Abstract An obligately anaerobic, nitrate-reducing bacterial strain (MJB2^T) was isolated from sediments of saline in Xinjiang province of China. Cells were Gram-stain-positive rods and motile by means of flagella and formed endospores. The novel strain MJB2^T was able to grow at 15–37 °C (optimum 28–30 °C), pH 5.8–9.4 (optimum 7.8) and with 1.0–7.0% NaCl (optimum 5.0–6.0%, w/v). Sulfate, sulfite, thiosulfate, elemental sulfur, nitrite and Fe(III) were not used as terminal electron acceptors. Oxidase

and catalase reactions were positive. H₂S was produced from L-cystine. Complex substrates such as beef extract, peptone and yeast extract can be used as sole energy sources. The DNA G+C content was 29.4 mol%. The major cellular fatty acids (> 10%) were C_{14:0}, C_{16:1} cis 7 and C_{16:1} cis 9. The main polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, three unidentified amino lipids, one unidentified amino glycolipid, two unidentified glycolipid, one unidentified aminophospholipid and two unidentified lipids. No respiratory quinones were detected. According to phylogenetic analysis based on 16S rRNA gene sequences, strain MJB2^T was affiliated to the family *Clostridiaceae* (order *Clostridiales*) with highest 16S rRNA gene sequence similarity of 95.3% to *Crasamicella profunda* Ra1766H^T. Strain MJB2^T exhibited 74.9% ANI values to *C. profunda*

The GenBank/EMBL/DDBJ Accession Number for the 16S rRNA gene sequence of strain MJB2^T is MH802515. The GenBank Accession Numbers for the whole genome sequences of strain MJB2^T is RYYS01000000.

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Ra1766H^T. In silico DNA–DNA relatedness value between strain MJB2^T and *C. profunda* Ra1766H^T was 19.5%. The distinct biochemical, chemotaxonomic and phylogenetic differences from the previously described taxa supported that strain MJB2^T represents a novel species of a new genus, for which the name *Anaerophilus nitritogenes* gen. nov., sp. nov. is proposed. The type strain is MJB2^T (=KCTC 15800^T=MCCC 1K03631^T).

Keywords *Anaerophilus* · Polyphasic taxonomy · 16S rRNA gene · Taxonomy

Abbreviations

ANI Average nucleotide identity
DDH DNA–DNA hybridization

Introduction

During an investigation of the bacterial diversity of lakes in Xinjiang province, north-western China, a novel strain designated MJB2^T was isolated from the sediment of Manasi Lake. Preliminary analysis of the 16S rRNA gene sequence of strain MJB2^T indicated it was related to members of the family *Clostridiaceae*. The family *Clostridiaceae* is the first of 19 families within the order *Clostridiales* of the class “*Clostridia*” of the phylum *Firmicutes* (Vos et al. 2009). At present, the family *Clostridiaceae* comprises over 50 genera (<https://www.ncbi.nlm.nih.gov/taxonomy>) that were isolated from various environments, such as soil, manure piles, freshwater, and marine sediments (Kuhner et al. 2000; Zhu et al. 2018; Klouche et al. 2007; Kim et al. 2007; Pi et al. 2013). Members of the family *Clostridiaceae* are generally obligately anaerobic rods and stain Gram-positive. Most of the proposed species belonging to the *Clostridiaceae* form endospores or have been shown to contain sporulation-specific genes (Onyenwoke et al. 2004). Based on polyphasic taxonomic approach, we conclude that strain MJB2^T represents a novel species of a novel genus *Anaerophilus*, for which the name *Anaerophilus nitritogenes* gen. nov. sp. nov. is proposed.

Materials and methods

Isolation, cultivation and maintenance

Strain MJB2^T was isolated from a sediment sample from Manasi Lake, Xinjiang, China (45° 45' N, 85° 45' E), in summer 2017. Samples for this study were collected in sterile serum bottles which were completely filled with water of the site and sealed with butyl stoppers, transported to the laboratory in an icebox and stored at 4 °C.

The medium for enrichment cultivation contained the following composition (L⁻¹): NaCl 19.45 g, MgCl₂·7H₂O 12.6 g, MgSO₄·7H₂O 6.64 g, CaCl₂ 1.8 g, KCl 0.55 g, NaHCO₃ 0.16 g, KBr 0.08 g, H₃BO₃ 22 mg, NaSiO₃·9H₂O 9.3 mg, NaF 2.4 mg, NH₄NO₃ 2.4 mg, Na₂HPO₄ 8 mg, SrCl₂·6H₂O 57 mg, ferric citrate 0.1 g, Bacto peptone 5 g, Bacto yeast extract 1 g, 1 mg resazurin and 0.4 g cysteine (the pH was adjusted to 7.0 with NaOH). The medium was prepared under anaerobic conditions in an anaerobic workstation (Coylab Vacuum Airlock S/N) containing a gas phase of N₂/H₂/CO₂ (80:10:10%, by vol.). After cultivation at 30 °C for 5 days, a white-colored colony was collected and named as MJB2^T. The colony was picked and purified by repeated restreaking. The strain was preserved at – 80 °C with 30% (v/v) glycerol supplemented with cysteine (0.05%, w/v) and Na₂S·9H₂O (0.05%, w/v) for further study. Strain MJB2^T has been deposited at the Marine Culture Collection of China and the Korean Collection for Type Cultures.

Physiological and biochemical analyses

Growth experiments were performed using Hungate tubes with medium of the same composition as used for the enrichment cultivation procedure. Temperature range for growth was respectively tested at 10, 15, 20, 25, 28, 30, 35, 37, 40, 42, 45 and 50 °C. Na⁺ tolerance was investigated in the enrichment cultural medium without Na⁺ ions at various NaCl concentrations (0, 0.25, 0.5 and 1.0–9.0%, at increments of 1%, w/v). The pH range (from pH 5.0 to 10.0, at intervals of 0.5 pH units) was determined using the buffer system described by Zhang et al. (2018).

After incubation for 3 days at 30 °C, OD₅₉₀ values were measured with a UV/visible spectrophotometer (Ultrospec 6300 pro; Amersham Biosciences) to

determine the optical growth, and the growth limits were tested after 14 days of incubation.

Cell morphology, motility and spores forming were observed by using optical microscope (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) after incubation on the enrichment cultural medium ager at 30 °C for 2 days (Huo et al. 2010). Catalase activity was determined by production of bubbles after the addition of a drop of 3% H₂O₂. Oxidase activity was observed by using the oxidase reagent (Dong and Cai 2001). Gram staining was performed by following the method outlined by Claus (1992).

H₂S production was tested using L-cystine as a substrate and determined according to Wu et al., (2010). Other enzyme activities and biochemical characteristics were tested using API 20A and API Rapid 32A (bioMérieux) test kits following the manufacturer's instructions.

Substrate utilization tests were performed using the enrichment cultural medium (without peptone and with 0.1% yeast extract as growth factors). D-arabinose, beef extract powder, erythritol, D-galactose, glucose, inosine, inositol, α -ketopamyl diacid, D-lactose, D-mannose, peptone, pyruvate, D-raffinose, rhamnose, D-ribose, sorbin, sorbitol, D-xylose and yeast extract were tested as growth substrates. Each substrate was added to the enrichment cultural medium at a final concentration of 20 mM. For analyses of electron acceptors, elemental sulfur (1%, w/v), thiosulfate (20 mM), sodium sulfite (2 mM), sodium sulfate (20 mM), sodium nitrite (2 mM), sodium nitrate (10 mM) and Fe(OH)₃ (13 mM) were added individually to the modified enrichment cultural medium (without peptone and with 0.1% yeast extract as growth factors). Susceptibility to antibiotics was determined on the enrichment cultural medium plates after incubation for 3 days at 30 °C using antibiotic discs (Hangzhou Microbial Reagent Co. Ltd, HangweiTM) which containing (per piece): chloramphenicol (30 μ g), ciprofloxacin (5 μ g), erythromycin (15 μ g), kanamycin (30 μ g), novobiocin (30 μ g), penicillin (10 U), polymyxin B (30 μ g), rifamcin (5 μ g), tetracycline (30 μ g), streptomycin (10 μ g). Sensitivity to oxygen was tested by inoculating the bacterium to the enrichment cultural medium without deoxidization.

Chemotaxonomic characterisation

For the preparation of cellular fatty acid methyl esters (FAMES), cells of strain MJB2^T and reference strain *Crassaminicella profunda* Ra1766H^T were harvested and freeze-dried at the exponential stage of growth after cultivated for 28 h at 30 °C on the enrichment cultural medium. Whole cell fatty acids were extracted and analyzed according to the instructions of the Microbial Identification System (Sherlock Version 6.0; MIDI database: ANAER6). Cells of the strain MJB2^T and the related strain *C. profunda* Ra1766H^T which grown on the enrichment cultural medium for 2 days at 30 °C were used for isoprenoid quinones and polar lipids analysis. Polar lipids were extracted by 80 ml of chloroform/methanol/water (1:2:1, by vol) and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) as described previously (Zhang et al. 2019). Isoprenoid quinones were analyzed as described by Komagata and Suzuki (1988) using HPLC–MS.

16S rRNA gene sequencing and phylogenetic analysis

We used a quick bacteria genomic DNA extraction kit (DongSheng Biotech) to obtain high quality genomic DNA of strain MJB2^T. The 16S rRNA gene was amplified, cloned and sequenced according to Zhang et al. (2018). The almost-complete 16S rRNA gene sequence (1495 bp) was subjected to pairwise sequence alignment by the BLASTN program (<http://www.ncbi.nlm.nih.gov>). Multiple sequences were aligned with Clustal W program of the MEGA 7 package (Kumar et al. 1994). The neighbour-joining (NJ, Saitou and Nei 1987), maximum-evolution (ME, Saitou and Nei 1987) and maximum-likelihood (ML, Felsenstein 1981) algorithms were used in phylogenetic tree-building. Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura 1980) for the neighbour-joining tree. The topology of the phylogenetic trees was evaluated by using the bootstrap based on 1000 replicates (Felsenstein 1985).

Genome sequencing and analysis

As described by Chun et al. (2018), combination of 16S similarity and OGRI can be used in a systematic process to identify and recognize a new species. Considering strain MJB2^T highest 16S rRNA gene sequence similarity is 95.3% which is lower than the recommended threshold value 98.7% (Chun et al. 2018), strain MJB2^T and *C. profunda* Ra1766H^T genome sequences were both sequenced and analysed. The genome of strain MJB2^T and *C. profunda* Ra1766H^T was sequenced by Solexa PE150 sequencing technology with the HiSeq platform (Beijing Genomics Institute). The de novo assembly of the reads was performed using ABySS 1.5.2 (Simpson et al. 2009). The assembly k-value was tested from 40 to 64 to find the optimal one using abyss-pescript. The quality of microbial genomes was assessed using the bioinformatics tool CheckM 1.0.8 (Parks et al. 2015). The open reading frames (ORFs) were predicted and annotated by Rapid Annotation using Subsystem Technology (RAST) server online (Overbeek et al. 2014). The average nucleotide identity (ANI) was calculated using the OrthoANIu algorithm of the Chun lab's online Average Nucleotide Identity calculator (Lee et al. 2016). In silico DNA–DNA hybridization (*is*DDH) values were calculated by genome-to-genome distance calculator (GGDC) (Meier-Kolthoff et al. 2013a, b). The DNA G+C content of strain MJB2^T was calculated from the draft genome sequence.

Results and conclusion

Morphological, physiological, and biochemical analyses

Cells of strain MJB2^T were Gram-stain-positive, strictly anaerobic, rod-shaped (2.3–3.5 µm long and 0.3–0.7 µm wide) (Supplementary Fig. S1), motile by flagella and formed endospores. Colonies were circular, 0.5–1 mm in diameter with white, flat, and rough surface after incubation at 30 °C for 48 h on the enrichment cultural medium agar. Strain MJB2^T grew optimally at pH 7.8, 28–30 °C and in the presence of 5.0–6.0% (w/v) NaCl. The strain was tested positive for catalase, oxidase activities and H₂S production. Nitrate was reduced to nitrite as electron acceptor, but

sulfate, sulfite, elemental sulfur, Fe(III) and thiosulfate were not used as terminal electron acceptors.

Strain MJB2^T can assimilate beef extract powder, peptone and yeast extract while not use D-arabinose, erythritol, D-galactose, glucose, inosine, inositol, α-ketopamyl diacid, D-lactose, D-mannose, pyruvate, D-raffinose, rhamnose, D-ribose, sorbin, sorbitol and D-xylose. Strain MJB2^T was sensitive to streptomycin (10 µg), but resistant to chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), kanamycin (30 µg), novobiocin (30 µg), penicillin (10 U), polymyxin B (30 µg), rifamcin (5 µg) and tetracycline (30 µg). All the enzyme tests showed negative results in the API 20A and API Rapid 32A kit except arginine arylamidase. Detailed physiological, biochemical and chemotaxonomic characteristics of strain MJB2^T are presented in Table 1 and the species description.

Chemotaxonomic characteristics

The principal cellular fatty acids of strain MJB2^T was C_{14:0}, C_{16:1} cis 7 and C_{16:1} cis 9 and the profiles shows some major differences with *C. profunda* Ra1776H^T. Strain MJB2^T lacked C_{16:1} cis 9 DMA which was detected as major ones in *C. profunda* Ra1776H^T (13.42%). The complete fatty acid profiles of strain MJB2^T and the type strains are summarised in Table S1. The respiratory quinone was absent in strain MJB2^T which is identical with *C. profunda* Ra1776H^T. Strain MJB2^T consist of phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), three unidentified amino lipids (AL-1, AL-2, AL-3), one unidentified amino glycolipid (AGL), two unidentified glycolipid (GL-1, GL-2), one unidentified aminophospholipid (APL) and two unidentified lipids (L-1, L-2). The polar lipids profile of strain MJB2^T was slightly different from that of *C. profunda* Ra1776H^T. An unidentified phospholipid (PL in Fig. S2) which was presented in *C. profunda* Ra1776H^T, was not detected in strain MJB2^T.

Molecular characterisation and phylogenetic analysis

The almost complete 16S rRNA gene (1495 bp) sequence of strain MJB2^T was determined (GenBank/EMBL/DDDBJ accession number MH802515). In phylogenetic trees generated using NJ, ML, and ME algorithms, strain MJB2^T was included within the

Table 1 Differential characteristics among strains MJB2^T and related type strain

Characteristic	1	2
Cell size (µm)	0.3–0.7 × 2.3–3.5	0.5–1 × 6–10 ^a
Growth range		
Temperature range (optimum) (°C)	15–37 (28–30)	25–45 (30) ^a
pH range (optimum)	5.8–9.4 (7.8)	6.7–8.1 (7.5)
NaCl range (optimum) (% w/v)	1–7 (5–6)	0.5–6 (3) ^a
Electron acceptors used		
Nitrate	+	–
Utilization of:		
D-arabinose	–	+
Erythritol	–	+
D-galactose	–	+
Glucose, inosine, inositol	–	+
D-lactose, D-mannose, pyruvate	–	+
D-raffinose, rhamnose, D-ribose	–	+
Sorbitol, sorbitol, D-xylose,	–	+
API 20A		
D-lactose	–	+
D-saccharose	–	+
L-arabinose	–	+
D-mannose	–	+
API 32A		
Mannose fermentation	–	+
Raffinose fermentation	–	+
Alkaline phosphatase	–	+
Leucine arylamidase	–	+
Histidine arylamidase	–	+
DNA G+C content (mol%)	29.4	33.7
Polar lipids	DPG, PG, PE, AGL, APL, GL-1, GL-2, AL-1, AL-2, AL-3, L-1, L-2	DPG, PG, PE, AGL, APL, GL-1, GL-2, AL-1, AL-2, AL-3, L-1, L-2, PL

Taxa: 1, strain MJB2^T; 2, *C. profunda* Ra1766H^T. All data were from this study unless otherwise indicated. Both strains are strictly anaerobic, Gram-stain-positive and positive for catalase, oxidase and H₂S production. Both strains form endospores and motile by means of flagella. Both strains have no quinone detected. –, negative; +, positive

^aData was taken from Fardeau et al. (2015)

family *Clostridiaceae*. Analysis using BLASTN program (<http://www.ncbi.nlm.nih.gov>) revealed that strain MJB2^T exhibits highest 16S rRNA gene sequence similarity to *C. profunda* Ra1776H^T

(95.3%). Sequence similarities with all other members of the family *Clostridiaceae* were < 95%. Phylogenetic analysis based on the multiple sequences alignment indicated that strain MJB2^T formed a separated

lineage away from other related members of the family *Clostridiaceae* (Figs. 1, 2).

Genome properties and comparison

The draft genome of strain MJB2^T was deposited at DDBJ/EMBL/GenBank under the Accession Number RYYS00000000. Draft genome sequences of strain MJB2^T and *C. profunda* Ra1776H^T generated 2.287 and 1.642 GB of clean data respectively. The genome completeness of strain MJB2^T and *C. profunda* Ra1776H^T was 98.6% and 99.3%, with a contamination percentage of 1.8% and 1.8%, respectively, which could be considered as excellent reference genome sequence for deeper analyses (Parks et al. 2015). Draft genome sequences of strains MJB2^T and *C. profunda* Ra1776H^T yielded genome size of 3,065,888 bp and 4,463,735 bp and produced 114 and 79 contigs after assembly respectively. N50 values of strains MJB2^T and *C. profunda* Ra1776H^T were 51,602 bp with the

largest contig of 230,820 bp, and 118,922 bp with the largest contig of 312,283 bp, respectively. The quality of the genome is high enough and suitable for taxonomic analysis (Chun et al. 2018). Coding Sequences of strain MJB2^T and *C. profunda* Ra1776H^T was 3,099 and 4,241, respectively and both have dormancy and sporulation gene. The genomic DNA G+C content of strain MJB2^T calculated from the draft genome sequence was 29.4 mol%, slightly lower than that of *C. profunda* RA1776H^T (33.7%). Strain MJB2^T showed ANI values of 74.9% to *C. profunda* RA1776H^T. The in silico DDH values (the recommended results from formula 2) indicated that strain MJB2^T shared 19.5% DNA relatedness with *C. profunda* RA1776H^T. The ANI and in silico DDH values were significantly lower than the recommended threshold value of 95–96% (Yoon et al. 2017) and 70% (Wayne et al. 1987) (Meier-Kolthoff et al. 2013a, b), strongly supporting the conclusion that strain MJB2^T

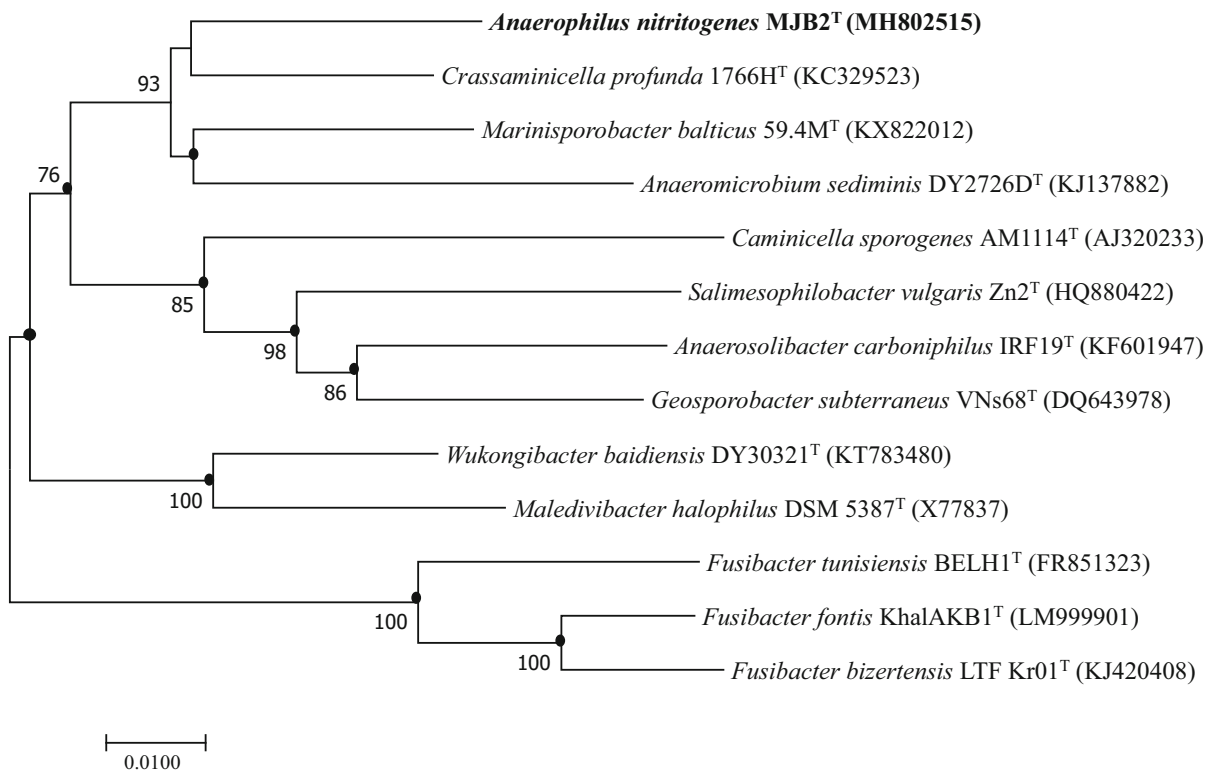


Fig. 1 Maximum-likelihood tree based on 16S rRNA gene sequences, showing the relationships of strain MJB2^T and related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values

above 50% are shown. Filled circles indicate that the corresponding nodes were recovered in maximum-likelihood, maximum-evolution and neighbor-joining phylogenetic trees. Bar, 0.01 substitutions per nucleotide position

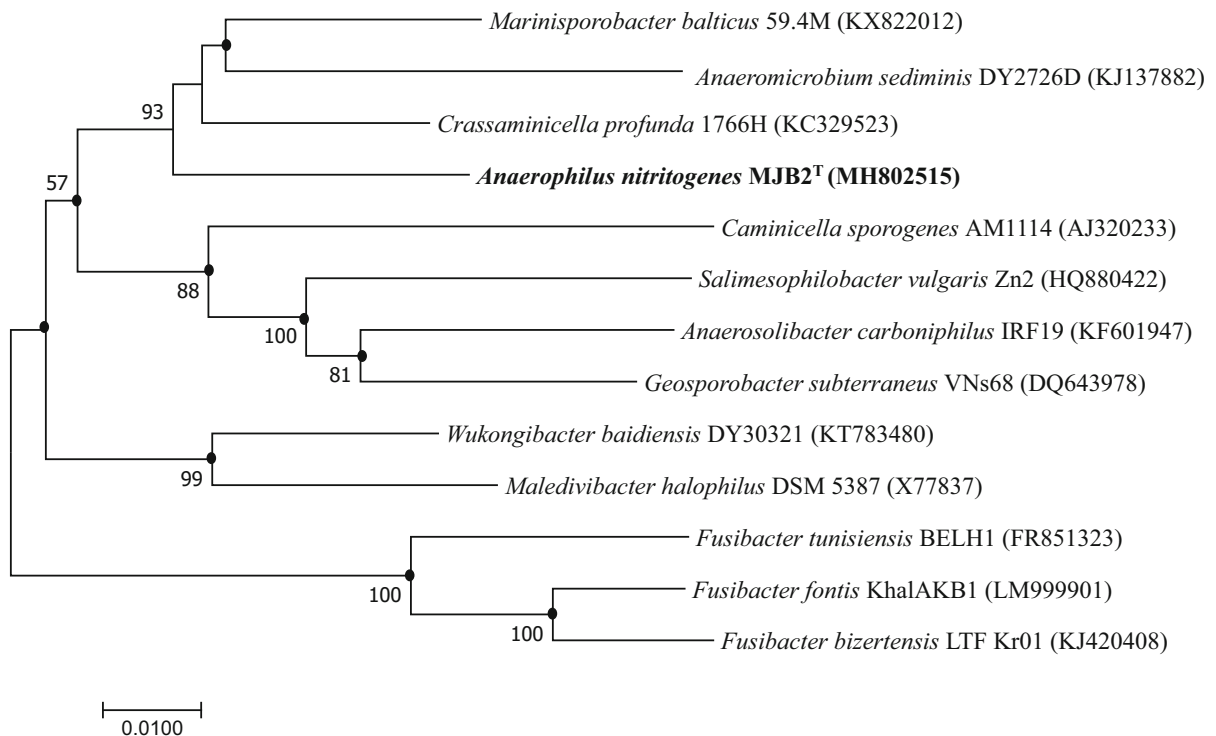


Fig. 2 Neighbour-joining tree based on 16S rRNA gene sequences, showing the relationships of strain MJB2^T and related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values

above 50% are shown. Filled circles indicate that the corresponding nodes were recovered in maximum-likelihood, maximum-evolution and neighbor-joining phylogenetic trees. Bar, 0.01 substitutions per nucleotide position

represents a novel species within the family *Clostridiaceae*.

Taxonomic conclusion

Based on the phylogenetic analyses, strains MJB2^T was found to be affiliated with the family *Clostridiaceae* and formed a clade with *C. profunda* Ra1776H^T. However, there were a number of differences between strain MJB2^T and the reference genera. For example, strain MJB2^T showed high Na⁺ tolerance and low G+C content than strains in genus *Crassaminicella* and *Marinisorobacter*; Nitrate cannot be utilized in genus *Crassaminicella* and *Marinisorobacter*. Glucose cannot be assimilated by strain MJB2^T (Table 2). Moreover, besides the low 16S rRNA gene sequence similarities, comparison in physiological, biochemical and chemotaxonomic characteristics, suggesting that strain MJB2^T represents a novel species that is distinguished from the recognized *Clostridiaceae* species. On the basis of

these data, strain MJB2^T merits recognition as a member of a novel species of a new genus within the family *Clostridiaceae* for which the name *Anaerophilus nitritogenes* gen. nov., sp. nov. is proposed. The TaxoNumber of strain MJB2^T in Digital Protologue database is TA00991.

Description of *Anaerophilus* gen.nov

Anaerophilus [Gr. pref. *an*, not; Gr. masc. n. *aer*, air; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*), friend, loving; N.L. masc. adj. *anaerophilus*, not air-loving.].

Cells are rod-shaped with a Gram-positive-type cell wall. Terminal spores and flagella are formed. Catalase, oxidase and H₂S production are positive. No respiratory quinone is detected. The major cellular fatty acid ($\geq 10\%$) are C_{14:0}, C_{16:1} cis 7 and C_{16:1} cis 9. The major polar lipids consist of diphosphatidylglycerol, phosphatidylglycerol and phosphatidylcholine. The genus is affiliated to the family *Clostridiaceae* of

Table 2 Differential phenotypic characteristics of strain MJB2^T and closely related genera of the family *Clostridiaceae*

	1	2	3
Isolation source	Sediment of salt lake	Sediments of the Guaymas basin	Subsurface sediments of the Baltic Sea
Growth aerobic	–	–	+
Temperature optimum (°C)	28–30	30	25
pH optimum	7.8	7.5	7.0–7.3
NaCl optimum (%)	5–6	3	0.5
Nitrate reduction	+	–	–
Assimilation of			
Glucose	–	+	+
Major fatty acids (> 10%)	C _{14:0} , C _{16:1} cis 7, C _{16:1} cis 9	C _{14:0} , C _{16:1} ω7, C _{16:1} ω7 DMA, C _{16:0}	C _{14:0} , ai-C _{15:0} , C _{16:1} ω9c, C _{16:0} , C _{18:1} ω9c
DNA G+C content (mol%)	29.4	33.7	42.9

Taxa: 1, strain MJB2^T; 2, *Crassaminicella* (data from Fardeau et al. 2015); 3, *Marinisporobacter* (data from Vandieken et al. 2017). All the taxa are gram-stain-positive, and endospore-forming

+, Positive; –, negative

the order *Clostridiales*. The type species is *Anaerophilus nitritogenes*.

Description of *Anaerophilus nitritogenes* sp. nov

Anaerophilus nitritogenes (N.L. n. *nitris* -itis, nitrite; Gr. v. *gennaô*, produce, engender; N.L. adj. *nitritogenes*, nitrite-producing).

Following characters are displayed in addition to those given in the genus description. Cells are 2.3–3.5 µm long, 0.3–0.7 µm wide. After incubation on the enrichment cultural medium agar at 30 °C for 2 days, colonies were 0.5–1 mm in diameter with white, flat, circular and rough surface. Growth occurs at 15–37 °C (28–30 °C), pH 5.8–9.4 (optimum 7.8). NaCl growth at 1–7% (w/v), with an optimum at 5–6% (w/v). Growth detected on beef extract powder, peptone and yeast extract, while not on D-arabinose, erythritol, D-galactose, glucose, inosine, inositol, α-ketopamyl diacid, D-lactose, D-mannose, pyruvate, D-raffinose, rhamnose, D-ribose, sorbin, sorbitol, D-xylose. Nitrate was reduced to nitrite as electron, but sulfate, sulfite, elemental sulfur, Fe(III) and thiosulfate were not used as terminal electron acceptors. The main polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, three unidentified amino lipids, one unidentified amino

glycolipid, two unidentified glycolipid, one unidentified aminophospholipid and two unidentified lipids. The principal cellular fatty acids are C_{14:0}, C_{16:1} cis 7 and C_{16:1} cis 9. The DNA G+C content was 29.4 mol% (as determined by genome). All substrates in the API 20A system were not used. Positive for activity of arginine arylamidase by using the API 32A systems.

The type strain MJB2^T (=KCTC 15800^T=MCCC 1K03631^T) was isolated from sediments of saline, Xinjiang province, China. The GenBank accession number of the 16S rRNA gene sequence of the type strain is MH802515.

Supplementary material

The transmission electron micrograph of the cell, polar lipids and fatty acid profiles are available as supplementary materials. Supplementary data associated with this article can be found in the online version.

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Compliance with ethical standards

Conflict of interest Authors declare that there is no conflict of interest.

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