

# *Nisaea sediminum* sp. nov., a Heavy Metal Resistant Bacterium Isolated From Marine Sediment in the East China Sea

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## Research Article

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# Abstract

A Gram-negative, rod-shaped, motile and strictly aerobic bacterium, designated NBU1469<sup>T</sup>, was isolated from marine sediment sampled on Meishan Island located in the East China Sea. Strain NBU1469<sup>T</sup> grew optimally at temperature of 40°C, NaCl concentration of 2.0% (w/v) and pH 7.5. Catalase and oxidase activities, H<sub>2</sub>S production, nitrate reduction and hydrolysis of Tween 20 were positive. Indole, methyl red reaction, urease, hydrolysis of gelatin, starch, casein, Tweens 40, 60 and 80 were negative. The major cellular fatty acids were C<sub>16:0</sub>, C<sub>19:0</sub> cyclo  $\omega$ 8c and summed feature 8 (C<sub>18:1</sub>  $\omega$ 7c and/or C<sub>18:1</sub>  $\omega$ 6c). The only respiratory quinone was ubiquinone-10 (Q-10). The major polar lipids were phosphatidylglycerol, two unidentified amino-phospholipids and two unidentified phospholipids. Comparative analysis of the 16S rRNA gene sequence showed highest similarities to the species with validated name *Nisaea nitritireducens* DR41\_18<sup>T</sup> (98.1%) and *Nisaea denitrificans* DR41\_21<sup>T</sup> (97.6%). Phylogenetic analyses indicated that strain NBU1469<sup>T</sup> formed a distinct lineage with strains *Nisaea nitritireducens* DR41\_18<sup>T</sup> and *Nisaea denitrificans* DR41\_21<sup>T</sup> within the genus *Nisaea*. The average nucleotide identity and digital DNA-DNA hybridization values between strain NBU1469<sup>T</sup> and related species of genus *Nisaea* were well below the threshold limit for prokaryotic species delineation. The DNA G+C content was 63.6 mol%. Based on its phenotypic, chemotaxonomic and genotypic data, strain NBU1469<sup>T</sup> is considered to be a representative of a novel species in the genus *Nisaea*, for which the name *Nisaea sediminum* sp. nov. is proposed. The type strain is NBU1469<sup>T</sup> (=KCTC 82224<sup>T</sup>=MCCC 1K04763<sup>T</sup>).

## Introduction

The genus *Nisaea*, belonging to the family *Rhodospirillaceae* in the class *Alphaproteobacteria*, was originally proposed by Urios et al. (2008) with the description of *Nisaea nitritireducens* DR41\_18<sup>T</sup> and *Nisaea denitrificans* DR41\_21<sup>T</sup>. At the time of writing, the genus *Nisaea* only contained the above two validly published species (<https://www.bacterio.net/genus/Nisaea>), which were reported from coastal, surface waters (Urios et al., 2008). Cells of *N. nitritireducens* DR41\_18<sup>T</sup> and *N. denitrificans* DR41\_21<sup>T</sup> were reported as Gram-negative, motile, rod-shaped, catalase- and oxidase- positive, containing ubiquinone-10 (Q-10) as the predominant quinone, with very high amounts of C<sub>18:1</sub>  $\omega$ 7c and the DNA G+C content was 60 mol%. Genus *Nisaea*, identified as hubs in most of subnetworks of microbial communities, may have the potential to synchronize ecological processes over broad ecosystems (Ma et al., 2020). In this paper, we describe a novel strain, designated NBU1469<sup>T</sup>, isolated from a marine sediment sample collected from Meishan Island in the East China Sea. Following the polyphasic taxonomic approach, we propose that strain NBU1469<sup>T</sup> represents a novel species of the genus *Nisaea*.

## Materials And Methods

### *Bacterial strains and culture condition*

Sediment samples were collected from the Meishan Island located in the East China

Sea, Ningbo, China (121°56' E, 29°45' N) in August 2019. Marine broth 2216 (MB, Difco) was used for isolation. The medium was solidified with 2.0% agar (MA). About 3.0 g sediment sample was serially diluted to  $10^{-4}$  with MB, and 200  $\mu$ l of each diluted sample was spread on MA plates. After 3 days of incubation at 26°C, a cream-colored colony was picked and purified by restreaking. The isolate was routinely cultured in MB at 26°C and stored at -80°C with 25% (v/v) glycerol. The reference type strains *Nisaea nitritireducens* DSM 19540<sup>T</sup> and *Nisaea denitrificans* DSM 18348<sup>T</sup> were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ; Germany). Two reference type strains were cultured under the identical experimental conditions as strain NBU1469<sup>T</sup> for comparative analysis.

### *Morphological, physiological and biochemical characterization*

Cell morphology was observed by using an optical microscope (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL). Exponentially growing cells incubated on MA plates were suspended and stained with uranyl acetate and then fixed on the copper mesh before observed with transmission electron microscopy. Gram staining was performed according to Dong and Cai (2001). Motility was examined by microscopic observation and inoculation on semi-solid MB medium with 0.5% agar (w/v). To determine the growth conditions of strain NBU1469<sup>T</sup>, the temperature range for growth was determined 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. The NaCl concentration range for growth was determined at 0, 0.5 and 1-10.0% (at intervals of 1%, w/v) NaCl in modified MB (with original Na<sup>+</sup> and Cl<sup>-</sup> removed). All tests of growth conditions were performed in quadruplicate and OD<sub>600</sub> measurements were taken after 24h incubation at 26°C with shaking at 140 rpm.

The following biochemical and physiological tests were carried out on strain NBU1469<sup>T</sup>, *N. nitritireducens* DSM 19540<sup>T</sup> and *N. denitrificans* DSM 18348<sup>T</sup> in MB, unless otherwise indicated. Catalase activity was detected via bubble production in 3% (v/v) H<sub>2</sub>O<sub>2</sub> solution. Oxidase activity was assessed by oxidation of 1% *p*-aminodimethylaniline oxalate. Indole production, methyl red, Voges-Proskauer test, H<sub>2</sub>S production, and hydrolysis of starch, casein, Tween 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 in which yeast extract and peptone were replaced by 0.02 g/L yeast extract and 0.01 g/L phenol red. Anaerobic growth was determined with an AnaeroPack-MicroAero (2.5 l; MGC, Japan) anaerobic system by using sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM) and sodium nitrate (20 mM) as electron acceptors,

respectively. Same media under aerobic condition were used as control. Heavy metal tolerance was studied in MB supplemented with different concentrations of  $\text{Co}^{2+}$  (0, 0.5, 1.0, 2.0, 3.0, 5.0 and 10.0 mM),  $\text{Cd}^{2+}$  (0, 0.5, 1.0, 2.0 and 5.0 mM),  $\text{Cu}^{2+}$  (0, 0.5, 1.0, 2.0, 5.0 and 10.0 mM),  $\text{Mn}^{2+}$  (0, 0.5, 1.0, 2.0, 5.0 and 10.0 mM) or  $\text{Zn}^{2+}$  (0, 0.5, 1.0, 2.0, 5.0 and 10.0 mM). 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 tested antibiotics were ( $\mu\text{g}$  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) (Zhang et al., 2020).

### *Chemotaxonomic characterization*

Biomass for chemotaxonomic and molecular studies was obtained by cultivation in MB at 26°C for 3 days, with shaking at 140 rpm. All the following tests for chemotaxonomic characterization were performed on strain NBU1469<sup>T</sup>, *N. nitritireducens* DSM 19540<sup>T</sup> and *N. denitrificans* DSM 18348<sup>T</sup> 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 the 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<sub>254</sub> 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% in ethanol to reveal total lipids,  $\alpha$ -naphthol/ $\text{H}_2\text{SO}_4$  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 (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Sun et al., 2017). Purified PCR products were cloned into the vector pMD19-T (TaKaRa). The recombinant plasmid was transformed into *Escherichia coli* DH5 $\alpha$  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., 2017) and BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple sequence 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 et al., 1987), maximum-

parsimony (Fitch et al., 1971) and maximum-likelihood (Felsenstein et al., 1981) methods. Evolutionary distances were calculated using the Kimura 2-parameter model (Kimura et al., 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 genome of strain NBU1469<sup>T</sup> and *Nisaea nitritireducens* DSM 19540<sup>T</sup> were sequenced using an Illumina HiSeq 4000 system (Illumina) at the Beijing Genomics Institute (Shenzhen, China) (Zhang et al., 2020). 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 antiSMASH 6.0 program was used as a tool for the identification of the secondary metabolism gene clusters (Kai et al., 2019). Genome data publicly available of *Nisaea denitrificans* DSM 18348<sup>T</sup> (AUFM000000000) was retrieved from the NCBI Genome database. The average nucleotide identity (ANI) values between strain NBU1469<sup>T</sup> and two reference species were calculated using the ANI calculator online service (Yoon et al., 2017). 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).

## Results And Conclusion

### *Morphological, physiological and biochemical characteristics*

Cells of strain NBU1469<sup>T</sup> were Gram-negative, rod-shaped, non-sporulating and motile (Fig. 1). Colonies were 0.3 mm in diameter, circular, elevated and cream-colored after growing on MA at 26°C for 3 days. Strain NBU1469<sup>T</sup> grew at 0.0-14.0% (w/v) NaCl (optimum 2.0%, w/v), pH 6.0-9.5 (optimum 7.5) and 20-40°C (optimum 40°C), but not at 45°C. Strain NBU1469<sup>T</sup> was able to grow in MB containing high concentrations of heavy metals, including Cd<sup>2+</sup> (0.5 mM), Cu<sup>2+</sup> (5.0 mM), Mn<sup>2+</sup> (2.0 mM), Zn<sup>2+</sup> (0.5 mM) or Co<sup>2+</sup> (2.0 mM). No growth was detected under anaerobic conditions on modified MA with the addition of different electron acceptors even after two weeks. Other physiological and biochemical characteristics of strain NBU1469<sup>T</sup> are given in the species description.

### *Chemotaxonomy results*

The predominant fatty acid of strain NBU1469<sup>T</sup> were C<sub>16:0</sub> (10.5%), C<sub>19:0</sub> cyclo  $\omega$ 8c (27.0%) and summed feature 8 (50.5%). The complete fatty acid profiles are summarized in Table 2. Ubiquinone-10 (Q-10) was the only detected respiratory quinone. The polar lipids included phosphatidylglycerol (PG), two unidentified amino-phospholipids (PN), three unidentified phospholipids (PL) and two unidentified lipids (L) (Fig. S1).

### *Phylogenetic analysis and genome characterization*

Based on the result of 16S rRNA gene sequence alignment, strain NBU1469<sup>T</sup> shared high sequence similarities of 98.1% and 97.6% to the species with validated name *N. nitritireducens* DR41\_18<sup>T</sup> and *N. denitrificans* DR41\_21<sup>T</sup>, respectively. Phylogenetic analysis revealed that strain NBU1469<sup>T</sup> was affiliated with *N. nitritireducens* DR41\_18<sup>T</sup> and *N. denitrificans* DR41\_21<sup>T</sup> on the different phylogenetic trees (Fig. 2, Fig. S2 and Fig. S3). Result of phylogenomic analysis also supported that strain NBU1469<sup>T</sup> closely related to *N. nitritireducens* DSM 19540<sup>T</sup> and *N. denitrificans* DSM 18348<sup>T</sup> (Fig. S4).

The draft genome sequence of strain NBU1469<sup>T</sup> and *N. nitritireducens* DSM 19540<sup>T</sup> were composed of 22 contigs for 5,022,713 bp with 63.6 mol% G+C content and 97 contigs for 4,845,838 bp with 60.6 mol% G+C content, respectively. The genome of strain NBU1469<sup>T</sup> contained 4,835 protein-coding genes and 77 RNA genes while *N. nitritireducens* DSM 19540<sup>T</sup> contained 4,506 protein-coding genes and 76 RNA genes. The result of antiSMASH 6.0 indicated that the novel isolate contained 1 gene cluster for terpenoid type, 1 gene cluster for ectoine type, 1 gene cluster for non-ribosomal peptide synthetase (NRPS) which is a multifunctional key enzyme in the process of biosynthesis of secondary metabolites which shown only 11% similarity to cupriachelin and 1 gene cluster belong to Type III PKS (polyketide synthase) (Fig. S5). The ANI and the dDDH values between strain NBU1469<sup>T</sup> and *N. nitritireducens* DSM 19540<sup>T</sup> were 78.8% and 21.1%, and the ANI and the dDDH values between strain NBU1469<sup>T</sup> and *N. denitrificans* DSM 18348<sup>T</sup> were 78.5% and 21.0%, which were well below the threshold value of 95% ANI relatedness for species delineation (Richter et al., 2009) and the proposed cut-off borderline of 70% (Wayne et al., 1987).

The experiments showed the strain NBU1469<sup>T</sup> could resist high concentrations of Cu<sup>2+</sup> (5.0 mM) and Co<sup>2+</sup> (2.0 mM). The adaption mechanism to heavy metal resistant was analyzed. According to the genome annotation results, strain NBU1469<sup>T</sup> harbored various putative proteins related to heavy metals resistance. Resistance to Cu<sup>2+</sup> was mainly based on genes involved in copper homeostasis in the genome of strain NBU1469<sup>T</sup>. There were 13 genes involved in copper homeostasis: 3 multicopper oxidase, 1 cytochrome c heme lyase subunit CcmF, 2 copper-translocating P-type ATPase, 3 multidrug resistance transporter, Bcr/CflA family, 1 copper resistance protein B, 1 copper tolerance protein, 1 magnesium and cobalt efflux protein CorC and 1 copper homeostasis protein CutE (Fan et al., 2018). The genome of strain NBU1469<sup>T</sup> also contained 7 genes related to Co<sup>2+</sup> uptake and resistance, containing 2 magnesium and cobalt transport protein CorA which help to uptake and accumulate Co<sup>2+</sup>, and 1 magnesium and cobalt efflux protein CorC which involved in the Co<sup>2+</sup> efflux function (Wu et al., 2018). In addition, 3 MerR family regulators and 1 transcriptional regulator HmrR were found in the genome. These proteins were regulators of various environmental stimuli, particularly, under high concentrations of heavy metals and oxidative stresses (Wu et al., 2016). All above genes may contribute to the phenotype of strain NBU1469<sup>T</sup> in copper and cobalt resistance.

## Taxonomic conclusion

The major cellular fatty acids (C<sub>16:0</sub>, C<sub>19:0</sub> cyclo  $\omega$ 8c and summed feature 8), predominant respiratory quinone (ubiquinone Q-10), major polar lipids (PG, PN1, PN2, PL1 and PL2), phylogenetic trees and phylogenomic tree supported that strain NBU1469<sup>T</sup> should be classified into the genus *Nisaea*. However, there are some additional differences between strain NBU1469<sup>T</sup> and the related type strains of genus *Nisaea*. The detailed fatty acid profile showed that strain NBU1469<sup>T</sup> possessed a higher amount of C<sub>19:0</sub> cyclo  $\omega$ 8c than reference species, while possessing lower amount of summed feature 3 and 8 than reference species (Table 2). The detailed polar lipid profile showed strain NBU1469<sup>T</sup> contained PL3, while not in strain *N. nitritireducens* DSM 19540<sup>T</sup> (Fig. S1). L1, L2, L3 and L4 were detected in strain *N. nitritireducens* DSM 19540<sup>T</sup> but not in strain NBU1469<sup>T</sup> and *N. denitrificans* DSM 18348<sup>T</sup>. Among these three strains, only NBU1469<sup>T</sup> possessed L5 and L6. The DNA G+C content of strain NBU1469<sup>T</sup> (63.6 mol%) was higher than that of *N. nitritireducens* DSM 19540<sup>T</sup> (60.6 mol%) and *N. denitrificans* DSM 18348<sup>T</sup> (60.0 mol%). There were also several phenotypic differences between NBU1469<sup>T</sup> and related type strains. Strain NBU1469<sup>T</sup> could tolerate NaCl concentration up to 14.0%, but two type strains could not grow at NaCl concentration higher than 6.0%. Hydrolysis of tween 20 and nitrate reduction were positive for NBU1469<sup>T</sup> but negative for two type strains (Table 1). Among these three species, only strain *N. denitrificans* DSM 18348<sup>T</sup> was facultatively anaerobic. Meanwhile, the ANI values (78.5-78.8%) and dDDH values (21.0-21.1%) between strain NBU1469<sup>T</sup> and the two reference species were below the thresholds recommended for species delineation 95% (ANI) and 70% (dDDH). All of the above confirmed that strain NBU1469<sup>T</sup> represented a novel species within the genus *Nisaea*.

Based on the phenotypic, chemotaxonomic and genotypic characteristics described above, we identified strain NBU1469<sup>T</sup> as the type strain of a novel species of the genus *Nisaea*, for which the name *Nisaea sediminum* sp. nov. is proposed.

### **Description of *Nisaea sediminum* sp. nov.**

*Nisaea sediminum* (se.di.mi'num. L. gen. pl. n. *sediminum* of sediments, pertaining to source of the isolate).

Cells are Gram-negative, aerobic, rod-shaped and motile. The cell size is 0.4-1.0×0.9-2.9  $\mu$ m. Colonies on Marine agar 2216 are 0.3 mm in diameter, circular, elevated and cream-colored after 3 days 26°C. The temperature range for growth is 20-40°C (optimum 40°C), but not at 45°C. Growth occurs at 0-14.0% NaCl and pH 6.0-9.5 (optimum, 2% NaCl and pH 7.5). Positive for catalase and oxidase activities, nitrate reduction, H<sub>2</sub>S production and hydrolysis of Tween 20. Negative for methyl red, indole production, urease, fermentation of <sub>D</sub>-glucose, arginine dihydrolase,  $\beta$ -galactosidase, hydrolysis of starch, casein, gelatin, aesculin, Tweens 40, 60 and 80. In the API ZYM kit, positive for activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valin arylamidase, acid phosphohydrolase and naphthol-AS-BI-phosphohydrolase. In the API 50CH kit, positive for acid production from glycerol, <sub>D</sub>-arabinose, <sub>L</sub>-xylose, <sub>D</sub>-galactose, <sub>L</sub>-fucose, <sub>D</sub>-Ribose, <sub>D</sub>-lyxose, <sub>L</sub>-Arabinose, <sub>D</sub>-xylose, <sub>L</sub>-rhamnose, <sub>D</sub>-fucose, 2-

ketogluconate and 5-ketogluconate. Sensitive to gentamicin, cefradine, clindamycin, nalidixic acid, vancomycin, oxacillin, doxycycline, neomycin, kanamycin, ofloxacin, amikacin, rifampicin, novobiocin and minocycline. The major fatty acid are C<sub>16:0</sub>, C<sub>19:0</sub> cyclo  $\omega$ 8c and summed feature 8 (C<sub>18:1</sub>  $\omega$ 7c and/or C<sub>18:1</sub>  $\omega$ 6c). Q-10 is the only detected respiratory quinone. The polar lipids include phosphatidylglycerol, two unidentified amino-phospholipids, three unidentified phospholipids and two unidentified lipids. The DNA G+C content of the genomic DNA of the type strain is 63.6 mol%.

The type strain NBU1469<sup>T</sup> (=KCTC 82224<sup>T</sup>=MCCC 1K04763<sup>T</sup>) was isolated from a marine sediment sample taken from the Meishan Island in the East China Sea. The GenBank accession numbers for the 16S rRNA gene and the draft genome data of strain NBU1469<sup>T</sup> are MT525301 and JACZCQ000000000, respectively.

## Declarations

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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 NBU1469<sup>T</sup> are MT525301 and JACZCQ000000000, respectively.

A transmission electron micrograph of the cells, polar lipids, phylogenetic trees (maximum-likelihood and maximum-parsimony trees) and phylogenomic tree are available as supplementary materials. Supplementary data associated with this article can be found in the online version.

### Authors' contributions

W.Y.Z. and S.H. conceived the study. S.T.Z., Y.P.C., C.B.G. and F.L.X. performed the research. D.J analyzed data. S.T.Z. wrote the paper. All authors read and approved the manuscript.

### Compliance with ethical standards

### Conflicts 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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## Table

**Table 1.** Differential characteristics of strain NBU1469<sup>T</sup> and related type strains of the genus *Nisaea*

Strains: 1, strain NBU1469<sup>T</sup>; 2, *N. nitritireducens* DSM 19540<sup>T</sup>; 3, *N. denitrificans* DSM 18348<sup>T</sup>. All data were taken from this study unless otherwise indicated. Data marked with <sup>a</sup> was taken from Urios *et al.* [1]. -, negative; +, positive; R, resistant; S, susceptible. The same characteristics shared by these three strains were listed in Table S1.

Characteristic	1	2	3
Habitat	marine sediment	water column <sup>a</sup>	water column <sup>a</sup>
Cell size (µm)	0.4-1.0×0.9-2.9	0.9±0.2×2.5±0.6 <sup>a</sup>	0.9±0.2×2.5±0.6 <sup>a</sup>
Oxygen requirement	Aerobic	Aerobic <sup>a</sup>	Facultative anaerobic <sup>a</sup>
Temperature range (optimum, °C)	20-40 (40)	15-44 (30) <sup>a</sup>	15-44 (30) <sup>a</sup>
pH range (optimum)	6.0-9.5 (7.5)	5.0-9.0 (6.0) <sup>a</sup>	5.0-9.0 (6.0) <sup>a</sup>
NaCl range (optimum) (% w/v)	0-14.0 (2.0)	0-6.0 (2.0) <sup>a</sup>	0-6.0 (2.0) <sup>a</sup>
Hydrolysis of tween 20	+	-	-
API 20NE test results:			
Nitrate reduction	+	-	-
Urease	-	-	+
API 50CH			
Glycerol, <sub>D</sub> -arabinose, <sub>L</sub> -xylose, <sub>D</sub> -galactose, <sub>L</sub> -fucose, 5-ketogluconate	+	-	-
<sub>D</sub> -Ribose, <sub>D</sub> -lyxose	+	-	+
<i>N</i> -acetyl-β- <sub>D</sub> -glucosamine, <sub>D</sub> -glucose, <sub>D</sub> -mannitol, maltose, raffinose, glycogen	-	+	+
<sub>D</sub> -Fructose, <sub>L</sub> -sorbose, inositol, amygdalin, salicin, cellobiose, lactose, melezitose	-	+	-
Sucrose, trehalose, <sub>D</sub> -arabitol	-	-	+
Susceptibility to			
Penicillin G, chloramphenicol, cephalixin, cefoxitin, amoxicillin,	R	S	R

cefamezin, carbenicillin, ciprofloxacin, ampicillin			
Streptomycin, tetracycline, erythromycin, norfloxacin	R	S	S
Polymyxin B	R	R	S
Gentamicin, cefradine	S	S	R
Clindamycin, nalidixic acid, vancomycin, oxacillin, doxycycline	S	R	R
Neomycin	S	R	S
DNA G+C content (mol%)	63.6	60.6	60.0 <sup>a</sup>

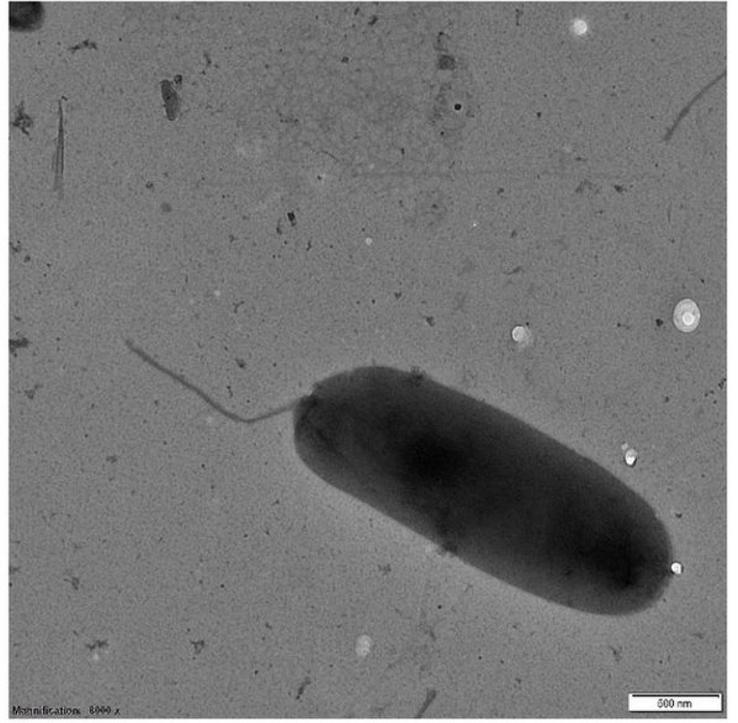
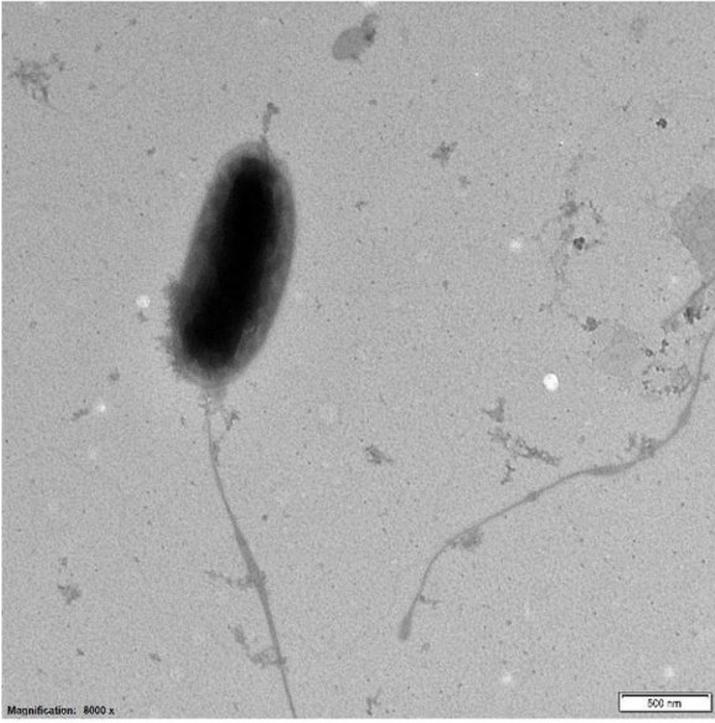
**Table 2.** Cellular fatty acids for strain NBU1469<sup>T</sup> and related type strains of the genus *Nisaea*

Strains: 1, strain NBU1469<sup>T</sup>; 2, *N. nitritireducens* DSM 19540<sup>T</sup>; 3, *N. denitrificans* DSM 18348<sup>T</sup>. 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. Abbreviations: tr, trace component (<0.5%); -, not detected.

Fatty acid	1	2	3
<b>Saturated</b>			
C <sub>16:0</sub>	10.5	9.2	9.6
C <sub>18:0</sub>	0.7	tr	tr
C <sub>20:0</sub>	0.6	-	-
C <sub>19:0</sub> 10-methyl	tr	0.9	0.8
<b>Hydroxy</b>			
C <sub>8:0</sub> 3-OH	tr	tr	0.5
C <sub>16:0</sub> 3-OH	0.5	0.9	0.8
<b>Unsaturated</b>			
C <sub>18:1</sub> $\omega$ 7c 11-methyl	0.6	-	0.6
C <sub>19:0</sub> cyclo $\omega$ 8c	27.0	tr	2.0
<b>Summed Features*</b>			
<b>2</b>	tr	0.7	0.6
<b>3</b>	7.0	24.5	13.9
<b>8</b>	50.5	61.4	70.0

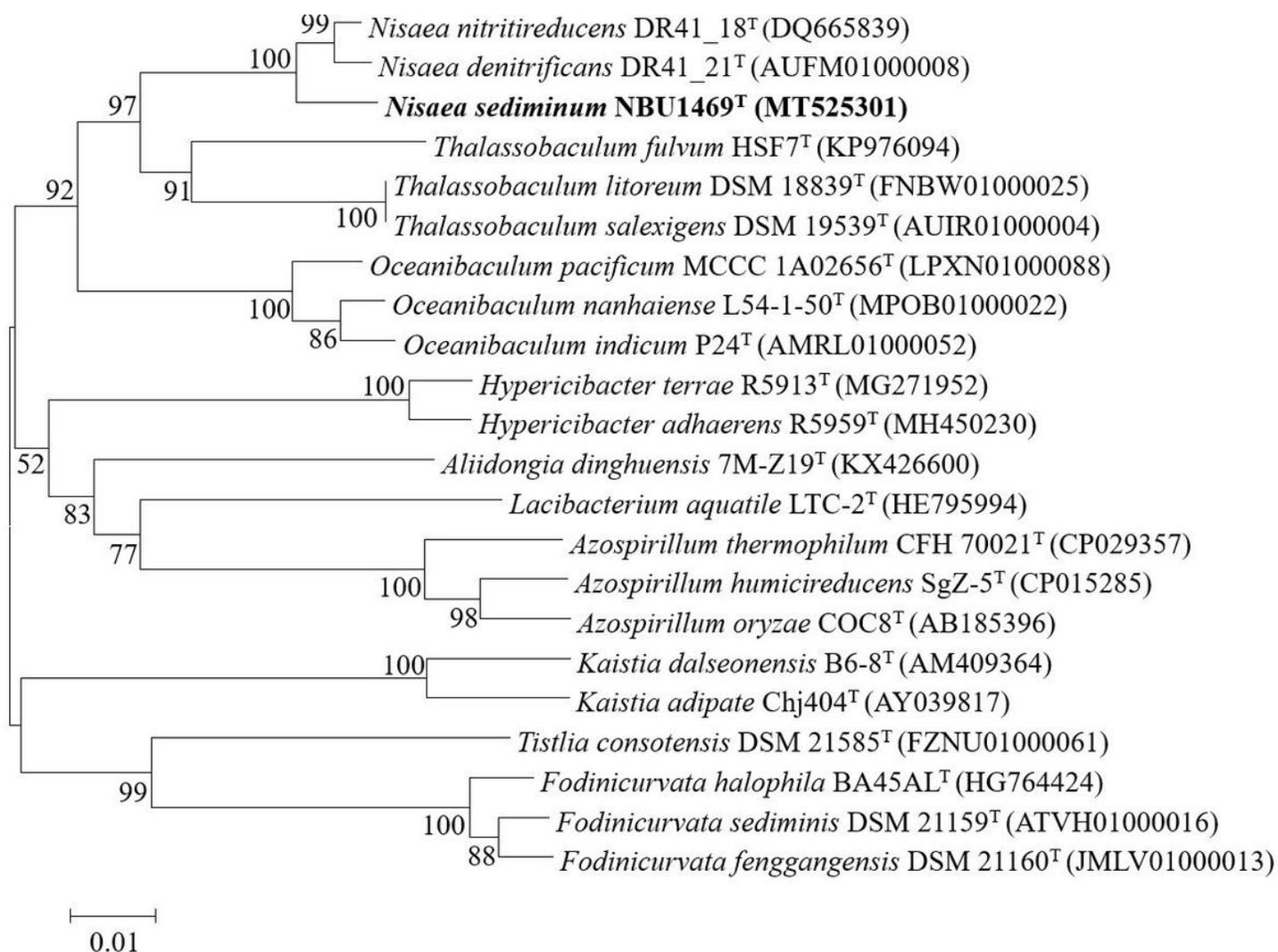
\* Summed feature 2 contained C<sub>14:0</sub> 3-OH and/or C<sub>16:1</sub> iso I; Summed feature 3 contained C<sub>16:1</sub>  $\omega$ 7c and/or C<sub>16:1</sub>  $\omega$ 6c; Summed feature 8 contained C<sub>18:1</sub>  $\omega$ 7c and/or C<sub>18:1</sub>  $\omega$ 6c.

## Figures



## Figure 1

Electron micrographs of cells of strain NBU1469T growing on MA medium, cells are rod-shaped with a monopolar flagellum. Bar, 500 nm.



**Figure 2**

Neighbor-joining phylogenetic tree based on the 16S rRNA gene sequences, showing the phylogenetic relationships of strain NBU1469T 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.

## Supplementary Files

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

- [SupplementarymaterialofNBU1469.docx](#)