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# *Algoriphagus resistens* sp. nov., isolated from marine sediment

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**Abstract**

Strain NH1$^T$, a pink-pigmented, facultatively anaerobic, heterotrophic, catalase-positive and oxidase-negative, Gram-stain-negative marine bacterium, was isolated from marine sediment on the coast of Weihai, China. Cells of strain NH1$^T$ were rod-shaped, 0.8–2.0 µm in length and 0.5–1.0 µm in width. The strain was able to grow at 13–37 °C, pH 5.5–8.5, in the presence of 0.0–8.0 % (w/v) NaCl. Optimal growth was observed at 28 °C, with 3.0 % (w/v) NaCl and pH 6.5–7.0. Nitrate was reduced. The G+C content of the DNA was 41.9 mol%. The major isoprenoid quinone was MK-7 and the main cellular fatty acids (>10 %) were summed feature 3 (33.6 %) comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$, and iso-$C_{15:0}$ (19.2%). The major polar lipids in strain NH1$^T$ were phosphatidylethanolamine, unidentified lipids, phospholipid and aminolipids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain NH1$^T$ was highly related to the type strains of *Algoriphagus antarcticus* (97.87 % 16SrRNA gene sequence similarity) and *Algoriphagus ratkowskyi* (97.56 %). On basis of the phenotypic and phylogenetic data, strain NH1$^T$ should be classified as representing a novel species of the genus *Algoriphagus*, for which the name *Algoriphagus resistens* sp. nov. is proposed. The type strain is NH1$^T$ (=MCCC 1H00140$^T$=KCTC 52228$^T$).

At the time of writing, the genus *Algoriphagus* [1] of the family *Cyclobacteriaceae* in phylum *Bacteroidetes* includes 32 species (LPSN, http://www.bacterio.net/-classifphyla.html). The type species of the genus is *Algoriphagus ratkowskyi*. Most member species have been isolated from marine environments, for example *A. ratkowskyi* was isolated from sea ice samples, *A. lutimaris* [2], *A. chungangensis* [3], *A. boseongensis* [4] and *A. taeanensis* [5] were all isolated from marine sediments. The novel strain NH1$^T$, described here was also isolated from marine sediment. Most species of the genus *Algoriphagus* are Gram-stain-negative, heterotrophic, aerobic, non-motile and rod-shaped. In this study, the novel isolate was characterized by phenotypic and phylogenetic analyses and is proposed to represent a novel species of the genus.

During a study into the discovery of multi-drug resistant bacteria from a marine environment, a novel facultatively anaerobic, pink-pigmented, Gram-stain-negative marine bacterium, designated NH1$^T$, was isolated at 28 °C in marine broth (MB medium), which consisted of the following ingredients in 1000 ml artificial seawater: 1 g yeast extract, 5 g peptone and 0.1 g ferric citrate. The artificial seawater was prepared with artificial sea salt [Sigma, 3.0 % (w/v)] and distilled water. The pH was adjusted to 7.0 and then autoclaved. This medium was used for all studies unless otherwise indicated; 1.8 % (w/v) agar was added for solid media (MA medium). The sample was collected from a marine environment (37° 31′ 04″ N 122° 01′ 18″ E) from the Weihai coast, Shandong Province, PR China. For isolating bacterial strains, 1 g wet sediment was blended in 99 ml sterilized seawater with glass beads and vigorously shaken. The suspension was gradient diluted to $10^{-6}$ with sterilized seawater and 0.1 ml aliquots of each dilution were spread onto solid media with/without different kinds of antibiotics. The plates were incubated at 28 °C for 5–7 days. Strain NH1$^T$ was isolated and stored at −80 °C with 1 % (w/v) NaCl and 15 % (w/v) glycerol. *Algoriphagus ratkowskyi* DSM 22686$^T$ and *Algoriphagus antarcticus* DSM 15986$^T$, obtained from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) were used as reference strains and were cultured under the same conditions as strain NH1$^T$ unless otherwise specified.

Routine growth of strains was performed at 28 °C using MA medium. On MA, optimum growth levels could be obtained after 2–3 days. Colony morphology examination of strain NH1$^T$ was performed on cultures from MA medium after incubation for 3 days at 28 °C. Cell size, morphology and motility were observed using light microscopy (Ci-L; Nikon); the presence of flagella on cells was tested by using

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Keywords: *Algoriphagus resistens*; marine sediment.
Abbreviation: OrthoANI, orthologous average nucelotide identity.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NH1$^T$ is KX852396. The whole-genome shotgun project of *Algoriphagus resistens* NH1$^T$ has been deposited at GenBank/EMBL/DDBJ under the accession number LMXN00000000.
One supplementary table and three supplementary figures are available with the online Supplementary Material.

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flagella staining kits (Solarbio). Motility was assessed with the hanging-drop method. Gram staining was carried out as described by Smibert and Krieg [6]. The antibiotic sensitivity profile of the isolate was determined with the Kirby–Bauer disc diffusion method on MA plates (since strain NH1$^T$ showed poor growth on Mueller–Hinton agar) using various antibiotics. For this method, bacterial lawns were first made from bacterial suspensions of 0.5 McFarland standards in 0.9 % (w/v) NaCl. Two or three discs of antibiotic were then applied to each plate. The result was determined from the measurements of the inhibition zones produced after 1–2 days of incubation according to CLSI/NCCLS guidelines. For determination of the effects of different growth temperatures, bacteria were inoculated on MA media and incubated for about 7 days at various temperatures (4, 8, 13, 17, 24, 28, 30, 37 and 42 °C) until growth could be indicated by visible colonies. To test for effects of pH on growth, the standard medium was separately modified by addition of buffers MES (for pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0 and 9.5) (Sangon) at concentrations of 20 mM. The pH of the medium was adjusted by addition of 1M HCl or NaOH before autoclaving. Tolerance to NaCl was tested using MA-NaCl media (1.8 % agar, 0.5 % peptone, 0.1 % yeast extract, 0.32 % $MgSO_4$, 0.12 % $CaCl_2$, 0.07 % KCl, 0.02 % $NaHCO_3$, w/v), with the NaCl concentrations ranging from 0.0–9.0 % (w/v) at intervals of 0.5 %. The standard solid medium was utilized to determine the hydrolysis of agar. In addition, hydrolysis of starch, lipid, cellulose and alginate was determined by supplementing the standard solid medium with 0.2 % (w/v) soluble starch, 1 % (v/v) Tweens 20, 40 and 80, 0.5 % (w/v) carboxymethylcellulose and 0.5 % (w/v) sodium alginate, respectively [7].

Physiological and biochemical tests were performed on strain NH1$^T$ and the two reference strains at the same time using the API 20E and API ZYM kits (bioMérieux) according the manufacturer's instructions. The results of these tests are given in the species description and Table S1 (available in the online Supplementary Material). Utilization of different compounds as sole carbon and energy sources was determined using the Biolog GEN III microplates. Tests of acid production from carbohydrates were performed by using the API 50CHB fermentation kit (bioMérieux) according the instructions of the manufacturer. The API 50CHB strips were incubated at 28 °C and read every 24 h. All the tests were performed in duplicate, and appropriate positive and negative controls were included. Reduction of nitrate and oxidation-fermentation tests were performed as described by Dong and Cai [8]. Growth under anaerobic conditions was determined after cultivation in an anaerobic chamber on the solid medium mentioned above with or without 0.1 % (w/v) $KNO_3$ for at least 2 weeks at 28 °C. Oxidase activity was tested by using the bioMérieux oxidase reagent kit according the manufacturer's instructions, and catalase activity was evaluated based on the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Further information on the morphological, physiological and biochemical characteristics of strain NH1$^T$ are given in the species description.

Respiratory isoprenoid quinones were extracted and separated according Tindall [9], and analysed by HPLC [10] Cultures for fatty acid analysis were incubated on MA plates at 28 °C for 3 days. The fatty acid method used was described by Sasser [11]. Extracts were separated using the Sherlock Microbial Identification System (MIS; MIDI). Peaks were automatically integrated and fatty acid names and percentages calculated by the MIS Standard software (Microbial ID). Polar lipids were separated by two-dimensional silica gel thin-layer chromatography. Total lipid material was detected using molybdatophosphoric acid and discrete functional groups were detected using spray reagents specific for defined functional groups. Full details are given in Tindall [9]. Cellular polar lipid analysis was carried out by the Identification Service of the DSMZ.

The genomic DNA of the strain NH1$^T$ was extracted and purified using a bacterial genomic DNA mini kit (Takara). The draft genome of strain NH1$^T$ was sequenced by Shanghai Personal Biotechnology (Shanghai, China) using Solexa paired-end sequencing technology. A library with a fragment length of 400 bp was constructed, and a total of 758 434 670 clean paired-end reads were generated to reach a 132-fold depth of coverage with an Illumina MiSeq. The final draft genome was assembled using SOAPdenovo version 2.04. The DNA G+C mol% of strain NH1$^T$ was determined from the mean G+C content of the draft genome. Orthologous Average Nucleotide Identity (OrthoANI) between strain NH1$^T$ and *A. antarcticus* DSM 15986$^T$ was calculated using OAT (Orthologous Average Nucleotide Identity Tool) [12]. The 16S rRNA gene sequence was amplified by PCR with two universal primers 27f and 1492r according to the method described by Liu *et al.* [13]. The purified PCR product was ligated to the vector pGM-T (Tiangen) and cloned according the instructions of the manufacturer. Sequencing was performed by Shanghai Sunny Biotechnology . The nearly complete 16S rRNA gene sequence of strain NH1$^T$was submitted to the GenBank database to search for similar sequences using the BLAST and EzBioCloud algorithm. 16S rRNA gene sequences of several closely related species were edited by using the BioEdit program [14] and multiple alignments were performed with the CLUSTAL X program [15]. Phylogenetic analyses were performed using the neighbour-joining [16], maximum-likelihood [17] and maximum-parsimony [18] methods with MEGA software version 6.0 [19]. Evolutionary distances were calculated by using Kimura's two-parameter model [20]. Bootstrap analysis based on 1000 replicates was also conducted in order obtain confidence levels for the branches [21].

After 3 days of culture on MA agar, strain NH1$^T$ formed colonies that were circular with an entire edge, pink-pigmented and opaque. Strain NH1$^T$ exhibited a number of phenotypic similarities with respect to species of the genus *Algoriphagus*, including negative Gram-staining behaviour, no flagella or motility, the presence of rod-shaped cells and MK-7 as the sole ubiquinone. The novel strain NH1$^T$ was resistant to

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colistin, erythromycin, kanamycin, penicillin, ampicillin, streptomycin, lincomycin, clindamycin, tetracycline and chloramphenicol. Strain NH1$^T$ can be distinguished from *A. ratkowskyi* DSM 22686$^T$, *A. antarcticus* DSM 15986$^T$ and other species of the genus *Algoriphagus* based on physiological and biochemical characteristics. Strain NH1$^T$ is not able to grow at temperatures below 10 °C; is negative for the oxidase test and lipase activity; is positive for catalase activity; and reduces nitrate to nitrite. On the contrary, almost all other species of the genus *Algoriphagus* can grow at a low temperature under 10 °C and have positive results in the oxidase test; *A. ratkowskyi*, *A. locisalis*, *A. chordae* and *A. winogradskyi* can hydrolyse Tweens 20 and 40; furthermore, *A. ratkowskyi* DSM 22686$^T$ is negative for catalase activity. The details of characteristics that differentiate strain NH1$^T$ from the similar species are shown in Table 1.

The major polar lipids in strain NH1$^T$ were phosphatidylethanolamine, unidentified lipids, phospholipid and aminolipids (Fig. S1). Although the polar lipid profile of strain NH1$^T$ was generally similar to that of *A. ratkowskyi* DSM 22686$^T$, with phosphatidylethanolamine and an unidentified lipid identified as the major polar lipids in both of these strains, minor amounts of an unidentified aminolipid was only detected in NH1$^T$. The predominant cellular fatty acids (>10 % of total) of strain NH1$^T$ were summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$, 33.6 %) and iso-$C_{15:0}$ (19.2%), which are also the dominant fatty acids in the other two reference strains. However, *A. antarcticus* DSM 15986$^T$also contained iso-$C_{15:1}$ G

**Table 1.** Differential phenotypic characteristics of Strain NH1$^T$ and some closely related species of the genus *Algoriphagus*

Strains: 1, NH1$^T$ (data from this study); 2, *A. ratkowskyi* DSM 22686$^T$ (this study); 3, *A. antarcticus* DSM 15986$^T$ (this study); 4, *A. locisalis* MSS-170$^T$ [25]; 5, *A. chordae* KMM 3957$^T$ [26]; 6, *A. winogradskyi* KMM 3956$^T$ [26]; 7, *A. trabzonensis* MS7$^T$ [27]. All species are Gram-stain-negative. +, Positive; −, negative; V, variable; ND, not determined.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| Nitrate reduction | + | − | − | − | − | + | − |
| Colour of cell mass | Pink | Pink | Orange-red | Orange | Orange-red | Bright pink | Reddish-orange |
| NaCl requirement for growth | − | + | − | + | + | − | − |
| NaCl range for growth (%, w/v) | 0.0–8.0 | 0.5–6.0 | 0.0–5.0 | 1.0–9.0 | 1.0–10.0 | 0.0–6.0 | 0.0–2.0 |
| Temperature for growth (°C) | | | | | | | |
| Range | 13–37 | −2–25 | 5–25 | 4–35 | 4–32 | 4–39 | 10–40 |
| Optimum | 28 | 16–19 | 20 | 30 | 23–25 | 25–28 | 28 |
| Hydrolysis of: | | | | | | | |
| Tween 20 | − | + | − | + | + | + | ND |
| Tween 40 | − | + | − | + | + | + | ND |
| Tween 80 | − | − | − | + | − | − | ND |
| Acid production from: | | | | | | | |
| L-Arabinose | − | + | − | + | − | − | + |
| Cellobiose | + | + | − | + | + | + | + |
| D-Fructose | − | + | − | + | ND | ND | − |
| D-Galactose | − | + | − | + | + | + | − |
| D-Glucose | + | + | − | + | + | + | + |
| Maltose | + | + | − | + | + | + | + |
| D-Mannose | + | + | − | + | + | + | + |
| Trehalose | − | + | − | V | ND | ND | + |
| D-Xylose | + | + | − | + | + | + | + |
| Utilization of: | | | | | | | |
| D-Galactose | + | + | + | + | + | + | ND |
| D-Glucose | + | + | − | + | + | + | ND |
| D-Lactose | + | + | + | + | + | + | ND |
| Maltose | + | + | − | + | + | + | ND |
| D-Mannose | + | + | − | + | + | + | − |
| D-Mannitol | − | + | − | − | − | − | ND |
| Sorbitol | − | + | + | ND | − | − | − |
| Glycerol | − | − | − | − | − | − | ND |
| Production of: | | | | | | | |
| Catalase | + | − | + | + | + | + | + |
| Oxidase | − | + | + | + | + | + | + |
| DNA G+C content (mol%) | 41.9 | 35–37 | 40–41 | 42 | 40 | 39–42 | 41.6 |

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(15.7 %) as one of the predominant cellular fatty acids. Comparison of major fatty acid profile of strain NH1$^T$ and reference strains is outlined in Table 2.

The 16S rRNA gene sequence of strain NH1$^T$ was obtained and used for phylogenetic analyses. The sequences obtained were aligned with sequences deposited in the GenBank database through BLAST software [22]. The highest degree of sequence similarity for strain NH1$^T$ was found to be with *A. antarcticus* DSM 15986$^T$ [23] (98.19 %). Alignment of the 16S rRNA gene sequence with that of *A. antarcticus* DSM 15986$^T$ using the EzTaxon Server 2.0 [24] gave 97.87 % similarity. Draft genome sequencing of strain NH1$^T$yielded a genome of 6 131 579 bp in length after assembly, producing 43 contigs, and the N50 value is 346 892. All contigs were larger than 1218 bp, the largest being 782 639 bp. The calculated G+C mol% of strain NH1$^T$ was 41.9 mol%. The OrthoANI value between strain NH1$^T$ and *A. antarcticus* DSM 15986$^T$ was 78.7 %, which was lower than the standard OrthoANI value criteria for species identity (95–96 %), supporting that strain NH1$^T$represents a novel species. Phylogenetic trees obtained by using the neighbour-joining method (Fig. 1) revealed that strain NH1$^T$ was a member of the genus *Algoriphagus* and allowed us to assign the novel isolate to a novel species, for which we propose the name *Algoriphagus resistens* sp. nov. The other two tree-making algorithms (maximum-likelihood and maximum-parsimony) resulted in trees showing similar topologies (Figs S2 and S3).

## DESCRIPTION OF *ALGORIPHAGUS RESISTENS* SP. NOV.

*Algoriphagus resistens* (re.sis′tens. L. pres. part. *resistens* being resistant, referring to the multidrug resistance).

Cells were approximately 0.8–2.0 µm in length and 0.5–1.0 µm in width. Colonies on MA are pink-pigmented, circularand about 2 mm in diameter after 3 days of growth at 28 °C. Able grow at 13–37 °C, pH 5.5–8.5, in the presence of 0.0–8.0 % (w/v) NaCl. Optimal growth was observed at 28 °C, with 3.0 % (w/v) NaCl and pH 6.5–7.0. No flagella or motility. Catalase-positive and oxidase-negative. Nitrate is reduced. Acid is produced with fermentation of glucose under anaerobic conditions, but no gas. Tweens 20, 40 and 80, starch, carboxymethylcellulose, sodium alginate and agar are not hydrolysed. Acid is produced from D-xylose, D-glucose, D-mannose, methyl $\alpha$-D-mannopyranoside, aesculin, salicin, cellobiose and maltose. Acid and alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, $\alpha$-chymotrypsin and *N*-acetylglucosaminidase are produced. Positive oxidations are observed with dextrin, maltose, trehalose, cellobiose, gentiobiose,

**Table 2.** Major cellular fatty acid contents of strain NH1$^T$ and its closest phylogenetic relatives

Strains: 1, NH1$^T$; 2, *A. ratkowskyi* DSM 22686$^T$; 3, *A. antarcticus* DSM 15986$^T$. All data were obtained in this study. Only fatty acids amounting to at least 1.0 % of the total cellular fatty acids of at least one of the strains are shown. TR, Trace (<1.0 %).

| Fatty acid | 1 | 2 | 3 |
|---|---|---|---|
| Straight-chain | | | |
| $C_{15:0}$ | TR | 1.8 | TR |
| $C_{16:0}$ | 1.8 | 1.9 | 1.1 |
| Unsaturated | | | |
| $C_{15:1}\omega 6c$ | TR | 2.3 | 1.0 |
| $C_{16:1}\omega 5c$ | 7.1 | 8.4 | 6.4 |
| $C_{17:1}\omega 6c$ | 1.3 | 1.3 | TR |
| $C_{17:1}\omega 9c$ | 6.6 | 2.7 | 4.1 |
| Branched | | | |
| iso-$C_{14:0}$ | TR | 1.38 | TR |
| iso-$C_{15:1}$ G | 4.3 | 7.9 | 15.7 |
| iso-$C_{15:0}$ | 19.2 | 17.9 | 20.4 |
| anteiso-$C_{15:0}$ | 1.8 | 6.2 | 4.5 |
| iso-$C_{16:0}$ | 3.3 | 4.1 | 3.5 |
| iso-$C_{16:1}$ h | 4.5 | 4.9 | 3.6 |
| Hydroxy | | | |
| iso-$C_{15:0}$ 3-OH | 2.2 | 1.7 | 2.6 |
| iso-$C_{16:0}$ 3-OH | TR | 1.7 | 1.5 |
| iso-$C_{17:0}$ 3-OH | 3.0 | 4.2 | 6.1 |
| Summed features* | | | |
| 3 | 33.6 | 24.0 | 17.2 |
| 4 | 1.0 | 1.1 | 1.3 |

*Summed features represent groups of one or two fatty acids that could not be separated using the MIDI system. Summed feature 3 comprised iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$; summed feature 4 comprised anteiso-$C_{17:1}$ B.

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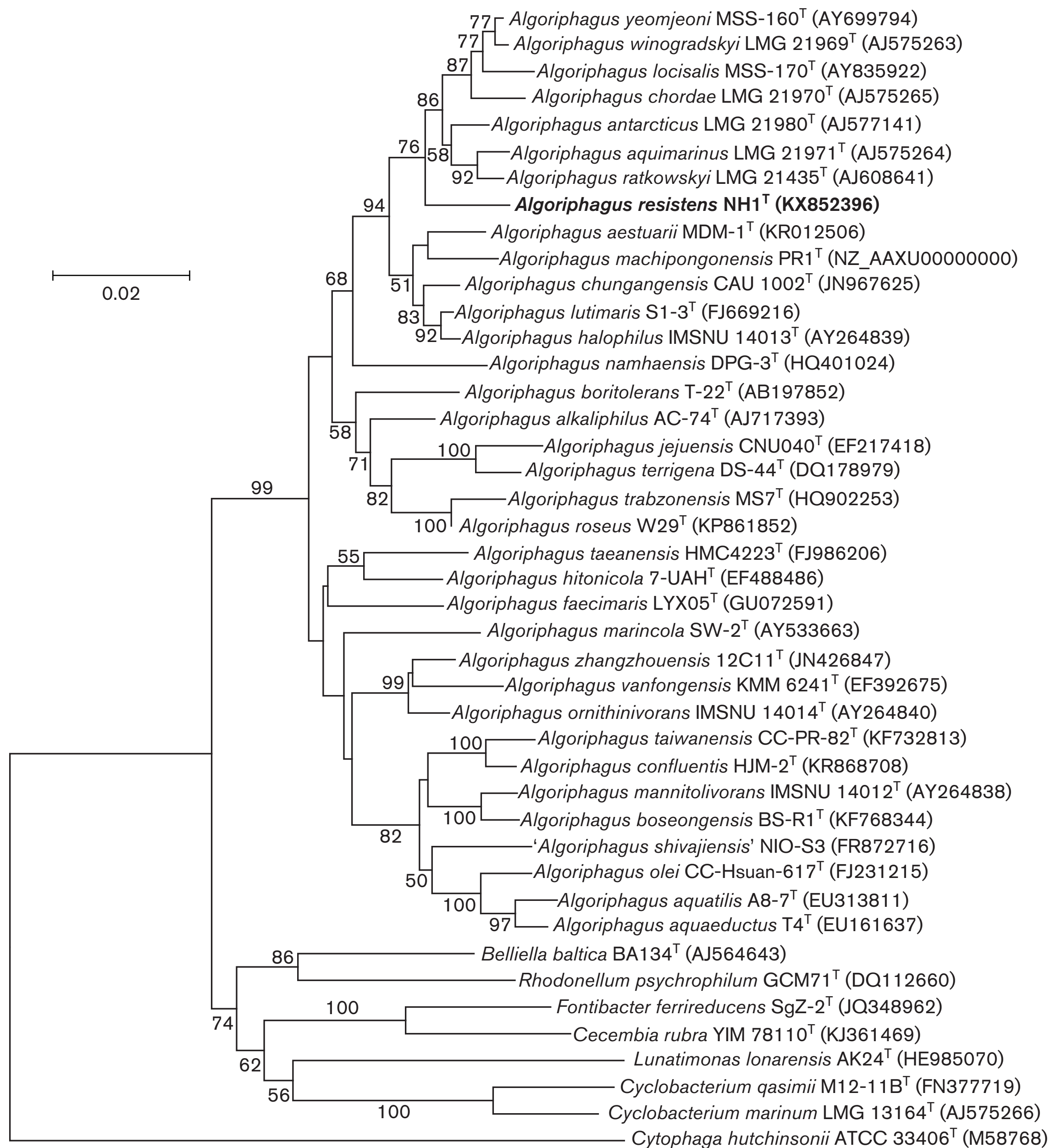


**Fig. 1.** Phylogenetic tree reconstructed with 16S rRNA gene sequences using the neighbour-joining method and showing the position of strain NH1$^T$ among related taxa. The strain characterized in this study is shown in bold type. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. Numbers at nodes are bootstrap values (>50 %) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 0.02 substitutions per nucleotide position.

raffinose, $\alpha$-D-lactose, D-melibiose, methyl $\alpha$-D-mannopyranoside, D-salicin, *N*-acetyl-D-glucosamine, $\alpha$-D-glucose, D-mannose, D-fructose, D-galactose, D-fucose, pectin, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, acetoacetic acid, sucrose, D-turanose, stachyose, L-fucose, L-rhamnose, D-serine, L-histidine, acetic acid and formic acid. The main fatty acids are iso-$C_{15:0}$ and iso-$C_{15:0}$ 2-OH/$C_{16:1}$ $\omega7c$ (summed feature 3). The polar lipids are phosphatidylethanolamine, unidentified lipids, phospholipid and aminolipids.

The type stain NH1$^T$ (=MCCC 1H00140$^T$=KCTC 52228$^T$), was isolated from marine sediment on the coast of Weihai, China. The DNA G+C content of the type strain is 41.9 mol%.

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Conflicts of interest

The authors declare that they have no conflict of interest

Ethical statement

This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

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