Korean Journal of Microbiology (2022) Vol. 58, No. 4, pp. 255-265
DOI https://doi.org/10.7845/kjm.2022.2066

pISSN 0440-2413
eISSN 2383-9902

Nisaea acidiphila sp. nov., isolated from a marine algal debris, emended description of the genus *Nisaea* Urios *et al*. 2008, and the emendation of *Thalassobaculum salexigens* as *Thalassobaculum litoreum* subsp. *salexigens* comb. nov.

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해조류 잔해로부터 분리된 신종 세균 *Nisaea acidiphila*에 대한 보고, *Nisaea* Urios *et al*. 2008에 대한 개정 기술 및 *Thalassobaculum salexigens*의 *T. litoreum* subsp. *salexigens*로의 재분류

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(Received September 26, 2022; Revised November 19, 2022; Accepted November 21, 2022)

A Gram-negative, facultatively anaerobic, rod-shaped (1.4 ± 0.46 μm × 0.53 ± 0.17 μm) and motile marine bacterium designated as MEBiC11861^T was isolated from a marine algal debris collected from Kosrae, Federation State of Micronesia (162°57'23.1"E, 5°21'13.0"N). Based on the 16S rRNA gene sequence analysis strain MEBiC11861^T identified as a novel species in the genus *Nisaea*, it showed high similarity to members of the genus *Nisaea* (97.0–98.4%). Strain MEBiC11861^T was growing at 10–42°C (optimum 26–29°C), at pH 4.0–8.5 (optimum pH 5.0) and with 0–10% (optimum 0.5%) NaCl. The $C_{12:0}$ (5.6%), $C_{16:0}$ (29.0%), $C_{12:0}$ 3-OH (4.3%), summed feature 3 ($C_{16:1}$ ω7*c* and/or $C_{16:1}$ ω6*c*; 9.9%), summed feature 8 ($C_{18:1}$ ω7*c* and/or $C_{18:1}$ ω6*c*; 31.2%), and $C_{19:0}$ cyclo ω8*c* (10.6%) were determined to be as predominant cellular fatty acids. The DNA G + C content is 63.0 mol%. The major respiratory quinone is Q-10. Several phenotypic characteristics such as utilization of gluconate, malate, adipate, arabinose etc., DNA G + C ratio, cellular fatty acids composition, and growth range of pH and salinity differentiate strain MEBiC11861^T from members of the genus *Nisaea*. On the basis of this polyphasic taxonomic data, strain MEBiC11861^T should be classified as a novel species in the genus *Nisaea* and it is proposed as *Nisaea acidiphila* sp. nov. The type strain is MEBiC11861^T (= KCCM 43219^T = JCM 31589^T). Emended description of the genus *Nisaea* Urios *et al*. 2008 and emendation of *Thalassobaculum salexigens* were also given.

Keywords: *Thalassobaculaceae*, marine algal debris, micronesia, polyphasic taxonomy

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Family *Rhodospirillaceae* (Garrity and Holt, 2001) was one of the major marine bacterial lineages that including phylogenetically heterogenic and physiologically diverse groups including photosynthetic to chemoorganotrophic, aerobic to anaerobic, etc. Consequently, the family had been divided into 9 families by Hördt *et al.* (2020) based on the phylogenomic

analysis. Amongst, family *Thalassobaculaceae* comprised of the genera *Nisaea*, *Oceanibaculum*, and *Thalassobaculum* and all members were isolated from marine environments (Su *et al*., 2016; Du *et al*., 2017; Zhu *et al*., 2021). The genus *Nisaea* was first proposed by Urios *et al.* (2008) with two novel species isolated from the water column in Mediterranean Sea and these species showing denitrifying activity (Urios *et al*., 2008). Recently a metal resistant novel species named *N. sediminum* (Zhu *et al*., 2021) from marine sediments was added. Basically, members of the genus *Nisaea* were reported as Gram-negative, motile by polar falgellum, rod-shaped, catalase- and oxidase-positive, containing ubiquinone-10 (Q-10) as the predominant quinone, with very high amounts of $C_{18:1}\omega7c$ and around 60–63% of the DNA G + C content (Zhu *et al*., 2021). Similar phylotypes has been reported from the diverse marine environments such as seawater of Hawaian Archipelago (Donachie *et al*., 2004), Monterey Bay (Suzuki *et al*., 2004), Antarctic or Arctic sea (Galand *et al*., 2010; Singh *et al*., 2015), and north west Mediterranean Sea (Obernosterer *et al*., 2010), marine organisms such as gut of *Ciona intestinalis* (Dishaw *et al*., 2014), healthy corals (Klaus *et al*., 2007; Sunagawa *et al*., 2009; de Castro *et al*., 2010), microalgae (Green *et al*., 2015), oil polluted environments (Kellermann *et al*., 2013; JQ712086&115, unpublished), marine sediment (OK067311), etc. Ecologically, members of the genus *Nisaea* possibly occupying hub position of microbial communities and predicted to play synchronize ecological processes over broad ecosystem (Ma *et al*., 2020). Still the ecological role of members in the genus *Nisaea* is not well explored due by small number of reported species. During the exploration of diverse marine microorganisms from tropic area, a novel strain affiliated with the genus *Nisaea* was isolated, its taxonomic properties was investigated and reported as a novel species in here.

Materials and Methods

Sampling, isolation, and culture conditions

Strain MEBiC11861^T was isolated from an algal debris collected at coastal area of the Kosrae, Federation State of the Micronesia (162°57’23.1"E, 5°21’13.0"N). A small piece of algal debris was taken into pre-sterilized microtube, vacuum packing, and transported into Korea. A tiny part of the sample was inoculated into sterilized seawater with 0.1% yeast extract and incubated at room temperature for 14 days, then, spread on R2A agar (BD) prepared with seawater (designated as MR2A) and cultivated at 25°C for 5 days. Individual colonies formed on MR2A medium were separated depend on morphological difference and then, purification process was conducted. The purified strain MEBiC11861^T was routinely cultivated at 25°C on the marine agar 2216 (BD; MA) for biochemical and physiological characterization after confirming that the growth of strain was better on MA, and stored at -80°C in marine broth (MB) supplemented with 20% (v/v) glycerol. For phenotypic comparisons, *N. nitritireducens* DSM 19540^T (= DR41_18^T) and *N. denitrificans* DSM 18348^T (= DR41_21^T) were purchased from DSM (Deutsche Sammlung von Microorganismen und Zellkulturen GmbH) and *N. sediminum* KCTC 82224^T (= NBU 1469^T) from KCTC (Korea Collection of Type Culture) and grown on MA at 25°C.

Phylogenetic and genomic analysis

Genomic DNA was extracted using a commercial DNA extraction kit (MoBio) after five days of cultivation under optimal growth condition. The 16S rRNA gene was amplified using 27F and 1492R bacterial primer sets (Giovannoni, 1991) and sequenced using an ABI 3730xl automatic sequencer. The sequences obtained were assembled using the Vector NTI ver. 9.1 (Life Technologies) and were than compared using BLAST pairwise alignment with sequences in the EzBiocloud database (Yoon *et al*., 2017a). Phylogenetic analysis based on the unambiguous 16S rRNA gene sequence (1,318 bp) of the isolated strain with those of closely related members of the *Thalassobaculaceae* was conducted using MEGA ver. 5.2 (Tamura *et al*., 2011). A phylogenetic tree using 1,000 replicated bootstrap analysis and complete deletion option was constructed using the neighbor-joining method (Saitou and Nei, 1987; NJ) inferred by the Jukes and Cantor distance model (Jukes and Cantor, 1969) as well as maximum-likelihood (Felsenstein, 1981; ML) and maximum-parsimony (Fitch, 1971; MP) methods. The sequences of *Inquilinus limosus* DSM 1600^T (AUHM01000026) and *Fodinicurvata fenggangensis* K17-16^T (FJ357427) were used as outgroups.

The whole genome sequence of strain MEBiC11861^T was

Table 1. Genomes used in the calculation of ANI and AAI values of the species in the family *Thalassobaculaceae*

No.	Genus	Strain	Accession No.	Size (Mb)	Contigs	Protein	RNAs (r/t/o)	Genes	G + C ratio
1	*Nisaea acidiphila*	MEBiC11861	CP102480	4.75	1	4,369	9/51/4	4,448	63.0
2	*Nisaea sediminum*	NBU1469	JACZCQ00000000	5.02	22	4,641	3/51/4	4,777	63.6
3	*Nisaea denitrificansns*	DSM 18348	AUFM00000000	4.63	22	4,234	8/45/4	4,313	60.5
4	*Nisaea nitritireducens*	DSM 19540	JACZFS00000000	4.84	97	4,348	13/49/4	4,491	60.6
5	*Thalassobaculum fulvum*	HSF7	BMZS00000000	5.96	33	5,481	4/45/4	5,578	70.8
6	*Thalassobaculum litoreum*	DSM 18839	FNBW00000000	5.36	53	4,991	12/59/4	5,122	67.1
7	*Thalassobaculum salexigens*	DSM 19539	AUIR00000000	5.08	17	4,671	9/51/4	4,768	67.4
8	*Oceanibaculum indicum*	P24	AMRL00000000	3.95	71	3,744	3/43/3	3,838	65.5
9	*Oceanibaculum nanhaiense*	L54-1-50	MPOB00000000	3.84	40	3,593	3/46/3	3,677	65.1
10	*Oceanibaculum pacificum*	MC2UP-L3	LPXN00000000	3.89	181	3,675	3/45/4	3,761	65.7
11	*Fodinicurvata fengganensis*	YIM D812	JMLV00000000	3.77	37	3,510	9/52/4	3,622	61.0
12	*Acetobacter aceti*	ATCC 23746	ARBD00000000	3.69	8	3,261	9/50/4	3,411	57.1

obtained using single-molecule real-time technology. A phylogenomic tree drawn with the genomes of closely related genera was produced using TYGS (Meier-Kolthoff and Göker, 2019) according to the provider's instructions. Additionally, ANI and AAI values between the genomes of strain MEBiC11861^T and species in the family *Thalassobaculaceae* with two reference species (Table 1) were calculated using Chunlab's online ANI calculator (Yoon *et al*., 2017b) and AAI calculator by Kostas lab (Luo *et al*., 2014), respectively. Gene clusters for the synthesis of secondary metabolites were searching by using antiSMASH ver 6.0 webservice (Blin *et al*., 2021).

Phenotypic, physiological, and biochemical properties

Unless otherwise stated, the physiological and morphological characterization of the strain was conducted according to methods described by Yang *et al*. (2006). Transmission electron micrographs were taken using a LIBRA120 (Carl-Zeiss) electron microscope with fixed cells that were negatively stained with 2% phosphotungstic acid at pH 7.0. The range and optimal growth temperatures were determined in MB at 12 different temperatures (10, 16, 20, 23, 26, 29, 32, 36, 39, 42, 46, and 50°C) in a temperature gradient incubator (TVS126MA; Adaventec) for up to 3 days and separately incubated at 4°C for 1 week. The tolerance range for NaCl was tested in modified ZoBell 2216 broth (ZoBell, 1941) prepared with distilled water and supplemented with the NaCl (Sigma; 0, 0.5, 1, 2, 3, 3.5, 4, 6, 10, 15, and 20%, w/v) and separately with 11% NaCl for 1 week. The tolerance range for pH was determined (pH 4, 5, 6, 6.5, 7, 7.5, 8, 9, and 10) in MB with the pH adjusted using 10 mM MES (pH 4–6), 10 mM HEPES (pH 6–8) or 10 mM AMPSO (pH 8–10) as biological buffers and separately cultivated under pH 3 and 3.5 with malate buffer for 1 week. The tolerance ranges for NaCl and pH were also determined in a temperature gradient incubator and the OD_{600} was monitor at 10 min intervals. The bacterial suspension used to inoculate API 20E, 20NE, API ZYM kit (bioMérieux) and a Microlog GN2 system (Biolog) was prepared in a 2% sea salt (Sigma) solution. API and Microlog panels were recorded after incubation at 25°C for 2 days. To confirm anaerobic growth, cells were inoculated with 0.3% nitrate and 0.5% yeast extract in the serum vial capped with aluminum seal, purged with deoxygenated nitrogen gas until color of indicator (final 0.2 mg/L of resazurin) was disappeared, and cultivated at 25°C for 5 days and observing growth.

Chemotaxonomic analysis

The cellular fatty acid profiles of strain MEBiC11861^T and reference strains were determined using the MIDI/Hewlett Packard Microbial Identification System (MIS) (Sasser, 1990) with Sherlock version 6.2 and the TSBA6 database according to the manufacturer's instructions at KCCM (Korea Culture Center of Microorganisms). Briefly, strains were streaked on trypticase soy broth agar to produce four segments, and the third segment was sampled when growth was observed at the fourth segment. Then, the samples were saponified, methylated, extracted, purified, and analyzed by gas chromatograph. Polar lipids were extracted using a chloroform/methanol system and

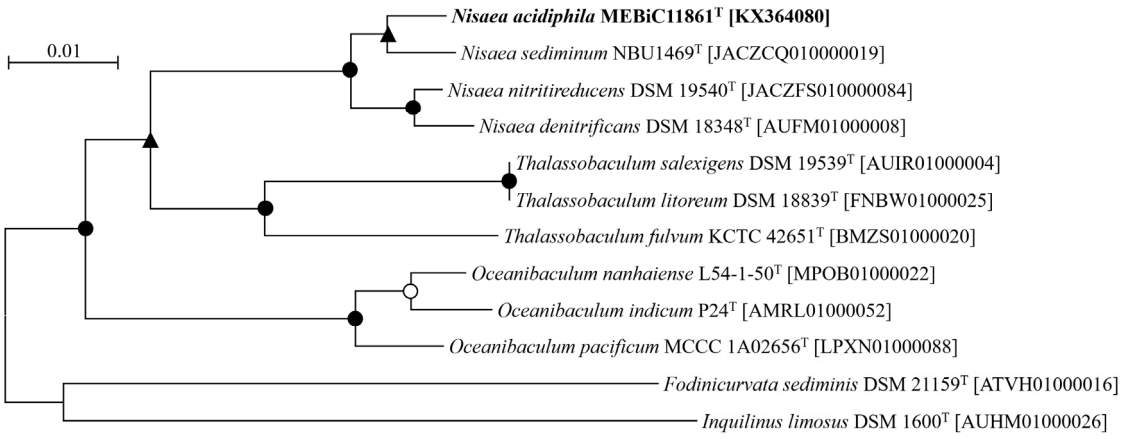

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1,318 bp between positions 99 and 1414 of *E. coli* numbering system) showing the relationship between strain MEBiC11861^T and members of the family *Thalassobaculaceae*. The tree is based on the Juke & Cantor distances model and the neighbour-joining algorithm. All nodes were recovered with 90% < (●), with 70% < (○), or one method with lower than 70% (▲) Bootstrap values by NJ, ML, and MP methods. Scale bar, 0.01 nucleotide substitutions per nucleotide position.

separated by two-dimensional TLC using silica gel 60 F_{254} aluminum-backed thin-layer plates (Merck) (Minnikin *et al*., 1984). The detailed procedure is described in Yang *et al.* (2013). After 2 dimensional development, each component was visualized using the following reagents; all lipids – 10% (w/v) molybdatophosphoric acid, free amino groups – 0.2% ninhydrin solution (Consden and Gordon, 1948), phosphorus – Zinzadze reagent (Dittmer and Lester, 1964), sugar groups - α -naphthol reagent (Jacin and Mishkin, 1965), and phosphatidylcholine (PC) – Dragendorff's reagent. The major respiratory quinone in strain MEBiC11861^T was determined by HPLC analysis according to the method described by Collins (1985).

Nucleotide sequence accession numbers

The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene sequence of strain MEBiC11861^T is KX364080 and that of the genome sequence is CP102480.

Results and Discussion

Isolation and 16S rRNA gene phylogeny of strain MEBiC11861^T

After purification of the strain using standard agar plating method, a cream-coloured, circular, convex, and opaque colony that was butyrous and entire edges was selected and designated as strain MEBiC11861^T. Strain MEBiC11861^T was found to be closely related to '*Nisaea sediminum*' NBU 1469^T, *N. nitritireducens* DR41_18^T and *N. denitrificans* DR41_21^T with 98.39%, 97.48% and 96.97% 16S rRNA gene sequence similarity, respectively, and all other type species showing lower than 94% similarity. The 16S rRNA gene sequence similarity values were lower than the cut-off value (98.65%) of the novel species suggested by Kim *et al*. (2014). The NJ tree revealed that strain MEBiC11861^T formed a coherent clade with members of the genus *Nisaea* but phyletic line was distinguished from other species. The clade was well recovered in ML and MP (Fitch, 1971) trees (Fig. 1). This result implied that strain MEBiC11861^T could be a separate species in the genus *Nisaea*.

Whole genome analysis of strain MEBiC11861^T

The 4.75 Mb (4,746,001 bp) draft genome of strain MEBiC11861^T analyzed by PacBio System was assembled into 1 contig and showed 371.93× coverage. Briefly, the genome contains 4,369 protein-coding genes (CDSs) with 3 set of rRNAs, 51 tRNAs, 4 ncRNAs and 15 pseudogenes. The DNA G + C content is estimated to be 63.0 mol% (Table 1). The 3 set of 16S rRNA genes encoded in the genome were identical but shows 4 bp difference against that by Sanger method and this result implied that the genome was not contaminated. Among the CDSs, 4,051 were assigned to COG but 1,230 revealed unknown functions. Considering the phylogenetic tree, species in the genus were divided into two groups depending on G + C ratio

(Fig. 1 and Table 1). The ANI and AAI values between strains showed that novel strain MEBiC11861^T should be identified as a novel species in the genus *Nisaea*, both values were lower than species delineation cut-off values (Table 2). However, values between *Thalassobaculum litoreum* DSM 18839^T and *T. salexigens* DSM 19539^T were 98.89 and 98.95, respectively, and this implied that two species should be unified to one. Therefore, *T. salexigens* should be reclassified as a member of the *T. litoreum.* Phylogenomic tree inferred by TYGS reflected above situation, however, phylogenetic position of the genus *Oceanibaculum* was out of the phyletic line of the family *Thalassobaculaceae* (Fig. 2). Discrepancy between 16S rRNA gene sequence phylogeny and genome-based phylogeny was also shown in previous report (Zhu *et al*., 2021). However, this discrepancy was not shown in the phylogenomic tree with whole members of *Rhodospirillaes* (Hördt *et al*., 2020), this might be due to the range of organisms used in analysis.

The genome of strain MEBiC11861^T contained a number of genes related to metal resistance, which is consistent with the results of Zhu *et al*. (2021). Similar to *N. sediminum* NBY1469^T, strain MEBiC11861^T also contained 1 terpene synthase, 1 NRPS (8% similarity with cystobactamide), 1 T3PKS, and an ectoine synthase clusters. Highly similar ectoine synthase gene cluster was common in the genus *Nisaea* but not in the genera

Table 2. ANI and AAI values between 10 species in the family *Thalassobaculaceae* and two outgroups. Species numbers are as designated in the Table 1. Species affiliated with the same genus were shaded with same color. The values exceeded the cut-off of species delineation were underlined.

ANI \ AAI	1	2	3	4	5	6	7	8	9	10	11	12
1		**85.50**	**80.16**	**80.58**	59.09	58.59	58.65	55.10	55.05	54.33	51.84	46.15
2	**83.21**		**80.14**	**80.03**	59.26	58.61	58.86	55.18	54.98	54.19	51.76	46.03
3	**78.63**	**78.54**		**94.22**	59.04	58.53	58.59	54.83	54.80	54.26	51.75	46.07
4	**78.70**	**78.76**	**91.64**		59.19	58.69	58.86	54.76	54.88	54.07	51.73	45.86
5	72.54	72.81	71.64	71.72		**66.80**	**66.94**	56.89	56.69	55.67	52.97	46.62
6	72.31	72.80	71.70	71.68	**76.92**		**98.95**	55.63	55.64	54.76	51.60	46.10
7	72.49	72.58	71.74	71.60	**76.80**	**98.89**		55.80	55.60	54.73	51.74	46.23
8	71.03	70.94	70.07	70.13	72.05	71.67	71.63		**90.65**	**74.96**	54.22	47.26
9	70.64	70.78	69.71	69.98	71.51	71.54	71.39	**88.39**		**75.34**	54.15	47.37
10	70.93	70.73	69.72	70.00	71.74	71.82	71.60	**80.48**	**80.59**		53.16	47.52
11	68.61	69.22	67.87	67.97	70.19	69.37	69.52	69.71	69.61	69.61		46.11
12	67.32	67.23	66.99	67.76	67.89	67.75	68.00	68.00	67.96	67.66	66.85	

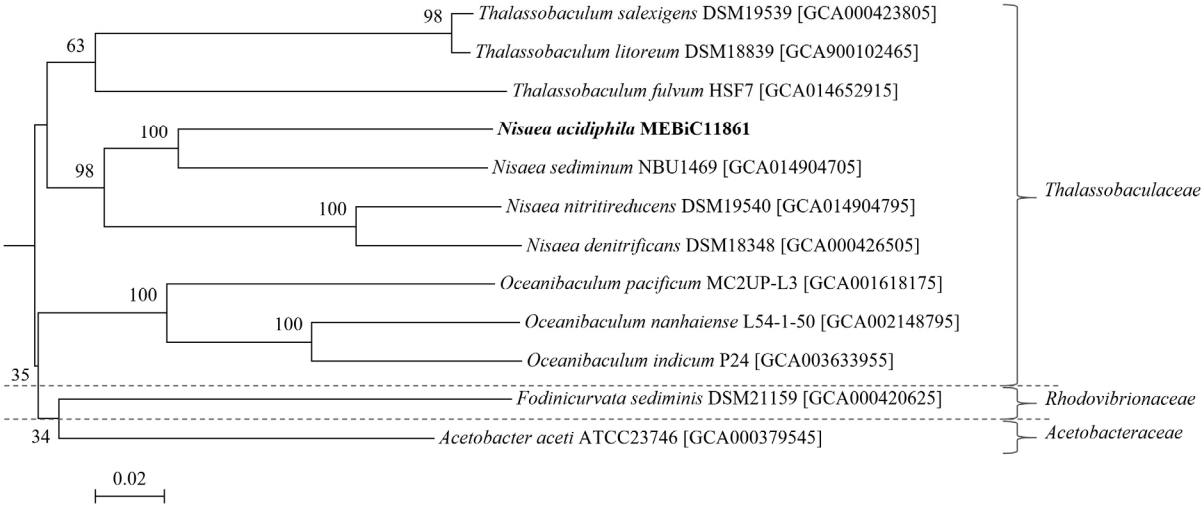

Fig. 2. Phylogenomic tree inferred by TYGS showing relationship between strain MEBiC11861^T and the members of the family *Thalassobaculaceae*. Members affiliated with different families were divided by dash.

Oceanibaculum and *Thalassobaculum*. The gene clusters of terpene, RiPP-like NRPS, and T3PKS also showed high similarity in the genus *Nisaea*. The similarity of above gene clusters of the genera *Oceanibaculum* and *Thalassobaculum* was very low compared to those of the genus *Nisaea* and the structure of the products looks also quite different. The genome contents on secondary metabolites implied that members in the genus *Nisaea* shared similar types but are not common in the family. Ability to synthesize ectoine may confer resistance to osmotic stress. Additionally, gene clusters for secondary metabolites also divided depending on the phylogenetic lines in the genus *Nisaea*.

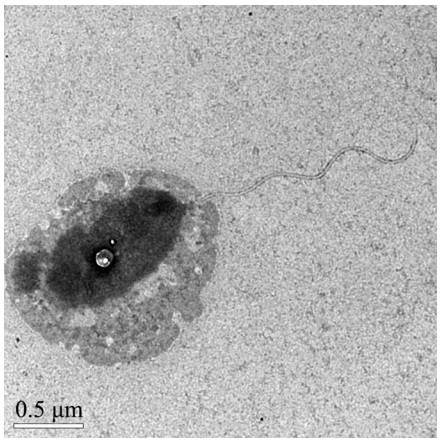

Fig. 3. Transmission electron micrograph images of strain MEBiC11861^T. The cells possess single polar flagellum and covered with thick extracellular sheath.

Phenotypic, physiological and biochemical analysis of strain MEBiC11861^T

The cell image obtain by SEM was rod shaped (0.5 ± 0.2 μm × 1.4 ± 0.5 μm) with a polar flagellum (Fig. 3) and this result is well matched with previously reported species (Zhu *et al*., 2021). The cell wall of strain was stained Gram-negative. Growth observed at temperature between 10 and 42°C (optimum 26–29°C), pH between 4.0 and 8.5 (optimum pH 5.0) and in the presence of 0–10% (w/v, optimum 0.5%) NaCl. No growth was

Table 3. Differential phenotypic characteristics of strain MEBiC11861^T and the species of the genus *Nisaea*. Strains: 1, MEBiC11681^T (data were obtained in the present study); 2, *Nisaea denitrificans* DSM 18348^T (data from this study and from Urios *et al*., 2008); 3, *Nisaea nitrireducens* DSM 19540^T (data from this study and from Urios *et al*., 2008); 4, *Nisaea sediminum* NBU1469^T (data from this study and from Zhu *et al*., 2021). +, Positive reaction; -, negative reaction. All strains are Gram-stain negative and produces acetoin, catalase, and oxidase. Negative for activity of gelatinase, arginine dihydrolase, lysine decarboxylase, ornithine decarbozylase, tryptophane deaminase and urease, and production of H_2S and indole. Nitrate reduction is positive. Enzyme activities for alkaline- and acid-phosphatases, esterase (C4), esterase-lipase (C8), leucine- and valine-arylamidases, and Naphtol-AS-BI phosphohydrolase were positive in all strains but trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, and α-fucosidase were negative (API ZYM).

Characteristics	1	2	3	4
Cell size (μm)	0.5 ± 0.2 × 1.4 ± 0.5	0.9 ± 0.2 × 2.5 ± 0.6[b]	0.9 ± 0.2 × 2.5 ± 0.6	0.7 ± 0.3 × 1.9 ± 1.0[c]
Growth conditions[a]				
Temperature (°C)	10–42 (26–29)	15–44 (30)[b]	15–44 (30)[b]	20–44 (40)[c]
pH	4–8 (5.0)	5.0–9.0 (6.0)[b]	5.0–9.0 (6.0)[b]	6.0–9.5 (7.5)[c]
NaCl (%)	0–10 (0.5)	0–6 (2)[b]	0–6 (2)[b]	0–14 (2)[c]
Enzyme activities				
Lipase(C14), cystine-arylamidases, α-and β-glucosidase, α-mannosidase	+	+	+	-
Utilization of:				
Tween 40, D-arabitol	+	-	-	-
Gluconate	-	+	+	-
Malate, citrate	-	+	+	-
Adipate	-	-	+	-
Arabinose	+	-	-	-
Glucose, maltose, mannitol, mannose	+	+	+	-
DNA G + C content (mol%)	63.0	60.5	60.6	63.6

[a] Values in parentheses are the optimum range.
[b] Data from Urios *et al*. (2008); [c] Data from Zhu *et al*. (2021).

Table 4. Fatty acid compositions (%) of strain MEBiC11681^T and the species of the genus *Nisaea*. Strains: 1, MEBiC11681^T; 2, *Nisaea denitrificans* DSM 18348^T; 3, *Nisaea nitrireducens* DSM 19540^T; 4, *Nisaea sediminum* NBU1469^T. Characters are scored as: -, not detected; tr, trace amount (< 1%); Lower than 1% in all strains are not recorded. All data were obtained in the present study.

Fatty acid	1	2	3	4
Saturated				
$C_{10:0}$	2.7	-	-	-
$C_{12:0}$	5.6	-	-	-
$C_{14:0}$	1.1	tr	tr	-
$C_{16:0}$	29.0	15.6	15.9	10.2
$C_{17:0}$	1.3	tr	tr	tr
$C_{18:0}$	tr	3.7	tr	tr
Summed feature				
Summed feature 2	-	tr	1.4	tr
Summed feature 3	9.9	13.1	14.9	6.1
Summed feature 8	31.2	58.3	56.7	54.8
Hydroxy & Cyclo				
$C_{19:0}$ $\omega 8c$ cyclo	10.6	1.2	6.4	21.6
$C_{12:0}$ 3-OH	4.3	-	-	-
Unsaturated				
$C_{18:1}$ $\omega 7c$ 11 methyl				1.7
$C_{20:2}$ $\omega 6,9c$				1.0

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system.
*Summed feature 2 contained one or more of the following fatty acids: $C_{12:0}$ aldehyde, $C_{14:0}$ 3-OH/iso-$C_{16:1}$.
*Summed feature 3 contained one or more of the following fatty acids: $C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c$.
*Summed feature 8 contained one or more of the following fatty acids: $C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$.

observed at 4°C and 46°C, with 11% NaCl, and at pH 3.5 and 9.0 (Table 3). Anaerobic growth was observed by reducing nitrate to nitrite. Enzyme activities and utilization of carbon and energy sources, as measured using commercial kits, are summarized in Table 3 and in the description of the species. Briefly, strain MEBiC11861^T was found to be mesophilic, slightly acidophilic and able to utilize a variety of substrates such as carbohydrates, short chain organic acids, and amino acids. Compared with the previously reported species, basic physiological properties were similar. However, cell size of strain MEBiC11861^T was smaller than others and growth ranges for temperature, NaCl, and pH revealed to be lower than previously reported species (Table 3).

The dominant fatty acids of strain MEBiC11861^T were determined to be $C_{12:0}$ (5.6%), $C_{16:0}$ (29.0%), $C_{19:0}$ $\omega 8c$ cyclo (10.6%), summed feature 3 ($C_{16:1}$ $\omega 6c$ and/or $C_{16:1}$ $\omega 7c$; 9.9%), and summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; 31.2%) (Table 4). The cellular fatty acids profile was not quite different from previously reported species, however, presence of relatively short-chain fatty acids such as $C_{10:0}$, $C_{12:0}$, and $C_{12:0}$ 3-OH was one of the distinguishable features from other species. The predominant polar lipids of strain MEBiC11861^T were phosphatidylglycerol (PG), phosphatidylethanolamine (PE), two unidentified lipids (L), one unidentified aminophospholipid (AL), and two unidentified aminophospholipids (PL) (Fig. 4). Considering the genome information APL1 could be identified as a phosphatidylserine. The major respiratory quinone of strain MEBiC11861^T was determined to be Q10 by HPLC analysis according to Collins (1985). The DNA G + C content deduced from genome sequence was 63.0 mol%.

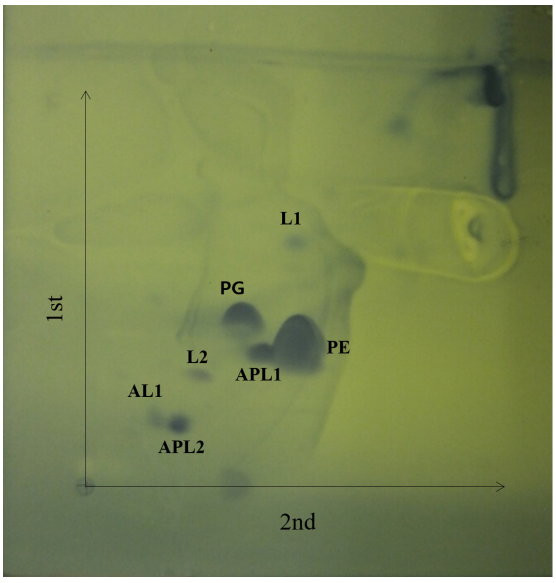

Fig. 4. Two-dimensional TLC after staining with molybdatophosphoric acid showing the total polar lipid profiles of strain MEBiC11861^T. PE, Phosphatidylethanolamine; PG, phosphatidylglycerol; APL, unidentified aminophospholipids; AL, unidentified aminolipid; L1-2, unidentified lipids.

Taxonomic conclusion

Strain MEBiC11861^T could be classified into the genus *Nisaea* on the basis of the phylogenetic and phylogenomic trees (Figs. 1 and 2), fatty acids profile (Table 4), types of respiratory quinone, DNA G + C ratio, reduction of nitrate, enzyme activities determined by API ZYM kit (Table 3) etc. However, the isolate could be distinguished from three type strains in the

existence of some short-chain fatty acids (Table 4), range and optimum of pH and NaCl for growth (Table 3), and assimilation of some carbon sources such as gluconate and malate (Table 3). Additionally, ANI and AAI values of strain MEBiC11861^T against members in the genus *Nisaea* were lower than species delineation range (Richter and Rosselló-Móra, 2009; Kim *et al*., 2014) (Table 2). On the basis of this polyphasic taxonomical evidence, it was concluded that strain MEBiC11861^T is a novel species in the genus *Nisaea* and proposed it as a name *Nisaea acidiphila* sp. nov. Emendation of the genus based on newly added species was also given. Additionally, ANI and AAI values demonstrated that *Thalssobaculum litoreum* and *T. salexigens* should be integrated into one species (Table 2). However, the two strains showed phenotypic differences (Urios *et al*., 2010), consequently transfer of *T. salexigens* to *T. litoreum* subsp. *salexigens* comb. nov. is proposed.

Emended description of the genus *Nisaea* Urios *et al*. 2008

The description given by Urios *et al.* (2008) is emended as follows. Cells are Gram-negative rod-shaped and motile by single polar flagellum. Physiologically mesophilic, slightly acidophilic to neutrophilic, and showed good growth with 0–4% NaCl. Basically, aerobic but some species are facultatively anaerobic by respire nitrate. The common predominant fatty acids were $C_{16:0}$, summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$), and summed feature 8 ($C_{18:1}$ $\omega7c$ and/or $C_{18:1}$ $\omega6c$). Possess Q-10 as respiratory quinone. Major polar lipids are phosphatidylglycerol and an unidentified aminophospholipid (possibly phosphatidylethanolamine). The DNA G + C contents ranged 60–66 mol%. The genus was phylogenetically affiliated with the family *Thalassobaculaceae* within the class *Alphaproteobacteria*. The type species is *Nisaea denitrificans*.

Description of the *Thalassobaculum litoreum* subsp. *salexigens* comb. nov.

The description same to that given by Urios *et al.* (2010) except the DNA G + C ratio, that is 67.4 mol%.

The type strain CZ41_10a^T (= DSM 19539^T = CIP 109064^T = MOLA 84^T), was isolated from the water column in the bay of Banyuls-sur-Mer. The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is EU008565 and that of the genome is AUIR00000000.

Description of *Nisaea acidiphila* sp. nov.

Nisaea acidiphila (a.ci.di'phi.la. N.L. n. *acidum* acid from L. adj. *acidus* sour; Gr. adj. *philos* loving; N.L. fem. adj. *acidiphila* acid-loving)

In addition to the characteristics of the genus, the species characteristic based on the type strain MEBiC11861^T is as follows. The Cells are rod-shaped with 1.4 ± 0.5 μm × 0.5 ± 0.2 μm in width. Colonies are cream-coloured with circular, convex, opaque and butyrous with entire edges, and 0.5–1.0 μm in diameter on marine agar after 2–3 days cultivation. Growth observed at temperature between 10 and 42°C (optimum 26–29°C), pH between 4.0 and 8.5 (optimum pH 5.0) and presence of 0–10% (w/v, optimum 0.5%) NaCl. No growth was observed at 4°C and 46°C, with 11% NaCl, and at pH 3.5 and 9.0. Nitrate is reduced to nitrite. Produces acetoin but could not produce H_2S and indole. Oxidase, catalase, β-galactosidase, and β-glucosidase activities are present but gelatinase and urease activities are negative in API20E and 20NE kits. When assayed with the API ZYM system alkaline- and acid-phosphatases, leucine-, valine-, and cystine arylamidases, esterase (C4), esterase lipase (C8), lipase (C14), β-galactosidase, α- and β-glucosidases, α-mannosidase, and naphthol-AS-BI-phosphohydrolase activities are present. Assimilates glucose, arabinose, mannose, mannitol, and maltose in API 20E kit. Oxidizes Tween 80, L-fucose, α-D-glucose, D-galactose, m-inositol, D-raffinose, D-galacturonic acid, D-gluconic acid, g-hydroxy butyric acid, α-keto glutaric acid, D,L-lactic acid, propionic acid, glucuron amide, L-arabinose, gentiobiose, α-D-lactose, lactulose, mannose, β-methyl-D-glucoside, D-psicose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, methyl pyruvate, cis-aconitic acid, citrate, D-galactonic acid lactone, D-glucosaminic acid, D-glucuronic acid, β-hydroxy butyric acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, succinic acid, succinamic acid, alaninamide, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-serine, and D,L-carnitine on Microlog GN2 plate. Major fatty acids are $C_{12:0}$, $C_{16:0}$, $C_{12:0}$ 3-OH, summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$

$\omega 6c$), summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$), and $C_{19:0}$ cyclo $\omega 8c$ when grown at 25°C. The predominant polar lipids are phosphatidyl glycerol, phosphatidylethanolamine, two unidentified lipids, two unidentified aminophospholipids, and one unidentified aminolipid. The DNA G + C content is 63.0 mol%.

Type strain MEBiC11861^T (= KCCM 43046^T = JCM 30369^T) was isolated from an algal debris collected at Kosrae State, Federation State of Micronesia. The GenBank/DDBJ/EMBL accession number for the 16S rRNA gene is KX364080 and that of the genome is CP102480.

적 요

그람 음성의 통성 혐기성의 이동성을 지닌 간균(1.4 ± 0.46 μm × 0.53 ± 0.17 μm) MEBiC11861^{T}은 미크로네시아 연방 코스래주(162°57'23.1"E, 5°21'13.0"N)에서 채취된 미동정 해조류 잔해에서 분리되었다. 16S rRNA 유전자 서열 분석 결과 MEBiC11861^T 균주는 *Nisaea*속 균주들과 높은 유사성(97.0~98.4%)을 보였다. MEBiC11861^T 균주는 섭씨 10~42°C (최적 26~29°C), pH 4.0~8.5 (최적 pH 5.0) 및 0~10% (최적 0.5%) NaCl 농도 조건에서 성장이 관찰되었다. 주요 세포 지방산은 $C_{12:0}$(5.6%), $C_{16:0}$(29.0%), $C_{12:0}$ 3-OH (4.3%), summed feature 3($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 9.9%), summed feature 8 ($C_{18:1}$ $\omega 7c$ and/or $C_{18:1}$ $\omega 6c$; 31.2%) 그리고 $C_{19:0}$ cyclo $\omega 8c$ (10.6%) 였으며 DNA G + C 함량은 65.6 mol%이다. 주요 호흡기 퀴논은 Q-10이며 글루콘산염, 말린산염, 아디핀산염, 아라비노스 등을 탄소원으로 이용한다. DNA G + C 비율, 세포지방산 조성, pH 및 염도의 성장 범위 등 여러 표현형 특성이 *Nisaea* 속의 다른 균주들과 구별된다. 이와 같은 다상분류 결과에 기초하여 균주 MEBiC11861^{T}는 *Nisaea*속의 새로운 종으로 분류되어야 함을 제안하며 이를 *Nisaea acidipila* sp. nov.로 제안하였다. 표준균주는 MEBiC11861^T (= CJM 43219^T = JCM 31589^T)이다. 균주 MEBiC11861^T 균주에서 확인된 새로운 사실들을 감안하여 *Nisaea* Urios *et al*. 2008에 대한 개정 설명과 *Thalassobaculum salexigens*를 *T. litoreum* subsp. *salexigens*로 재분류제안도 제시하였다.

Acknowledgments

This work was supported by the KIOST in-house program (PEA0021) and National Marine Biodiversity Institute of Korea (2022M01100) to KKK. We thank the Kosrae State Government, Federated States of Micronesia, for allowing marine organism research.

Conflict of Interest

The authors have no conflict of interest to report.

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