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TAXONOMIC DESCRIPTION

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Pseudoalteromonas piratica sp. nov., a budding, prosthecate bacterium from diseased *Montipora capitata*, and emended description of the genus *Pseudoalteromonas*

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Abstract

A Gram-stain-negative, motile, rod-shaped bacterium designated OCN003^T was cultivated from mucus taken from a diseased colony of the coral *Montipora capitata* in Kāne'ohe Bay, O'ahu, Hawai'i. Colonies of OCN003^T were pale yellow, 1–3 mm in diameter, convex, smooth and entire. The strain was heterotrophic, strictly aerobic and strictly halophilic. Cells of OCN003^T produced buds on peritrichous prosthecae. Growth occurred within the pH range of 5.5 to 10, and the temperature range of 14 to 39 °C. Major fatty acids were 16 : 1ω7*c*, 16 : 0, 18 : 1ω7*c*, 17 : 1ω8*c*, 12 : 0 3-OH and 17 : 0. Phylogenetic analysis of 1399 nucleotides of the 16S rRNA gene nucleotide sequence and a multi-locus sequence analysis of three genes placed OCN003^T in the genus *Pseudoalteromonas* and indicated that the nearest relatives described are *Pseudoalteromonas spongiae*, *P. luteoviolacea, P. ruthenica* and *P. phenolica* (97–99 % sequence identity). The DNA G+C content of the strain's genome was 40.0 mol%. Based on *in silico* DNA–DNA hybridization and phenotypic differences from related type strains, we propose that OCN003^T represents the type strain of a novel species in the genus *Pseudoalteromonas*, proposed as *Pseudoalteromonas piratica* sp. nov. OCN003^T (=CCOS1042^T=CIP 111189^T). An emended description of the genus *Pseudoalteromonas* is presented.

On the basis of 16S rRNA gene nucleotide sequences, Gauthier *et al.* [1] revised the genus *Alteromonas* to contain only *Alteromonas macleodii* and reclassified the remaining species in the genus *Pseudoalteromonas*. The genus *Pseudoalteromonas* comprises 39 species at the time of writing, some of which are in mutualistic or pathogenic relationships with marine organisms. Some strains influence the metamorphosis of marine invertebrates, including polychaetes [2], corals [3], sea urchins [4] and bryozoan larvae [5], and some are also thought to be important in the survival and fitness of host organisms [6]. Species of the genus *Pseudoalteromonas* have been described as etiological agents of diseases in fish [7], crustaceans [8] and sponges [9].

As part of a study of culturable bacteria in the Hawaiian reef coral *Montipora capitata* (Smith AM, *et al.*, unpublished data), mucus from a tissue-loss disease lesion on a *M. capitata* colony in Kāneʻohe Bay, Oʻahu, was spread on marine agar (MA, Difco) and incubated at 30 °C for 24 h. Strain OCN003^T arose as a pale yellow colony, which was purified by streaking on MA. Purity was assessed by microscopic observation (e.g. of Gram-stained cells) and consistency of colony morphology on MA. Unless otherwise stated, all characteristics described herein are based on cultures grown on MA or in marine broth (MB, Difco) for 48 h at 30 °C. On MA, OCN003^T grew as pale yellow, smooth, convex colonies with an entire, translucent margin. No diffusible pigment was observed.

Genomic DNA was extracted from OCN003^T with the MoBio PowerSoil DNA isolation kit (MoBio) and used in a PCR with primers 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [10] to amplify a fragment of the 16S rRNA gene. The PCR product was purified with the QIAquick PCR Purification kit (QIAGEN) and sequenced by Sanger sequencing in the

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Keywords: budding; prosthecate; *Gammproteobacteria*; *Pseudoalteromonas piratica* sp. nov.

Abbreviations: GASW, glycerol artificial seawater; MA, marine agar; MB, marine broth; MLSA, multi-locus sequence analysis; TEM, transmission electron microscopy.

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The GenBank/EMBL/DDBJ accession numbers of nucleotide sequences generated in this study are as follows: 16S rRNA, KF042038; chromosome I, CP009888; chromosome II, CP009889.

Two supplementary tables are available with the online Supplementary Material.

Advanced Studies in Genomics, Proteomics and Bioinformatics facility at the University of Hawai'i at Mānoa using the same primers. In a BLAST alignment, the 1399 nucleotides in the 16S rRNA gene of OCN003^T shared 99.4 % identity with the 16S rRNA gene sequence of *Pseudoalteromonas spongiae* UST010723-006^T, its nearest neighbour on this basis, and only ~93–97 % with other type strains of species of the genus *Pseudoalteromonas*. This nucleotide sequence and those of 14 other species in the genus were aligned in MEGA v7.0.20 [11], and a maximum-likelihood phylogenetic tree was generated. Strain OCN003^T and *P. spongiae* UST010723-006^T were adjacent in the tree (Fig. 1). A modified multi-locus sequence analysis (MLSA) based on a method to distinguish vibrios was used to further investigate the relationship between OCN003^T and other members of the genus *Pseudoalteromonas* [12]. Nucleotide sequences from the protein-coding housekeeping genes *recA*, *gapA* and *ftsZ* were retrieved from the complete genome sequence of OCN003^T [CP009888, CP009889] [13] and from those of 15 other species of the genus *Pseudoalteromonas* (Table S1, available in the online Supplementary Material). Individual sequences were retrieved from whole genomes annotated in RAST [14], concatenated and aligned in MEGA using the CLUSTALW program [11], and confirmed to be homologous to those of strain OCN003^T. A maximum-likelihood tree based on the MLSA showed that OCN003^T did not cluster robustly with any type strain of species of the genus *Pseudoalteromonas* (Fig. 2). An *in silico* whole-genome DNA–DNA hybridization of OCN003^T with its closest phylogenetic neighbours, *P. spongiae*, *P. ruthenica*, *P. phenolica* and *P. luteoviolacea*, using publicly available genome sequences, reflected the outcome of the MLSA analysis, with identities ranging from 22.8 to 25.5 %, which suggests that OCN003^T is a representative of a novel species in the genus *Pseudoalteromonas* (Table 1).

Dominant fatty acids in OCN003^T were 16 : 1ω7*c*, 16 : 0, 18 : 1ω7*c*, 17 : 1ω8*c*, 12 : 0 3-OH and 17 : 0 (comprising 82.17 % of the total), according to the MIDI Sherlock Microbial Identification System v. 6.2, by Microbial ID [15]. This fatty acid profile was similar to those of *P. spongiae* and *P. ruthenica* (Table 2). No fatty acid profiles have been reported for *P. phenolica* or *P. luteoviolacea*. The DNA G+C content of OCN003^T was 40.0 mol% [13], in line with reported DNA G+C contents of *P. spongiae*, *P. ruthenica*, *P. phenolica* and *P. luteoviolacea* (Table 1).

Characteristics of OCN003^T, *P. spongiae*, *P. luteoviolacea*, *P. ruthenica* and *P. phenolica* were measured as described below and described in Table 3. Whether or not OCN003^T could grow anaerobically was examined in the GasPak 100 System (BD), according to the manufacturer's instructions. The concentration of sodium chloride required for growth was tested using a previously described protocol in a medium containing 5.0 g $MgCl_2$, 2.0 g $MgSO_4$, 0.5 g $CaCl_2$, 1.0 g KCl, 5.0 g peptone (per litre) and various NaCl concentrations (0–80 g l^{-1}) and a pH adjusted to 7.5 with KOH [16]. The pH range for growth was determined in MB using the following buffers: pH 3.0–4.0, glycine/HCl; pH 4.0–8.0, citrate/Na_2HPO_4; pH 6.0–8.0, phosphate buffer; pH 9.0–11, glycine/NaOH [17]. The pH was adjusted prior to sterilization, verified after sterilization prior to inoculation and re-verified at the end of the incubation. Cell morphology was

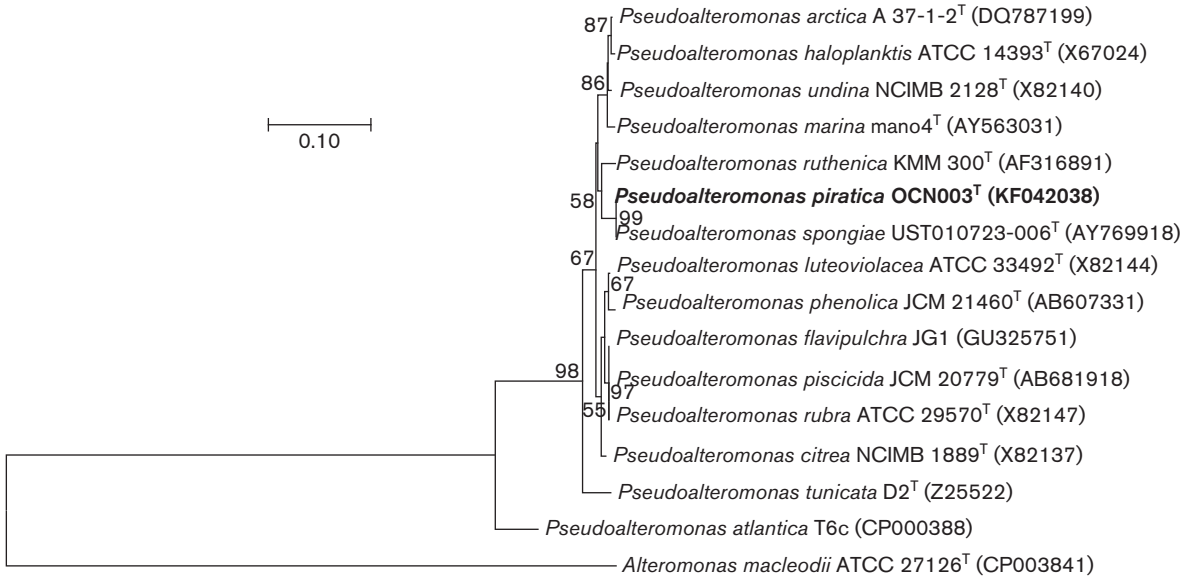

Fig. 1. Maximum-likelihood dendrogram displaying the phylogenetic relationships between *Pseudoalteromonas piratica* sp. nov. OCN003^T and related species of the genus *Pseudoalteromonas* on the basis of 16S rRNA gene sequences. *Alteromonas macleodii* ATCC 27126^T was chosen as the outgroup. Bar, one nucleotide substitution per 10 nucleotides. Bootstrap values >50 % (500 replicates) are indicated at nodes. GenBank accession numbers for each reference strain are in parentheses.

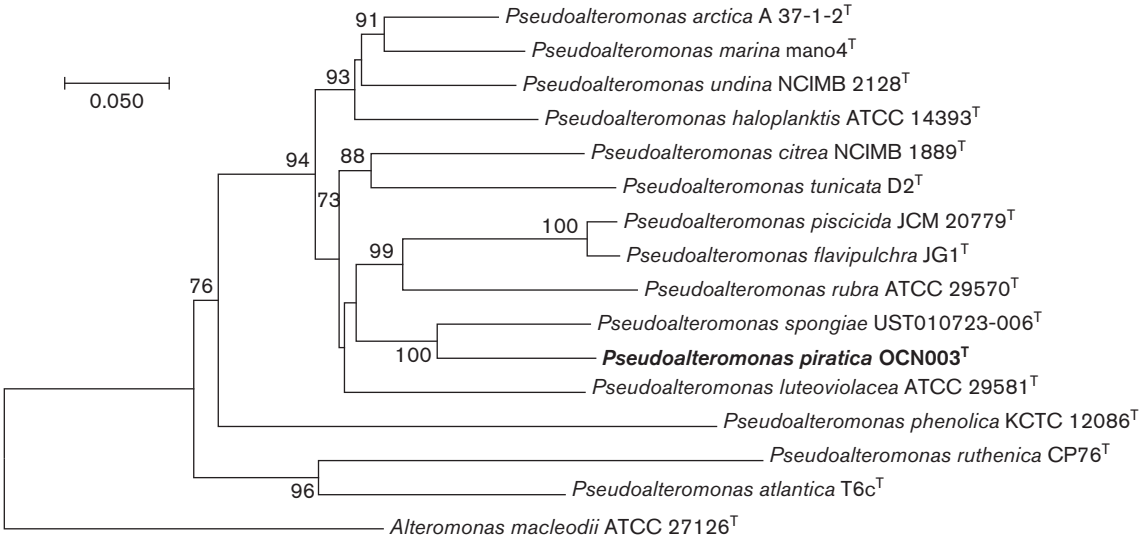

Fig. 2. Maximum-likelihood dendrogram displaying the phylogenetic relationships between *Pseudoalteromonas piratica* sp. nov. OCN003^T and related species of the genus *Pseudoalteromonas* based on a modified multi-locus sequence analysis (MLSA). Analysis was based on nucleotide sequences of the housekeeping genes *recA*, *gapA* and *ftsZ*. *Alteromonas macleodii* ATCC 27126^T was chosen as the outgroup. Bar, five nucleotide substitutions per 100 nucleotides. Bootstrap values >50 % (500 replicates) are indicated at nodes.

examined by transmission electron microscopy (TEM). An overnight culture of OCN003^T was deposited on a Formvar-coated copper grid, fixed with 1 % uranyl acetate and viewed on a Hitachi HT7700 TEM at 100 kV. Flagella, buds and prosthecae were observed (Fig. 3). Motility was determined by light microscopy of a hanging-drop preparation and observation of growth in motility medium [16]. Susceptibility to antibiotics was tested in a liquid medium inoculated with 10^4 c.f.u. ml^{-1} of OCN003^T. If the optical density at 600 nm (OD_{600}) was less than 0.05 after 24 h of incubation, OCN003^T was considered susceptible to the antibiotic. Oxidase and catalase activities were determined using commercially available reagent stains and droppers (BD Difco). Enzymic activity and single carbon source utilization were determined using API 20E (bioMérieux) and GN2 MicroPlate (Biolog) kits. API 20E strips were scored after 24 h of incubation at 30 °C, and the GN2 MicroPlates were scored after 24 and 48 h at 30 °C. All other tests were performed according to the manufacturers' recommendations, with the exception that the final NaCl concentration of the suspension medium for the API 20E and Biolog GN2 kits was adjusted to 2 % (w/v) NaCl.

OCN003^T differed from type strains of existing species of the genus *Pseudoalteromonas* by the presence of prosthecae

Table 1. Characteristics of sequenced genomes of the genus *Pseudoalteromonas*

Accession numbers: *Pseudoalteromonas piratica* sp. nov. OCN003^T (CP009888, CP009889), *P. spongiae* UST010723-006^T (AHCE02000000), *P. ruthenica* CP76 (AOPM00000000), *P. phenolica* KCTC 12086^T (CP013187, CP013188) and *P. luteoviolacea* B (=ATCC 29581; CAPN00000000). DDH, DNA–DNA hybridization.

Organism	DNA G+C content (mol%)	DDH estimate with OCN003^T (%)
P. piratica sp. nov. OCN003^T	40.0	100.0
P. spongiae UST010723-006^T	40.6	25.5
P. ruthenica CP76	47.6	22.8
P. phenolica KCTC 12086^T	40.6	25.3
P. luteoviolacea B (=ATCC 29581)	41.9	25.0

Table 2. Major cellular fatty acids of OCN003^T, *P. spongiae* and *Pseudoalteromonas. ruthenica*

Numbers represent percentages of total fatty acids. Data for *P. ruthenica* and *P. spongiae* are from Ivanova *et al.* [20] and Lau *et al.* [21], respectively. NR, Not reported.

Dominant fatty acid	Content (%)		
	OCN003^T	*P. spongiae*	*P. ruthenica*
16 : 1ω7*c*	26.4	29.1	31.0–42.0
16 : 0	20.7	18.4	13.8–18.7
18 : 1ω7*c*	15.8	6.4	4.0–16.0
17 : 1ω8*c*	8.1	9.8	4.0–8.0
12 : 0 3-OH	5.7	6.9	NR
17 : 0	5.5	NR	NR
18 : 0	2.9	NR	NR
14 : 0	2.2	5.3	2.0–11.0

Table 3. Differentiation of OCN003^T from other type strains of species of the genus *Pseudoalteromonas*

Data for *P. spongiae*, *P. ruthenica*, *P. phenolica* and *P. luteoviolacea* are from Lau *et al.* [21], Ivanova *et al.* [20], Isnansetyo and Kamei [22] and Gauthier [23], respectively. +, Positive; −, negative; ND, not described. All strains are oxidase- and gelatinase-positive. All strains utilize α-D-glucose, but not glycerol, L-arabinose, rhamnose or sorbitol.

Characteristic	OCN003^T	*P. spongiae*	*P. ruthenica*	*P. phenolica*	*P. luteoviolacea*
Colour	Pale yellow	Pale orange	Pale orange	Brown	Purple/yellow
Gas bubble formation	−	+	ND	ND	ND
Flagella	+	−	+	+	+
Motility	+	−	+	+	+
Prosthecae	+	−	ND	ND	−
Budding	+	−	ND	ND	−
NaCl (%) range for growth	1.0–6.0	2.0–6.0	1.0–9.0	1.0–5.0	3.0–6.0
Temp. (°C) range for growth	14.0–39.0	8.0–44.0	10.0–35.0	18.0–37.0	10.0–30.0
pH range for growth	5.5–10.0	5.0–10.0	6.0–10.0	6.5–9.5	>6.0
DNA G+C content (mol%)	40.0	40.6	48.4–48.9	39.9–40.6	40.9–42.2
Production of:					
Catalase	+	+	+	−	−
Lipase	+	−	+	+	+
Arginine decarboxylase	+	−	−	−	−
Tryptophan deaminase	+	−	ND	ND	−
Indole	+	−	ND	ND	−
Gelatinase	+	+	+	+	+
Acetoin	+	−	ND	ND	ND
Biolog GN2					
Amygdalin	+	−	ND	ND	ND
Citrate	+	−	−	−	ND
Cellobiose	−	−	+	ND	−
D-Fructose	+	+	−	ND	−
D-Mannose	+	+	−	+	−
Trehalose	−	−	−	+	+
Glycogen	+	−	ND	ND	ND
Inositol	+	+	−	−	−
L-Leucine	+	ND	ND	−	−
L-Threonine	+	ND	ND	−	+
Maltose	+	+	−	+	+
Mannitol	+	−	−	−	−
N-Acetyl-D-glucosamine	+	+	−	+	ND
Sucrose	+	−	+	+	−
Susceptible to:					
Kanamycin	− (100 µg)	− (100 µg)	+/−	ND	−
Chloramphenicol	+ (30 µg)	+ (0.1 µg)	ND	ND	+
Ampicillin	+ (100 µg)	+ (0.1 µg)	ND	ND	ND
Streptomcyin	+ (50 µg)	− (100 µg)	− (10 µg)	ND	−

and buds (Fig. 3) [1]. OCN003^T could also be distinguished from its four closest phylogenetic neighbours (*P. spongiae*, *P. ruthenica*, *P. phenolica* and *P. luteoviolacea*) on the basis of 16S rRNA gene nucleotide sequence and by 13–29 physiological traits, particularly arginine decarboxylase production and mannitol utilization (Table 3). OCN003^T could be differentiated from its nearest phylogenetic neighbour, *P. spongiae*, based on culture-independent characteristics, specifically motility, 16S rRNA gene sequence and *in silico* DNA–DNA hybridization. Molecular evidence, together with phenotypic characteristics, support OCN003^T representing the type strain of a novel species in the genus *Pseudoalteromonas*.

EMENDED DESCRIPTION OF THE GENUS *PSEUDOALTEROMONAS* GAUTHIER *ET AL.* 1995 EMEND. BEURMANN *ET AL.*

The description is as described by Gauthier *et al.* [1], with the following additional morphological features. When grown in

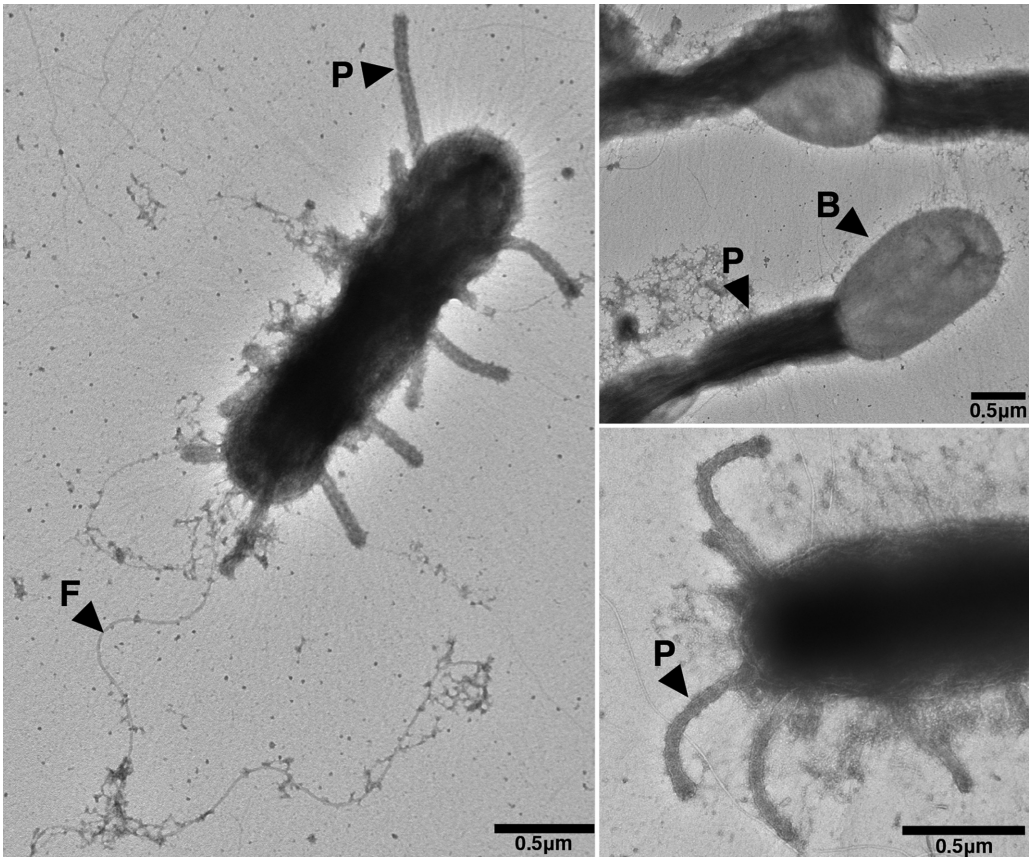

Fig. 3. Electron micrographs of uranyl acetate fixed cells of OCN003^T, showing polar flagellum (F), prosthecae (P) and buds (B). Bars, 0.5 µm.

marine or glycerol artificial seawater (GASW; [18]) broth at temperatures between 25 and 30 °C for 24 to 48 h, some strains can produce buds and prosthecae (see Fig. 3).

DESCRIPTION OF *PSEUDOALTEROMONAS PIRATICA* SP. NOV.

Pseudoalteromonas piratica (pi.ra′ti.ca. L. fem. adj. *piratica* of or belonging to pirates, referring to the prosthecae and buds on the cells that resemble arms and fists of marauding pirates).

Cells are Gram-stain-negative rods, 1.4 to 2.6 µm in length and 0.5 to 0.8 µm in width. Cells are motile by a single polar flagellum, and prosthecae are produced peritrichously. Buds can be formed at the end of prosthecae, which form on both mother and daughter cells. Colonies on marine agar are pale yellow, 1–3 mm in diameter and convex, with a smooth surface and entire, translucent margin. Neither diffusible pigment nor gas bubbles form on marine agar. Grows aerobically exclusively between 14 and 39 °C (but not at 13 or 40 °C) and only between pH 5.5 and 10 (but not at pH 5.0 or 10.5). Requires NaCl (1.0–6.0 %) for growth. The dominant fatty acids are 16 : 1ω7*c*, 16 : 0, 18 : 1ω7*c*, 17 : 1ω8*c*, 12 : 0 3-OH and 17 : 0. Susceptible to chloramphenicol (30 µg), spectinomycin (50 µg), gentamicin (30 µg), ampicillin (100 µg), streptomycin (50 µg) and neomycin (90 µg), but resistant to kanamycin (100 µg). Produces oxidase, catalase, esterase, lipase, arginine decarboxylase, tryptophan deaminase, indole, gelatinase and acetoin. However, lysine decarboxylase, ornithine decarboxylase, sulfide, urease and β-galactosidase are not produced. In Biolog GN2, positive for acetic acid, amygdalin, citrate, D-fructose, D-mannose, glycogen, glycyl-L-aspartic acid, hydroxyl-L-proline, inosine, inositol, L-alanine, L-alanyl-glycine, L-aspartic acid, L-glutamic acid, L-leucine, L-proline, L-serine, L-threonine, maltose, mannitol, *N*-acetyl-D-glucosamine, propionic acid, sucrose, α-D-glucose, β-hydroxylbutyric acid and γ-hydroxylbutyric acid (Table S2).

The type strain, OCN003^T (=CCOS1042^T=CIP 111189^T), was isolated from the mucus of *Montipora capitata* displaying signs of the disease acute *Montipora* white syndrome in Kāneʻohe Bay, Oʻahu, Hawaiʻi [19]. The DNA G+C content of the type strain is 40.0 mol%

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Conflicts of interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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