Leeuwenhoekiella palythoae sp. nov., a new member of the family *Flavobacteriaceae*

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The taxonomic status of a novel, heterotrophic, strictly aerobic, gliding and yellow–orange-pigmented bacterium (strain KMM 6264^T), associated with the coral *Palythoa*, was determined. The 16S rRNA gene sequence analysis indicated that strain KMM 6264^T clustered with the recognized species of the genus *Leeuwenhoekiella* of the family *Flavobacteriaceae* with 96.4–98.2 % sequence similarity. DNA–DNA reassociation levels between the isolate and the type strains of *Leeuwenhoekiella* species were 15–22 %. The DNA G+C content was 41.2 mol%. The phylogenetic evidence and the results of genomic and phenotypic analyses showed that the isolate should be classified as a member of a novel species of the genus *Leeuwenhoekiella*, for which the name *Leeuwenhoekiella palythoae* sp. nov. is proposed. The type strain is KMM 6264^T (=KCTC 22020^T=LMG 24856^T).

The genus *Leeuwenhoekiella* was created to accommodate heterotrophic, strictly aerobic, yellow-pigmented and gliding marine bacteria (Nedashkovskaya *et al.*, 2005). It belongs to the family *Flavobacteriaceae* (phylum *Bacteroidetes*) (Bernardet *et al.*, 2002) and comprises three recognized species, *Leeuwenhoekiella aequorea*, *Leeuwenhoekiella marinoflava* (formerly *Cytophaga marinoflava*) and *Leeuwenhoekiella blandensis* (Reichenbach, 1989; Nedashkovskaya *et al.*, 2005; Pinhassi *et al.*, 2006). It should be noted that five strains of *L. aequorea* were isolated from an Antarctic seawater sample and one strain was recovered from a common inhabitant of the East Sea, the sea urchin *Strongylocentrotus intermedius*.

Strain KMM 6264^T was isolated from a coral of the genus *Palythoa*, which had been collected from Vanfong Bay, South China Sea, Vietnam. For strain isolation, 0.1 ml tissue homogenate was transferred onto medium containing (l^{-1}): 5.0 g Bacto peptone (Difco), 5.0 g sucrose, 1.0 g glucose, 2.5 g yeast extract (Difco), 0.1 g KH_2PO_4, 0.1 g $MgSO_4$ and 15.0 g Bacto agar (Difco), using 30 % (v/v) natural seawater and 70 % (v/v) distilled water. After primary isolation and purification, the strain was cultivated at 28 °C on the same medium or on marine agar 2216 (MA) and stored at –80 °C in marine broth 2216 (both from Difco) supplemented with 20 % (v/v) glycerol.

An almost-complete 16S rRNA gene sequence (1480 nucleotides) of strain KMM 6264^T was determined by following the procedure described previously (Vancanneyt *et al.*, 2004). The sequence data were aligned with those of representative members of the family *Flavobacteriaceae*, retrieved from GenBank, and the construction of a neighbour-joining (Saitou & Nei, 1987) phylogenetic tree and a bootstrap analysis were carried out as described by Cho *et al.* (2006). The maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) algorithms gave similar results (data not shown).

The phylogenetic analysis indicated that strain KMM 6264^T forms a distinct lineage within the genus *Leeuwenhoekiella* (Fig. 1). The 16S rRNA gene sequence similarities between the coral isolate and *Leeuwenhoekiella* species with validly published names ranged from 96.4 to 98.2 %. *L. blandensis* MED 217^T, which had been isolated from a seawater sample collected from the Mediterranean Sea, was the closest relative of strain KMM 6264^T, with a sequence similarity of 98.2 %. Based on the results of this analysis and according to the recommendation of Stackebrandt &

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 6264^T is FJ405187.

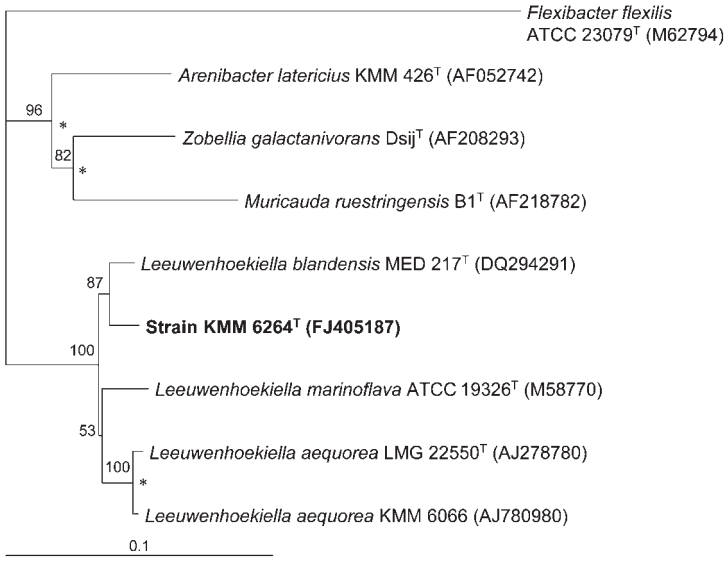

Fig. 1. Phylogenetic tree of *Leeuwenhoekiella* species and related taxa based on 16S rRNA gene sequences. Percentages at nodes are levels of bootstrap support based on 1000 resamplings. Asterisks indicate branches that were also recovered in maximum-likelihood and maximum-parsimony trees. The sequence of *Flexibacter flexilis* ATCC 23079^T was used as an outgroup. Bar, 0.1 substitutions per nucleotide position.

Ebers (2006), strain KMM 6264^T may be considered as a member of a novel species of the genus *Leeuwenhoekiella*.

Genomic DNA was isolated following the method of Marmur (1961) and the G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of strain KMM 6264^T was 41.2 mol%.

DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were determined as described by De Ley *et al.* (1970). The DNA–DNA reassociation values between strain KMM 6264^T and the type strains of the recognized *Leeuwenhoekiella* species varied from 15 to 22 %. These data clearly indicated that the isolate constitutes a novel species within the genus *Leeuwenhoekiella* (Wayne *et al.*, 1987).

For determination of whole-cell fatty acid profiles, strain KMM 6264^T was grown at 28 °C for 48 h on MA. Fatty acid methyl esters were extracted and analysed as described previously (Nedashkovskaya *et al.*, 2006). The cellular fatty acids of strain KMM 6264^T that accounted for more than 1 % of the total fatty acids were iso-$C_{15:1}$ (25.2 %), iso-$C_{15:0}$ 3-OH (21.6 %), iso-$C_{15:0}$ (18.6 %), iso-$C_{17:1}$ (9.8 %), iso-$C_{17:0}$ 3-OH (6.9 %), $C_{15:0}$ (2.9 %), $C_{16:0}$ (2.4 %), $C_{16:1}\omega7c$ (1.8 %), iso-$C_{16:0}$ (1.2 %) and $C_{14:0}$ 3-OH (1.0 %).

Phenotypic analysis of strain KMM 6264^T was performed using previously described methods (Nedashkovskaya *et al.*, 2003, 2004). API 20NE and API ZYM galleries (bioMérieux) were also used according to the manufacturer's instructions except that the galleries were incubated at 28 °C. The physiological, biochemical and morphological characteristics of strain KMM 6264^T are listed in the species description and in Table 1. Strain KMM 6264^T shared many features with the recognized *Leeuwenhoekiella* species. Cells are motile by gliding, produce alkaline phosphatase, catalase, oxidase and β-galactosidase, utilize L-arabinose, D-glucose, lactose, D-mannose and sucrose, hydrolyse gelatin, starch and Tweens 40 and 80 and do not require NaCl for growth. However, the isolate differed from *Leeuwenhoekiella* species by the presence of acid production from L-arabinose, cellobiose, lactose, maltose and L-rhamnose, by its inability to hydrolyse casein and Tween 20 and by its susceptibility to gentamicin, kanamycin, neomycin and polymyxin. Strain KMM 6264^T could be differentiated from each of the recognized species of the genus *Leeuwenhoekiella* by means of a set of phenotypic properties (Table 1).

On the basis of the data presented above, we propose a novel species, *Leeuwenhoekiella palythoae* sp. nov.

Description of *Leeuwenhoekiella palythoae* sp. nov.

Leeuwenhoekiella palythoae (pa.ly.tho′a.e. N.L. gen. n. *palythoae* of *Palythoa*, the genus of coral from which the type strain was isolated).

Cells are Gram-negative rods, motile by gliding, 0.4–0.5 μm in width and 1.4–3.2 μm in length. On MA, colonies are 2–3 mm in diameter, circular with entire edges and yellow–orange in colour. Optimal growth is observed with 3–4 % NaCl. Decomposes aesculin. Does not hydrolyse agar. Forms acid from trehalose, but not from L-sorbose, *N*-acetylglucosamine, citrate, adonitol, dulcitol or inositol. Does not utilize inositol, sorbitol, malonate or citrate. According to API ZYM, esterase lipase (C8), leucine and valine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α- and β-glucosidase, *N*-acetylglucosaminidase and α-mannosidase activities are present, but esterase (C4), lipase (C14), cystine arylamidase, α-chymotrypsin, β-glucuronidase and α-fucosidase activities are not present. Other biochemical

Table 1. Differential phenotypic characteristics of *Leeuwenhoekiella* species

Taxa: 1, KMM 6264^T (*Leeuwenhoekiella palythoae* sp. nov.); 2, *L. aequorea* (*n*=5); 3, *L. blandensis* (*n*=1); 4, *L. marinoflava* (*n*=1). Data were taken from Pinhassi *et al.* (2006), Nedashkovskaya *et al.* (2005) and this study. All taxa were positive for the following: gliding motility; alkaline phosphatase, catalase, oxidase and *β*-galactosidase activities; utilization of L-arabinose, D-glucose, lactose, D-mannose and sucrose; hydrolysis of gelatin, starch and Tweens 40 and 80; acid production from glycerol; and susceptibility to carbenicillin, chloramphenicol, doxycycline and erythromycin. All taxa were negative for the following: nitrate reduction; production of flexirubin-type pigments; hydrolysis of DNA, urea, cellulose and chitin; acid production from melibiose, raffinose and *N*-acetylglucosamine; and H_2S and indole production. +, Positive; −, negative; V, variable; *n*, number of strains.

Characteristic	1	2	3	4
Growth conditions				
Salinity range (%)	0–12	0–15	0–17	0–15
Temperature range (°C)	4–38	4–37	10–41	4–37
Temperature optimum (°C)	23–25	23–25	28–30	21–23
Acid formation from:				
L-Arabinose, cellobiose, lactose, maltose, L-rhamnose	+	−	−	−
D-Galactose	+	+	−	+
D-Glucose	+	−	+	−
Sucrose	+	+	−	−
DL-Xylose	−	−	+	−
Mannitol	−	+	−	−
Utilization of mannitol	−	+	−	−
Hydrolysis of casein, Tween 20	−	+	+	+
Susceptibility to:				
Ampicillin, oleandomycin, streptomycin	+	V	−	−
Benzylpenicillin	+	V	−	+
Gentamicin, kanamycin, neomycin, polymyxin	+	−	−	−
Lincomycin	−	+	+	+
Tetracycline	+	+	+	−
DNA G+C content (mol%)	41.2	35–36	42.5	38

and physiological characteristics are listed in Table 1. Predominant fatty acids (>5 %) are iso-$C_{15:1}$, iso-$C_{15:0}$ 3-OH, iso-$C_{15:0}$, iso-$C_{17:1}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content of the type strain is 41.2 mol%.

The type strain, KMM 6264^T (=KCTC 22020^T=LMG 24856^T), was isolated from the coral *Palythoa*, collected from Vanfong Bay, South China Sea, Vietnam.

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