International Journal of Systematic and Evolutionary Microbiology (2006), **56**, 365–368 DOI 10.1099/ijs.0.63800-0

Thalassomonas loyana sp. nov., a causative agent of the white plague-like disease of corals on the Eilat coral reef

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The taxonomic position of the coral pathogen strain CBMAI 722^T was determined on the basis of molecular and phenotypic data. We clearly show that the novel isolate CBMAI 722^T is a member of the family *Colwelliaceae*, with *Thalassomonas ganghwensis* as the nearest neighbour (95 % 16S rRNA gene sequence similarity). CBMAI 722^T can be differentiated from its nearest neighbour on the basis of phenotypic and chemotaxonomic features, including the utilization of cellobiose and L-arginine, the production of alginase and amylase, but not oxidase, and the presence of the fatty acids 12 : 0 3-OH and 14 : 0, but not 10 : 0 or 15 : 0. The DNA G+C content of CBMAI 722^T is 39·3 mol%. We conclude that this strain represents a novel species for which we propose the name *Thalassomonas loyana* sp. nov., with the type strain CBMAI 722^T (=LMG 22536^T). This is the first report of the involvement of a member of the family *Colwelliaceae* in coral white plague-like disease.

There is growing concern for the health of coral reefs worldwide (Rosenberg & Loya, 2004). Global climate changes, sea water pollution from aquaculture, oil spills and urban sewage, coral bleaching and other infectious diseases have been deemed to be the main causes of the decline of coral reefs (Hoegh-Guldberg, 2004; Hughes *et al.*, 2003; Knowlton & Rohwer, 2003; Rosenberg & Ben-Haim, 2002; Sutherland *et al.*, 2004). Recent reports suggest that bacteria may also play a role in the development of coral tumours (Breitbart *et al.*, 2005). Coral reefs may well serve as indicators of both local environmental degradation and global climate changes.

In the present study, we analysed the taxonomic position of a novel isolate from the coral *Favia favus* suffering from white plague in the Eilat coral reef (Barash *et al.*, 2005). Pure cultures of the strain caused the coral disease in controlled aquarium experiments (Barash *et al.*, 2005). 16S rRNA gene sequence data positioned the novel isolate in the neighbourhood of the genus *Thalassomonas* (Macian *et al.*, 2001). The two currently recognized species of this genus, *Thalassomonas viridans* and *Thalassomonas ganghwensis*, have been isolated from oysters in the Mediterranean sea and flat tide sediments in Korea, respectively, but have not, to date, ever been implicated in coral disease. Our data suggest that strain CBMAI 722^T represents a novel species of the genus *Thalassomonas*.

Strain CBMAI 722^T was isolated from diseased *Favia favus* by crushing the coral in 10 ml sterile artificial sea water with the aid of a mortar and pestle. The diseased *Favia favus* was collected by SCUBA diving at Eilat in the Gulf of Aqaba, Red Sea. Strain CBMAI 722^T was grown on ZoBell 2216E agar medium (MA; Oppenheimer & ZoBell, 1952) at 20 °C for 48 h unless otherwise stated. Colony morphology was examined on cultures grown on MA by using a stereoscopic microscope. Cell morphology was examined on wet mounts via a phase-contrast microscope. Exponentially growing cells

Published online ahead of print on 13 October 2005 as DOI 10.1099/ijs.0.63800-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Thalassomonas loyana* sp. nov. CBMAI 722^T is AY643537.

Additional phylogenetic trees and an electron micrograph of cells of *Thalassomonas loyana* sp. nov. are available as supplementary figures in IJSEM online.

in marine broth (MB) medium were negatively stained with 1 % (w/v) uranyl acetate for electron microscopy (840A; JEOL). Sequences for 16S rRNA genes were generated on a DNA sequencer (ABI Prism 3100; Applied Biosystems) and analysed as described by Thompson *et al.* (2001). The consensus sequences were assembled and phylogenetic trees were constructed based on the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony and maximum-likelihood methods using KODON 2.0 software (Applied Maths). The DNA G+C content was determined by HPLC (Tamaoka & Komagata, 1984).

Phenotypic characterization of the isolate was performed using API 20NE (bioMérieux) and Biolog GN2 metabolic fingerprinting kits following the manufacturers' instructions with some modifications. The bacterial inocula were suspended in saline solution (3 % NaCl) containing 10 % MB for Biolog tests, whereas cell suspensions (2 % NaCl) were used for API 20NE tests. Reactions were recorded after 48 h at 30 °C. Carbon source utilization was also checked with standard basal medium as described previously (Baumann *et al.*, 1984; Leifson, 1963; Oppenheimer & ZoBell, 1952). Salt tolerance was evaluated in a medium consisting of 0·5 g yeast extract and 2·5 g peptone per litre of sterile distilled water (Hidaka & Sakai, 1968). Alginate hydrolysis activity was determined using previously described methods (Sawabe *et al.*, 1995). Antibiograms were determined using the disc diffusion method of Acar & Goldstein (1996) using commercial discs (Oxoid). Analysis of fatty acid methyl esters was carried out as described by Huys *et al.* (1994). For fatty acid analysis, cells were grown on MA for 48 h at 28 °C.

According to our 16S rRNA gene sequence analysis, strain CBMAI 722^T is a member of the family *Colwelliaceae* (Ivanova *et al.*, 2004). The closest phylogenetic neighbours of the novel isolate were *T. ganghwensis* KCTC 12041^T and *T. viridans* CECT 5083^T, with 95 and 94 % 16S rRNA gene sequence similarity, respectively (Fig. 1). This low level of similarity suggests that strain CBMAI 722^T represents a novel branch within the family *Colwelliaceae*. Strain CBMAI 722^T consistently grouped with *Thalassomonas* species even when different tree-building methods were used (see Supplementary Fig. S1 in IJSEM Online).

Strain CBMAI 722^T is a Gram-negative, motile, rod-shaped bacterium with a single polar flagellum (see Supplementary Fig. S2 in IJSEM Online). Many of the cells appear to divide asymmetrically. The strain does not possess some of the main characteristics of the genus *Thalassomonas* such as oxidase activity and a DNA G+C value of between 42 and 48 mol%. Strain CBMAI 722^T grew well in synthetic basal sea water supplemented only with carbon sources and without the addition of yeast extract, indicating that this strain does not require organic factors for growth. Several phenotypic and chemotaxonomic features were found that can be used to discriminate strain CBMAI 722^T from its nearest phylogenetic neighbours (Table 1). For instance, strain CBMAI 722^T does not produce oxidase, does not possess pigment and does not grow at 15 or 37 °C, all of which are characteristics common to the other *Thalassomonas* species. It produces alginase, in contrast with the other *Thalassomonas* species. Strain CBMAI 722^T is able to utilize cellobiose and L-arginine, but *T. ganghwensis* is not. Previously recognized *Thalassomonas* species contain the fatty acid 15:0, but this fatty acid is not found in strain CBMAI 722^T. In addition, strain CBMAI 722^T has a higher content of fatty acid 14:0 than the other *Thalassomonas* species. The 16S rRNA sequence analysis appears to suggest that strain CBMAI 722^T may represent a new genus, but this remains to be determined in future studies with a broader collection of isolates of this novel taxon. We conclude that strain CBMAI 722^T represents a novel species for which we propose the name *Thalassomonas loyana* sp. nov.

Description of *Thalassomonas loyana* sp. nov.

Thalassomonas loyana (loy′an.a. N.L. fem. adj. *loyana* named in honour of the Israeli biologist Y. Loya).

Cells are 0·5–0·8 μm in width and 1–2 μm in length. They form translucent, convex, smooth-rounded colonies with an entire margin that are cream-coloured and 3 mm in size on MA after 3 days incubation at 25 °C. No growth occurs on 0 or ⩾ 10·0 % NaCl. No growth occurs below 15 °C or at 37 °C or above. Tests negative for indole, arginine dihydrolase, oxidase and urease activities. Weakly positive for gelatinase activity and nitrate reduction. Does not ferment glucose, but hydrolyses aesculin. According to Biolog tests,

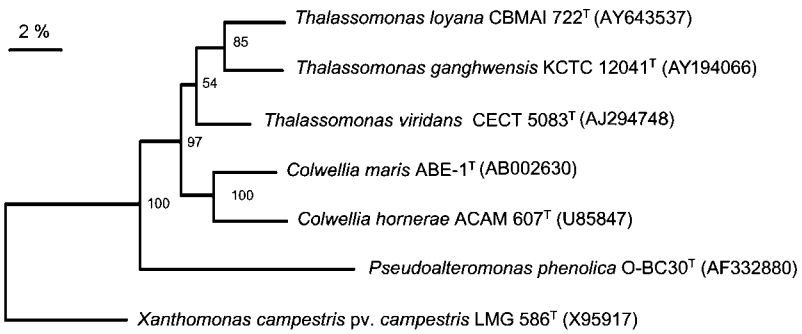

Fig. 1. Phylogenetic tree with the estimated position of *Thalassomonas loyana* sp. nov. using the neighbour-joining method with the Kimura two-parameter model based on the almost-complete 16S rRNA gene sequences with 500 bootstrap replications. Bar, 2 % estimated sequence divergence.

Table 1. Characteristics that differentiate *Thalassomonas loyana* sp. nov. from other *Thalassomonas* species

Characteristics listed in this table were obtained from Macian *et al.* (2001) and Yi *et al.* (2004). According to tests performed in Baumann's basal medium, *T. loyana* utilizes D-galactose, maltose, *N*-acetylglucosamine, D-glucose, acetate, D-glucosamine, pyruvate, D-cellobiose, L-proline, L-glutamate, inulin, DL-lactate, L-arginine and histidine as carbon sources. *T. loyana* does not utilize D-mannose, D-fructose, sucrose, melibiose, lactose, D-gluconate, succinate, fumarate, citrate, aconitate, *myo*-erythritol, D-mannitol, glycerol, δ-aminobutyrate, L-tyrosine, D-sorbitol, DL-malate, 2-oxoglutarate, xylose, trehalose, glucuronate, putrescine, propionate, arabinose, *myo*-inositol, D-raffinose, rhamnose, D-ribose, salicin, sarcosine, tartrate, L-alanine, L-asparagine, L-citrulline, glycine, L-leucine, L-ornithine and L-serine.

Characteristic	*T. loyana*	*T. ganghwensis*	*T. viridans*
Pigment	−	Yellow	Green
Production of:			
Alginase	+	−	−
Amylase	+	−	+
Oxidase	−	+	+
Utilization of:			
Acetate	+	+	−
L-Arginine	+	−	+
D-Cellobiose	+	−	+
D-Galactose	+	+	−
D-Ribose	−	−	+
L-Tyrosine	−	+	−
Fatty acid content (%):			
12 : 0 3-OH	6	−	5–6·1
14 : 0	13	−	2–3·1
10 : 0	−	4·9	−
15 : 0	−	1·2	6–11
16 : 0	5	22	11–13·7
16 : 1ω9*c*	5·7	4·7	−
17 : 1ω8*c*	12	4·4	14–19·7
16 : 1ω7*c* and/or 15 iso 2-OH	31·3	20·6	21·2–28·4
Growth at 15 and 37 °C	−	+	+
DNA G+C content (mol%)	39·3	42	48·4

is able to utilize α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-glucosamine, D-arabitol, D-cellobiose, i-erythritol, D-galactose, α-D-glucose, maltose, D-raffinose, turanose, acetate, citrate, D-gluconic acid, D-glucosaminic acid, α-ketoglutaric acid, DL-lactate, malonate, propionate, succinate, L-alanylglycine, L-glutamate, glycyl-L-aspartate, glycyl-L-glutamate, L-histidine, L-proline, DL-carnitine, α-D-glucose 1-phosphate and D-glucose 6-phosphate as sole carbon sources. Does not utilize Tween 40, Tween 80, L-arabinose, L-fucose, gentiobiose, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, methyl β-D-glucoside, D-psicose, L-rhamnose, D-sorbitol, D-trehalose, xylitol, pyruvic acid methyl ester, succinic acid monomethylester, *cis*-aconitic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, quinic acid, D-saccharic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, L-asparagine, L-aspartic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenyethylamine, putrescine, 2,3-butanediol, glycerol or DL-α-glycerol phosphate as sole carbon source. Weakly positive for utilization of *N*-acetyl-D-galactosamine, D-fructose, D-mannose, D-melibiose, sucrose, formic acid, D-alanine, L-alanine and 2-aminoethanol according to the Biolog system. The major fatty acids are summed feature 3 (31·3 %; comprising 16 : 1ω7*c* and/or 15 iso 2-OH), 14 : 0 (13·1 %), 17 : 1ω8*c* (11·7 %), 12 : 0 3-OH (6·3 %), 18 : 1ω7*c* (6·6 %), 16 : 1ω9*c* (5·7 %), 16 : 0 (4. 6 %), 15 : 1ω8*c* (3·1 %), 11 : 0 3-OH (2·5 %), 12 : 0 (2·3 %), 18 : 1ω9*c* (1·9 %), 13 : 0 (1·2 %), 14 : 1ω5*c* (1·2 %) and 15 : 1ω6*c* (1·2 %). Produces amylase, alginase, DNase and β-galactosidase, but not agarase or κ-carragenase. Sensitive to (μg per disc) erythromycin (10), kanamycin (10), gentamicin (10), ampicillin (10) and tetracycline (10). The DNA G+C content of the type strain is 39·3 mol%.

The type strain of this species, CBMAI 722^T (=LMG 22536^T), was isolated from diseased coral in Eilat, Israel.

Acknowledgements

F. L. T. acknowledges a young researcher grant (2004/00814-9) from FAPESP, Brazil. J. S. acknowledges grants from the Fund for Scientific Research (FWO), Belgium. E. R. acknowledges support from the Israel Center for the Study of Emerging Diseases.

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