Loktanella hongkongensis sp. nov., a novel member of the α -*Proteobacteria* originating from marine biofilms in Hong Kong waters

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A Gram-negative, non-motile, non-spore-forming, short rod-shaped bacterium (UST950701-009P^T) was isolated from a marine biofilm in Hong Kong waters. Colonies are pink in colour, convex with a smooth surface and entire edge. Brown diffusible pigment is produced. Whitish colonies, with otherwise identical morphology, emerge from every culture upon ageing. The white colonies can be maintained as separate cultures (UST950701-009W) without turning pink. UST950701-009P^T and UST950701-009W have identical 16S rRNA gene sequences and similar G + C (65.9–66.2 mol%) and fatty acid (86.22–88.52 % 18 : 1 ω 7c) contents. Phylogenetic analysis of the 16S rRNA gene sequence places UST950701-009P^T within the *Rhodobacter* group of the α -subclass of the *Proteobacteria*. The nearest neighbours belong to the genus *Loktanella*, with similarity values ranging from 94.5 to 95.5 %. Data on G + C and fatty acid contents support the affiliation to the genus *Loktanella*. UST950701-009P^T and -009W are heterotrophic, strictly aerobic and require NaCl for growth (2.0–14.0 %). Both grow in pH 5.0–10.0 and at 8–44 °C. Both are positive in oxidase, catalase and β -galactosidase tests, but they differ in the pattern of carbohydrate oxidation and assimilation. Molecular evidence together with phenotypic characteristics shows that UST950701-009P^T constitutes a novel species within the genus *Loktanella*. The name *Loktanella hongkongensis* sp. nov. is proposed; the type strain is UST950701-009P^T (=NRRL B-41039^T=JCM 12479^T) and a morphovar is UST950701-009W (=NRRL B-41040=JCM 12480).

The α -subclass of the *Proteobacteria* comprises one of the largest fractions of heterotrophic bacteria in the marine environment (Cottrell & Kirchman, 2000; Hagström *et al.*, 2002). Among the three major groups of α -*Proteobacteria* (the SAR11, SAR116 and *Roseobacter* clades), only the *Roseobacter* clade contains culturable members. Amongst the 13 recognized genera of the *Roseobacter* clade, *Loktanella* Van Trappen *et al.* 2004 is the most recently established. *Loktanella* already has three described species, *Loktanella fryxellensis*, *Loktanella vestfoldensis* and *Loktanella salsilacus* (Van Trappen *et al.*, 2004); all have been recovered from

microbial mats in Antarctic lakes. In this study, we propose a novel species of *Loktanella* originating from a marine biofilm in subtropical water.

During the characterization of bacteria isolated from a 7-day-old marine biofilm in Hong Kong waters, strain UST950701-009P^T was isolated on marine agar 2216 (Difco) at 30 °C. UST950701-009P^T grows as pink, convex colonies with an entire margin and a smooth and shiny surface. Unless otherwise specified, all characteristics described hereafter were based on cultures grown on marine agar 2216 for 48 h at 30 °C. Brown diffusible pigment is produced under all culture conditions. Whitish colonies, with otherwise identical colony morphological characteristics, emerge from every culture upon ageing (3 days or beyond). The whitish colonies can be maintained as separate cultures without returning to the pink colour and are therefore regarded as a morphovar (UST950701-009W) of UST950701-009P^T.

The nearly complete 16S rRNA gene sequences of

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains UST950701-009P^T and UST950701-009W are AY600300 and AY600301.

An expanded phylogenetic tree, scanning electron micrographs and details of sequencing primers and API test results are available as supplementary material in IJSEM Online.

Fig. 1. Neighbour-joining dendrogram showing the estimated phylogenetic relationships among UST950701-009P^T, UST950701-009W and related genera in the α -Proteobacteria on the basis of 16S rRNA gene sequences. *Paracoccus kocurii* JCM 7684^T was chosen as the outgroup. Bar, 1 nucleotide substitution per 10 nucleotides. Bootstrap values >50% (500 replicates) are indicated at nodes. GenBank accession numbers are shown in parentheses. Refer to Supplementary Fig. A for a fuller tree.

UST950701-009P^T and -009W (1407 and 1336 bp, respectively) were resolved on a capillary genetic analyser (Megabace) using a dye terminator method (Rosenblum *et al.*, 1997). Primers used in the sequencing reactions are detailed in Supplementary Table A available in IJSEM Online. Fragments of DNA sequences obtained from individual primers with replications ($n=3$) were assembled using

the Sequencher software package (Gene Codes). Sequence alignment using the bl2seq function at the NCBI server indicated full identity in the 1333 overlapping nucleotide positions of the UST950701-009P^T and -009W 16S rRNA gene sequences with the exception of an ambiguous nucleotide position in UST950701-009W. A phylogenetic tree (Fig. 1; see also Supplementary Fig. A for a fuller tree) was constructed using neighbour-joining in the ARB software package (Ludwig *et al.*, 2004). The stability of grouping was estimated by the bootstrap analysis (500 replications). The closest relatives of UST950701-009P^T were *L. vestfoldensis* (95.5%), *L. salsilacus* (95.0%) and *L. fryxellensis* (94.5%). A tree based on maximum-parsimony method showed the same topology (data not shown).

The DNA G+C contents of strains UST950701-009P^T and -009W as determined by the HPLC method described in Mesbah *et al.* (1989) were respectively 65.9 ± 0.4 and 66.2 ± 0.4 mol%. These values are within the range of the DNA G+C contents described for the genus *Loktanella* (Van Trappen *et al.*, 2004). UST950701-009P^T and -009W showed highly similar cellular fatty acid profiles, as determined using the Sherlock Microbial Identification Systems according to the manufacturer's protocols (Table 1). 18:1 ω 7c is the predominant fatty acid in both strains, comprising 84.5–87.0% of the total fatty acid, which is congruent with the characteristics of the genus *Loktanella*. On the other hand, the presence of 12:0 3-OH and the absence of summed features 2 and 7 distinguish the two strains from the already described members of the genus *Loktanella* (Table 1).

Table 1. Fatty acid composition of UST950701-009P^T, UST950701-009W and the three previously described species of the genus *Loktanella*

Species/strains: 1, UST950701-009P^T; 2, UST950701-009W; 3, *L. salsilacus*; 4, *L. fryxellensis*; 5, *L. vestfoldensis*. Numbers given are average percentages of total fatty acids $\pm$ range of values ($n=2$). –, Not detectable; tr, trace amounts (<1% of total). Unknown fatty acids are indicated by their equivalent chain length relative to the chain lengths of known straight-chain saturated fatty acids. Data for reference species are from Van Trappen *et al.* (2004).

Fatty acid	1	2	3	4	5
10:0 3-OH	2.0 $\pm$ 0.1	1.7 $\pm$ 0.2	2.4 $\pm$ 0.7	3.7 $\pm$ 1.1	6.1 $\pm$ 1.5
12:1 3-OH	tr	tr	–	–	5.6 $\pm$ 1.4
12:0 3-OH	1.9 $\pm$ 0.1	1.7 $\pm$ 0.1	–	–	–
14:0	–	tr	–	–	–
16:0	5.8 $\pm$ 2.1	4.6 $\pm$ 4.6	2.9 $\pm$ 0.9	2.7 $\pm$ 1.1	2.9 $\pm$ 0.7
17:0	tr	tr	–	–	–
18:0	3.5 $\pm$ 0.6	2.0 $\pm$ 0.2	1.4 $\pm$ 0.8	1.6 $\pm$ 0.9	1.8 $\pm$ 0.3
18:1 ω 7c	84.5 $\pm$ 2.5	87.0 $\pm$ 2.1	87.7 $\pm$ 1.9	84.9 $\pm$ 3.7	74.1 $\pm$ 3.1
18:1 ω 7c 11-methyl	tr	1.1 $\pm$ 1.2	tr	–	1.9 $\pm$ 0.8
Summed feature 2*	–	–	tr	1.7 $\pm$ 0.7	–
Summed feature 3	tr	tr	2.8 $\pm$ 0.9	–	–
Summed feature 7	–	–	1.2 $\pm$ 1.0	4.7 $\pm$ 2.0	4.7 $\pm$ 0.7
Unknown 11:799	tr	tr	–	–	2.3 $\pm$ 1.2

*Summed features represent the following combinations of fatty acids: 2, 12:0 aldehyde, 16:1 iso I, 14:0 3-OH and/or unknown 10:928; 3, 16:1 ω 7c and/or 15 iso 2-OH; 7, 19:1 ω 6c, 19:0 cyclo ω 10c and/or unknown 18:846.

The phenotypic characteristics of UST950701-009P^T and -009W are given in the species description. Anaerobic growth was examined in the Oxoid anaerobic system. Cell morphology was examined using a scanning electron microscope according to procedures in Neu *et al.* (2001) (Supplementary Fig. B). Cell motility and reaction to Gram stain were determined using light microscopy at $\times 1000$ according to Smibert & Krieg (1994). Susceptibility to antibiotics was tested according to Acar (1980). The production of bacteriochlorophyll *a* was examined according to Lafay *et al.* (1995). Casein hydrolysis and catalase and oxidase activities were tested according to Smibert & Krieg (1994). The hydrolysis of Tween 80 was tested according to Baumann & Baumann (1988). Tests for indole, acetoin and H₂S production, citrate utilization and β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase activities were performed using the API 20E system (bioMérieux) according to the manufacturer's instructions. The metabolism of 49 different carbohydrates was tested using both the API 20E and API 50CH systems according to the manufacturer's instructions.

Strains UST950701-009P^T and -009W, which have identical 16S rRNA gene sequences and highly similar phenotypic characteristics, can only be distinguished by colony colour, the metabolism of carbohydrates (detailed in Supplementary Table B) and the presence/absence of trace amounts of the fatty acid 14:0 (Table 1). The two strains differ from the three already described species of *Loktanella* by their fatty acid profiles (Table 1) and a number of physiological characteristics (Table 2). Based on tests using the API 20NE and API 20E systems, Van Trappen *et al.* (2004) reported that the three already described members of *Loktanella* did not metabolize any of the carbohydrates included in the tests. We

observed essentially the same negative result in our API 20E tests (see Supplementary Table B). However, strains UST950701-009P^T and -009W appeared to be able to metabolize a wide range of carbohydrates in the API 50CH tests, including some of those that gave negative results in the API 20E tests such as L-arabinose, D-glucose and D-melibiose. Given the variability of results using different methods, the carbohydrate metabolism of the three already described members of *Loktanella* and the two strains in the present study is ambiguous.

Description of *Loktanella hongkongensis* sp. nov.

Loktanella hongkongensis (hong.kong.en'sis. N.L. fem. adj. *hongkongensis* referring to Hong Kong, the isolation source of the type strain).

Cells of the type strain are Gram-negative, short rods, immotile and not forming spores. Colonies are pink in colour, convex with entire margins and have smooth and shiny surfaces. The colony size is 1–2 mm after 48 h of cultivation on marine agar 2216 at 30 °C. White colonies, with morphological characteristics otherwise identical to those of UST950701-009P^T, emerge from every culture of UST950701-009P^T after 3 days. The whitish colonies can be maintained as separate cultures without returning to the pink colour and are therefore regarded as a morphovar (UST950701-009W) of UST950701-009P^T. The remaining characteristics refer to the type strain. Growth is strictly aerobic and occurs at 8–44 °C (but not at 4 or 48 °C) and at pH 5.0–10. Requires NaCl for growth and can grow on marine agar 2216 supplemented with NaCl up to 14 % final concentration. Cannot grow on nutrient agar or trypticase soy agar (both from Oxoid). The DNA G+C content is 65.9 mol% and the dominant fatty acid is 18:1 ω 7c (84.5 % of total). Susceptible to streptomycin (50 μ g), penicillin (0.1 μ g), chloramphenicol (0.5 μ g), ampicillin (0.1 μ g) and tetracycline (0.1 μ g) but not kanamycin (tested up to 100 μ g). Bacteriochlorophyll *a*, indole, acetoin and H₂S are not produced. Catalase, oxidase and β -galactosidase activities are positive, whereas arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase and gelatinase are negative. Casein and Tween 80 are not hydrolysed. Citrate is not utilized. A wide range of carbohydrates are metabolized (acid production and/or assimilation) such as arabinose, fructose, glucose, mannitol, sucrose and xylose (see Supplementary Table B for details).

The type strain is UST950701-009P^T (=NRRL B-41039^T =JCM 12479^T), which was isolated from a marine biofilm in Hong Kong waters. A morphovar of UST950701-009P^T is UST950701-009W (=NRRL B-41040=JCM 12480).

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Table 2. Differentiation of UST950701-009P^T and -009W from the three previously described members of *Loktanella*

Species/strains: 1, UST950701-009P^T; 2, UST950701-009W; 3, *L. salsilacus*; 4, *L. fryxellensis*; 5, *L. vestfoldensis*. Data for reference species are from Van Trappen *et al.* (2004). +, Positive; (+), weakly positive; –, negative; ND, not described; V, variable.

Characteristic	1	2	3	4	5
Colony colour	Pink	White	Beige	Pink–beige	Pink
Pigment	Brown	Brown	ND	ND	ND
Conditions for growth:					
NaCl (%)	2–14	2–14	0–10	0–5	0–10
Temperature (°C)	8–44	8–44	5–30	5–25	5–37
Nutrient agar	–	–	–	–	(+)
Trypticase soy agar	–	–	–	–	(+)
Hydrolysis of Tween 80	–	–	+	+	+
Carbohydrate metabolism*	v ^a	v ^a	– ^b	– ^b	– ^b

*Results based on API 20E and API 50CH tests (a) or API 20E and API 20NE tests (b).

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