Fangia hongkongensis gen. nov., sp. nov., a novel gammaproteobacterium of the order *Thiotrichales* isolated from coastal seawater of Hong Kong

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A Gram-negative, coccobacillus-shaped, aerobic bacterium, designated strain UST040201-002^T, was isolated in February 2004 from seawater at the outlet of a sandfilter in Port Shelter, Hong Kong SAR, China. This strain possessed ubiquinone-8; its 16S rRNA gene sequence shared only 91 % similarity with the sequence from *Caedibacter taeniospiralis* and 89–90 % similarity with sequences from *Francisella tularensis*, *Francisella novicida*, *Francisella philomiragia* and *Wolbachia persica*. 16S rRNA gene sequence analysis showed that the strain formed a distinct clade with *C. taeniospiralis*. This subcluster formed a tight coherent group with members of the family *Francisellaceae* and *W. persica*. Combined phylogenetic and physiological data suggest that strain UST040201-002^T represents a novel genus and species within the order *Thiotrichales*. The name *Fangia hongkongensis* gen. nov., sp. nov. is proposed; the type strain is UST040201-002^T (=JCM 14605^T=NRRL B-41860^T).

During a study of the diversity of marine bacteria in coastal seawater of Hong Kong, a pale-yellow-pigmented bacterium, UST040201-002^T, was isolated and analysed using a polyphasic taxonomic approach.

Seawater was sampled in February 2004 from the outlet of a tank storing sand-filtered seawater that was pumped from a depth of 5 m adjacent to the Coastal Marine Laboratory of Hong Kong University of Science and Technology. Aliquots of 100 µl were spread onto YPS-SW agar (Lau *et al.*, 2005) and incubated at 30 °C for 3 days. A pale yellow colony was selected and purified by repeated restreaking on YPS-SW agar. It was cultivated in marine broth 2216 (MB) and stored in MB supplemented with 50 % (v/v) glycerol at −80 °C.

Colony morphology was observed on YP-SW (YPS-SW without starch) agar plates that had been incubated at 30 °C for 3 days. Cell morphology was examined using a Zeiss MC100 Spot microscope at 1000 × magnification. Cell motility was determined in motility agar [0.5 % marine agar (MA) with 0.005 % 2,3,5-triphenyltetrazolium chloride]. Gram-reaction was assessed according to Collins *et al.* (1989). Growth was evaluated at various temperatures (4, 16, 20, 25, 30, 33, 37, 40 and 42 °C) in YP-SW medium (Lau *et al.*, 2005). Growth at various pH values (3.0–9.0 in single unit steps) was evaluated in artificial seawater (ASW; Lewin & Lounsbery, 1969) containing 0.1 % yeast extract and buffered with 10 mM sodium acetate (pH 3.0–4.0), 10 mM MES (pH 5.0–6.0) or 10 mM Tris/HCl (pH 7.0–9.0). pH values of the media were measured before and after autoclaving and only slight changes of 0.0–0.2 units were observed. Salinity requirements were determined in YP-SW prepared with 0, 5, 15, 20, 40, 75 or 100 % filtered seawater. Salt tolerance was tested in ASW containing 0.4 % yeast extract plus 0, 1, 2, 3, 4, 5, 7.5, 10 or 15 % (w/v) NaCl. NaCl and KCl requirements were tested in a medium containing 0.1 % yeast extract supplemented with NaCl or KCl at 0, 0.2, 0.4, 0.6, 0.8 or 1.0 %. The requirement for magnesium ions was tested in ASW with 0.2 % yeast extract, with magnesium sulfate replaced by equimolar levels of potassium sulfate. Anaerobic growth was examined in the Oxoid anaerobic system on YP-SW agar supplemented with 0.1 % $NaNO_3$, 0.1 % glucose or 0.1 % meat extract. Acid production from glucose, sucrose, mannose, maltose and lactose was determined in

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Photomicrographs of strain UST040201-002^T are available as a supplementary figure with the online version of this paper.

Leifson's modified O/F medium with 0.1 % cysteine-HCl (Smibert & Krieg, 1994). Carbohydrate assimilation was determined with API 50 CH strips using ASW supplemented with 0.05 % yeast extract for 2 weeks at 30 °C. Fermentation of (+)-D-glucose, (−)-D-mannitol and sucrose, and hydrolysis of chitin and Tweens 20, 40, 60 and 80 were carried out according to Baumann & Baumann (1981). Catalase, oxidase, lecithinase (observed after 2 weeks) and nitrate reductase activities, indole production, H_2S generation from cysteine or thiosulfate, and hydrolysis of casein, cellulose, starch and gelatin were performed according to Smibert & Krieg (1994). DNA hydrolysis was performed according to Lau *et al.* (2005). Haemolytic activity was investigated using defibrinated rabbit blood (5 %, v/v) prepared with blood agar base (BBL) dissolved in filtered seawater. Degradation of dead yeast cells was tested on VY/2 agar (Reichenbach, 1989) prepared with 100 % filtered seawater. Other biochemical characteristics were determined with the API ZYM, API 20E, VITEK ANI and VITEK NHI systems (bioMérieux). Isoprenoid quinone analysis was performed by the HPLC method (Collins, 1994) using ubiquinone-8 extracted from *Escherichia coli* (strain XL1 Blue) as a reference. Fatty acid methyl ester analysis was determined by the MIDI Sherlock Microbial Identification System (Microbial ID) with cells grown on heart-infusion blood agar supplemented with 3 % NaCl at 35 °C for 24 h. Genomic DNA was extracted using a TaKaRa MiniBEST Bacterial Genomic DNA Extraction kit and DNA base composition was determined by using the HPLC method (Mesbah *et al.*, 1989). The 16S rRNA gene was amplified using the primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1525R (5′-AAGGAGTGWTCCARCC-3′) (Lane, 1991) with Vent DNA polymerase (NEB) and sequenced using an Applied Biosystems 3100 automated DNA sequencer. Related 16S rRNA gene sequences were retrieved from the NCBI nucleotide database after searching with BLAST (Altschul *et al.*, 1997). The sequences of strain UST040201-002^T and related species were aligned with CLUSTAL_X (Thompson *et al.*, 1997) and edited with the BioEdit sequence alignment editor V5.0.9 (Hall, 1999; www.mbio.ncsu.edu/BioEdit/bioedit.html). 16S rRNA gene sequence similarity values were calculated with 1255 aligned nucleotides, after removal of columns containing gaps or ambiguous nucleotides [positions 29–1419; *Escherichia coli* numbering (Brosius *et al.*, 1978)], using BioEdit. Evolutionary distances were computed using the Kimura two-parameter model (Kimura, 1980) and phylogenetic trees were generated by MEGA version 2.1 (Kumar *et al.*, 2001) using the neighbour-joining method (Saitou & Nei, 1987) or the minimum-evolution algorithm and evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

At 30 °C, strain UST040201-002^T grew on MA, YP-SW agar, heart-infusion agar with 5 % rabbit blood (HIAB; prepared with or without filtered seawater) and medium with 2.5 % KCl or NaCl plus 0.1 % yeast extract, but not on nutrient agar. At 25 °C, the bacterium grew on horse blood agar and chocolate agar, but not MacConkey agar. It did not grow on blood agar, chocolate agar or MacConkey agar, with or without CO_2, at 37 °C. Colonies were 0.5–2.0 mm in diameter, pale yellow, circular, convex, smooth, glistening, translucent and mucoid with entire margins on YP-SW after 3 days incubation at 30 °C. On horse blood agar or chocolate agar, colonies appeared grey in colour. On HIAB agar (prepared in seawater), α-haemolysis was observed after 4 days incubation at 30 °C. In MB overnight cultures, cells of strain UST040201-002^T were short rods or coccobacilli (0.35–0.70 × 0.70–1.50 μm), occurring singly

Table 1. Phenotypic characteristics of strain UST040201-002^T

Positive for alkaline phosphatase, esterase (C4, C8), lipase, leucine arylamidase, proline arylamidase, γ-glutamyl arylamidase, acid phosphatase, naphthol-AS-Bl-phosphorylase, β-galactosidase, *N*-acetyl-β-glucosaminidase, tryptophan deaminase, reduction of triphenyl tetrazolium and reduction of resazurin. Negative for valine/cystine arylamidase, trypsin, α-chromotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and the Voges–Proskauer test. Produces acid from glucose, sucrose and maltose. Does not assimilate glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, ferric citrate, salicin, D-cellobiose, maltose, D-lactose, D-melibiose, sucrose, trehalose, inulin, D-melezitose, D-raffinose, glycogen, xylitol, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate or potassium 5-ketogluconate. +, Positive; −, negative; W+, weakly positive.

Characteristic	UST040201-002^T
Salinity range (% seawater)	15–100
Oxidase	W+
Catalase	+
Denitrification	−
Lecithinase	+
Hydrolysis of:	
Agar	−
Casein	+
Cellulose	−
Chitin	−
DNA	+
Aesculin	−
Gelatin	+
Starch	−
Tween 20, 40, 60, 80	+
Production of:	
H_2S from cysteine or thiosulfate	−
Indole	−
Haemolysis in rabbit blood agar	+
Hydrolysis of dead yeast cells	−

Table 2. Whole-cell fatty acid profiles of strain UST040201-002^T and species of the genus *Francisella*

Taxa: 1, strain UST040201-002^T; 2, *F. tularensis*/*F. novicida* (14 strains); 3, *F. philomiragia* (16 strains). Values given are percentages of total fatty acids. Values in parentheses indicate the range. Fatty acids are designated as total number of carbon atoms : number of double bonds, followed by the position of the double bond from the aliphatic end of the molecule; i, a and OH represent iso-branched, anteiso-branched and hydroxy fatty acids, respectively. Data are from this study and Hollis *et al.* (1989). Strain UST040201-002^T was grown on HIAB + 3 % NaCl at 35 °C for 24 h. Other strains were grown on HIAB at 35 °C for 24 h (Hollis *et al.*, 1989). *c* represents a *cis* isomer. ECL, Equivalent chain-length; tr, less than 0.8 %.

Fatty acid	1	2	3
Saturated straight-chain			
10 : 0	–	30 (10–58)	13 (10–58)
12 : 0	–	tr (0–1)	tr (0–1)
14 : 0	tr	11 (4–15)	16 (8–25)
16 : 0	2.9	10 (5–13)	9 (6–15)
16 : 0 aldehyde	–	1 (0–4)	2 (0–5)
17 : 0	tr	–	–
18 : 0	4.7	3 (1–4)	9 (6–13)
19 : 0	tr	–	–
20 : 0	tr	1 (0–3)	3 (2–4)
22 : 0	–	5 (3–10)	6 (4–8)
24 : 0	–	5 (0–10)	4 (0–9)
26 : 0	–	tr (0–1)	tr (0–1)
Saturated branched-chain			
a-15 : 0	19.0	–	–
a-17 : 0	25.1	–	–
a-19 : 0	3.7	–	–
i-14 : 0	3.9	–	–
i-15 : 0	tr	–	–
i-16 : 0	–	–	–
17 : 0 10-methyl	tr	–	–
i-17 : 0	tr	–	–
i-18 : 0	3.0	–	–
Unsaturated/hydroxy			
13 : 1 AT 12–13	tr	–	–
18 : 1ω9*c*	7.9	7 (4–8)	12 (9–17)
18 : 2	–	2 (0–3)	2 (1–3)
20 : 1	–	tr (0–1)	tr (0–1)
22 : 1	–	1 (0–2)	1 (1–2)
24 : 1	–	5 (0–8)	9 (5–14)
26 : 1	–	tr (0–1)	tr (0–2)
10 : 0 2-OH	–	1 (0–4)	tr (0–1)
10 : 0 3-OH	tr	–	–
12 : 0 2-OH	tr	–	–
12 : 0 3-OH	3.5	–	–
i-14 : 0 3-OH	tr	–	–
15 : 0 2-OH	tr	–	–
16 : 0 3-OH	0.8	3 (0–6)	3 (2–4)
i-16 : 0 3-OH	0.9	–	–
17 : 0 3-OH	tr	–	–
18 : 0 3-OH	1.4	12 (7–24)	9 (6–11)
14 : 0 3-OH/16 : 1 ISO I	5.5	–	–
Unknown			
ECL 10.928	1.0	–	–
ECL 12.484	tr	–	–
ECL 14.502	tr	–	–
ECL 14.959	1.0	–	–
ECL 15.669	tr	–	–

or in pairs. In the stationary phase, spherical cells (2.5–5.7 μm) were occasionally seen. Cells were non-motile. No flagella were observed when cells were stained with 1 % phosphotungstic acid and examined by TEM. Colony morphology and phase-contrast and SEM micrographs are available as Supplementary Fig. S1a–c in IJSEM Online. Strain UST040201-002^T was mesophilic and grew at 16–40 °C with optimal growth at 30–33 °C. It grew at pH 5.0–8.8, with optimal growth around pH 4.9–6.8. The strain required either Na^+ or K^+, but not Mg^{2+}, for growth. It grew in 0.4–7.5 % (w/v) NaCl, with optimal growth between 2 and 3 % (w/v) NaCl. The minimum amount of KCl that supported growth was about 0.6 %. The isoprenoid quinone of strain UST040201-002^T was ubiquinone-8. The DNA G + C content of strain UST040201-002^T was 53.9 ± 0.4 mol%. Table 1 lists the phenotypic characteristics of strain UST040201-002^T that were analysed.

The fatty acid profile of strain UST040201-002^T was very different from those of other members of the genus *Francisella* (Table 2). Strain UST040201-002^T contained a-17 : 0, a-15 : 0, 18 : 1ω9*c* and 14 : 0 3-OH/16 : 1 ISO I as major fatty acids, whereas members of the genus *Francisella* contained 10 : 0, 14 : 0, 16 : 0, 18:0, 18 : 0 3-OH and 18 : 1ω9*c* as major fatty acids.

The nearly complete 16S rRNA gene sequence of strain UST040201-002^T (1515 nt positions) shared only 91 % similarity with that of the type strain of *Caedibacter taeniospiralis*, an obligate intracellular parasite of *Paramecium tetraurelia* stock 51k (Beier *et al.*, 2002) and 89–90 % similarity with *Francisella tularensis* subsp. *tularensis* (Olsufiev *et al.*, 1959; Larsson *et al.*, 2005), *Francisella tularensis* subsp. *mediasiatica* and *Francisella tularensis* subsp. *holarctica* (Olsufjev & Mescheryakova, 1983), and *Francisella novicida* and *Francisella philomiragia* (Hollis *et al.*, 1989). The 16S rRNA gene sequence of strain UST040201-002^T shared similarities of only 84–85 % and 83–84 % with members of the families *Piscirickettsiaceae* and *Thiotrichaceae*, respectively.

Phylogenetic analysis using the neighbour-joining algorithm showed that strain UST040201-002^T formed a distinct lineage within the order *Thiotrichales* and clustered with *C. taeniospiralis* with a 100 % bootstrap value; this clade was coherently linked to the lineage of *Francisellaceae* at 100 % bootstrap value (Fig. 1). This tight coherent clustering of strain UST040201-002^T with *C. taeniospiralis* and members of the family *Francisellaceae* was confirmed in phylogenetic trees generated from minimum-evolution or maximum-parsimony algorithms (data not shown) and agreed with the findings of Beier *et al.* (2002) that *C.*

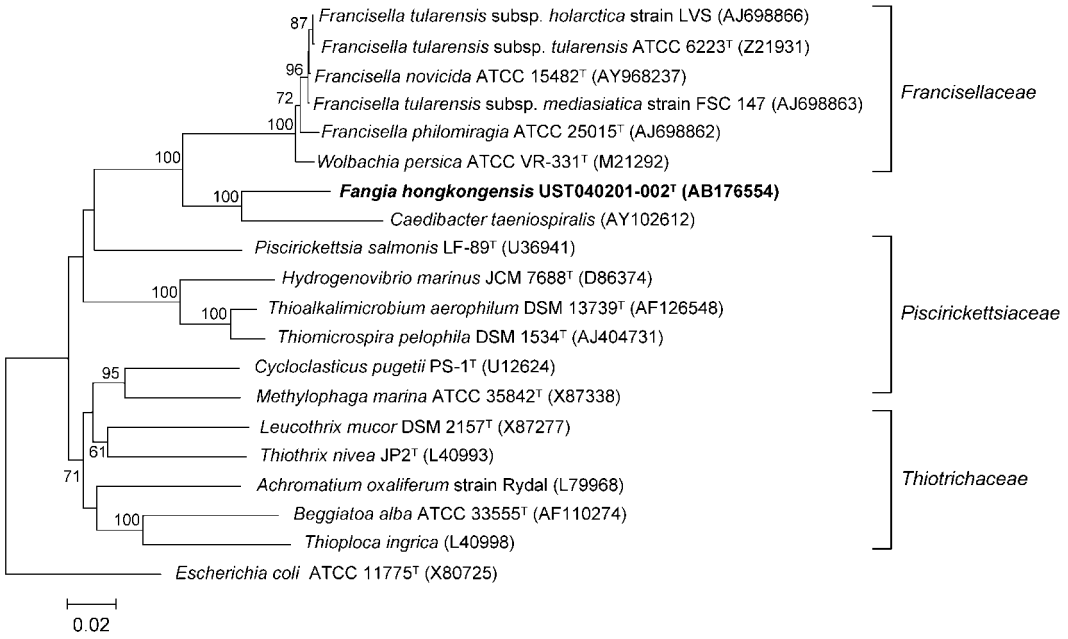

Fig. 1. Phylogenetic relationship between strain UST040201-002^T and related taxa within the order *Thiotrichales* based on 16S rRNA gene sequences. The tree was created by using the neighbour-joining method; numbers at nodes represent bootstrap percentages from 1000 resampled datasets. *Escherichia coli* ATCC 11775^T (GenBank accession no. X80725) was used as the outgroup. Bar, 0.02 nt substitutions per position.

taeniospiralis is closely affiliated with members of the family *Francisellaceae*.

Strain UST040201-002^T shared many common phenotypic traits with members of the family *Francisellaceae*: Gram-negative, short rod or coccobacilli morphology, aerobic metabolism, catalase activity, lack of flagella and spores, ability to form acid from glucose but not from lactose, β-lactamase-positive, urease-negative, and susceptible to tetracycline and chloramphenicol. However, strain UST040201-002^T differed from current members of the genus *Francisella* by its higher G+C content and its inability to produce H_2S from cysteine (Table 3). In particular, strain UST040201-002^T could be distinguished from *F. tularensis*/*F. novicida* by its production of oxidase and gelatinase and from *F. philomiragia* by its inability to produce indole. On the basis of all characteristics described above, it is proposed that strain UST040201-002^T be placed in a new genus in the order *Thiotrichales* as a representative of a novel species, *Fangia hongkongensis* gen. nov., sp. nov.

Description of *Fangia* gen. nov.

Fangia (Fan′gi.a. N.L. fem. n. *Fangia* named after Professor Xinfang Fang, founder of the Institute of Microbiology of the Chinese Academy of Sciences).

Gram-negative, short rods to coccobacilli, occurring singly or in pairs, non-motile. Non-sporulating and non-flagellated. Divides by binary fission. Strictly aerobic, chemoheterotrophic, requiring sodium or potassium ions and organic growth factors such as yeast extract for growth. Produces acid from glucose. Catalase-positive; positive or weakly positive for oxidase. Major respiratory quinone is Q-8. Predominant fatty acids are a-17:0, a-15:0 and 18:1ω9*c*. Phylogenetically, *Fangia* is a member of the order *Thiotrichales*. The type species is *Fangia hongkongensis*.

Table 3. Characteristics that differentiate strain UST040201-002^T from related species of the family *Francisellaceae*

Taxa: 1, strain UST040201-002^T; 2, *F. tularensis*/*F. novicida*; 3, *F. philomiragia*. Data are from Sjöstedt (2005) and this study. +, Positive; −, negative; w+, weakly positive; v, variable.

Characteristic	1	2	3
Size (μm)	0.35–0.7 × 0.7–1.5*	0.2–0.7 × 0.2	0.7 × 1.7
Optimum growth temperature (°C)	30–33	37	25 or 37
Oxidase	w+	−	+
Gelatinase	+	−	v
H_2S production from cysteine	−	+	+
Indole production	−	−	+
DNA G+C content (mol%)	54	33–36	33–34

*Size of bacteria from an overnight MB culture.

Description of *Fangia hongkongensis* sp. nov.

Fangia hongkongensis (hong.kong.en′sis. N.L. fem. adj. *hongkongensis* pertaining to Hong Kong SAR, PR China, where the bacterium was first isolated).

In addition to the characteristics given in the genus description, exhibits the following properties. In MB, cells are short rods about 0.35–0.70 μm in diameter and 0.7–1.5 μm in length. In half-strength MB, spherical and pleomorphic cells of 0.6–4.8 × 0.9–4.8 μm are seen. Colonies are circular, pale yellow, translucent, smooth, shiny, convex and mucoid with entire margins. Colonies are about 0.5–2.0 mm in diameter after 3 days culture on YP-SW agar at 30 °C. Cells are mesophilic. Grows at 16–40 °C and pH 4.9–8.8, with optimal growth at 30–33 °C and around pH 4.9–6.8. Requires sodium or potassium ions for growth. Grows in 0.4–7.5 % (w/v) NaCl, with optimal growth at 2–3 % (w/v) NaCl. Physiological and biochemical properties are listed in Table 1. Fatty acid profile is given in Table 2. Resistant to ampicillin (10 μg), polymyxin B (300 U) and penicillin G (2 U) and sensitive to chloramphenicol (3 μg), tetracycline (30 μg), streptomycin (10 μg), gentamicin sulfate (10 μg) and kanamycin (20 μg).

The type strain is UST040201-002^T (=JCM 14605^T=NRRL B-41860^T), which was isolated from a seawater sample collected from sand-filtered seawater, in the Port Shelter adjacent to the Coastal Marine Laboratory, Hong Kong University of Science and Technology.

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