Stenothermobacter spongiae gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from a marine sponge in the Bahamas, and emended description of *Nonlabens tegetincola*

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A bacterial strain, UST030701-156^T, was isolated from a marine sponge in the Bahamas. Strain UST030701-156^T was orange-pigmented, Gram-negative, rod-shaped with tapered ends, slowly motile by gliding and strictly aerobic. The predominant fatty acids were a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 0 3-OH, i17 : 1ω9*c* and summed feature 3, comprising i15 : 0 2-OH and/or 16 : 1ω7*c*. MK-6 was the only respiratory quinone. Flexirubin-type pigments were not produced. Phylogenetic analysis based on 16S rRNA gene sequences placed UST030701-156^T within a distinct lineage in the family *Flavobacteriaceae*, with 93·3 % sequence similarity to the nearest neighbour, *Nonlabens tegetincola*. The DNA G+C content of UST030701-156^T was 41·0 mol% and was much higher than that of *N. tegetincola* (33·6 mol%). Strain UST030701-156^T can be distinguished from other members of the *Flavobacteriaceae* by means of a number of chemotaxonomic and phenotypic characteristics. It is proposed, therefore, that UST030701-156^T represents a novel taxon designated *Stenothermobacter spongiae* gen. nov., sp. nov. The type strain is UST030701-156^T (=NRRL B-41138^T=JCM 13191^T). Carbon-source utilization by *N. tegetincola* was re-examined and an emended description is therefore included.

The family *Flavobacteriaceae*, belonging to the phylum '*Bacteroidetes*', accommodates a large number of bacteria isolated from marine eukaryotes (Bernardet *et al.*, 1996). For example, *Salegentibacter holothuriorum* is from a sea cucumber (Nedashkovskaya *et al.*, 2004), *Mesonia algae* and *Formosa algae* are from macroalgae (Nedashkovskaya *et al.*, 2003; Ivanova *et al.*, 2004), current members of the genus *Winogradskyella* are from macroalgae and sponges (Nedashkovskaya *et al.*, 2005a; Lau *et al.*, 2005a) and *Gramella echinicola*, *Leeuwenhoekiella aequorea* KMM 6066 and *Salegentibacter mishustinae* are from the sea urchin *Strongylocentrotus intermedius* (Nedashkovskaya *et al.*, 2005b, c, d). On the basis of the polyphasic taxonomic data from the present study, we propose that the bacterial strain UST030701-156^T, originating from a marine sponge, represents a novel genus of the *Flavobacteriaceae*.

The bacterial strain UST030701-156^T was isolated from tissue of the marine sponge *Lissodendoryx isodictyalis* in the Bahamas after 48 h cultivation at 30 °C on an agar medium composed of 5 g peptone l^{-1}, 3 g yeast extract l^{-1} and 0·22-μm-filtered sea water (hereafter referred to as marine agar). Colonies of UST030701-156^T were circular, 2–4 mm in diameter and convex with smooth surfaces, entire margins and a non-diffusible orange pigment. Unless otherwise specified, all characteristics described hereafter are based on cultures grown on marine agar for 48 h at 30 °C. The optimum temperature for the growth of UST030701-156^T was 28–30 °C.

The nearly complete 16S rRNA gene sequence of UST030701-156^T (1399 bp) was obtained bidirectionally

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UST030701-156^T is DQ064789.

A neighbour-joining dendrogram for strain UST030701-156^T and related species and the results of API 20E, API 20NE, API ZYM, API 50 CH and MicroLog 3 tests are available as supplementary material in IJSEM Online.

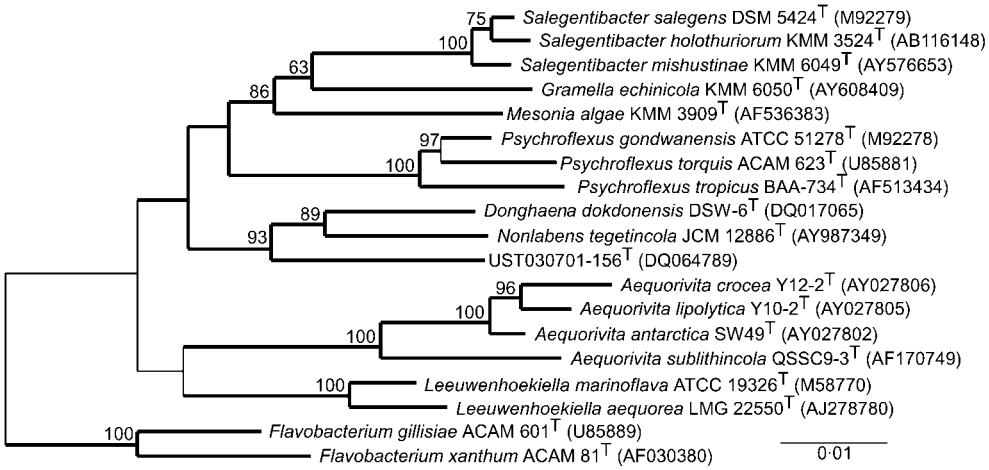

Fig. 1. Neighbour-joining dendrogram showing the estimated phylogenetic relationships among UST030701-156^T and related species on the basis of 16S rRNA gene sequences. Strains belonging to the genus *Flavobacterium* served as outgroups. Thicker lines indicate branches also found in maximum-likelihood and maximum-parsimony trees. Bootstrap values >50 % (500 replicates) are indicated at nodes. GenBank accession numbers are shown in parentheses. Bar, 1 nucleotide substitution per 100 nucleotides.

with replications ($n=3$) as described elsewhere (Lau *et al.*, 2004). Comparison of the nearly complete 16S rRNA gene sequence of UST030701-156^T with those available from GenBank revealed that UST030701-156^T is a member of the family *Flavobacteriaceae*. UST030701-156^T shared 93·3 and 93·6 % sequence similarity with the two most closely related species, *Nonlabens tegetincola* (Lau *et al.*, 2005b) and *Donghaena dokdonensis* (Yoon *et al.*, 2006), respectively. The sequence similarity to other species was ⩽90·9 %. A neighbour-joining phylogenetic tree constructed using the ARB software package (Ludwig *et al.*, 2004) showed that UST030701-156^T belonged to a distinct branch, which clustered robustly (93 %, 500 replications) with *N. tegetincola* and *D. dokdonensis* (Fig. 1). Trees based on maximum-parsimony and maximum-likelihood methods showed essentially the same topology (Fig. 1). The results of phylogenetic analysis suggest that UST030701-156^T represents a novel genus within the family *Flavobacteriaceae*. The distinction of UST030701-156^T from the closest relative, *N. tegetincola*, is further supported by the substantial difference in their DNA G+C contents: the value for UST030701-156^T is 41·0±0·2 mol% (three replicates) while that for *N. tegetincola* is 33·6±0·2 mol% (Lau *et al.*, 2005b). The DNA G+C content was determined by using an HPLC method according to Mesbah *et al.* (1989).

The predominant fatty acids (>5 %) of UST030701-156^T were a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 0 3-OH, i17 : 1ω9*c* and summed feature 3 (comprising i15 : 0 2-OH and/or 16 : 1ω7*c*) (altogether representing 76·2 % of the total), as determined using the Sherlock Microbial Identification System according to the manufacturer's protocol (Table 1). This fatty acid profile differed from that described for *N. tegetincola* by the absence of 18 : 0 and by having a smaller amount of i16 : 0 (Table 1). MK-6 was the only respiratory quinone in UST030701-156^T, as determined using an HPLC method according to Collins (1994). Menaquinones extracted from *Cellulophaga lytica* ATCC 23178^T (Johansen *et al.*, 1999) and *Pedobacter heparinus* ATCC 13125^T (Steyn *et al.*, 1998) served as references for MK-6 and MK-7, respectively.

Anaerobic growth was examined using the Oxoid Anaerobic System. The requirement for NaCl was tested in a medium containing (l^{-1}) 5 g $MgCl_2$, 2 g $MgSO_4$, 0·5 g $CaCl_2$, 1 g

Table 1. Comparison of major cellular fatty acids of UST030701-156^T and *N. tegetincola* UST030701-324^T

Both strains were grown at 30 °C on marine agar for 48 h. Values are mean percentages±SD of total fatty acids. Data for *N. tegetincola* are from Lau *et al.* (2005b). −, Not detected.

Fatty acid	Strain UST030701-156^T	*N. tegetincola* UST030701-324^T
Unknown 13·6	4·0±1·1	6·1±1·8
i14 : 0	0·5±0·0	2·1±0·8
a15 : 0	6·2±0·7	3·8±2·3
i15 : 0	38·1±0·5	33·1±6·4
i15 : 0 3-OH	6·2±0·3	5·4±3·0
i16 : 0	1·9±0·4	6·8±0·4
i16 : 0 3-OH	3·4±0·3	5·7±3·1
17 : 1ω6*c*	1·7±0·3	2·2±0·4
i17 : 1ω9c	5·7±1·4	3·5±0·8
i17 : 0 3-OH	11·2±0·3	13·7±5·5
18 : 0	−	2·5±0·0
Summed feature 3*	8·8±1·2	7·5±3·6

*Comprises i15 : 0 2-OH and/or 16 : 1ω7*c*.

KCl, 5 g peptone and various amounts of NaCl, adjusted to pH 7·5 using KOH (Isnansetyo & Kamei, 2003). Cell morphology was examined using scanning electron microscopy (JEOL 7600F) according to the procedures described by Neu *et al.* (2001) (see Supplementary Fig. S1, available in IJSEM Online, for a scanning electron micrograph). The Gram-stain reaction was determined using light microscopy according to Smibert & Krieg (1994). Gliding motility was determined using phase-contrast light microscopy after growth on quarter-strength marine 2216 medium solidified with 1 % agar according to Bowman (2000). Susceptibility to antibiotics was tested according to Acar (1980). Flexirubin-type pigment production and carboxylmethylcellulose hydrolysis were determined according to Bernardet *et al.* (2002). Casein hydrolysis was determined according to Norris *et al.* (1985); hydrolysis of chitin and Tweens 20, 40 and 80 was determined according to Baumann & Baumann (1988). Oxidase and catalase activities and the hydrolysis of agar, DNA and starch were tested according to Smibert & Krieg (1994). Other enzymic activities, the substrate-utilization pattern, nitrate reduction and the production of H_2S, indole and acetoin were determined by using the commercial systems API 20E, API 20NE, API 50 CH, API ZYM (bioMérieux) and MicroLog 3 (Biolog). Cells for inoculation to the API systems were suspended in a sterile solution of sea-water mix at 22 ‰ salinity (MacDonell *et al.*, 1982). The phenotypic characteristics of UST030701-156^T are given in the species description. Detailed results obtained from the API and MicroLog 3 systems are provided in Supplementary Tables S1–S3. The MicroLog 3 system was also used to test for the utilization of carbon sources by *N. tegetincola* UST030701-324^T. Results are given in the emended description of *N. tegetincola* and Supplementary Table S3.

Chemotaxonomic and phenotypic characteristics that distinguish UST030701-156^T from other genera of the *Flavobacteriaceae* are given in Tables 1 and 2. Strain UST030701-156^T differs from *N. tegetincola* by (i) having a higher DNA G+C content, (ii) not being able to hydrolyse DNA, (iii) having gliding motility and a different cell morphology, (iv) growing in a narrower temperature range, (v) having α-glucosidase and β-galactosidase activities and (vi) having a different carbon-source utilization pattern (refer to Supplementary Tables S2 and S3 for a detailed comparison). Strain UST030701-156^T differs from the members of *Psychroflexus* by (i) having different cell morphology and a different fatty acid profile, (ii) having a higher DNA G+C content, (iii) being less halotolerant and psychrotolerant and (iv) having β-galactosidase activity but not β-glucosidase activity. Additionally, UST030701-156^T can be distinguished from the members of *Mesonia*, *Gramella* and *Salegentibacter* by means of the traits detailed in Table 2. Molecular evidence, together with the chemotaxonomic and phenotypic characteristics, suggests that strain UST030701-156^T constitutes a novel genus within the family *Flavobacteriaceae*.

Description of *Stenothermobacter* gen. nov.

Stenothermobacter (Ste.no.ther′mo.bac′ter. Gr. adj. *stenos* narrow; Gr. adj. *thermos* hot; N.L. masc. n. *bacter* rod; N.L. masc. n. *Stenothermobacter* a rod with narrow temperature range, pertaining to the narrow temperature range that supports growth of UST030701-156^T).

Table 2. Differentiation of UST030701-156^T from closely related genera

+, Positive; −, negative; ND, not determined; V, variable. Data for reference taxa are from Lau *et al.* (2005b), Bowman *et al.* (1998), Donachie *et al.* (2004), McCammon & Bowman (2000) and Nedashkovskaya *et al.* (2003, 2004, 2005b, c).

Characteristic	UST030701-156^T	*Nonlabens*	*Psychroflexus*	*Mesonia*	*Gramella*	*Salegentibacter*
DNA G+C content (mol%)	41·0	33·6	32·0–36·0	32·7–34·0	39·6	36·8–38·0
NaCl range for growth (%)	2·0–6·0	2·0–4·0	0–20·0	1·0–15·0	1·0–15·0	0–20·0
Temperature range for growth (°C)	20·0–36·0	12·0–44·0	−16·0 to 43·0	4·0–34·0	4·0–37·0	4·0–36·0
Pigmentation	Orange	Orange	Orange	Yellow/white	Yellow/orange	Yellow
Cell shape	Rod, tapered ends, in chains	Rod	Coccoid, rod, filament	Rod	Rod	Rod
Motility	Slow gliding	Non-motile	Gliding/non-motile	Non-motile	Gliding	Non-motile
Hydrolysis of:						
DNA	−	+	V	−	+	+
Casein	−	−	−	+	+	V
Production of:						
Acetoin	+	+	ND	−	−	−
H_2S	−	−	−	+	−	+
Enzyme activity						
α-Glucosidase	+	−	+	ND	ND	ND
β-Glucosidase	−	−	+	ND	ND	ND
β-Galactosidase	+	−	−	ND	+	+

Cells are Gram-negative rods (>2·5 μm in length) with tapered ends forming chains of up to four cells. Strictly aerobic. Chemo-organotrophic. MK-6 is the only respiratory quinone. Oxidase-positive. Catalase activity is very weak. Phylogenetic analysis based on the 16S rRNA gene sequence indicates that *Stenothermobacter* is a member of the family *Flavobacteriaceae*. The genus contains one species, *Stenothermobacter spongiae*, which is the type species.

Description of *Stenothermobacter spongiae* sp. nov.

Stenothermobacter spongiae (spon′gi.ae. L. gen. n. *spongiae* of a sponge, pertaining to the isolation source of the type strain).

The description is as for the genus, with the following additions. Cells are slowly motile by gliding. Colonies on marine agar are orange in colour, circular, 2·0–4·0 mm in diameter and convex with smooth surfaces and entire margins. No diffusible pigment. Flexirubin-type pigments are not produced. Growth occurs at 20·0–36·0 °C (28·0–30·0 °C optimum), at pH 6·0–10·0 and at 2·0–6·0 % NaCl. Susceptible to ampicillin, chloramphenicol, penicillin, streptomycin and tetracycline, but not to kanamycin. The DNA G+C content is 41·0 mol%. The predominant fatty acids (>5 %) are a15 : 0, i15 : 0, i15 : 0 3-OH, i17 : 0 3-OH, i17 : 1ω9*c* and summed feature 3 (comprising i15 : 0 2-OH and/or 16 : 1ω7*c*) (altogether representing 76·2 % of the total). Produces acetoin, but not indole or H_2S. Nitrate is not reduced. Hydrolyses gelatin, starch and Tweens 20, 40 and 80, but not agar, casein, carboxylmethylcellulose, chitin or DNA. Positive for acid phosphatase, alkaline phosphatase, β-galactosidase, α-glucosidase, α-chymotrypsin, cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin and naphthol-AS-BI-phosphohydrolase activities. Negative for *N*-acetyl-β-glucosaminidase, arginine dihydrolase, α-fucosidase, α-galactosidase, β-glucosidase, β-glucuronidase, α-mannosidase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease activities. Growth occurs on the following sole carbon sources in the API 20E, API 20NE and API 50 CH systems: D-arabinose, D-galactose, D-glucose, glycerol, D-mannitol, D-melibiose, D-sorbitol, starch and D-sucrose. No acid is produced from these carbon sources. The following carbon sources in the MicroLog 3 system are utilized: D-maltose, D-melibiose, D-raffinose, sucrose, monomethyl succinate, acetic acid, α-ketoglutaric acid, α-ketovaleric acid, propionic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine and L-proline. Refer to Supplementary Tables S2 and S3 for full lists of carbon sources included in the API and MicroLog 3 systems.

The type strain is UST030701-156^T (=NRRL B-41138^T=JCM 13191^T), isolated from tissue of the marine sponge *Lissodendoryx isodictyalis* in the Bahamas.

Emended description of *Nonlabens tegetincola*

The description remains as given by Lau *et al.* (2005b), but with the following modifications: able to utilize 57 carbon sources in the MicroLog 3 system, including α-cyclodextrin, dextrin, glycogen, adonitol, D-arabitol, L-erythritol, D-fructose, L-fucose, D-galactose, α-D-glucose, *myo*-inositol, α-D-lactose, lactulose, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, D-trehalose, turanose, methylpyruvate, monomethyl succinate, acetic acid, citric acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, succinamic acid, glucuronamide, L-alaninamide, L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-ornithine, L-proline, L-serine, L-threonine and uridine. A full list of carbon sources included in the MicroLog 3 test system is available in Supplementary Table S3.

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