Zhouia amylytica gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from sediment of the South China Sea

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Three Gram-negative, non-spore-forming strains were isolated from sediment from the South China Sea, China, and their taxonomic positions were investigated using a polyphasic approach. Strains HN-171^T, HN-172 and HN-181 grew optimally at 30 °C, in the presence of 4.5–5.0 NaCl % (w/v) and at pH 7.2–7.4. They contained MK-6 as the predominant respiratory quinone and contained iso-C_{15:1} G, iso-C_{15:0}, summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7c/t) and C_{15:0} as the major fatty acids. The DNA G + C content of strain HN-171^T was 34.5 mol%. Phylogenetic analyses based on 16S rRNA gene sequences demonstrated that strain HN-171^T, together with strains HN-172 and HN-181, formed a distinct evolutionary lineage within the family *Flavobacteriaceae*. The 16S rRNA gene sequences of strains HN-171^T, HN-172 and HN-181 shared 99.8–100 % similarity with each other, and the sequence of strain HN-171^T exhibited similarity values below 90.2 % with those of other members of the family *Flavobacteriaceae*. The closest relative of HN-171^T was *Coenonia anatina* LMG 14382^T (90.2 %). On the basis of their phenotypic and phylogenetic properties, the three isolates represent a novel genus and a novel species, for which the name *Zhouia amylytica* gen. nov., sp. nov. is proposed. The type strain is HN-171^T (= CGMCC 1.6114^T = JCM 14016^T).

The family *Flavobacteriaceae* was proposed by Jooste (1985), and its description has since been subjected to repeated emendation (Bernardet *et al.*, 1996, 2002). In addition to providing phylogenetic data, Bernardet *et al.* (2002) proposed minimal standards for separating the genera classified as belonging to the family *Flavobacteriaceae*: these standards included characteristics relating to pigmentation, gliding motility, the seawater requirement, growth at different temperatures, cellular fatty acid composition and DNA G + C content. Currently, the family *Flavobacteriaceae* contains 53 genera with validly published names. Twenty-two of these genera were established within the last 5 years, being based on bacterial isolates from marine environments: *Aequorivita*, *Algibacter*, *Aquimarina*, *Arenibacter*, *Bizionia*, *Croceibacter*, *Dokdonia*, *Donghaeana*, *Gaetbulibacter*, *Gaetbulimicrobium*, *Gramella*, *Lacinutrix*, *Maribacter*, *Mesonina*, *Muricauda*, *Olleya*, *Robiginitalea*, *Stenothermobacter*, *Subsaxibacter*, *Subsaximicrobium*, *Ulvibacter* and *Winogradskyella* (Bowman & Nichols, 2002, 2005; Bruns *et al.*, 2001; Cho & Giovannoni, 2003, 2004; Ivanova *et al.*, 2001; Jung

et al., 2005; Lau *et al.*, 2006; Mancuso *et al.*, 2005; Nedashkovskaya *et al.*, 2003, 2004a, c, d, 2005a, b, c, d; Yoon *et al.*, 2005, 2006a, b). The abundance of marine members of the *Flavobacteriaceae* suggests that the family potentially plays an important role in marine ecosystems and in biogeochemical cycles, in interaction with other marine organisms.

During a survey of the ecology and bacterial diversity of the South China Sea, many bacterial strains were isolated. Three of them, designated HN-171^T, HN-172 and HN-181, form a deep branch within the family *Flavobacteriaceae*. In this communication, we describe the isolation of these three bacterial strains and our study using polyphasic taxonomy.

A sediment sample was collected from the South China Sea, China (sample site GPS position, 115° 15' 90" E 21° 18' 65" N); the salinity of the overlying water was 3 ‰. Strains were isolated by spreading sample dilutions on marine agar 2216 (MA; Difco) and incubating the plates at 30 °C. The cell morphology was examined by transmission electron microscopy (H600; Hitachi) and scanning electron microscopy (200; FEI Quanta). Gliding motility and flexirubin pigments were investigated according to the methods

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HN-171^T is DQ423479.

specified in the minimal standards for describing new taxa of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). The Gram reaction was determined using cells grown on MA at 30 °C for 24 h, according to the method described by Gerhardt *et al.* (1994). Endospore formation was determined after malachite-green staining (Dong & Cai, 2001) of cells grown on MA. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (pH 4.4–9.0, in increments of 0.2 pH units) with HCl or NaOH prior to sterilization. Growth in the presence of various NaCl concentrations was investigated in MB: the final concentrations (i.e. including the original concentration in MB) are given below. Growth at various temperatures (4–45 °C, in increments of 3 °C) was measured on MA. Anaerobic growth was determined in MB in an anaerobic test tube filled with nitrogen gas. Catalase activity was assessed from the formation of bubbles after a 3% H₂O₂ solution was dropped onto a fresh colony. Oxidase activity and the hydrolysis of casein, starch, gelatin and Tweens 20, 40, 60 or 80 were determined as described by Dong & Cai (2001) with artificial seawater. The ability of the three isolates to utilize a battery of 95 different carbon sources was tested using GN MicroPlates (Biolog) inoculated with cells suspended in sterile artificial seawater. The artificial seawater contained the following (l⁻¹ distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). Cells for the analysis of cellular fatty acids were grown on MA for 2 days at 30 °C, and the cellular fatty acid composition was determined as described previously (Hu *et al.*, 2004, and references therein). Biomass for the analysis of isoprenoid quinones was grown in MB; the quinones were determined according to Collins (1985) and Wu *et al.* (1989). DNA G+C contents were determined by thermal denaturation (Marmur & Doty, 1962) using the DNA of *Escherichia coli* DH5 α as a standard for calibration of the *T_m* value. The 16S rRNA gene was amplified and sequenced as described previously (Zhang *et al.*, 2003) and sequences were aligned using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) with the Kimura two-parameter model in TREECON W, version 1.3b (Van de Peer & De Wachter, 1994) and the maximum-parsimony method (Fitch, 1971).

The morphological, physiological and biochemical characteristics of strain HN-171^T are given in the species description. The differentiating features for the three isolates and phylogenetically related genera are listed in Table 1. The almost-complete 16S rRNA gene sequence (1478 nt) of strain HN-171^T was determined. Phylogenetic trees based on 16S rRNA gene sequences showed that strains HN-171^T, HN-172 and HN-182 form a distinct phylogenetic lineage within the family *Flavobacteriaceae* (Fig. 1); strain HN-171^T exhibits the highest similarity to *Coenonia anatina* LMG 14382^T (90.2%).

The isoprenoid quinone of strains HN-171^T, HN-172 and HN-181 was MK-6. All three strains contained branched,

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences (1394 bp), showing the phylogenetic position of strain HN-171^T and representatives of some related taxa. Bootstrap values (expressed as percentages of 1000 replications) > 50% are shown at branching points. *Bacteroides fragilis* ATCC 25285^T was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.1 substitutions per nucleotide position.

hydroxyl, straight-chain and unsaturated fatty acids. The fatty acid profile of strain HN-171^T (> 1% of total fatty acids) comprised iso-C_{15:1} G (24.2%), iso-C_{15:0} (14.9%), summed feature 4 (iso-C_{15:0} 2-OH and/or C_{16:1} ω7clt) (10.7%), C_{15:0} (9.4%), C_{16:0} (4.9%), iso-C_{15:0} 3-OH (3.9%), C_{14:0} (2.5%), C_{15:0} 2-OH (1.4%), iso-C_{13:0} 3-OH (1.4%) and anteiso-C_{15:0} (1.1%). The DNA G+C content of strain HN-171^T was 34.5 mol% (*T_m*).

The three isolates differed from their close phylogenetic neighbours in the family *Flavobacteriaceae* in terms of a number of phenotypic and chemotaxonomic properties (Table 1). In particular, the novel isolates contained the characteristic fatty acid summed feature 4. On the basis of the phenotypic and phylogenetic data, we propose the creation of a novel genus and species in the family *Flavobacteriaceae*, *Zhouia amylolytica* gen. nov., sp. nov., to accommodate the three strains HN-171^T, HN-172 and HN-181.

Description of *Zhouia* gen. nov.

Zhouia (Zhou'i.a. N.L. fem. n. *Zhouia* named after Professor Pei-Jin Zhou, a pioneer of environmental microbiology in China).

Cells are Gram-negative, non-motile, non-spore-forming rods. Strictly aerobic. NaCl is required for growth. Positive for oxidase and catalase. The menaquinone is MK-6. The

Table 1. Differential characteristics of strains HN-171^T, HN-172 and HN-181 and related genera

Taxa: 1, strains HN-171^T, HN-172 and HN-181; 2, *Coenonia* (Vandamme *et al.*, 1999); 3, *Gaetbulimicrobium* (Yoon *et al.*, 2006a); 4, *Cellulophaga* (Johansen *et al.*, 1999; Bowman, 2000; Nedashkovskaya *et al.*, 2004b); 5, *Stanierella* (Reichenbach, 1989; Nedashkovskaya *et al.*, 2005b); 6, *Winogradskyella* (Nedashkovskaya *et al.*, 2005a; Lau *et al.*, 2005); 7, *Psychroflexus* (Bowman *et al.*, 1998; Donachie *et al.*, 2004). All are positive for alkaline phosphatase (not determined for *Stanierella*) and catalase activities. All are negative for urease activity and flexirubin pigments. Symbols: +, positive; –, negative; v, variable; w, weakly positive; ND, not determined.

Characteristic	1	2	3	4	5	6	7
Gliding motility	–	–	+	+	–	+	v
Pigmentation	Yellow/pale yellow	Whitish	Orange	Yellow to orange	Orange to red	Yellow	Orange
Oxidase	–	–	–	–	–	–	v
NaCl requirement	+	–	+	+	+	+	+
Growth with >8% NaCl	+	ND	–	v	–	v	v
Optimal NaCl concentration (%)	4.5–5.0	ND	2–3	v	2	v	2–10
Growth at:							
–2 °C	–	ND	ND	v	–	–	–
42 °C	+	+	–	–	–	v	v
Optimal temperature (°C)	30	37	37	15–25	25	21–25	10–25
Hydrolysis of:							
Agar	–	–	–	+	+	v	–
Casein	–	–	+	v	+	v	–
Starch	+	ND	+	+	–	v	+
Gelatin	+	–	+	+	+	+	+
Tween 40	–	+	+	+	+	v	+
Nitrate reduction	–	–	–	v	–	–	v
Acid production from:							
D-Glucose	–	–	–	v	–	–	–
D-Cellobiose	+	–	+	v	–	v	–
Utilization of:							
D-Glucose	+	+	+	+	ND	v	+
D-Mannose	+ /w	+	–	v	ND	v	+
Sucrose	+	+	–	v	ND	v	+
Major fatty acids (>10% of total)	iso-C _{15:1} ; iso-C _{15:0} ; summed feature 4*	iso-C _{15:0} ; iso-C _{13:0}	iso-C _{17:0} 3-OH; iso-C _{15:0}	C _{16:1} ω7c; C _{15:0} ; iso-C _{15:1}	iso-C _{17:0} 3-OH; iso-C _{15:0} ; iso-C _{17:1} ω7c	iso-C _{15:0} ; anteiso-C _{15:0} ; iso-C _{15:1} ; iso-C _{16:0} 3-OH; iso-C _{17:0} 3-OH	anteiso-C _{15:1} ω10c; iso-C _{16:0} ; iso-C _{16:0} 3-OH; anteiso-C _{17:0} 3-OH; anteiso-C _{15:0} ; iso-C _{15:0} ; iso-C _{15:1} ω10c; iso-C _{17:0} 3-OH
DNA G + C content (mol%)	34.5	35–36	36	32–38	34	34–36	32–36

*Summed feature 4 contains iso-C_{15:0} 2-OH and/or C_{16:1}ω7c/t.

major fatty acids are iso-C_{15:1}, iso-C_{15:0} and summed feature 4. The type species is *Zhouia amylolytica*.

Description of *Zhouia amylolytica* sp. nov.

Zhouia amylolytica (a.my.lo.ly'ti.ca. Gr. n. *amylos* starch; N.L. adj. *lyticus* from Gr. adj. *lutikos* dissolving; N.L. fem. adj. *amylolytica* dissolving starch, pertaining to the ability of the bacterium to hydrolyse starch).

Exhibits the following properties in addition to those given in the genus description and in Table 1. Cells are 0.25–0.3 × 1.3–3.0 µm in size; devoid of flagellar and gliding motility. Colonies on MA are circular, slightly raised, smooth, yellow to pale-yellow in colour and 2.0–3.0 mm in diameter after incubation for 2 days at 30 °C. Growth occurs at 7–42 °C, with an optimum at 30 °C; growth does not occur at 4 or 45 °C. Growth is observed at pH 6.0–8.0, with an optimum at pH 7.2–7.4; growth does

not occur at pH 5.8 or 8.2. Optimal growth occurs in the presence of 4.5–5% (w/v) NaCl; growth does not occur in the absence of NaCl or in the presence of >9% (w/v) NaCl. Nitrate is not reduced. Starch and gelatin are hydrolysed, but agar, casein and Tweens are not hydrolysed. Dextrin, α -cyclodextrin, glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α -D-lactose, maltose, D-melibiose, methyl β -D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, D-trehalose, turanose, succinic acid monomethyl ester, acetic acid, D-galactonolactone, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, DL-lactic acid, propionic acid, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-ornithine, L-proline, L-threonine, urocanic acid, inosine, uridine, D-glucose 1-phosphate and D-glucose 6-phosphate are utilized. Tweens 40 and 80, adonitol, L-arabinose, D-arabitol, i-erythritol, *myo*-inositol, D-mannitol, xylitol, methyl pyruvate, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α -hydroxybutyric acid, β -hydroxybutyric acid, γ -hydroxybutyric acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-alaninamide, D-alanine, L-histidine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, DL-carnitine, γ -aminobutyric acid, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol and DL- α -glycerol phosphate are not utilized. The major fatty acids are iso-C_{15:1} G (24.2%), iso-C_{15:0} (14.9%), summed feature 4 (10.7%) and C_{15:0} (9.4%). The DNA G+C content of the type strain is 34.5 mol%.

The type strain, HN-171^T (=CGMCC 1.6114^T=JCM 14016^T), was isolated from a sediment sample from the South China Sea, China.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30230010). Our thanks go to Professor W.-Y. Zhuang at the Institute of Microbiology, Chinese Academy of Sciences, for her help with nomenclature.

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