lamia majanohamensis gen. nov., sp. nov., an actinobacterium isolated from sea cucumber *Holothuria edulis*, and proposal of *lamiaceae* fam. nov.

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A novel, Gram-positive bacterial strain, F12^T, was isolated from the abdominal epidermis of a sea cucumber, *Holothuria edulis*, collected from seawater off the coast of Japan. According to 16S rRNA gene sequence analysis, this strain represents a novel, deep-rooting lineage within the class *Actinobacteria* and clusters with uncultured bacteria and *Acidimicrobium ferrooxidans*.

Compared to species with validly published names, the highest 16S rRNA gene sequence similarity (89.8%) was to *Acidimicrobium ferrooxidans* DSM 10331^T. Phylogenetic analyses showed that strain F12^T represents a distinct phylogenetic lineage related closely to the genus *Acidimicrobium*. Strain F12^T contained MK-9(H₈) as the major menaquinone, whilst 17:0, 17:1 ω 8c, 15:0 and 16:0 were the major cellular fatty acids. The cell-wall peptidoglycan of strain F12^T was composed of meso-diaminopimelic acid as the diagnostic diamino acid, alanine and glutamic acid (1:2:1). The cell-wall sugars detected were rhamnose, mannose, arabinose, galactose and xylose. The G+C content of the DNA was 74.4 mol%. From the taxonomic data obtained in this study, the name *lamia majanohamensis* gen. nov., sp. nov. is proposed for the isolate, with type strain F12^T (=NBRC 102561^T=DSM 19957^T). The name *lamiaceae* fam. nov. is also proposed for the distinct phyletic line represented by the genus *lamia*.

We have turned our attention to the intestine and epidermis of marine creatures as a source of novel lineages of bacteria. In our previous studies, we have mainly used marine agar 2216 (MA; Becton Dickinson) as the isolation and cultivation medium. As a result, we isolated several novel-lineage bacteria: *Agarivorans albus* (Kurahashi & Yokota, 2004), *Pseudovibrio ascidiaceicola* (Fukunaga *et al.*, 2006), *Endozoicomonas elysicola* (Kurahashi & Yokota, 2007a) and *Tateyamaria omphalii* (Kurahashi & Yokota, 2007b). In this study we used SN medium, which is commonly used for algal cultivation, and a novel bacterial strain (F12^T), forming a deep branch with *Acidimicrobium ferrooxidans* in the order *Acidimicrobiales*, was isolated.

Acidimicrobium strains are Gram-positive, rod-shaped bacteria that are found in warm, acidic, sulphur- or mineral sulphide-rich environments. *Acidimicrobium ferrooxidans* was originally described by Clark & Norris (1996) and was subsequently placed in the subclass *Acidimicrobidae* (Stackebrandt *et al.*, 1997), which contains only the order *Acidimicrobiales*, family *Acidimicrobiaceae*, genus *Acidimicrobium* and one species, *Acidimicrobium ferrooxidans*. Interestingly, Rheims *et al.* (1999) carried out molecular environmental studies and discovered the existence of several novel 16S rRNA gene sequence clusters with probable worldwide distribution in different terrestrial environments, forming several lineages deeply rooted to the order *Acidimicrobiales*. However, no novel species belonging to these clusters have been reported.

The strain used in this study was obtained from the abdominal epidermis of a sea cucumber, *Holothuria edulis*, which was collected off the coast of Aka Island, Okinawa prefecture, Japan, at a depth of 6 m, as described previously (Kurahashi & Yokota, 2004). The medium used for isolation of strain F12^T was SN medium, consisting of

Abbreviations: DAP, diaminopimelic acid; ML, maximum likelihood; NJ, neighbour joining; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain F12^T is AB360448.

A supplementary figure showing the general morphology of cells of strain F12^T is available with the online version of this paper.

750 p.p.m. NaNO₃, 15.9 p.p.m. K₂HPO₄, 5.6 p.p.m. disodium EDTA dihydrate, 10.4 p.p.m. Na₂CO₃, 1.0 p.p.m. vitamin B₁₂ and 1.0 p.p.m. Cyano trace metal solution [(1 distilled water)⁻¹: 6.25 g citric acid.H₂O, 6.0 g ferric ammonium citrate, 1.4 g MnCl₂.4H₂O, 0.39 g Na₂MoO₄.2H₂O, 0.025 g Co(NO₃)₂.6H₂O and 0.222 g ZnSO₄.7H₂O] in filtered 75 % seawater. The strain cultures were maintained routinely on MA at 25 °C.

Phenotypic and chemotaxonomic characteristics were determined by using MA at 30 °C unless otherwise indicated. Colony morphology, size and colour were examined by using cultures grown aerobically after 3 weeks incubation. Salt tolerance was tested in R2AM medium [(1 distilled water)⁻¹: 18.2 g R2A agar, 10.0 g MgCl₂.6H₂O, 6.0 g MgSO₄.7H₂O, 2.0 g CaCl₂.2H₂O and 0.6 g KCl] supplemented with 0–12 % (w/v) NaCl after 3 weeks incubation at 30 °C. Gram reaction was determined by using a Gram Stain kit (Becton Dickinson), following the manufacturer's instructions. Cell motility and morphology were studied by using phase-contrast microscopy and transmission electron microscopy (TEM), respectively. Specimens for TEM were negatively stained with phosphotungstic acid. Catalase activity was determined by dropping 3 % H₂O₂ onto colonies, and oxidase-detection strips (bioMérieux) were used for the oxidase test. Biochemical analyses were performed by inoculating API 20E strips (bioMérieux), a microbial identification product. Instructions of the manufacturer were followed, except that inocula for API 20E were prepared in artificial seawater. Results are given in the species description below. Respiratory quinones were extracted with chloroform:methanol (2:1, v/v), fractionated on a spin column and determined by LC-MS using an LCMS-QP8000 α (Shimadzu). Chemotaxonomic analyses of the G+C content of the DNA and whole-cell fatty acids were performed as described previously (Kurahashi & Yokota, 2004). The whole-cell fatty acid methyl ester profile was determined by using the MIDI Sherlock Microbial Identification system (Microbial ID). Note, however, that *Acidimicrobium ferrooxidans* DSM 10331^T was cultured on *Acidimicrobium* medium [(1 distilled water)⁻¹: 0.5 g MgSO₄, 0.4 g (NH₄)₂SO₄, 0.2 g K₂HPO₄ and 0.1 g KCl, adjusted to pH 2.0 with H₂SO₄] at 45 °C. Fatty acids unidentified by MIDI were analysed by using GC-MS (Agilent). After cells were cultured for 3 weeks at 30 °C on MA, cell walls were isolated and purified by using the method of Schleifer & Kandler (1972). The amino acid and sugar compositions of cell-wall hydrolysates (Becker *et al.*, 1965) were analysed by TLC (Hasegawa *et al.*, 1983) and HPLC as described by Takeuchi *et al.* (1999). The *N*-acyl type of muramic acid was determined by the colorimetric method of Uchida & Aida (1977).

Sequencing of the 16S rRNA gene was carried out as described previously (Kurahashi & Yokota, 2004). 16S rRNA gene sequence similarity was investigated by using the FASTA program (Pearson, 1994). Alignments of 16S rRNA gene sequences of the isolate and related strains were

carried out with CLUSTAL W software (version 1.8) (Thompson *et al.*, 1994). The evolutionary-distance matrix was calculated by using the Kimura two-parameter method (Kimura, 1980). Phylogenetic trees were constructed by using two different methods, the neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-likelihood (ML; Felsenstein, 1981) algorithms. The topology of the phylogenetic tree was evaluated by bootstrap analysis with 1000 sample replications according to the method of Felsenstein (1993).

Cells of strain F12^T possessed a Gram-positive cell wall and were non-spore-forming and non-motile. Cells were rod-shaped and had lengths ranging from 1.2 to 1.7 μ m and widths from 0.3 to 0.5 μ m. Colonies on MA after 3 weeks at 30 °C were circular, convex, white in colour, nearly opaque and usually 0.2–0.3 mm in diameter. Growth was observed at 15–40 °C, with an optimum between 28 and 30 °C. Growth occurred at pH 6–9, with an optimum at pH 7. Electron microscopic investigations suggested that the isolate showed transverse and binary fission in cells, as a result of cell division (see Supplementary Fig. S1, available in IJSEM Online).

Strain F12^T contained MK-9(H₆) as the predominant menaquinone (78.3 %) and MK-9(H₄), MK-9(H₈), MK-9(H₂) and MK-9(H₀) as the minor menaquinones (5.6, 2.4, 1.0 and 0.6 %, respectively). The DNA G + C content of F12^T was found to be 74.4 mol%, higher than that of *Acidimicrobium ferrooxidans* (67.0–68.5 mol%). The cellular fatty acid composition of strain F12^T was dominated by 17:0 (18.4 %), 17:1 ω 8c (13.1 %), 15:0 (9.9 %) and 16:0 (9.7 %), a profile different from that of the closely related genus *Acidimicrobium* (Table 1). The cell wall of strain F12^T consisted of glutamic acid:alanine:meso-diaminopimelic acid (DAP) in a ratio of 1:2:1; this corresponds to peptidoglycan type A1 γ according to Schleifer & Kandler (1972). The cell-wall muramic acid was of the glycolyl type. The cell-wall sugars of strain F12^T were rhamnose, mannose, arabinose, galactose and xylose.

An almost-complete 16S rRNA gene sequence for strain F12^T was determined and the FAST search in GenBank showed that the strain belongs to the class *Actinobacteria*. All species with validly published names exhibited <90 % sequence similarity to the newly determined sequence. Strain F12^T displayed closest sequence similarity to *Acidimicrobium ferrooxidans* DSM 10331^T (GenBank accession no. U75647, 89.8 % similarity). Isolate F12^T is equally closely related to '*Candidatus Microthrix parvicella*' EU19 (GenBank accession no. DQ147285, 89.8 % similarity; Blackall *et al.*, 1995). However, '*Candidatus Microthrix parvicella*' could not be stored successfully and physiological tests failed, due to its poor growth on artificial media. Phylogenetic trees obtained by using the NJ (Fig. 1) and ML (data not shown) methods revealed clear affiliations of the novel isolate with the uncultured isolates 101-26 (GenBank accession no. EF157223, 96.3 % similar-

Table 1. Cellular fatty acid compositions (%) of strain F12^T and *Acidimicrobium ferrooxidans* DSM 10331^T

Strains: 1, F12^T; 2, *Acidimicrobium ferrooxidans* DSM 10331^T (data from present study). —, <0.1 % or not detected.

Fatty acid	1	2
Saturated straight-chain		
14:0	1.6	0.2
15:0	9.9	—
16:0	9.7	1.0
17:0	18.4	—
18:0	8.2	—
19:0	0.7	—
Unsaturated straight-chain		
16:1 ω 7c	2.3	—
16:1 ω 5c	8.5	—
17:1 ω 9c	0.4	—
17:1 ω 8c	13.1	—
17:1 ω 6c	1.8	—
18:1 ω 9c	4.3	—
18:1 ω 7c	3.2	—
18:1 ω 5c	0.6	—
18:3 ω 6c (6, 9, 12)		0.1
Branched		
iso 14:0	—	0.3
iso 15:0	—	4.1
anteiso 15:0	—	1.3
iso h 16:1	1.7	—
iso 16:0	8.2	83.3
iso 17:0	—	1.3
anteiso 17:0	—	7.8
iso 18:0	1.3	—
cyclo 19:0 ω 8c	1.1	—
10-methyl 17:0	1.4	—
Hydroxy branched		
iso 14:0 3-OH	—	0.4
Unknown		
ECL 14.959*	3.7	—
Summed feature 3†	—	0.1

*ECL, Equivalent chain length.

†Summed feature 3 comprises C16:1 ω 7c and/or iso C15:0 2-OH.

ity; Kim & Crowley, 2007) and AKIW1115 (GenBank accession no. DQ129371, 94.8 % similarity; Brodie *et al.*, 2007) and placed the novel strain on a separate branch within the order *Acidimicrobiales*.

Characteristics that differentiate strain F12^T from members of phylogenetically related genera are shown in Table 2. Strain F12^T differs from members of the genera *Frankia* and *Geodermatophilus* in several characteristics. Strain F12^T contains *meso*-DAP in the cell-wall peptidoglycan and MK-9(H₆) as the predominant menaquinone in the cell membrane, whereas *Acidimicrobium ferrooxidans* possesses MK-9(H₈) and members of the genus *Sporichthya* possess LL-DAP. There is >10 % difference in the DNA G+C

content of strain F12^T and those of members of the genus *Acidothermus*. The results of the treeing analyses showed that isolate F12^T formed a distant monophyletic clade with cloned sequences within the order *Acidimicrobiales*, as indicated by the 100 % bootstrap value, and this clade was not closely associated with any other taxon.

On the basis of the phenotypic and genotypic characteristics described above, strain F12^T is proposed as the type strain of *Iamia majanohamensis* gen. nov., sp. nov. We also propose *Iamiaceae* fam. nov., based on the distinct phylogenetic position of *Iamia majanohamensis* gen. nov., sp. nov. within the order *Acidimicrobiales*.

Description of *Iamia* gen. nov.

Iamia (I.a'mi.a. N.L. fem. n. *Iamia* arbitrary name formed from the acronym of the Institute of Applied Microbiology at the University of Tokyo, which has made significant contributions to microbiology).

Cells are Gram-positive, non-endospore-forming rods that are non-motile. Colonies grow aerobically, but not anaerobically. In media devoid of sodium chloride, growth is observed. Oxidase- and catalase-positive. The predominant menaquinone is MK-9(H₆). Cell wall contains glutamic acid, alanine and *meso*-DAP. The peptidoglycan type is presumed to be A1 γ . The peptidoglycan is of the glycolyl type. The major cellular fatty acids are 17:0, 17:1 ω 8c, 15:0 and 16:0. The type species is *Iamia majanohamensis*.

Description of *Iamia majanohamensis* sp. nov.

Iamia majanohamensis (ma.ja.no.ha.men'sis. N.L. fem. adj. *majanohamensis* pertaining to Majanohama, the site on the coast of Aka Island, Japan, where the type strain was isolated).

In addition to the properties given in the genus description, cells are 1.2–1.7 $\times$ 0.3–0.5 μ m in size. Colonies on MA are small, white, circular, convex, smooth, shiny and 0.2–0.3 mm in diameter after 3 weeks incubation at 30 °C. Optimal growth temperature is 28–30 °C. No growth occurs at temperatures above 45 °C or below 10 °C. Hydrolysis of gelatin is observed. Cell-wall sugars are rhamnose, mannose, arabinose, galactose and xylose. Positive for catalase, oxidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, production of acetoin and gelatinase and reduction of nitrate to N₂, but negative for β -galactosidase, urease, tryptophan deaminase, utilization of citrate, production of H₂S and indole and assimilation of amygdalin, arabinose, D-glucose, inositol, D-mannose, melibiose, rhamnose, sucrose and sorbitol. The DNA G+C content is 74.4 mol%.

The type strain, F12^T (=NBRC 102561^T=DSM 19957^T), was isolated from the ventral epidermis of the sea cucumber *Holothuria edulis* at Aka Island, Okinawa, Japan.

Fig. 1. NJ tree showing the phylogenetic positions of strain F12^T and related organisms, inferred from 16S rRNA gene sequence analyses. Bootstrap values >70% (percentage of 1000 bootstrap resamplings) are indicated. ● indicates recovered nodes with >70% bootstrap value in an ML tree. *Flavobacterium aquatile* ATCC 11947^T (M62797) was used as an outgroup to define the root of the tree. Bar, 0.02 substitutions per nucleotide position.

Description of *Iamiaceae* fam. nov.

Iamiaceae (I.a.mi.a'ce.ae. N.L. fem. n. *Iamia* type genus of the family; -aceae ending to denote a family; N.L. fem. n. *Iamiaceae* the family of *Iamia*).

Segregation of these organisms into a novel family is justified by their distinct phyletic lineage based on the 16S rRNA gene. The pattern of 16S rRNA gene sequence signature nucleotides of members of the family consists of 408–434 (G–C), 722–733 (G–G), 1118–1155 (U–A), 443–

Table 2. Differential characteristics of strain F12^T and members of related genera

Taxa: 1, strain F12^T (data from this study); 2, *Acidimicrobium* (Clark & Norris, 1996; Normand, 2006); 3, *Frankia* (Normand *et al.*, 1996); 4, *Geodermatophilus* (Normand *et al.*, 1996); 5, *Sporichthya* (Rainey *et al.*, 1993; Tamura *et al.*, 1999); 6, *Acidothermus* (Mohagheghi *et al.*, 1986). ND, No data available; –, negative; +, positive.

Characteristic	1	2	3	4	5	6
Hyphae	–	–*	+	+	+ / –	–
Motility	–	–*	–	+ / –	+ / –	–
Flagellum	–	–*	–	Polar	Polar	–
Growth at pH 2	–	+	–	ND	ND	–
Growth temperature (°C)						
Optimum	25–30	45–50	25–30	24–28	20–30	50–60
Range	15–40	25–50*	10–37	10–37	15–37	37–70
Oxidase	+	+	ND	ND	ND	–
Catalase	+	–*	+	+	ND	+
Nitrate reduction	+	ND	–	+ / –	+	–
Cell-wall type	meso-DAP	meso-DAP	meso-DAP	meso-DAP	LL-DAP	meso-DAP
Major menaquinone	MK-9(H ₆), MK-9(H ₄), MK-9(H ₈)	MK-9(H ₈)*	MK-9(H ₄)	MK-9(H ₄)	MK-9(H ₆), MK-9(H ₈)	ND
DNA G + C content (mol%)	74.4	67.0–68.5	66–71	73–75	70–71	61
Habitat	Sea cucumber epidermis	Warm, acidic, iron-, sulphur- or mineral sulphide-rich environments	Soil, plant roots	Desert soil, sea	Soil	Hot spring

*This study.

491 (U–A), 1165–1171 (G–C) and 1263–1272 (A–U). The type genus is *Iamia*.

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