Ferrimonas senticii sp. nov., a novel gammaproteobacterium isolated from the mucus of a puffer fish caught in Kaneohe Bay, Hawai'i

Sonia Campbell, Renee M. Harada and Qing X. Li

Correspondence

Qing X. Li
qingl@hawaii.edu

Department of Molecular Biosciences and Bioengineering, University of Hawai'i at Manoa, 1955 East West Road, Honolulu, HI 96822, USA

A novel species, strain P2S11^T, was isolated from the mucus of a puffer fish caught off the coast of Kaneohe Bay, O'ahu, Hawai'i. A phylogenetic analysis based on 16S rRNA gene sequences showed that the novel strain was most closely related to *Ferrimonas marina* DSM 16917^T and *Ferrimonas balearica* DSM 9799^T with 93.5% and 82.9% sequence similarities, respectively, which established the novel strain as belonging to the genus *Ferrimonas*. The strain formed off-white coloured colonies on marine agar and cells were Gram-negative, non-motile rods. H₂S was produced when strain P2S11^T was grown on TSI medium with added salt. Strain P2S11^T had a DNA G+C content of 54.9 mol% and the dominant fatty acids were C_{16:1}ω9c, C_{16:0} and C_{17:1}ω8c. On the basis of this polyphasic study, strain P2S11^T (=ATCC BAA-1480^T=DSM 18821^T) represents a novel species of the genus *Ferrimonas*, for which the name *Ferrimonas senticii* sp. nov. is proposed.

The puffer fish *Arothron hispidus* is commonly found in the warm tropical waters around the Hawaiian islands. Bacterial strain P2S11^T was isolated from the mucus of a specimen of *A. hispidus* that was caught off the windward side of the island of O'ahu in August 2005. Strain P2S11^T was isolated on half-strength marine agar 2216 (1/2 MA; Difco). The off-white colonies were further purified and maintained in 10% glycerol:90% half-strength marine broth at −80 °C.

The genus *Ferrimonas* was established in 1995 with the description of the type species *Ferrimonas balearica* (Rosselló-Mora *et al.*, 1995). To date, three other species, *Ferrimonas marina* (Katsuta *et al.*, 2005), *Ferrimonas futtsuensis* and *Ferrimonas kyonanensis* (Nakagawa *et al.*, 2006), have been isolated, characterized and described.

The male puffer fish weighed 306 g. It was dissected into separate organs and body parts. Subsamples of each organ, as well as 5–10 g samples of the mucus, were homogenized with sterile artificial seawater (50–100 ml; Coralife) and serial dilutions were plated onto ORI agar (Simidu & Tsukamoto, 1985) and 1/2 MA (Difco). The media were incubated at 30 °C for 1–5 days. Genomic DNA was isolated from pure cultures using a phenol/chloroform mixture (Marmur, 1961). Amplification of the 16S rRNA gene from genomic DNA was performed by PCR using primers 27F and 1492R (Lane, 1991) and Mastermix *Taq*

DNA polymerase (Eppendorf). Amplification of the gyrase subunit B gene (*gyrB*) was achieved using the UP1Ei and UP2ri primers as described in Katsuta *et al.* (2005). All PCR products were purified using the Ultraclean PCR purification kit (Mo Bio Lab) and sequenced by the Advanced Studies of Genomics, Proteomics and Bioinformatics Center at the University of Hawai'i at Manoa. For *gyrB* gene sequencing, a different set of primers was used from the amplification primers: UP1s and UP2rs were used as described in Katsuta *et al.* (2005). Sequences were edited manually and assembled using Seqman II (Lasergene) and subjected to a BLAST search for comparison with sequences in the public domain (Altschul *et al.*, 1997). Sequence sizes were 1405 and 1063 bp for the 16S rRNA and *gyrB* genes, respectively.

The phylogenetic relationship of strain P2S11^T to other species of the genus *Ferrimonas* was determined based on comparative 16S rRNA and *gyrB* gene sequence analyses. These analyses were performed with programs in the PHYLIP 3.63 package (Felsenstein, 2004). Pairwise similarities were calculated using CLUSTAL W multiple sequence alignment software (Chenna *et al.*, 2003). Evolutionary distances were calculated by the maximum-likelihood method with DNADIST. Bootstrap analyses were based on 100 replicates using SEQBOOT, DNADIST and CONSENSE. Phylogenetic trees were constructed with jumbled orders of sequences and the neighbour-joining method (Saitou & Nei, 1987).

Fig. 1 shows the phylogenetic trees based on the 16S rRNA (Fig. 1a) and *gyrB* (Fig. 1b) gene sequences. The 16S rRNA gene sequence of strain P2S11^T was most closely related to

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA and *gyrB* gene sequences of strain P2S11^T are DQ778094 and EF015634, respectively.

Fig. 1. Neighbour-joining trees based on the 16S rRNA (a) and *gyrB* (b) gene sequences showing the relationships between strain P2S11^T and members of the genus *Ferrimonas*. Numbers at the nodes are bootstrap percentages based on the maximum-likelihood analysis of 100 resampled datasets. *Escherichia coli* strains ATCC 11775^T and ATCC 25922 were used as the outgroups for the 16S rRNA and *gyrB* gene trees, respectively. Bars, 0.01 nucleotide substitutions per site (a), 0.02 nucleotide substitutions per site (b).

that of *F. marina* DSM 16917^T (Katsuta *et al.*, 2005) with 93.5% similarity. For the *gyrB* gene sequence, strain P2S11^T was most closely related to *F. balearica* DSM 9799^T with 82.9% similarity (Rosselló-Mora *et al.*, 1995). Based on these low sequence similarities (<97%), strain P2S11^T should be considered distinct from other species of the genus *Ferrimonas* (Stackebrandt & Goebel, 1994).

The DNA G+C content of strain P2S11^T was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) using a HPLC method (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984). The DNA G+C content of strain P2S11^T was 55 mol%, a value lower than that of all other species of the genus *Ferrimonas*, for which the DNA G+C content varies from 57 to 60 mol%. Fatty acid methyl ester analysis for strain P2S11^T was performed by MIDI Laboratories using whole cells grown on MA for 24 h at 28 °C (Sasser, 1997). The fatty acid content of strain P2S11^T and that of other species of the genus *Ferrimonas* that are available are summarized in Table 1. The major fatty acids for *F. balearica* DSM 9799^T and *F. marina* DSM 16917^T were C_{18:1}ω9c (17.6 and 16.3%, respectively), C_{16:0} (13.4 and 13.9%, respectively) and C_{15:0} iso (9.8 and 11.5%, respectively). The major fatty acids for *F. futtsuensis* FUT3661^T and *F. kyonanensis* Asr22-7^T were C_{16:1}ω9c (20.4 and 28.5%, respectively), C_{16:0} (19.4 and 15.6%, respectively) and C_{18:1}ω9c (15.9 and 10.9%, respectively). For strain P2S11^T, the main three fatty acids were C_{16:1}ω9c (16.9%), C_{16:0} (13.2%) and C_{17:1}ω8c (10.6%). Fatty acid C_{16:1}ω9c was not detected in the analysis of *F. balearica* and *F. marina*.

Quinone analysis was performed using fresh whole cells of strain P2S11^T grown in marine broth for 2–3 days. The cells were extracted using a mixture of chloroform/methanol (2:1, v/v; Hiraishi, 1988). After filtration and dilution in acetone, the extract was analysed by LC-MS using a HPLC (1100 series; Agilent) coupled to a diode array detector and a single quadrupole mass spectrometer (model SL; Agilent). The HPLC column used was a Vydac C18 Mass Spec column. The most abundant quinone found in *F. balearica* and *F. marina* was menaquinone MK7 (Rosselló-Mora *et al.*, 1995; Katsuta *et al.*, 2005), similar to *F. futtsuensis* and *F. kyonanensis* (Nakagawa *et al.*, 2006). MK7 was also the most abundant menaquinone in strain P2S11^T.

Cells of strain P2S11^T were Gram-negative rods and non-motile. Motility was checked by the hanging drop method using light microscopy under a 100× objective with oil immersion and by stab inoculation into modified motility test agar (1⁻¹: 10 g tryptone, 20 g NaCl and 5 g agar). After two days growth on MA at 30 °C, cells were around 1.7 μm in diameter. Salt was required for growth and the range of salt tolerance was 1.5–7.0% (w/v), with an optimum value of 2.0%. The cells grew within a temperature range of 20 °C (slow growth, 2–3 days) to 37 °C. The temperatures tested were 5, 20, 25, 30, 37 and 42 °C on MA plates over 1 week. Other strains of the genus *Ferrimonas* are facultative anaerobes capable of Fe(III) reduction. Strain P2S11^T grew anaerobically on MA (in a Gas-Pak Pouch; BD). The iron-reducing capability of strain P2S11^T was tested anaerobically with lactate and iron (III) citrate (Lovley & Phillips, 1986), but the novel strain did not grow under these conditions.

Table 1. Fatty acid content (%) of species of the genus *Ferrimonas*

Taxa: 1, strain P2S11^T; 2, *F. marina* DSM 16917^T (Katsuta *et al.*, 2005); 3, *F. balearica* DSM 9799^T (Katsuta *et al.*, 2005); 4, *F. futtsuensis* FUT3661^T (Nakagawa *et al.*, 2006); 5, *F. kyonanensis* Asr22-7^T (Nakagawa *et al.*, 2006). Taxon 1 was grown in marine broth for 24 h at 28 °C, taxa 2 and 3 were grown in marine broth for 24 h at 25 °C, and taxa 4 and 5 were grown in marine broth at 25 °C. Only values greater than 0.99 % are listed. ND, No data.

Fatty acid	1	2	3	4	5
C _{12:0}	2.7	ND	ND	3.7	5.0
C _{13:0}	1.5	ND	ND	ND	ND
C _{12:0} 3-OH	3.6	ND	ND	6.7	8.1
C _{14:0} anteiso	ND	1.2	ND	ND	ND
C _{14:0} iso	1.3	ND	ND	ND	ND
C _{14:0}	2.6	ND	ND	3.6	5.1
C _{15:0} iso	1.8	11.5	9.8	2.2	1.4
C _{15:1} ω8c	2.1	ND	ND	ND	ND
C _{15:0}	3.2	2.1	1.8	ND	ND
C _{16:0} iso	1.3	2.8	ND	ND	ND
C _{16:1} iso ω9c	ND	7.0	10.4	ND	ND
C _{16:1} iso ω7c	ND	6.3	5.2	ND	ND
C _{16:1} ω9c	16.9	ND	ND	20.4	28.5
C _{16:0}	13.2	13.9	13.4	19.4	15.6
C _{17:0} iso	0.2	4.2	2.1	ND	ND
C _{17:1} ω8c	10.6	7.3	12.6	2.2	1.8
C _{17:1} ω6c	1.1	ND	ND	ND	ND
C _{17:0}	6.0	5.5	8.0	ND	ND
C _{18:1} ω9c	9.8	16.3	17.6	15.9	10.9
C _{18:1} ω7c	3.5	9.4	4.9	4.0	1.9
C _{18:0}	2.0	3.6	3.9	1.9	ND
Summed features*					
1	2.6	ND	ND	ND	ND
2	2.2	ND	ND	ND	3.3
3	7.2	ND	ND	9.8	10.3

*Summed feature 1 contains C_{15:1} iso H and/or C_{13:0} 3-OH; summed feature 2 contains C_{16:1} iso I and/or C_{14:0} 3-OH; summed feature 3 contains C_{16:1}ω7c and/or C_{16:1}ω6c and/or C_{15:0} iso 2-OH.

Physiological and biochemical tests performed are summarized in Table 2. The presence or absence of constitutive enzymes was determined using API ZYM (bioMérieux) and the profile of substrates utilized was obtained with API 20NE (bioMérieux). The oxidation of carbon substrates was determined with Biolog GN microplates (Table 2). Hydrogen sulfide production was tested with modified triple-sugar iron (TSI) agar slants (EMD). Since TSI contains only 0.5 % NaCl, 2 % NaCl (w/v) was added to the medium for optimal growth. The TSI tubes were incubated at 30 °C. After 24 h, a black precipitate indicating the presence of H₂S was observed. No fermentation occurred. In comparison, *F. marina* does not produce H₂S and *F. balearica* does.

Table 2. Characteristics of species of the genus *Ferrimonas*

Taxa: 1, strain P2S11^T; 2, *F. marina* DSM 16917^T (Katsuta *et al.*, 2005); 3, *F. balearica* DSM 9799^T (Katsuta *et al.*, 2005); 4, *F. futtsuensis* FUT3661^T (Nakagawa *et al.*, 2006); 5, *F. kyonanensis* Asr22-7^T (Nakagawa *et al.*, 2006). ND, No data.

Characteristic	1	2	3	4	5
Growth at 42 °C	—	—	+	—	—
Growth in NaCl at (% v/v):					
0.5	—	—	+	—	—
5.5	+	—	+	—	—
H ₂ S production	+	—	+	+	+
Urease	—	—	—	ND	ND
Fe(III) reduction	—	—	+	+	+
Biolog profile:					
Acetate	+	+	+	—	—
L-Alanyl glycine	—	+	—	ND	ND
Dextrin	—	+	—	ND	ND
D-Glucose	—	+	—	—	—
L-Glutamate	—	+	+	—	—
Lactate	+	+	+	+	+
Maltose	—	+	+	—	—
DNA G + C content (mol%)	55	60	60	58	57

On the basis of the distinct 16S rRNA and *gyrB* gene sequences, physiology and fatty acid composition, strain P2S11^T represents a novel species of the genus *Ferrimonas*, for which the name *Ferrimonas senticii* sp. nov. is proposed.

Description of *Ferrimonas senticii* sp. nov.

Ferrimonas senticii (sen.ti.ci.i. N.L. gen. n. *senticii* of Sentic, named in honour of Sinisa Sentic for scientific and philosophical discussions).

Aerobic, facultatively anaerobic. Off-white colonies. Gram-negative rods, cell size around 1.7 µm in length and non-motile. Circular colonies form on MA at 30 °C after 1–2 days. Optimum temperature range is 20–37 °C. NaCl is required for growth in a range of 1.7–7.0 % (w/v), with an optimum at 2.0 %. Strain P2S11^T utilizes DL-lactic acid, acetic acid, Tween 80, inosine, uridine, thymidine and pyruvic acid methyl ester as carbon sources in the Biolog GN2 test. Produces H₂S on TSI + 2 % NaCl agar. The DNA G + C content is 54.9 mol%. The predominant fatty acids are C_{16:1}ω9c, C_{16:0} and C_{17:1}ω8c.

The type strain, P2S11^T (=ATCC BAA-1480^T=DSM 18821^T), was isolated from the slime of a puffer fish, *Arothron hispidus*, caught off the coast of the windward side of the island of O'ahu, Hawai'i.

Acknowledgements

This work was supported in part by a contractual agreement with the State of Hawai'i Department of Health - Office of Hazard Evaluation and Emergency Response. We thank Brian and Myrna Yamane for sample collection.

References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G. & Thompson, J. D. (2003). Multiple sequence alignment with the CLUSTAL series of programs. *Nucleic Acids Res* **31**, 3497–3500.
- Felsenstein, J. (2004). PHYLIP (phylogeny inference package), version 3.63. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.
- Hiraishi, A. (1988). Respiratory quinone profiles as tools for identifying different bacterial populations in activated sludge. *J Gen Appl Microbiol* **34**, 39–56.
- Katsuta, A., Adachi, K., Matsuda, S., Shizuri, Y. & Kasai, H. (2005). *Ferrimonas marina* sp. nov. *Int J Syst Evol Microbiol* **55**, 1851–1855.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Lovley, D. R. & Phillips, E. J. (1986). Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl Environ Microbiol* **51**, 683–689.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nakagawa, T., Iino, T., Suzuki, K. & Harayama, S. (2006). *Ferrimonas futtsuensis* sp. nov. and *Ferrimonas kyonanensis* sp. nov., selenate-reducing bacteria belonging to the *Gammaproteobacteria* isolated from Tokyo Bay. *Int J Syst Evol Microbiol* **56**, 2639–2645.
- Rosselló-Mora, R. A., Ludwig, W., Kämpfer, P., Amann, R. & Schleifer, K.-H. (1995). *Ferrimonas balearica* gen. nov., spec. nov., a new marine facultative Fe(III)-reducing bacterium. *Syst Appl Microbiol* **18**, 196–202.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1997). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Simidu, U. & Tsukamoto, K. (1985). Habitat segregation and biochemical activities of marine members of the family *Vibrionaceae*. *Appl Environ Microbiol* **50**, 781–790.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.