Maritimimonas rapanae gen. nov., sp. nov., isolated from gut microflora of the veined rapa whelk, *Rapana venosa*

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A yellow-pigmented, Gram-negative, aerobic bacterial strain comprising rod-shaped cells devoid of flagellar and gliding motility, designated strain A31T, was isolated from a veined rapa whelk (*Rapana venosa*) collected from the South Sea, Republic of Korea. Results from 16S rRNA gene sequence analysis indicated that the isolate belonged to the family *Flavobacteriaceae*; the highest level of nucleotide sequence similarity (92.6 %) was observed with *Tenacibaculum aestuarii* KCTC 12569T. The predominant cellular fatty acids were iso-C15 : 1G (24.2 %), iso-C15 : 0 (20.1 %) and iso-C17 : 0 3-OH (11.2 %). Flexirubin-type pigments were absent. The major isoprenoid quinone was MK-6. The DNA G+C content was 31.7 mol%. Data from a polyphasic taxonomic study suggested that the isolate represents a novel species in a new genus of the family *Flavobacteriaceae*, for which the name *Maritimimonas rapanae* gen. nov., sp. nov. is proposed. The type strain of *Maritimimonas rapanae* is A31T (KCTC 22186T = JCM 15075T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Reichenbach, 1989) is one of the largest phylogenetic groups within the phylum *Bacteroidetes* (Garrity & Holt, 2001). The family currently comprises more than 45 genera with validly published names, most of them originating from diverse environments including freshwater, brackish and marine waters, soil and epibenthic fauna. Members of the family are known to be proficient in degrading various biopolymers such as cellulose, chitin and pectin (Kirchman, 2002). Recently, phylogenetic analyses have revealed that many marine species in the family clustered together into a well-defined ‘marine clade’ (Bowman & Nichols, 2005). These marine flavobacteria are known to play a role in the utilization of high-molecular-mass dissolved organic matter (Kirchman, 2002). In the course of our study on gut microflora of the veined rapa whelk (*Rapana venosa*), a yellow-pigmented bacterial strain, designated A31T, was isolated and subjected to taxonomic investigation. In this study, the description of a novel *Flavobacterium*-like bacterium that showed relatively low levels of 16S rRNA gene sequence similarity to members of the family *Flavobacteriaceae* with validly published names is reported.

Strain A31T was isolated using the standard dilution plating technique from a veined rapa whelk sample collected from the South Sea (Yeooja Bay; 34° 44′ N 127° 44′ E) off the coast of the Republic of Korea during July 2008. Isolation was achieved using marine agar 2216 (MA; Difco) (Yang *et al.*, 2007) at 25°C for 7 days. The isolate was cultured routinely on MA and maintained at −80°C as a suspension in marine broth 2216 (MB; Difco) containing glycerol (20 %, w/v).

Bacterial DNA preparation, PCR amplification and sequencing of the 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The resultant sequence of strain A31T (1416 nt) was aligned manually against sequences obtained from GenBank. Phylogenetic trees were inferred from the regions available for all sequences (positions 22–1450; *Escherichia coli* numbering system) using the neighbour-joining (Saitou & Nei, 1987) and Fitch–Margoliash (Fitch & Margoliash, 1967) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by means of bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the program jPHYDIT (available at http://plaza.snu.ac.kr/~jchun/jphydit/) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000).
Preliminary sequence comparison with 16S rRNA gene sequences held in GenBank indicated that our isolate showed low levels of similarity to strains of known species of the family Flavobacteriaceae. Strain A31 T was most closely related to members of the genera Tenacibaculum (90.1–92.6 % 16S rRNA gene sequence similarity), Polaribacter (89.8–91.4 %), Lutibacter (90.9 %), Actibacter (91.2 %), Lutimonas (90.3 %) and Aestuariicola (90.2 %). No strains of other species with validly published names exceeded 90.5 % 16S rRNA gene sequence similarity with strain A31 T. To elucidate the phylogenetic relationship between the novel isolate and other species of the family Flavobacteriaceae, phylogenetic trees were constructed by using two different tree-making algorithms. The neighbour-joining tree (Fig. 1) showed that strain A31 T formed a very robust clade with strains of Tenacibaculum, Polaribacter, Lutibacter, Lutimonas, Actibacter and Aestuariicola species, but could not be linked to any of the known genera in the family Flavobacteriaceae. The tree based on the Fitch–Margoliash method showed a similar topology (see Supplementary Fig. S1, available in IJSEM Online). The tree suggested that strain A31 T formed a monophyletic clade with those genera with 100 % bootstrap support. Thus, strain A31 T should be recognized as a member of a new genus.

Cells grown for 7 days at 30 °C on MA were observed with phase-contrast (TMS-F; Nikon) and scanning electron (S-4800; Hitachi) microscopes. Gliding motility was investigated by examining the edge of a hanging drop of fresh MB culture, as recommended by Bernardet et al. (2002). The requirement for sea salts (Sigma) for growth was determined at various concentrations (final concentrations of 0–12 % in increments of 1 %) on nutrient agar (NA; Difco), tryptophan soy agar (TSA; Difco), R2A agar (Difco) and ZoBell’s agar (ZoBell, 1941; 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate and 15 g Bacto agar in 1 l distilled water). All media used in the physiological tests were supplemented with 4 % sea salts unless MA or MB was used. The pH range for growth was determined in ZoBell’s liquid medium. The medium was adjusted to pH 3–11 (in increments of 1 pH unit) by the addition of HCl/NaOH and sterilized by filtration. Growth temperatures (4–50 °C) and growth in an anaerobic chamber (CO₂/H₂/N₂, 10:10:80; Sheldon Manufacturing) were determined on MA after incubation for up to 1 week. The nature of growth on the surface, subsurface and bottom of tubes was determined in MB tubes cultivated for up to 14 days at 30 °C. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovács’ reagent (Kovács, 1956), respectively. Acid production from glucose was tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested in MB containing 0.2 % KNO₃ (Skerman, 1967). H₂S production was determined on Kligler iron agar (Difco). Hydrolysis of CM-cellulose (1 %, w/v), casein (2 % (w/v) skimmed milk), chitin from crab shell (0.5 %, v/v; Sigma), dextran (1 %, w/v), egg yolk (10 %, w/v), elastin (0.5 %, w/v), starch (0.2 %, w/v) and Tween 80 (1 %, w/v) was tested as described by Smibert & Krieg (1994) using MA as the basal medium.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain A31 T and members of the family Flavobacteriaceae. Numbers at nodes are levels of bootstrap support (%) based on neighbour-joining analyses of 1000 resampled datasets; only values greater than 50 % are shown. The sequence of Bacteroides fragilis ATCC 25285T (GenBank accession no. X83935) was used as an outgroup (not shown). Bar, 0.01 substitutions per nucleotide position.
Hydrolysis of L-tyrosine (0.5 %, w/v) and xylan (1 %, w/v) was tested using MA as the basal medium (Barrow & Feltham, 1993). Cellulase activity was tested by observing the disintegration of filter paper in an MB culture (Smibert & Krieg, 1994; Bernardet et al., 2002). DNase activity was determined with DNase test agar (Difco). Other biochemical tests and enzyme activity determinations were performed using the API 20E and API ZYM kits (bioMérieux) and GN2 MicroPlates (Biolog) prepared according to the manufacturers’ instructions except that bacterial strains were suspended in distilled water supplemented with 4 % sea salts. Antibiotic resistance was determined by the disc-diffusion method using commercial antibiotic-impregnated discs (BBL Becton Dickinson); results were interpreted according to the guidelines set forth by the CLSI (2003). The presence of flexirubin-type pigments was examined as described by Bernardet et al. (2002).

Colonies of strain A31T on MA were yellow-pigmented, convex and did not adhere to the agar. Flexirubin-type pigments were not detected. Physiological, morphological and biochemical characteristics are described in Table 1 and in the species description. A number of phenotypic characteristics clearly distinguished strain A31T from members of other genera in the family Flavobacteriaceae. Strain A31T was oxidase-positive, whereas strains of Lutibacter and Lutimonas are negative. Strain A31T was unable to hydrolyse aesculin or starch, unlike most members of related genera. Significant differences between strain A31T and members of other genera were found in the utilization of carbon sources.

### Table 1. Differential phenotypic characteristics of strain A31T and members of related genera of the family Flavobacteriaceae

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<td>V</td>
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*a*, Cocci; *f*, filaments; *o*, ovals; *r*, rods.

§All species except *P. glomeratus* are negative.


**All type strains for which data are available are negative apart from that of *T. skagerrakense*, which is weakly positive. Data not available for *T. gallaecium* or *T. soleae*.

| All type strains for which data are available are negative apart from that of *T. adriaticum*, which is positive. Data not available for *T. aesthes*, *T. aiptasiae*, *T. gallaecium*, *T. lutimaris* or *T. soleae*. |   |   |   |   |   |   |   |
Table 2. Cellular fatty acid compositions (%) of strain A31T and members of related genera in the family Flavobacteriaceae

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*Not detected in the following species: a, T. adriaticum; b, T. aiptasiae; c, T. litopenaei; d, T. litoreum; e, T. maritimum; f, T. skagerrakense; g, T. soleae; h, P. butkevichii; i, P. dokdonensis.

†Detected in the following species: a, T. adriaticum; c, T. litopenaei; d, T. litoreum; e, T. maritimum; g, T. soleae; h, P. butkevichii; i, P. dokdonensis.

‡Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 contained iso-C15 : 1 and/or C13 : 0 3-OH; summed feature 3 contained C16 : 0 7c and/or iso-C15 : 0 2-OH; summed feature 4 contained iso-C17 : 1 I and/or anteiso-C17 : 1 B.
Cells of strain A31T grown on MA for 2 days at 30 °C were prepared (in duplicate) and analysed for fatty acids by GLC according to the instructions of the Microbial Identification System (MIDI, 1999). For G+C content calculations, DNA samples were prepared in triplicate and G+C content was determined by the thermal denaturation method of Marmur & Doty (1962). Isoprenoid quinones were isolated by using the method of Minnikin et al. (1984) and were analysed by HPLC (Varian) as described by Collins (1985).

The fatty acid profiles of strain A31T and related members of the family Flavobacteriaceae are shown in Table 2. The fatty acid profile of strain A31T (>10% of total fatty acids) included the branched fatty acids iso-C15:0 (20.1%), iso-C16:0 (2.5%) and anteiso-C15:0 (1.3%), the hydroxy fatty acids iso-C17:0 3-OH (11.2%), iso-C15:0 3-OH (7.8%), iso-C16:0 3-OH (4.5%) and C16:0 3-OH (2.2%), the straight-chain fatty acids C16:0 (3.8%), C15:0 (3.3%) and C18:0 (3.1%), the unsaturated fatty acid iso-C15:1 G (24.2%) and summed feature 3 (comprising iso-C15:0 2-OH and/or C16:1ω7c 7.5%). Significant differences were found in the levels of iso-C15:1 G, anteiso-C15:0 and summed feature 3 in strain A31T and members of related genera. The DNA G+C content of strain A31T was 31.7 ± 0.4 mol%, a value within the range reported for members of the family Flavobacteriaceae. The predominant isoprenoid quinone of strain A31T was MK-6, and small amounts of MK-7 and MK-8 were also found (MK-6/MK-7/MK-8 ratio 91 : 5 : 4), as observed in all members of the family Flavobacteriaceae.

On the basis of the data from this polyphasic study, strain A31T represents a novel species in a new genus in the family Flavobacteriaceae, for which the name Maritimimonas rapanae gen. nov., sp. nov. is proposed.

Description of Maritimimonas gen. nov.

Maritimimonas (Ma.ri’ti.mi.mo’nas. L. adj. maritimus of the sea, marine; L. fem. n. monas a unit; M. fem. n. Maritimimonas a monad from the sea, pertaining to the habitat of the animal that harboured the type species, a marine rock).

Cells are rod-shaped, non-flagellated, yellow-pigmented and non-gliding. Gram-negative. Aerobic, chemoheterotrophic and mesophilic. Oxidase- and catalase-positive. Spores are not formed. Flexirubin-type pigments are weakly positive and x-arylamidase and trypsin activities are weakly positive and x-glucosidase, lipase (C14), x-galactosidase, β-galactosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, x-fucosidase and x-mannosidase activities are absent. Acid is produced from glucose. In GN2 MicroPlate (Biolog) tests, D-glucose, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl l-glutamic acid, hydroxy-L-proline, L-ornithine, L-serine and L-threonine are utilized. Sensitive to (μg per disc, unless indicated) chloramphenicol (30), erythromycin (15), tetracycline (30) and vancomycin (30); resistant to amikacin (30), ampicillin (10), gentamicin (10), kanamycin (30), nalidixic acid (30), penicillin (10 IU), polymyxin B (300 IU) and streptomycin (10). The predominant cellular fatty acids are iso-C15:1 G, iso-C15:0, iso-C17:0 3-OH, iso-C15:0 3-OH and summed feature 3 (comprising iso-C15:0 2-OH and/or C16:1ω7c 7.5%). The DNA G+C content of the type strain is 31.7 mol%.

The type strain is A31T (=KCTC 22186T =JCM 15075T), isolated from a sample from a veined rapa whelk (Rapana venosa) collected from the South Sea, Republic of Korea.

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References


