Marixanthomonas ophiurae gen. nov., sp. nov., a marine bacterium of the family *Flavobacteriaceae* isolated from a deep-sea brittle star

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An aerobic, Gram-negative, non-motile, yellow-pigmented bacterium, strain KMM 3046^T, was isolated from a deep-sea brittle star from the Fiji Sea and was subjected to a polyphasic taxonomic analysis. Strain KMM 3046^T grew at 5–32 °C and in the presence of 1–12 % (w/v) NaCl. It contained MK-6 as the predominant menaquinone and 3-OH i16 : 0, 3-OH i17 : 0 and 3-OH a17 : 0 as the major fatty acids. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain KMM 3046^T forms a distinct evolutionary lineage within the family *Flavobacteriaceae* (phylum *Bacteroidetes*), displaying 92.3–91.9 % sequence similarity with respect to *Salegentibacter* species. On the basis of the phenotypic and phylogenetic data, strain KMM 3046^T represents a novel genus and species of the family *Flavobacteriaceae*, for which the name *Marixanthomonas ophiurae* gen. nov., sp. nov. is proposed. The type strain of *Marixanthomonas ophiurae* is KMM 3046^T (= NRIC 0684^T = JCM 14121^T).

The family *Flavobacteriaceae*, belonging to the phylum *Bacteroidetes* (the *Cytophaga–Flavobacterium–Bacteroides* group), was proposed by Reichenbach (1992) and subsequently described and emended by Bernardet *et al.* (1996, 2002). The taxonomy of the family *Flavobacteriaceae*, as well as the ecology and biotechnological applications of its members, have been summarized by Bernardet *et al.* (2002) and Bernardet & Nakagawa (2003). The genus *Salegentibacter* (type species, *Salegentibacter salegens*) has been described by McCammon & Bowman (2000) as the result of the reclassification of the marine halotolerant bacterium [*Flavobacterium*] *salegens* described by Dobson *et al.* (1993). Four novel *Salegentibacter* species have been described subsequently: *Salegentibacter holothuriorum* (Nedashkovskaya *et al.*, 2004), *S. mishustinae* (Nedashkovskaya *et al.*, 2005a), *S. agarivorans* (Nedashkovskaya *et al.*, 2006) and *S. flavus* (Ivanova *et al.*, 2006). The genus *Salegentibacter* is one of the marine members of the family *Flavobacteriaceae*, which also includes the genera *Zobellia* (Barbeyron *et al.*, 2001), *Muricauda* (Bruns *et al.*, 2001), *Tenacibaculum* (Suzuki

et al., 2001), *Aequorivita* (Bowman & Nichols, 2002), *Croceibacter* (Cho & Giovannoni, 2003), *Algoriphagus*, *Brumimicrobium*, *Cryomorpha* and *Crocinitomix* (Bowman *et al.*, 2003), *Mesonina* (Nedashkovskaya *et al.*, 2003) and *Leeuwenhoekiella* (Nedashkovskaya *et al.*, 2005b).

During a survey of the biodiversity of micro-organisms associated with marine invertebrates in the Fiji Sea, an aerobic, Gram-negative, yellow-pigmented bacterium, strain KMM 3046^T, was isolated from an unidentified deep-sea brittle-star specimen and was characterized using phenotypic and phylogenetic analyses. 16S rRNA gene sequence analysis demonstrated that the isolate forms a distinct phylogenetic lineage within the family *Flavobacteriaceae*, adjacent to members of the genus *Salegentibacter* (92.3–91.9 % sequence similarities). A number of phenotypic properties were found that serve to discriminate strain KMM 3046^T from *Salegentibacter* species and from other phylogenetically related taxa. On the basis of these data, we concluded that the novel isolate KMM 3046^T represents a novel genus and species.

A brittle star (ophiuroid) specimen (Ophiuroidea, Echinodermata) was collected from the Fiji Sea at a depth of 480 m in December 1991. The specimen was rinsed carefully with sterile seawater and cut aseptically. A small amount of tissue was crushed using a sterile mortar and pestle, and an aliquot of the diluted homogenate was spread

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KMM 3046^T is AB261012.

A maximum-likelihood phylogenetic tree, a scanning electron micrograph and a two-dimensional thin-layer chromatogram for strain KMM 3046^T are available as supplementary material in IJSEM Online.

on seawater-medium agar plates containing the following (l^{-1}): peptone, 5.0 g; yeast extract, 2.5 g; glucose, 1.0 g; K_2HPO_4 , 0.2 g; $MgSO_4$, 0.05 g; and agar, 15.0 g; made up in a seawater/distilled water mixture (750 and 250 ml, respectively). The plates were incubated at 28 °C for 14 days. Each colony was picked up and restreaked on agar before being processed further. Strain KMM 3046^T grew aerobically on seawater-medium agar, marine agar 2216 (MA; Difco) or marine broth 2216 (Difco) at 25–28 °C, and was stored at –80 °C in marine broth 2216 supplemented with 30 % (v/v) glycerol. Flagellar motility was investigated by using the hanging drop method. Gliding motility was investigated using the same method (Bernardet *et al.*, 2002) and as described by Bowman (2000). Cell morphology was examined using scanning electron microscopy of cells grown on MA at 25 °C for 3 days. The cell suspension was diluted and the cells were fixed with 1 % glutaraldehyde and then filtered (0.2 µm pore size; Nuclepore). Subsequently, the filters were processed by sequential ethanol dehydration and drying with CO₂. The samples were then overlaid with gold and examined with a scanning electron microscope (S570; Hitachi). The presence of flexirubin pigments was investigated as described by Fautz & Reichenbach (1980). The Gram, oxidase and catalase reactions and the hydrolysis of casein, alginate, cellulose (CM-cellulose and filter paper), chitin, gelatin and Tweens 20, 40, 80 were tested according to the standard methods described by Smibert & Krieg (1994). The hydrolysis of starch was investigated, after 2 days incubation on MA containing 0.2 % (w/v) soluble starch, by flooding the plates with a 1 % (w/v) iodine solution. The formation of H₂S from thiosulfate was tested using a lead acetate paper strip. Acid production from carbohydrates was examined using an oxidation/fermentation medium for marine bacteria (Leifson, 1963). Growth at different temperatures and pH and in the presence of various NaCl concentrations, the hydrolysis of various substrates and antibiotic resistance were studied as described previously (Romanenko *et al.*, 2003, 2004). In addition, biochemical tests were carried out using API 20NE test kits as described by the manufacturer (bioMérieux), except that the culture was suspended in a 2 % (w/v) NaCl solution. The API test results were read after incubation at 25 °C for 24 and 48 h. DNA was isolated by using the procedure of Marmur (1961). The DNA G+C content was determined by using the method of Marmur & Doty (1962) as modified by Owen *et al.* (1969). For polar lipid and fatty acid analyses, strain KMM 3046^T was cultivated on MA at 25 °C for 3 days and the lipids were extracted using the chloroform/methanol method of Bligh & Dyer (1959). The polar lipids were analysed using the methods of Rowe *et al.* (2000) and Vaskovsky & Terekhova (1979). Two-dimensional TLC of the polar lipids was carried out using chloroform/methanol/benzene/28 % NH₄OH (65:30:10:6, by vol.) for the first direction and chloroform/methanol/benzene/acetone/acetic acid/water (70:30:10:5:4:1, by vol.) for the second direction. Non-specific TLC detection of lipids was conducted with 10 % H₂SO₄ in methanol at 180 °C. Amino-group-containing

lipids were determined with ninhydrin, phospholipids were determined with molybdate reagent, glycolipids were determined with anthrone spray and choline lipids were determined with Dragendorff's reagent. Fatty acid methyl esters were obtained by alkaline methanolysis (15 % NaOH/methanol) followed by acid methanolysis (2 M HCl/methanol) as described by Nichols *et al.* (1993). The resultant fatty acid methyl esters were extracted by hexane and analysed using a GLC-MS Hewlett Packard gas chromatograph (model 6890) equipped with a Hewlett Packard 5 MS 5 % phenyl methyl siloxane capillary column (30 m × 250 µm × 0.25 µm) and connected to a Hewlett Packard mass spectrometer (model 5973). Identification of the fatty acid methyl esters was achieved by using equivalent chain-length values and comparing the retention times of the samples with those of standards. Cellular quinones were determined by HPLC (LC-6A; Shimadzu) using a Cosmosil 5C₁₈ column (4.6 × 150 mm), with acetonitrile/2-propanol (50:50, v/v) as the eluant (0.5 ml min⁻¹) at a temperature of 40 °C and with an SPD-2AM (270 nm) UV detector. The yellow pigments were studied following chloroform/methanol (8:1, v/v) extraction. A non-polar pigment was isolated by preparative TLC and eluted with hexane; the visible spectrum of the hexane extract was examined with a spectrophotometer (model 7250; Cecil Instruments). The 16S rRNA gene sequence of strain KMM 3046^T was determined as described by Shida *et al.* (1997) and compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DDBJ databases, by using the FASTA program (Pearson & Lipman, 1988). Distances were calculated according to the method of Jukes & Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining method of Saitou & Nei (1987), with the CLUSTAL X program (version 1.8; Thompson *et al.*, 1997), and using the maximum-likelihood method, with the BioEdit program (Hall, 1999). Bootstrap analysis was applied with the programs implemented in the PHYLIP package (Felsenstein, 1993), using 1000 resamplings of the dataset.

Phylogenetic analysis based on the almost-complete 16S rRNA gene sequence (1397 nt) of strain KMM 3046^T revealed that *S. holothuriorum* KMM 3524^T (Nedashkovskaya *et al.*, 2004) and *S. flavus* KMM 6000^T (Ivanova *et al.*, 2006) are the closest phylogenetic neighbours, showing 92.3 and 92.1 % sequence similarity, respectively, and that the type strain of the type species of the genus, *S. salegens* DSM 5424^T, and the distantly related species *Mesonina algae* KMM 3909^T each show <92 % sequence similarity with respect to the isolate. The neighbour-joining tree (Fig. 1) showed that strain KMM 3046^T forms a separate evolutionary lineage between the *Salegentibacter* and *Aequorivita/Leeuwenhoekiella* lineages within the family *Flavobacteriaceae*. The maximum-likelihood tree showed a rather different topology, but the relationships between KMM 3046^T and *Mesonina algae* and *Salegentibacter* species were still apparent (see Supplementary Fig. S1 available in IJSEM Online). The

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships of strain KMM 3046^T and related taxa. Numbers at nodes indicate bootstrap values greater than 700 (from 1000 resamplings of the dataset). Bar, 0.02 substitutions per nucleotide position.

morphological, physiological, biochemical and chemotaxonomic characteristics of strain KMM 3046^T are given in Table 1 and in the genus and species descriptions (and in Supplementary Table S1 and Supplementary Fig. S2, available in IJSEM Online). Table 1 lists the phenotypic properties that serve to differentiate between strain KMM 3046^T and members of the genera *Salegentibacter*, *Mesonia*, *Aequorivita* and *Leeuwenhoekiella*. Because of the variety in the phenotypic characteristics among members of the genus *Salegentibacter*, all five species were included in Table 1. The major respiratory quinone of strain KMM 3046^T was MK-6 and its DNA G + C content was 37.3 mol%. These features are in accordance with those of related organisms, except with regard to the G + C contents of *S. flavus*, *Mesonia algae* and members of the genus *Leeuwenhoekiella*, which differ noticeably from that of strain KMM 3046^T.

The major cellular fatty acids of strain KMM 3046^T are listed in the species description, and its detailed fatty acid composition is shown in Supplementary Table S1. Strain KMM 3046^T is characterized by the presence of significant amounts of 3-OH i16:0 and 3-OH i17:0 (57.6 % of the total). The polar lipid composition of strain KMM 3046^T included phosphatidylethanolamine, lysophosphatidylethanolamine, aminophospholipid, an unknown lipid and sphingolipid, the latter component being predominant (see Supplementary Fig. S3 available in IJSEM Online). One non-polar pigment was found, characterized by absorption peaks at 422 and 445 nm, with a shoulder at 475 nm. Consequently, strain KMM 3046^T can be distinguished from members of the genera *Salegentibacter*, *Mesonia*, *Aequorivita* and *Leeuwenhoekiella* as well as from other members of the family *Flavobacteriaceae* on the basis of a combination of biochemical characteristics (Table 1), chemotaxonomic traits (Supplementary Table S1) and the phylogenetic distance (Fig. 1).

On the basis of these results, the novel isolate, strain KMM 3046^T, represents a novel genus and species, for which the

name *Marixanthomonas ophiurae* gen. nov., sp. nov. is proposed.

Description of *Marixanthomonas* gen. nov.

Marixanthomonas (Ma'ri.xan'tho.mo'nas. L. n. *mare* the sea; Gr. adj. *xanthos* yellow; Gr. n. *monas* a unit, monad; N.L. fem. n. *Marixanthomonas* a marine yellow monad).

Cells are Gram-negative, non-motile, non-spore-forming rods. Strictly aerobic. Oxidase- and catalase-positive. Yellow-pigmented. Flexirubin pigments are not produced. Chemoorganotrophic, with an absolute requirement for sodium ions. Halophilic. The predominant menaquinone is MK-6. Polar lipids consist of sphingolipid, phosphatidylethanolamine, lysophosphatidylethanolamine, aminophospholipid and an unknown lipid. The major cellular fatty acids are 3-OH i16:0, 3-OH i17:0, 3-OH a17:0, 16:0 and 18:0. Isolated from the marine environment. The DNA G + C content of the type species is 37.3 mol%. Analysis of the 16S rRNA gene sequence shows that the genus belongs to the family *Flavobacteriaceae*. The type species is *Marixanthomonas ophiurae*.

Description of *Marixanthomonas ophiurae* sp. nov.

Marixanthomonas ophiurae (o.phi.u'rae. N.L. gen. n. *ophiurae* of Ophiura, a class of invertebrates belonging to the Ophiuroidea, the source of isolation of the type strain).

Possesses the following traits in addition to those reported for the genus. Cells are 0.4–0.5 × 2.2–2.8 µm. On MA, colonies are round, 4–5 mm in diameter, smooth, shiny, opaque, slimy, light yellow (having a non-diffusible carotenoid pigment) and have regular edges. Grows in the presence of 1.0–12 % (w/v) NaCl (optimum, 3–5 %). Weak growth occurs with 1 and 12 % NaCl. Psychrotolerant. Grows at 5–32 °C (optimum, 25–28 °C) and grows slowly at 5 °C; no growth occurs above 33 °C. The optimal pH for

Table 1. Differential characteristics of strain KMM 3046^T and related taxa

Strains/species: 1, KMM 3046^T; 2, *S. salegens* DSM 5424^T; 3, *S. mishustinae* KMM 6049^T; 4, *S. holothuriorum* KMM 3524^T; 5, *S. flavus* KMM 6000^T; 6, *S. agarivorans* KMM 7019^T; 7, *Mesonina algae*; 8, *Leeuwenhoekiella* (type strains of the three species); 9, *Aequorivita antarctica*. Data are from Dobson *et al.* (1993), McCammon & Bowman (2000), Nedashkovskaya *et al.* (2003, 2004, 2005a, b), Ivanova *et al.* (2006), Pinhassi *et al.* (2006), Bowman & Nichols (2002) and this study. All taxa are negative for anaerobic growth, the production of flexirubin pigments, the hydrolysis of chitin and cellulose (CM-cellulose and filter paper) and the production of indole. All taxa are positive for gelatin hydrolysis, the production of oxidase and catalase and growth in 6% NaCl. Abbreviations: +, positive; –, negative; w, weak reaction; v, variable reaction between strains or species; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9
Gliding motility	–	–	–	–	–	+	–	+	–
Nitrate reduction	–	+	–	–	–	+	–	–	–
Na ⁺ requirement	+	–	+	+	+	+	+	–	+
Growth at:									
34 °C	–	+	+	+	+	+	+	+	–
41 °C	–	–	–	–	–	+	–	v	–
Tolerance of NaCl at:									
8 %	+	+	+	+	+	+	+	+	–
12 %	w	+	+	–	–	+	+	+	–
15 %	–	+	+	–	–	+	+	+	–
18 %	–	+	+	–	–	+	–	–	–
Hydrolysis of:									
Casein	+	–	+	–	–	–	+	+	–
Starch	–	+	+	+	+	+	–	+	+
Alginate	–	+	+	+	ND	+	–	ND	–
Agar	–	–	–	–	–	+	–	–	–
Urea	–	–	–	–	+	–	–	–	v
Acid from sugars	–	+	+	+	–	+	–	+	–
H ₂ S production	–	+	+	+	–	+	+	–	–
DNA G + C content (mol%)	37.3	37.8	37.5	37.5	40.4 ± 0.5	39.2	32.7–34.0	35–42.5	38–39

growth is 6.5–8.5. Negative results in the Simmons' citrate test and the Voges–Proskauer test; negative for H₂S, arginine dihydrolase, ornithine and lysine decarboxylases and phenylalanine deaminase; negative for nitrate reduction and for the utilization of D-glucose, L-arabinose, lactose, D-mannose, sucrose, inositol, sorbitol, mannitol, citrate and malonate (using conventional methods). Negative in API 20NE kits for the production of arginine dihydrolase, urease and indole, in the ONPG test, for the hydrolysis of aesculin and for the utilization of glucose, L-arabinose, D-mannose, N-acetylglucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate and phenylacetate. Starch, aesculin, alginate, agar, cellulose (CM-cellulose or filter paper) and chitin are not hydrolysed. Gelatin is hydrolysed in 3–4 days when tested using a conventional method, and in 24 h in the API 20NE test. Tweens 80, 40 and 20 are hydrolysed. Acid is not produced from D-glucose, D-xylose, rhamnose, fructose, galactose, maltose, sucrose, lactose, arabinose, N-acetylglucosamine, mannitol, inositol or glycerol. Other physiological characteristics are listed in Table 1. Susceptible to (content per disc) lincomycin (15 µg), erythromycin (15 µg), carbenicillin (100 µg), oleandomycin (15 µg), streptomycin (30 µg) (weakly) and chloramphenicol (30 µg), and resistant to ampicillin (10 µg), benzylpenicillin

(10 U), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), polymyxin B (300 U), tetracycline (30 µg) and doxycycline (10 µg). The major fatty acids are 3-OH i16:0 (41.2 %), 3-OH i17:0 (16.4 %), 3-OH a17:0 (5.1 %), 16:0 (6.9 %) and 18:0 (5.2 %). Other fatty acids present are as follows: i15:0 (2.9 %), 15:0 (2.4 %), 15:1 (1.3 %), i16:0 (1.8 %), 2-OH 15:0 (3.3 %), 3-OH 16:0 (4.4 %), 3-OH 17:0 (2.5 %) and 18:1ω9c (3.9 %). The polar lipid composition is as given in the genus description, with sphingolipid as the predominant compound. The non-polar pigment is characterized by absorption peaks at 422 and 445 nm, with a shoulder at 475 nm. The DNA G + C content is 37.3 mol%.

The type strain, KMM 3046^T (=NRIC 0684^T=JCM 14121^T), was isolated from an ophiuroid specimen collected from the Fiji Sea at a depth of 480 m.

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