Flagellimonas eckloniae gen. nov., sp. nov., a mesophilic marine bacterium of the family *Flavobacteriaceae*, isolated from the rhizosphere of *Ecklonia kurome*

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A marine bacterium, DOKDO 007^T, was isolated from the rhizosphere of the marine alga *Ecklonia kurome* collected from Dokdo Island, Korea, in October 2004. The strain produced orange-coloured colonies on marine agar 2216. 16S rRNA gene sequence analysis indicated that the novel isolate belonged to the family *Flavobacteriaceae* and showed relatively high sequence similarities with members of the genus *Muricauda* (92.0–94.0 %). Phylogenetic analysis based on nearly complete 16S rRNA gene sequences revealed that the novel isolate shared a lineage with members of the genera *Muricauda* and *Costertonia*. Cells were aerobic, Gram-negative rods producing non-diffusible carotenoid pigments. In contrast to all other members of the family *Flavobacteriaceae*, cells of DOKDO 007^T were motile by means of a polar flagellum. Optimal growth occurred in the presence of 3.5–4 % (w/v) sea salts (corresponding to 2.7–3.1 % NaCl), at pH 8 and at temperatures of 26–29 °C. The novel strain required Ca^{2+} ions in addition to NaCl for growth. The dominant fatty acids were iso-15 : 0, iso-15 : 1ω10c and 10-methyl-16 : 0. The major respiratory quinone was MK-6. The DNA G+C content was 56.3 mol%, an unusually high value for members of the family *Flavobacteriaceae*. On the basis of these polyphasic taxonomic data, strain DOKDO 007^T should be classified as representing a new genus and novel species in the family *Flavobacteriaceae*, for which the name *Flagellimonas eckloniae* gen. nov., sp. nov. is proposed. The type strain is DOKDO 007^T (=KCCM 42307^T=JCM 13831^T).

The family *Flavobacteriaceae* belongs to the phylum *Bacteroidetes* (previously known as *Cytophaga–Flavobacterium–Bacteroides*), which groups chemoorganotrophic, non-spore-forming, Gram-negative rods that are non-motile or motile by gliding. Members of the family can be non-pigmented or pigmented by carotenoid and/or flexirubin pigments depending on the genus. Menaquinone 6 serves as their only or major respiratory quinone (Bernardet *et al.*, 1996, 2002). Some members of the family, such as *Arenibacter latericius*, *Mesonia algae*, *Maribacter ulvicola* and *Zobellia galactanovorans* (Barbeyron *et al.*, 2001; Ivanova *et al.*, 2001; Nedashkovskaya *et al.*, 2003a, 2004b) have been isolated from a diverse range of marine macroalgae. Here, we propose that strain DOKDO 007^T, originating from the rhizosphere of a marine macroalga, represents a new genus in the family *Flavobacteriaceae*.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DOKDO 007^T is DQ191180.

Transmission and scanning electron micrographs of cells of strain DOKDO 007^T are available as supplementary material in IJSEM Online.

The marine macroalga *Ecklonia kurome* was collected along the seashore of Dokdo Island, Korea, in October 2004. A small piece of algal rhizosphere was crushed in 2 ml sterile seawater which was then spread onto marine agar 2216 (MA; Difco) and cultivated at 30 °C for 7 days. Among the morphologically distinct colonies that grew on MA, a tiny orange-coloured colony was isolated, designated DOKDO 007^T, and preserved in marine broth 2216 (MB; Difco) containing 20 % glycerol at −80 °C. The isolate was further cultivated on MA or in MB for morphological and biochemical characterization.

Unless otherwise stated, the minimal standards for describing novel taxa in the family *Flavobacteriaceae* proposed by Bernardet *et al.* (2002) were tested according to previously described methods (Bae *et al.*, 2005; Sohn *et al.*, 2004). Transmission and scanning electron micrographs were taken using JSM-6700F (JEOL) and JEM-2000EXII (JEOL) electron microscopes, respectively. Gliding motility was investigated according to the method described by Bowman (2000) on bacteria grown for 24 h at 20 °C. Flagellar motility was examined using a 24 h MB culture under a light

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microscope (Axioplan; Zeiss). Cellular pigments were extracted with 3 ml methanol/acetone mixture (1 : 1, v/v) from culture grown on MA and their absorption spectra were measured with a spectrophotometer (UV-2410PC; Shimadzu). Flexirubin-type pigments were detected by placing a drop of 20 % KOH on colonies (Fautz & Reichenbach, 1980). The degradation of starch and casein was tested according to Smibert & Krieg (1994). Bacterial suspensions used to inoculate API 20NE (bioMérieux) and Microlog GN2 (Biolog) systems were prepared in 3 % sea salts (Sigma) solution. The commercial sea salts preparation was also used to test the salt tolerance range of the novel strain. The physiological, biochemical and morphological characteristics of strain DOKDO 007^T are given below, in the genus and species descriptions and in Table 1.

Gliding motility was not observed. Flagellar motility was observed and confirmed by the presence of a single polar flagellum on some bacterial cells as shown by transmission electron microscopy (see Supplementary Fig. S1 in IJSEM Online). This is a very unusual characteristic in the family *Flavobacteriaceae*. Cells of *Polaribacter irgensii* have been shown to display a polar flagellum, although motility has never been observed (Gosink *et al.*, 1998). In contrast, the motility observed in cells of *Costertonia aggregata* is not of the gliding type, but the presence of flagella has not been reported (Kwon *et al.*, 2006).

The presence of NaCl alone in the medium did not support the growth of strain DOKDO 007^T. We therefore tested the requirement for three other seawater components; $CaCl_2.2H_2O$, KCl and $MgCl_2.6H_2O$. All combinations of these components at concentrations of 0.18 % $CaCl_2.2H_2O$, 0.055 % KCl and 0.59 % $MgCl_2.6H_2O$ were added to modified ZoBell 2216e medium containing 5 g yeast extract, 1 g peptone, 30 g NaCl and 0.01 g $FePO_4$ per litre of distilled water. Growth was observed only in the presence of Ca^{2+} ions, in addition to NaCl. Growth also occurred in the presence of 2.5–7 % of the commercial sea salts preparation.

The cellular fatty acid methyl ester profile was determined according to Sohn *et al.* (2004) on bacteria grown in MB for 3 days at 25 °C. The dominant fatty acids of strain DOKDO 007^T were iso-15 : 0 (41.8 %), iso-15 : 1ω10*c* (11.4 %), 10-methyl-16 : 0 (9.2 %), 15 : 0 (7.0 %), unidentified fatty acid ECL 18.056 (6.5 %) and 16 : 1ω7*c* (6.0 %). The strain also contained small amounts of anteiso-15 : 0 (2.5 %), iso-16 : 0 (1.5 %), 16 : 0 (1.4 %), iso-14 : 0 (1.3 %) and iso-13 : 0 (1.3 %). The novel strain contained a relatively large amount of iso-15 : 0, a characteristic shared with *C. aggregata* (Kwon *et al.*, 2006) (Table 1).

Using the HPLC analysis method of Collins (1985), the major respiratory quinone was determined to be MK-6. The DNA G+C content was 56.3 mol% as determined by HPLC using a symmetry reversed-phase C18 column (Waters; Stackebrandt & Liesack, 1993). *Robiginitalea biformata* is the only other member of the family *Flavobacteriaceae* that shows such a high DNA G+C content (Cho & Giovannoni, 2004).

Extraction of the genomic DNA and amplification of the 16S rRNA gene were conducted according to Sohn *et al.* (2004). A phylogenetic tree featuring strain DOKDO 007^T and closely related genera was generated based on Jukes and Cantor or maximum-likelihood distance models with neighbour-joining or maximum-parsimony algorithms. A total of 1309 unambiguously aligned sequences were compared. The closest neighbour was *Muricauda aquimarina* (94.0 % gene sequence similarity), followed by *Muricauda ruestringensis* (93.5 %), *Muricauda flavescens* (92.0 %) and *C. aggregata* KOPRI 13342^T (91.3 %). Phylogenetic analysis based on 16S rRNA gene sequences of previously published strains revealed that strain DOKDO 007^T shared a phyletic line with the genera *Muricauda* and *Costertonia*, though occupying a distinct position (Fig. 1).

Strain DOKDO 007^T shared many characteristics with closely related members of the family *Flavobacteriaceae*, including the type of major respiratory quinone, the range of temperature and pH supporting growth and the requirement for salt and oxygen. Some features, such as (i) the requirement of a seawater component (Ca^{2+}) in addition to NaCl for growth, the high amount of iso-15 : 0 and flagellar motility and (ii) the high DNA G+C content are only shared with *C. aggregata* and *Robinginitalea biformata*, respectively. However, the presence of 16 : 1ω7*c* (6.0 %) and 10-methyl-16 : 0 (9.2 %) and the absence of oxidase activity are major differences with closely related members of the family *Flavobacteriaceae* (Table 1). This new strain should therefore be recognized as representing a novel member of the family *Flavobacteriaceae*. We suggest a new genus, *Flagellimonas*, and propose the strain as *Flagellimonas eckloniae* gen. nov., sp. nov.

Description of *Flagellimonas* gen. nov.

Flagellimonas (Fla′gell.i.mon.as. L. n. *flagellum* a whip and in bacteriology, a flagellum; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Flagellimonas* a bacterium motile by means of a flagellum which is unusual for a member of the family *Flavobacteriaceae*).

Cells are strictly aerobic, motile, Gram-negative rods. Produce non-diffusible carotenoid pigments, but flexirubin-type pigments are absent. The major respiratory quinone is MK-6. The major cellular fatty acids are iso-15 : 0, iso-15 : 1ω10*c* and 10-methyl-16 : 0. Oxidase activity is absent, but catalase activity is present. The DNA G+C content of the type species is 56.3 mol%. As determined by 16S rRNA gene sequence analysis, the genus *Flagellimonas* is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *Flagellimonas eckloniae*.

Description of *Flagellimonas eckloniae* sp. nov.

Flagellimonas eckloniae (ec.klo.ni′a.e. N.L. fem. n. *Ecklonia* scientific genus name of the marine alga from which the bacterium was isolated; N.L. gen. n. *eckloniae* of *Ecklonia*).

Table 1. Phenotypic characteristics that differentiate strain DOKDO 007^T from closely related members of the family *Flavobacteriaceae*

Taxa: 1, DOKDO 007^T; 2, *Muricauda*; 3, *Costertonia aggregata* KOPRI 13342^T (only species in genus); 4, *Maribacter*; 5, *Zobellia*; 6, *Arenibacter*; 7, *Robiginitalea biformata* HTCC 2501^T (only species in genus). Data are from Barbeyron *et al.* (2001), Bruns *et al.* (2001), Cho & Giovannoni (2004), Ivanova *et al.* (2001), Kwon *et al.* (2006), Nedashkovskaya *et al.* (2003b, 2004a, b, c) and Yoon *et al.* (2005a, b). Fatty acid percentages amounting to <3 % of the total fatty acids in all strains are not included. For fatty acid analysis, some bacterial strains were not cultivated in the same conditions and different analysis procedures were used. All strains are positive for catalase activity, require oxygen for growth, have MK-6 as a major respiratory quinone and do not require specific growth factors or produce indole. +, Positive; −, negative; ND, not determined; V, variable.

Characteristic	1	2	3	4	5	6	7
Gliding motility	−	+/ND	−	+	+	−	−
Growth in/on:							
Temperature range (°C)	17–36	8–44	10–35	4–33	4–45	4–42	10–44
Optimum temperature (°C)	26–29	20–30, 30–37	26–32	21–24	21–35	28–30	30
pH range	7–9	6–8	6.5–9	5.5–10	ND	5.5–10	6–9
Optimum pH	8	6.5–7.5	7.5–8.0	7.5–8.5	ND	7.5–8.5	8–8.5
NaCl concentration range (%)	1.9–5.4*	0.5–9	1.2–9.3*	1–7	0.5–10	1–10	0.25–10
Optimum NaCl concentration (%)	2.7–3.1*	2–3	2.3*	1.5–2.0	2–3	ND	2.5
Seawater requirement†	+	−	+	−	−	−	−
Oxidase activity	−	V	+	+	+	+	+
Nitrate reduction	−	−	+	V	+	+	−
Acid from carbohydrates	−	V	−	V	V	V	−
Hydrolysis of:							
Agar	−	−	−	V	+	−	ND
Casein	+	−	−	−	−	−	−
Gelatin	−	−	+	V	+	V	−
Starch	−	−	−	V	V	−	+
Major fatty acids:							
i-15 : 0	41.8	14.7–23.8	39.7	10.6–20.5	16.8–22.5	8.5–17.3	24–28
i-15 : 1ω10c	11.4	19.5–21.6	22.4	10.1–18.9	8.8–14.9	14.3–19.3	14–21
a-15 : 0						6.6–8.6	3–4
15 : 0	7.0	5.1–13.2	7.8	3.5–14.5	7.5–14.4	13.3–29.0	5–6
16 : 1ω9c			4.6			<5–11.0	
16 : 1ω7c	6.0						
10M-16 : 0	9.2						
i-17 : 1				2.0–4.0	2.4–5.1		
i-15 : 0 3-OH		4.6–5.5		2.9–5.4	4.6–8.3		4.3
i-16 : 0 3-OH		1.7–4.6					
i-17 : 0 3-OH		17.3–20.9		11.6–29.2	15.1–25.9	<5–6.1	25–27
Summed feature 3‡		2.3–4.2		5.8–12.9	9.9–15.5		
ECL 18.056	6.5	7.0–8.8		2.7–10.3			
DNA G+C content (mol%)	56.3	41–45.4	35.8	35–39	36–43	37.5–40	55–56

*Sea salt concentration was converted to NaCl concentration.

†Requirement of seawater indicates that Na^+ alone does not support growth. The strain requires additional cations present in seawater for growth, such as Mg^{2+}, Ca^{2+} and/or K^+.

‡Summed feature 3 contains 16 : 1ω7c and/or iso-15 : 0 2-OH.

Displays the following properties in addition to those given in the genus description. Cells are 1.1–2.3 μm in length and 0.2–0.36 μm in diameter and motile by means of a single polar flagellum (see Supplementary Fig. S1 in IJSEM Online). Gliding motility is not observed. Cells form irregular aggregates during growth in MB. After 3 days of incubation on MA at 25 °C, colonies are orange-pigmented, opaque, convex and uniformly circular. Absorption maxima of the pigments extracted with solvent solution are observed at 450 and 473 nm. Growth occurs between 17 and 36 °C, at pH 7–9 and in the presence of 2.5–7 % sea salts. Requires Na^+ and Ca^{2+} ions for growth. Optimal growth requires the presence of 3.5–4 % (w/v) sea salts (corresponding to 2.7–3.1 % NaCl), pH 8 and 26–29 °C. Cells hydrolyse casein,

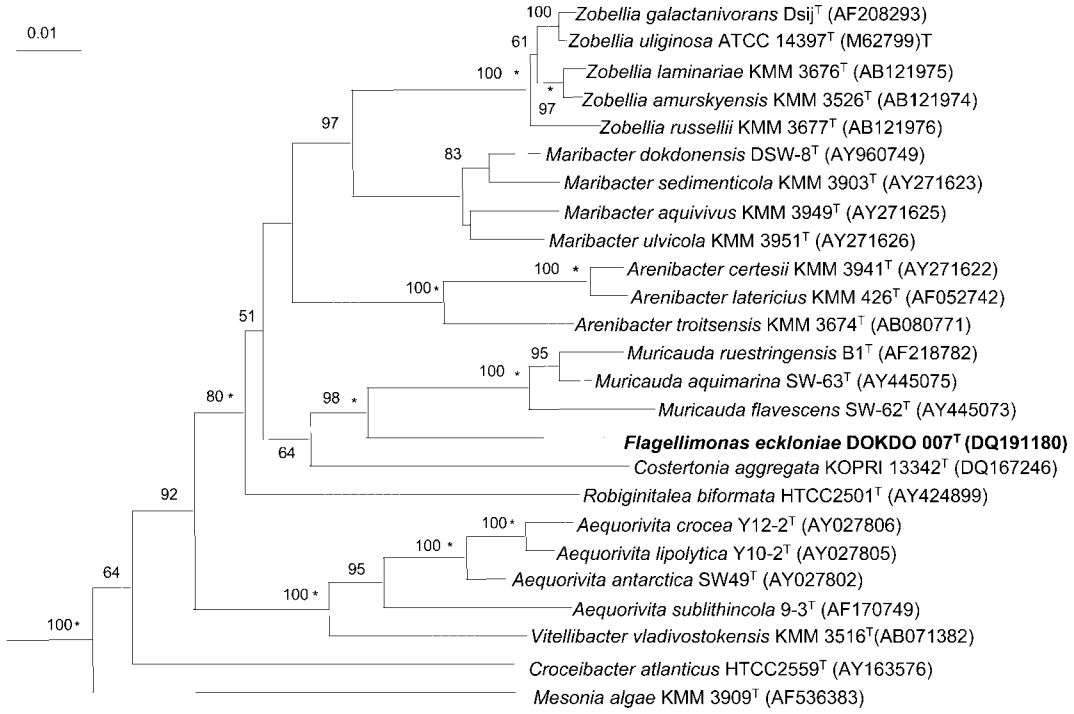

Fig. 1. Phylogenetic tree based on nearly complete 16S rRNA gene sequences (1309 unambiguously aligned base pairs) showing the relationship between strain DOKDO 007^T and related members of the family *Flavobacteriaceae*. The 16S rRNA gene sequences of *Bacteroides fragilis* ATCC 25285^T (GenBank accession no. M61006) and *Sphingobacterium spiritivorum* DSM 2582^T (AJ459411) served as outgroups (not shown). The tree is based on the Jukes and Cantor distance model and the neighbour-joining algorithm. Bootstrap values >50 % for 1000 resamples are shown. Asterisks indicate the bootstrap values >70 % for 1000 resamples that were also found in the tree generated by the maximum-likelihood distance model and maximum-parsimony algorithm. Bar, 0.01 nucleotide substitutions per nucleotide position.

but not agar, gelatin or starch. In API 20NE test strips, β-glucosidase, β-galactosidase and protease (gelatin hydrolysis) activities are positive, but reduction of nitrate to nitrogen, glucose acidification, production of H_2S and arginine dihydrolase and urease activity are negative; acetoin production is weakly positive. In Biolog GN2 MicroPlates, cells utilize dextrin, cellobiose, L-fucose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannose, sucrose, D-trehalose, turanose, D-glucuronic acid, DL-lactic acid and L-aspartic acid. Weakly positive results are recorded for the utilization of glycogen, D-fructose, D-melibiose, methyl β-D-glucoside, D-raffinose, L-rhamnose, α-ketobutyric acid, α-ketoglutaric acid, succinic acid, alaninamide, L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-glutamic acid, hydroxyl-L-proline, L-leucine, L-proline, L-threonine, urocanic acid, phenylethylamine and glucose 1-phosphate. The dominant fatty acids are iso-15 : 0 (41.8 %), iso-15 : 1ω10*c* (11.4 %), 10-methyl-16 : 0 (9.2 %), 15 : 0 (7.0 %), unidentified fatty acid ECL 18.056 (6.5 %,) and 16 : 1ω7*c* (6.0 %). Other characteristics are shown in Table 1.

The type strain, DOKDO 007^T (=KCCM 42307^T=JCM 13831^T), was isolated from the rhizosphere of the marine alga *Ecklonia kurome* collected on Dokdo Island, Korea.

Acknowledgements

This work was supported by the 'Marine and Extreme Genome Research Center' program of the Ministry of Maritime Affairs and Fisheries, Korea and the 'Dokdo' Program (PM40201).

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