Vitellibacter aestuarii sp. nov., isolated from tidal-flat sediment, and an emended description of the genus *Vitellibacter*

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A Gram-reaction-negative, aerobic, yellowish-orange-pigmented bacterial strain, designated JC2436^T, was isolated from tidal-flat sediment of Oi Island in Korea. Comparative 16S rRNA gene sequence analysis indicated its close affiliation to *Vitellibacter vladivostokensis*, with 96 % sequence similarity to the type strain. Cells grew with 2–6 % NaCl and at 10–41 °C. Orange flexirubin pigments were present. The major isoprenoid quinone was MK-6, the DNA G+C content was 48.7 mol% and the predominant fatty acids (>10 %) were iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH. The data obtained from this polyphasic study support the classification of this isolate within a novel species in the genus *Vitellibacter*, for which the name *Vitellibacter aestuarii* sp. nov. is proposed. The type strain is JC2436^T (=IMSNU 14137^T =KACC 13727^T =KCTC 22361^T =JCM 15496^T).

Members of the phylum *Bacteroidetes* (formerly the *Cytophaga–Flavobacterium–Bacteroides* group) are widespread and represent a large proportion of the microbial communities in marine environments. Some of these bacteria play important roles in the degradation of dissolved and particulate organic matter in nature (Cottrell & Kirchman, 2000; Devey *et al.*, 2001). Tidal-flat sediments represent a marine environment that has been recognized as an important place for bioremediation; they have recently yielded various novel bacterial taxa (Kim *et al.*, 2004; Yi & Chun, 2006), including many members of the family *Flavobacteriaceae* in the phylum *Bacteroidetes* (Choi *et al.*, 2006; Kim *et al.*, 2008). The genus *Vitellibacter* and species *Vitellibacter vladivostokensis* in the family *Flavobacteriaceae* were described to accommodate a strain isolated from a holothurian in the Sea of Japan (Nedashkovskaya *et al.*, 2003). Here, we present the polyphasic characterization of a bacterial isolate from tidal-flat sediment for which we propose a novel species in the genus *Vitellibacter*.

Strain JC2436^T was isolated from tidal flat sediment of Oi Island in Korea (37° 20.533′ N 126° 41.333′ E) using marine agar 2216 (MA; Difco) at 30 °C. The isolate was preserved as a glycerol suspension (20 %, w/v, in distilled water) at –80 °C.

The 16S rRNA gene was amplified and sequenced from a single colony as described previously (Chun & Goodfellow, 1995). Identification of phylogenetic neighbours was carried out by using the BLAST program (Altschul *et al.*, 1997) against the database of the EzTaxon server (http://www.eztaxon.org/; Chun *et al.*, 2007). The 100 sequences with the highest alignment scores were then selected for the calculation of pairwise sequence similarity using a global alignment algorithm implemented at the EzTaxon server. The nearly complete 16S rRNA gene sequence of strain JC2436^T (1395 bp) was obtained and aligned manually against those of members of the nearest genera of the family *Flavobacteriaceae* using a bacterial 16S rRNA secondary structure model. The regions available for all sequences (positions 96–1412; *Escherichia coli* numbering system) showed unambiguous alignment and were used to construct phylogenetic trees. Phylogenetic analyses were carried out using the JPHYDIT program (Jeon *et al.*, 2005; http://chunlab.snu.ac.kr/jphydit/) and PAUP 4.0 (Swofford, 1998) as described previously (Chun *et al.*, 2000; Yi & Chun, 2006). Phylogenetic trees were inferred by using the maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. The resultant tree topologies were evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Strain JC2436^T showed the highest 16S rRNA gene sequence similarity to *Vitellibacter vladivostokensis* KMM 3516^T (96 %), followed by the type strains of *Aequorivita* species (93.2–94 %). The neighbour-joining tree showed that the isolate formed a monophyletic clade with the type strain of *V. vladivostokensis* (Fig. 1) with 88 % bootstrap support. This relationship was also found in the maximum-parsimony and maximum-likelihood trees.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JC2436^T is EU642844.

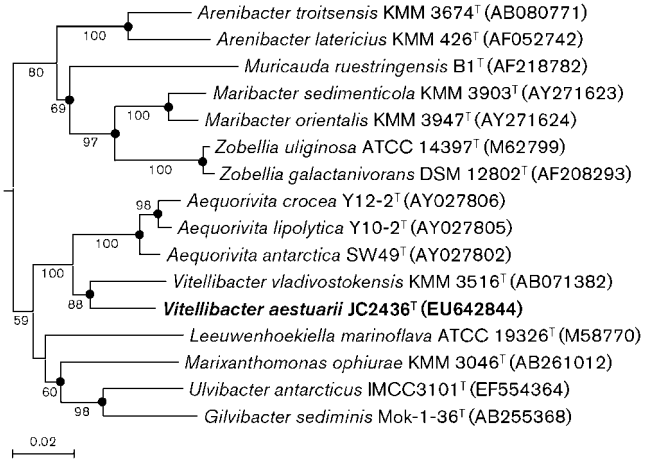

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences of strain JC2436^T and closely related members of the family *Flavobacteriaceae*. The sequence of *Chryseobacterium balustinum* ATCC 33487^T was used as the outgroup (GenBank accession no. M58771; not shown). Percentages at nodes are levels of bootstrap support (>50 %) from 1000 resampled datasets. Solid circles indicate that the corresponding nodes (groupings) were also recovered in the maximum-likelihood and maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position.

Growth at various NaCl concentrations was investigated in sea-salt-free Zobell's agar (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1000 ml distilled water). The temperature range for growth was determined optically in a temperature gradient incubator (model TVS 126MA; Advantec) using marine broth 2216 (MB; Difco) in the range 4–45 °C. Growth under anaerobic conditions was assessed in an anaerobic chamber on MA that had been prepared anaerobically under a nitrogen atmosphere. Gliding motility was determined as described by Bowman (2000). Strain JC2436^T did not grow under anaerobic conditions. The temperature range for growth was 10–41 °C (optimum, 30–35 °C). Growth occurred with 2–6 % NaCl (optimum, 2–3 %).

Comparative phenotypic characterization was carried out in parallel on strain JC2436^T and the reference strain *V. vladivostokensis* KMM 3516^T. Biochemical tests were performed using the API 20NE and API ZYM kits (bioMérieux) and GN2 MicroPlates (Biolog). A heavy cell suspension in AUX medium (bioMérieux) with 2.5 % NaCl was dispensed into each well of the API kits. All kits were incubated at 30 °C for 5 days. The oxidase test was performed using a commercial oxidase reagent (bioMérieux). Catalase activity was tested by addition of a 3 % (v/v) H_2O_2 solution to colonies. Flexirubin pigments were detected using 20 % (w/v) KOH, a colour change from yellow or orange to brown–red indicating the presence of pigments (Fautz & Reichenbach, 1980). Acid production from carbohydrates was examined using API 50 CHB kits (bioMérieux). Hydrolysis of starch (0.2 %, w/v), casein (50 %, v/v, skimmed milk), cellulose (0.5 %, w/v, CM-cellulose; Sigma), Tweens 20, 40, 60 and 80 (1 %, v/v), chitin (1 %, w/v) and elastin (2 %, w/v) was detected using MA as the basal medium (Nedashkovskaya *et al.*, 2003; Smibert & Krieg, 1994). Susceptibility to antibiotics [ampicillin (10 μg), benzylpenicillin (10 μg), carbenicillin (100 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), oleandomycin (15 μg), polymyxin B (300 U), streptomycin (10 μg) and tetracycline (30 μg); discs from Adventec] was examined according to Nedashkovskaya *et al.* (2003) using the disc diffusion method. The results of biochemical and physiological tests are shown in Table 1 and in the species description.

The cellular fatty acid compositions of the test strain and *V. vladivostokensis* KMM 3516^T were analysed by GLC according to the instructions of the Microbial Identification System (MIDI) using cells grown on MA at 30 °C for 5 days. The same growth conditions had originally been used to grow *V. vladivostokensis* KMM 3516^T, except that the temperature was 28 °C (Nedashkovskaya *et al.*, 2003). Overall, the fatty acid profiles of the two strains were very similar, with only slight difference in the proportions of some components (Table 2). The DNA G+C content of strain JC2436^T was determined by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). The G+C content of strain JC2436^T was 48.7 mol%, while that of *V. vladivostokensis* KMM 3516^T was reported as 41.3 mol% (Nedashkovskaya *et al.*, 2003). Menaquinones were extracted, purified and analysed by using a modification of the HPLC method (Collins, 1994).

On the basis of 16S rRNA gene sequence similarity and phylogenetic analysis, strain JC2436^T exhibited a close relationship to *V. vladivostokensis* KMM 3516^T. The two strains shared several important phenotypic characteristics, strongly suggesting that they belong to the same genus, such

Table 1. Characteristics that differentiate strain JC2436^T from *V. vladivostokensis* KMM 3516^T

Strains: 1, JC2436^T; 2, *V. vladivostokensis* KMM 3516^T. All data were obtained in this study except the DNA G+C content of *V. vladivostokensis* KMM 3516^T (from Nedashkovskaya *et al.*, 2003).

Characteristic	1	2
Catalase	−	+
Growth temperature (°C)		
Optimum	30–35	28
Range	10–41	4–43
NaCl concentration range for growth (%)	2–6	1–6
Susceptibility to:		
Ampicillin	−	+
Benzylpenicillin	+	−
Lincomycin	−	+
Hydrolysis of Tween 40	−	+
DNA G+C content (mol%)	48.7	41.3

Table 2. Fatty acid compositions of strain JC2436^T and *V. vladivostokensis* KMM 3516^T

Strains: 1, JC2436^T; 2, *V. vladivostokensis* KMM 3516^T. Values are percentages of total fatty acids and were obtained in this study. −, Not detected; fatty acids that amounted to <1 % in both strains are not shown.

Fatty acid	1	2
iso-$C_{15:1}$	3.3	2.4
iso-$C_{15:0}$	37.6	32.4
anteiso-$C_{15:0}$	3.6	3.8
$C_{15:0}$	4.2	4.3
iso-$C_{16:0}$	1.2	2.7
Summed feature 3*	9.3	6.9
$C_{16:0}$	1.6	1.7
iso-$C_{15:0}$ 3-OH	4.6	3.5
iso-$C_{17:1}\omega 9c$	5.1	9.0
iso-$C_{17:0}$	−	2.0
iso-$C_{16:0}$ 3-OH	1.5	1.4
iso-$C_{17:0}$ 3-OH	21.1	23.2

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7c$.

as the absence of flagella, the inability to produce endospores, the presence of MK-6 as the major quinone and the production of flexirubin pigments, oxidase and alkaline phosphatase. On the other hand, the two strains showed rather low 16S rRNA gene sequence similarity (96 %) and could be differentiated from each other by a number of phenotypic characteristics (Table 1). We therefore propose that strain JC2436^T be assigned to a novel species in the genus *Vitellibacter*, for which the name *Vitellibacter aestuarii* sp. nov. is proposed. We also emend the description of the genus *Vitellibacter* to accommodate the novel species.

Emended description of the genus *Vitellibacter* Nedashkovskaya *et al.* 2003

Cells are Gram-reaction-negative, strictly aerobic rods that produce non-diffusible yellow–orange flexirubin pigments. Cells do not form endospores, they are not flagellated and gliding motility is not observed. Cells require Na^+ for growth and are chemo-organotrophic. Cells are oxidase- and alkaline phosphatase-positive. The major respiratory quinone is MK-6. The main cellular fatty acids are iso-$C_{15:0}$ and iso-$C_{17:0}$ 3-OH. The DNA G+C content is approximately 41–49 mol%. The type species is *Vitellibacter vladivostokensis*.

Description of *Vitellibacter aestuarii* sp. nov.

Vitellibacter aestuarii (aes.tu.a′ri.i. L. gen. n. *aestuarii* of a tidal flat).

The description is the same as the emended description of the genus with the following additional characteristics. Cells are approximately 8 μm long. Colonies on MA are circular with regular edges, convex and yellowish orange. Growth occurs with 2–6 % (w/v) NaCl (optimum, 2–3 %) and at 10–41 °C (optimum, 30–35 °C). Cells are catalase-negative. Decomposes gelatin (API 20NE), casein, elastin, DNA and Tween 20, but not Tweens 40, 60 or 80, chitin, starch or CM-cellulose. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and cystine arylamidase activities are present, as are weak lipase (C14), naphthol-AS-BI-phosphohydrolase and acid phosphatase activities. Trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities are absent (API ZYM). Acid is not produced from any of the carbohydrates in API 50 CHB kits. Does not reduce nitrate to nitrite. Susceptible to carbenicillin and benzylpenicillin, but not to ampicillin, gentamicin, kanamycin, lincomycin, neomycin, oleandomycin, polymyxin B, streptomycin or tetracycline. The DNA G+C content of the type strain is 48.7 mol%. The detailed fatty acid composition of the type strain is given in Table 2.

The type strain, JC2436^T (=IMSNU 14137^T =KACC 13727^T =KCTC 22361^T =JCM 15496^T), was isolated from tidal-flat sediment of Oi Island, Korea.

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