Umboniibacter marinipuniceus gen. nov., sp. nov., a marine gammaproteobacterium isolated from the mollusc *Umbonium costatum* from the Sea of Japan

Lyudmila A. Romanenko,¹ Naoto Tanaka² and Galina M. Frolova¹

Correspondence

Lyudmila A. Romanenko
lro@piboc.dvo.ru

¹Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch, Russian Academy of Sciences, 690022 Vladivostok, Prospect 100 Let Vladivostoku, 159, Russia

²NODAI Culture Collection Center, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

Two bacterial strains, KMM 3891^T and KMM 3892, were isolated from internal tissues of the marine mollusc *Umbonium costatum* collected from the Sea of Japan. The novel isolates were Gram-negative, aerobic, faint pink–reddish-pigmented, rod-shaped, non-motile, stenohaline and psychrotolerant bacteria that were unable to degrade most tested complex polysaccharides. Polar lipids consisted of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. Fatty acid analysis revealed C_{17:1}ω6c, C_{17:0}, C_{16:0} and C_{16:1}ω7c as the dominant components. The major isoprenoid quinone was Q-7. The DNA G+C content of strain KMM 3891^T was 51.7 mol%. According to phylogenetic analysis of 16S rRNA gene sequences, strains KMM 3891^T and KMM 3892 were positioned within the *Gammaproteobacteria* as a separate branch, sharing <93% sequence similarity to their phylogenetic relatives including *Saccharophagus degradans*, *Microbulbifer* species, *Endozoicomonas elysicola*, *Simiduia agarivorans* and *Teredinibacter turnerae*. Based on phenotypic characterization and phylogenetic distance, the novel marine isolates KMM 3891^T and KMM 3892 represent a new genus and species, for which the name *Umboniibacter marinipuniceus* gen. nov., sp. nov. is proposed. The type strain of *Umboniibacter marinipuniceus* is KMM 3891^T (=NRIC 0753^T =JCM 15738^T).

The class *Gammaproteobacteria* includes a large cluster of halophilic, carbohydrate-degrading bacteria, including representatives of the genera *Microbulbifer* (Gonzalez *et al.*, 1997), *Saccharophagus* (Ekborg *et al.*, 2005), *Teredinibacter* (Distel *et al.*, 2002), *Endozoicomonas* (Kurahashi & Yokota, 2007) and *Simiduia* (Shieh *et al.*, 2008), which have been described from marine or saline environments. At the time of writing, the genus *Microbulbifer* includes eight species, *Microbulbifer hydrolyticus*, the type species of the genus (Gonzalez *et al.*, 1997), *M. salipaludis* (Yoon *et al.*, 2003a), *M. elongatus* (Yoon *et al.*, 2003b), *M. maritimus* (Yoon *et al.*, 2004), *M. celer* (Yoon *et al.*, 2007), *M. halophilus* (Tang *et al.*, 2008), *M. agarilyticus* and *M. thermotolerans* (Miyazaki *et al.*, 2008), which have been recovered from deep and intertidal sediments, seawater, solar salterns and saline soil. The carbohydrate-degrading bacterial group also includes

bacteria that are associated with marine biota, such as *Saccharophagus degradans* (Gonzalez & Weiner, 2000; Ekborg *et al.*, 2005), isolated from the salt-marsh grass *Spartina alterniflora*, and *Endozoicomonas elysicola* (Kurahashi & Yokota, 2007), isolated from a marine mollusc. *Teredinibacter turnerae* (Distel *et al.*, 2002) represents endosymbiotic micro-organisms that were retrieved from the gill tissue of a wood-boring mollusc, *Lyrodus pedicellatus* (Teredinidae), and are characterized by the ability to grow with cellulose as a sole carbon source and to fix nitrogen. The genus *Cellvibrio* is distantly related to the *Microbulbifer*/*Saccharophagus* group and includes *Cellvibrio mixtus*, the type species of the genus (containing the subspecies *C. mixtus* subsp. *dextranolyticus* and *C. mixtus* subsp. *mixtus*; Blackall *et al.*, 1985), *C. fibrivorans*, *C. gandavensis*, *C. ostraviensis* (Mergaert *et al.*, 2003), *C. fulvus*, *C. vulgaris* and *C. japonicus* (Humphry *et al.*, 2003). Members of the genus *Cellvibrio* have been reported to be soil inhabitants and are known degraders of cellulose, dextran, chitin, starch or amylose.

In the course of studying the biodiversity of micro-organisms associated with marine invertebrates, strains KMM 3891^T and KMM 3892 were isolated from an internal tissue of the sand snail *Umbonium costatum*

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 3891^T and KMM 3892 are AB467279 and AB467280.

Results of 2D TLC of polar lipids of strain KMM 3891^T and a 16S rRNA gene sequence-based maximum-likelihood tree are available as supplementary material with the online version of this paper.

(Gastropoda, Trochidae) collected from sediment offshore from the Russian coast of the Sea of Japan. The novel isolates were Gram-negative, aerobic, faint pink–reddish-pigmented, rod-shaped, non-motile, stenohaline and psychrotolerant bacteria that were unable to degrade most tested complex polysaccharides. Phylogenetic analysis based on 16S rRNA gene sequences placed the novel strains amongst the carbohydrate-degrading marine bacteria in the *Gammaproteobacteria* as a separate branch, and 16S rRNA gene sequence similarity to their closest relatives, *Microbulbifer* species, *E. elysicola*, *Simiduia agarivorans* and *T. turnerae*, did not exceed 93 %. On the basis of distinctive phenotypic characteristics and phylogenetic distance, a novel genus and species are described to accommodate the strains.

Strains KMM 3891^T and KMM 3892 were isolated as described previously (Romanenko *et al.*, 2004, 2007). They were grown aerobically on marine 2216 agar (MA) or marine broth (MB) at 25–28 °C and stored at –80 °C in liquid MB supplemented with 30 % (v/v) glycerol. Motility was observed by the hanging-drop method as described by Gerhardt *et al.* (1994). Gram staining, oxidase and catalase and hydrolytic reactions for gelatin, casein, chitin, CM-cellulose, DNA and Tweens 20, 40 and 80 were tested according to the standard methods described by Smibert & Krieg (1994). Hydrolysis of starch was determined after 2 days of incubation on MA containing 0.2 % (w/v) soluble starch by flooding the plates with 1 % (w/v) iodine solution. Formation of H₂S from thiosulfate was tested using a lead acetate paper strip. Acid production from carbohydrates was examined using the oxidation/fermentation medium of Leifson (1963) for marine bacteria. The ability of the strains to grow in the presence of organic substrates as sole carbon and energy sources was tested for 3 weeks on artificial seawater (ASW)-based medium supplemented with 0.2 g NH₄Cl and 0.05 g yeast extract l⁻¹ and 0.4 % carbon source. Growth was considered as negative if it was equal to or less than that in the negative control to which any carbon source had not been added. The ASW contained (l⁻¹) 30 g NaCl, 4.9 g MgCl₂, 3.9 g Na₂SO₄, 1.1 g CaCl₂, 0.66 g KCl, 0.2 g NaHCO₃, 0.096 g KBr, 0.026 g H₃BO₃, 0.024 g SrCl₂ and 0.003 g NaF in distilled water. The ability of the strains to grow without additional organic growth factors and the requirement for sodium ions were respectively tested on the basal medium of Baumann & Baumann (1981) supplemented with 0.1 % glycerol, 0.1 % potassium acetate and 0.1 % potassium succinate and on basal medium in which sodium salts had been replaced by equimolar amounts of potassium salts. Requirement for and tolerance of NaCl was tested on ASW-based medium using various concentrations of NaCl in the range 0–20 %, supplemented with (l⁻¹) 10.0 g Bacto peptone, 2.0 g yeast extract, 0.028 g FeSO₄ and 15.0 g agar. Growth at different temperatures and pH and antibiotic resistance were studied as described previously (Romanenko *et al.*, 2004, 2007). In addition, biochemical tests were carried out using API ZYM test kits

(bioMérieux) according to the manufacturer's instructions, except that the cultures were suspended in ASW. For polar lipid and fatty acid analyses, strains KMM 3891^T and KMM 3892 were cultivated on MA at 28 °C for 3 days and lipids were extracted using the chloroform/methanol extraction method of Bligh & Dyer (1959). Polar lipids were analysed as described by Vaskovsky & Terekhova (1979). Fatty acid methyl esters were obtained by alkaline methanolysis (15 % NaOH/methanol). The resultant fatty acid methyl esters were extracted with hexane and analysed using a GLC-MS Hewlett Packard model 6890 gas chromatograph equipped with an HP 5 MS 5 % phenyl methyl siloxane capillary column (30 m × 250 µm × 0.25 µm) and connected to a Hewlett Packard model 5973 mass spectrometer. The pigments were extracted from cellular biomass using chloroform/methanol (2:1, v/v) following hexane extraction. The visible spectra of extracts were analysed with a CECIL 7250 spectrophotometer. Cells of strains KMM 3891^T and KMM 3892 for respiratory lipoquinone analysis were obtained from cultures grown in MB supplemented with casein hydrolysate at 25 °C. Isoprenoid quinones were extracted using chloroform/methanol (2:1, v/v), purified by preparative TLC on silica gel 60 ADAMANT plates (Fluka) and analysed by HPLC (Agilent 1100 Series) using a reversed-phase column (Hepersil ODS, 5 µm; 40 × 250 mm). Methanol/isopropanol (65:35) was used as the mobile phase and quinones were detected by monitoring the absorbance at 270 nm. The DNA base composition was determined as described by Marmur & Doty (1962) and Owen *et al.* (1969). The photobiotin-labelled DNA probe microplate method of Ezaki *et al.* (1989) was used to determine DNA relatedness between strains KMM 3891^T and KMM 3892. The 16S rRNA gene sequences of strains KMM 3891^T and KMM 3892, containing 1508 and 1502 nt, respectively, were determined as described by Shida *et al.* (1997). The sequences obtained were compared with 16S rRNA gene sequences retrieved from the EMBL/GenBank/DDBJ databases by using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis of 16S rRNA gene sequences was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Phylogenetic trees were constructed by the neighbour-joining and maximum-parsimony methods and distances were calculated according to Kimura's two-parameter model. The robustness of phylogenetic trees was estimated by bootstrap analysis of 1000 replicates.

Cultural, physiological and metabolic properties are listed in Table 1 and in the genus and species descriptions. Strains KMM 3891^T and KMM 3892 were similar in their phenotypic characteristics (except for differences in sensitivity to antibiotics and pigment absorption peaks) and their chemotaxonomic characteristics. The polar lipid profiles were identical and included phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (Supplementary Fig. S1, available in IJSEM Online). The

Table 1. Differential characteristics for strains KMM 3891^T and KMM 3892 and members of related genera of the *Gammaproteobacteria*

Species: 1, *Umboniibacter marinipunicus* gen. nov., sp. nov. (strains KMM 3891^T and KMM 3892; data from this study); 2, *E. elysicola* (Kurahashi & Yokota, 2007); 3, *Simiduia agarivorans* (Shieh *et al.*, 2008); 4, *Saccharophagus degradans* (Gonzalez & Weiner, 2000; Ekborg *et al.*, 2005); 5, *T. turnerae* (Distel *et al.*, 2002); 6, *C. japonicus* (Humphry *et al.*, 2003); 7, *M. agarilyticus* (Miyazaki *et al.*, 2008); 8, *M. celer* (Yoon *et al.*, 2007); 9, *M. elongatus* (Yoon *et al.*, 2003b); 10, *M. halophilus* (Tang *et al.*, 2008); 11, *M. hydrolyticus* (Gonzalez *et al.*, 1997); 12, *M. maritimus* (Yoon *et al.*, 2004); 13, *M. salipaludis* (Yoon *et al.*, 2003a); 14, *M. thermotolerans* (Miyazaki *et al.*, 2008). +, Positive; –, negative; w, weak reaction; s, slow reaction; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Colony colour*	PR	BG	OW/C	BB	T/YB†	C	C	GY	YB	YB	C	YB	GY	B
Motility	–	+	+‡	+	+	+	–	–	+	+	–	–	–	+
Growth at 4 °C	–	+	–	+	–	–	ND	–	ND	–	–	–	–	ND
Maximal growth temperature (°C)	33	36	40	37	35	47	35–38	48	38	45	41	48	45	52–54
NaCl range for growth (%)	2–5	ND	0.5–7	1–10	0.6–3.6	ND	ND	ND	ND	1–20	0.6–6	ND	ND	ND
Tolerance of 10 % NaCl	–	ND	–	+	–	–	–	+	ND	+	–	+	+	–
Nitrate reduction	–	+	+	+	ND	+	+	–	–	–	–	–	ND	+
Acid from sugars	w	+	ND	+	+	+	+	+	+	+	–	+	+	+
Hydrolysis of:														
Gelatin	+	–	+	–	ND	–	+	–	+	+	+	+	ND	+
Casein	s	ND	+	ND	ND	ND	+	+	ND	–	ND	+	w	+
Tween 80	+	ND	–	+	ND	–	+	+	ND	–	+	+	+	+
Chitin	–	ND	+	+	ND	+	+	ND	+	ND	+	–	–	+
Agar	–	ND	+	+	–	ND	+	–	+	ND	–	–	w	+
Cellulose	–	ND	+	+	+	+	ND	ND	ND	ND	+	ND	ND	ND
Starch	w	ND	+	+	ND	ND	+	+	ND	–	+	+	+	+
Aesculin	–	–	+	ND	ND	+	–	+	ND	–	ND	–	+	+
Tyrosine	–	ND	ND	+	ND	ND	ND	–	ND	ND	ND	+	w	ND
Main fatty acids§	C _{17:1} ω6c, C _{17:0} , C _{16:0} , C _{16:1} ω7c	C _{16:1} ω7c, C _{16:0} , C _{14:0}	C _{16:1} ω7c/i-C _{15:0} , 2-OH, C _{16:0} , C _{17:1} ω8c, C _{18:1} ω7c	C _{16:0} , C _{14:0}	ND	C _{16:1} , C _{18:1} , C _{16:0}	i-C _{15:0} , i-C _{17:1} , C _{18:1}	i-C _{15:0} , C _{16:0} , i-C _{17:0}	i-C _{15:0} , i-C _{17:1} ω9c, C _{18:1} ω7c	i-C _{15:0} , i-C _{17:0} , C _{16:0} , i-C _{17:1} ω9c	i-C _{15:0} , C _{16:0}	i-C _{15:0} , i-C _{11:0} , 3-OH, i-C _{17:1} ω9c	i-C _{15:0} , C _{16:0}	i-C _{15:0} , i-C _{16:0} , i-C _{17:1} , C _{16:1} , C _{18:1}
DNA G + C content (mol%)	51.7	50.4	55.6	45.8	49–51	53.3	55.2–55.3	57.7	ND	63.2	57.7	59.9	59	56.0–56.2

*B, Brown; BB, black–brown; BG, beige; C, cream; GY, greyish yellow; OW, off-white; PR, pink–reddish; T, translucent; YB, yellowish brown.

†Pigmentation was reported to be age dependent (Distel *et al.*, 2002).

‡Motile cells were rarely observed (Shieh *et al.*, 2008).

§i, Iso-branched.

presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol in the polar lipid composition is in line with profiles reported for *Cellvibrio* species (Humphry *et al.*, 2003), *Simiduia agarivorans* (Shieh *et al.*, 2008) and other marine gammaproteobacteria (Romanenko *et al.*, 2003, 2004, 2005). Analysis of the respiratory lipoquinones revealed ubiquinone Q-7 as a major compound and trace amounts of Q-8 in both strains. Ubiquinone Q-7 alone or with Q-8 has been reported to be a major component for some *Shewanella* species (Venkateswaran *et al.*, 1999). The novel strains were distinguished from their phylogenetic relatives in the predominance of ubiquinone Q-7, as *E. elysicola* contains the major ubiquinone Q-9 (Kurahashi & Yokota, 2007), *Simiduia agarivorans* contains Q-10 together with MK-9 and MK-7 (Shieh *et al.*, 2008) and *M. elongatus*, *M. salipaludis*, *M. maritimus*, *M. halophilus*, *M. agarilyticus* and *M. thermotolerans* contain Q-8 as the major ubiquinone (Yoon *et al.*, 2003a, b, 2004; Tang *et al.*, 2008; Miyazaki *et al.*, 2008).

The fatty acid profiles of strains KMM 3891^T and KMM 3892 contained C_{11:0} (1.2 and 1.5 %, respectively), C_{12:0} (4.0 and 5.7 %), C_{13:0} (4.8 and 5.8 %), C_{14:0} (4.2 and 4.8 %), C_{15:0} (4.4 and 5.2 %), C_{16:0} (14.9 and 14.0 %), C_{17:0} (13.9 and 10.9 %), C_{18:0} (2.5 and 1.4 %), C_{10:0} 3-OH (1.7 and 2.3 %), C_{11:0} 3-OH (6.0 and 4.7 %), C_{12:0} 3-OH (3.1 and 2.3 %), C_{15:1} (1.0 and 0.6 %), C_{16:1}ω7c (11.3 and 11.6 %), C_{17:1}ω6c (21.6 and 14.1 %) and C_{18:1}ω9 (4.0 and 5.3 %). The fatty acid compositions of strains KMM 3892 and KMM 3891^T are characterized by the presence of large amounts of C_{17:1}ω6c, C_{17:0}, C_{16:0} and C_{16:1}ω7c (50.6 and 61.7 % of the total, respectively) and the presence of the hydroxy fatty acids C_{10:0} 3-OH, C_{11:0} 3-OH and C_{12:0} 3-OH (9.3 and 10.6 %, respectively). It should be noted that the predominant fatty acids C_{17:1}ω6c, C_{17:0}, C_{16:0} and C_{16:1}ω7c were each present in approximately equal amounts in both strains. As shown in Table 1, the fatty acid profiles of the novel bacteria differed substantially from those of *Microbulbifer* species in the absence of iso-C_{15:0}, iso-C_{17:0} and iso-C_{17:1} (Gonzalez *et al.*, 1997; Yoon *et al.*, 2003a, b, 2004, 2007; Miyazaki *et al.*, 2008; Tang *et al.*, 2008), from that of *Saccharophagus degradans* in the absence of C_{12:1} 3-OH and C_{10:0} (Gonzalez & Weiner, 2000) and from that of *E. elysicola* in the absence of C_{14:1} 3-OH and in the presence of C_{17:1}ω6c and C_{17:0} (Kurahashi & Yokota, 2007). The fatty acid profiles of strains KMM 3892 and KMM 3891^T could be distinguished from that of *C. japonicus* (Humphry *et al.*, 2003) in the minor content of C_{18:1}ω9 and in the significant amount of C_{17:0}. The fatty acid profiles of strains KMM 3892 and KMM 3891^T were similar to that of *Simiduia agarivorans* (Shieh *et al.*, 2008) in their major components, but differed in the presence of C_{11:0}, C_{12:0}, C_{11:0} 3-OH, C_{13:0} and C_{15:0}.

Polar lipids, ubiquinones and fatty acids have not been reported for *T. turnerae* (Distel *et al.*, 2002).

It is clear that the combination of C_{17:1}ω6c, C_{17:0}, C_{16:0} and C_{16:1}ω7c as predominant fatty acids and the presence of hydroxy fatty acids makes the fatty acid compositions of the novel isolates KMM 3892 and KMM 3891^T unique compared with those of their phylogenetic relatives, including *Microbulbifer* species, *Saccharophagus degradans*, *E. elysicola*, *Simiduia agarivorans* and *C. japonicus*.

The DNA G+C content of 51.7 mol% determined for strain KMM 3891^T clearly distinguished it from the members of the genus *Microbulbifer* (57.7–63.2 mol%). This value is slightly higher than the G+C content reported for *Saccharophagus degradans* (45.8 mol%) but is lower than the values reported for *C. japonicus* (53.3 mol%) and *Simiduia agarivorans* (55.6 mol%); it falls within the range of G+C contents found for *T. turnerae* (49–51 mol%) and *Endozoicomonas elysicola* (50.4 mol%) (Table 1). DNA–DNA relatedness of 90 % was obtained for strains KMM 3891^T and KMM 3892, indicating their affiliation to the same species in accordance with the cut-off value of 70 % recognized by Wayne *et al.* (1987) for the purpose of bacterial species discrimination.

Phylogenetic analysis based on 16S rRNA gene sequences showed that strains KMM 3891^T and KMM 3892 formed a separate branch within the *Gammaproteobacteria* adjacent to *E. elysicola* (Kurahashi & Yokota, 2007) (Fig. 1 and Supplementary Fig. S2). Their closest phylogenetic relatives were found to be the carbohydrate-degrading marine bacteria *Saccharophagus degradans* (92.7 % sequence similarity to the type strain), *M. salipaludis* (92.3 %), *M. hydrolyticus* (92.1 %), *M. agarilyticus* (92.0 %), *M. elongatus* (91.6 %), *M. celer* (91.4 %), *M. maritimus*, *M. halophilus*, *M. thermotolerans* (all 91.2 %), *C. japonicus* (91.1 %), *E. elysicola* (91.0 %), *T. turnerae* (90.9 %) and *Simiduia agarivorans* (90.4 %). It is interesting to note that the novel mollusc isolates clustered with *E. elysicola* MKT110^T, which was isolated from the gastrointestinal tract of the sea slug *Elysia ornata*, collected in seawater off Izu-Miyake Island, Japan, at a depth of 15 m (Kurahashi & Yokota, 2007). The ability to decompose complex polysaccharides has been not reported for *E. elysicola* (Kurahashi & Yokota, 2007). Strains KMM 3891^T and KMM 3892 are likely to be very tightly associated with their host mollusc *Umbonium costatum*, as they displayed characteristic physiological and metabolic properties and a unique phylogenetic position. In addition, we failed to detect the same or similar bacteria in the surrounding seawater or sediment samples.

The novel strains are phylogenetically distantly related to *E. elysicola*, sharing only 91.0 % 16S rRNA gene sequence similarity, and can be distinguished by a combination of phenotypic and chemotaxonomic traits (Table 1). The isolated phylogenetic position of strains KMM 3891^T and KMM 3892 is supported by their unique physiological and biochemical properties. The isolates were Gram-negative, aerobic, non-fermentative, heterotrophic, halophilic, stenohaline, faint pink–reddish-pigmented, non-motile, rod-shaped bacteria. They could be distinguished from their

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences available from the GenBank/EMBL/DBJ databases (accession numbers in parentheses) showing the relationships of isolates KMM 3891^T and KMM 3892 and related members of the *Gammaproteobacteria*. Phylogenetic analysis was performed using the software package MEGA 4 (Tamura *et al.*, 2007) after multiple alignment of the data by CLUSTAL_X (version 1.83; Thompson *et al.*, 1997). Bootstrap values based on 1000 replications are given as percentages at branching points; only values greater than 90% are shown. Bar, 0.02 substitutions per nucleotide position.

close relatives belonging to the genera *Saccharophagus*, *Microbulifer*, *Teredinibacter*, *Endozoicomonas* and *Simidiua* and *C. japonicus* in their minimal and maximal growth temperatures, salinity range for growth and metabolic properties and in not being able to degrade most tested polysaccharides, including agar, cellulose and chitin (Table 1). The strains exhibited only weak amylolytic activity. Starch hydrolysis determined by plating on agar medium was recorded as weak because the clearance zones that formed around bacterial spots were barely visible. Strains KMM 3891^T and KMM 3892 produced proteolytic enzymes in routine and API ZYM tests as displayed in Table 1 and in the species description. Characteristics that differentiate strains KMM 3891^T and KMM 3892 from related gamma-proteobacteria are listed in Table 1.

On the basis of phenotypic and phylogenetic evidence, strains KMM 3891^T and KMM 3892 could not be assigned to any known species or genus within the *Gammaproteobacteria*; we therefore propose to classify strains KMM 3891^T and KMM 3892 within a novel genus and species, as *Umboniibacter marinipuniceus* gen. nov., sp. nov.

Description of *Umboniibacter* gen. nov.

Umboniibacter (Um.bo.ni.i.bac'ter. N.L. n. *Umbonium* scientific name of a genus of marine mollusc; N.L. masc. n. *bacter* rod; N.L. masc. n. *Umboniibacter* a rod from *Umbonium*, referring to the isolation of the first strains from the sand snail *U. costatum*).

Gram-negative, aerobic, oxidase-positive, weakly catalase-positive, rod-shaped bacteria. Chemo-organoheterotrophic. Sodium ions are essential for growth. The predominant isoprenoid quinone is Q-7. Polar lipids include phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major fatty acids are C_{17:1}ω6c, C_{17:0}, C_{16:0} and C_{16:1}ω7c. The DNA G + C content of the type strain of the type species is 51.7 mol% (thermal denaturation

method). On the basis of 16S rRNA gene sequence analysis, the genus represents a separate branch within the *Gammaproteobacteria*, related to the genera *Endozoicomonas*, *Simidiua* and *Microbulifer*. Known strains have been isolated from the marine environment. The type species of the genus is *Umboniibacter marinipuniceus*.

Description of *Umboniibacter marinipuniceus* sp. nov.

Umboniibacter marinipuniceus (ma.ri.ni.pu.ni'ce.us. L. masc. adj. *marinus* marine; L. masc. adj. *puniceus* purple, red; N.L. masc. adj. *marinipuniceus* marine and red).

In addition to the properties given in the genus description, the species is characterized as follows. Cells are 0.4–0.6 μm in diameter and 1.5–2.5 μm long. Non-motile. Colonies on MA are faint pink–reddish pigmented, transparent, smooth and shiny with regular edges, 2–3 mm in diameter. Abundant growth is observed on/in MA or MB supplemented with casein hydrolysate and media containing natural or artificial seawater supplemented with 0.5% (w/v) peptone, tryptone or meat hydrolysate. No growth on basal medium supplemented with 0.1% glycerol, 0.1% potassium acetate and 0.1% potassium succinate or on basal medium when sodium salts are replaced by equimolar amounts of potassium salts. Weak growth when D-glucose or a mixture of amino acids (L-alanine, L-asparagine, L-arginine, L-phenylalanine, L-lysine, L-leucine, L-valine and L-histidine) is added. No growth on TSA, nutrient agar or R2A. Requires NaCl for growth; growth occurs at 2–5% NaCl (w/v) (optimum 2.5–3%). Weak growth in 2 and 5% NaCl. Grows well in/on peptone medium containing NaCl alone without addition of any of the components of sea salts (MgCl₂, KCl, CaCl₂, NaNO₃, K₂HPO₄, KCl, NaSO₄, NaHCO₃, NaF or FeSO₄). Psychrotolerant; temperature range for growth is 5–33 °C with optimum growth at 25–28 °C. Weak or no growth at 4 °C, slow growth at 5 °C and no growth above

33 °C. Grows at pH 6.5–10.0, with optimum growth at pH 8.0–8.5. Negative for H₂S production. Positive for hydrolysis of gelatin and Tweens 20, 40 and 80. Casein is hydrolysed within 3–5 days. Starch is hydrolysed weakly; clearance zones around bacterial spots on starch-containing agar are barely visible. Does not degrade CM-cellulose, agar, chitin or DNA. On L-tyrosine-containing medium, does not produce melanin-like pigments and/or clearance zones. Strain-dependent weak acid production is observed from D-glucose and maltose; the type strain is positive for D-glucose and negative for maltose. No acid formed from sucrose, lactose, D-galactose, D-mannose, cellobiose, D-xylose, L-arabinose, L-rhamnose, D-sorbitol or D-mannitol. In API 20NE tests, positive for gelatin hydrolysis and the PNPG test and negative for nitrate reduction, indole production, glucose acidification, arginine dihydrolase, urease production, aesculin hydrolysis and assimilation of substrates that are included in the API 20NE strip. Weak growth on D-glucose, maltose, sucrose, D-mannitol, citrate, L-alanine, L-asparagine, L-arginine, L-phenylalanine, L-valine and L-lysine in routine tests. No growth on D-xylose, D-galactose, N-acetylglucosamine, lactose, melibiose, raffinose, L-rhamnose, L-arabinose, D-ribose, D-mannose, cellobiose, glycerol, acetate, glutamic acid or DL-methionine. In API ZYM tests, positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl- β -glucosaminidase and negative for lipase (C14), trypsin, α -galactosidase, β -galactosidase, α -glucosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase. Detailed fatty acid profiles are given in the text; major fatty acids are as given in the genus description. The pigments are characterized by absorption peaks at 390 and 455 nm with a shoulder at 427 nm for strain KMM 3891^T and an absorption peak at 407 nm only for strain KMM 3892. Susceptible to (per disc) kanamycin (30 µg), nalidixic acid (30 µg), neomycin (30 µg), ofloxacin (5 µg), polymyxin (300 U), erythromycin (15 µg) and chloramphenicol (30 µg) and resistant to benzylpenicillin (10 U), carbenicillin (100 µg), oxacillin (10 µg), tetracycline (30 µg), cephalosin (30 µg) and cephalixin (30 µg). Susceptibility to ampicillin (10 µg), gentamicin (10 µg), lincomycin (15 µg), oleandomycin (15 µg), rifampicin (5 µg) and streptomycin (30 µg) is strain-dependent: KMM 3891^T is susceptible and KMM 3892 is resistant. Strain KMM 3892 is susceptible to vancomycin (30 µg), whereas KMM 3891^T is resistant.

The type strain, KMM 3891^T (=NRIC 0753^T =JCM 15738^T), and reference strain KMM 3892 were isolated from the marine mollusc *Umbonium costatum* (Gastropoda, Trochidae), collected from the southern coast of the Sea of Japan, Russia.

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