Pelagicoccus mobilis gen. nov., sp. nov., *Pelagicoccus albus* sp. nov. and *Pelagicoccus litoralis* sp. nov., three novel members of subdivision 4 within the phylum 'Verrucomicrobia', isolated from seawater by *in situ* cultivation

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Five Gram-negative, white-pigmented, spherical, chemoheterotrophic bacteria were isolated from seawater from Japan and the Republic of Palau by use of an *in situ* cultivation technique. Phylogenetic analyses based on 16S rRNA gene sequences indicated that the five novel isolates, 02PA-Ca-133^T, YM14-201^T, H-MN57^T, H-MN48 and MN1-156, were closely affiliated to members of subdivision 4 within the phylum 'Verrucomicrobia'. The novel isolates shared 96–100 % sequence similarity with each other and showed less than 90 % similarity with the cultivated strains of subdivision 4. DNA–DNA relatedness values between strains 02PA-Ca-133^T, YM14-201^T and H-MN57^T were less than 70 %; the value commonly accepted as the threshold for the phylogenetic definition of a species. Antibiotic susceptibility tests and amino acid analysis of cell-wall hydrolysates indicated that the novel isolates did not contain muramic acid or diaminopimelic acid in their cell walls, suggesting that these strains lack peptidoglycan. The DNA G+C contents of the five strains were 51–57 mol%. The major menaquinone was MK-7 and C_{16:0}, C_{16:1}ω7c and anteiso-C_{15:0} were the major fatty acids. On the basis of polyphasic taxonomic evidence, it is concluded that these strains should be classified as representing a new genus and three novel species in subdivision 4 of the phylum 'Verrucomicrobia', for which the names *Pelagicoccus mobilis* gen. nov., sp. nov. [type strain 02PA-Ca-133^T (=MBIC08004^T=IAM 15422^T=KCTC 13126^T)], *Pelagicoccus albus* sp. nov. [type strain YM14-201^T (=MBIC08272^T=IAM 15421^T=KCTC 13124^T)] and *Pelagicoccus litoralis* sp. nov. [type strain H-MN57^T (=MBIC08273^T=IAM 15423^T=KCTC 13125^T)] are proposed.

The phylum 'Verrucomicrobia' (Hedlund *et al.*, 1997; Hugenholtz *et al.*, 1998) is one of the primary lineages of the domain *Bacteria*. At present, these lineages comprise

six informal monophyletic subdivisions (Hugenholtz *et al.*, 1998; Vandekerckhove *et al.*, 2000) of which three of the subdivisions are recognized in the second edition of *Bergey's Manual of Systematic Bacteriology* as the families *Verrucomicrobiaceae* (subdivision 1), *Opitutaceae* (subdivision 4) and *Xiphinematobacteriaceae* (subdivision 2). Based on verrucomicrobial 16S rRNA gene sequences and other molecular phylogenetic studies, members of the phylum 'Verrucomicrobia' have been detected in a very wide range of quite different habitats within the global ecosystem (Hugenholtz *et al.*, 1998; Joseph *et al.*, 2003; Rappé *et al.*, 2003; Kanokratana *et al.*, 2004; Haukka *et al.*, 2005, 2006; Dedysh *et al.*, 2006). It has been estimated that members of the phylum 'Verrucomicrobia' comprise up to 10 % of the total bacteria in soil and contribute up to 9.8 % of the bacterial 16S rRNA in this system (Sangwan *et al.*, 2004).

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Abbreviations: ONPG, o-nitrophenol β-D-galactosidase; PUF, polyurethane foam; TEM, transmission electron microscopy.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains 02PA-Ca-133^T, YM14-201^T and H-MN57^T are AB286015, AB286016 and AB286017, respectively.

Details of the composition of the 'P' medium used in the paper are available as supplementary material with the online version of this paper.

Furthermore, it has also been reported that members of the phylum 'Verrucomicrobia' can thrive in marine environments (Polymenakou *et al.*, 2005). However, in spite of their wide ecological distribution in nature, so far there are few cultivated representatives of the phylum and most of them have yet to be cultured and described (Sakai *et al.*, 2003; Scheuermayer *et al.*, 2006).

Strain 02PA-Ca-133^T was isolated from seawater collected during December 2002 from a coral reef located in the Republic of Palau (GPS location, 7° 20' 4.5" N 134° 29' 47.5" E) at a depth of 5 m. Organisms present in the seawater samples were isolated using marine broth 2216 (Difco) supplemented with 1 % (w/v) CaCO₃ and 1.5 % (w/v) agar. Strains YM14-201^T, H-MN57^T, H-MN48 and MN1-156 were isolated by using an *in situ* cultivation technique (Yasumoto-Hirose *et al.*, 2006). Strain YM14-201^T was isolated from an artificial polyurethane foam (PUF) block supplemented with sterile seawater containing 0.1 % (w/v) 7-hydroxyflavone and 1.5 % (w/v) agar. The PUF block was placed in the sea (GPS location, 7° 20' 4.5" N 134° 29' 47.5" E, 5 m depth; Republic of Palau) for 3 days during September 2004. Strains H-MN57^T, H-MN48 and MN1-156 were isolated from PUF blocks supplemented with HV agar (Hayakawa & Nonomura, 1987) in 90 % seawater containing 0.1 % (w/v) collidine or 0.1 % (w/v) vanillin. The blocks were moored for 2 weeks at stations in Heita Bay, Kamaishi, Japan, in May 2005. After two weeks, pieces of the PUF blocks (0.5–1 cm³) were homogenized with a glass rod in 5 ml of sterile seawater. A 50–100 µl sample of the homogenate was applied to the surface of an agar isolation medium. Strain YM14-201^T appeared after 30 days incubation at 25 °C on 'P' medium (the composition of 'P' medium is provided as Supplementary Material in IJSEM Online). Strains H-MN57^T, H-MN48 and MN1-156 appeared as tiny white colonies after incubation for 10, 20 and 90 days, respectively, at 25 °C on HV medium containing 90 % seawater with 40 µg ml⁻¹ of nalidixic acid and 100 µg ml⁻¹ of cycloheximide. In the present study, we attempted to determine the phylogenetic position of strains 02PA-Ca-133^T, YM14-201^T, H-MN57^T, H-MN48 and MN1-156 using a polyphasic taxonomic approach, including 16S rRNA gene sequence analysis. In addition, we performed physiological, biochemical and chemotaxonomic analyses to characterize the five novel isolates. Based on these data, it is proposed that the isolates represent a new genus and three novel species within the phylum 'Verrucomicrobia'.

The temperature range and pH range for growth were determined by incubating the isolates on marine agar 2216 (Difco) and 1/2 strength R2A agar (Difco) containing 75 % artificial seawater (Lyman & Fleming, 1940). The NaCl concentration for growth was determined on marine agar 2216 and 1/2 strength R2A agar containing 0–10 % (w/v) NaCl. Gram-staining was performed as described by Murray *et al.* (1994). Cell morphology was observed using light microscopy (BX60; Olympus) and transmission electron microscopy (TEM). For TEM observations, cells were mounted on

Formvar-coated copper grids and negatively stained with 2 % (w/v) aqueous uranyl acetate. Grids were observed using a model H-7000 TEM (Hitachi) operated at 75 kV. In the course of TEM, various cell sizes and shapes were observed. Cells of strain 02PA-Ca-133^T grown on marine agar 2216 were coccoid and mostly 0.5–0.7 µm in diameter. The cells bore single (Fig. 1a) or multiple (Fig. 1b) flagella. Motility was observed by light microscopy. Cells divided by means of binary fission and appendages were observed on some cells (Fig. 1c). However, cells of strains YM14-201^T and H-MN57^T grown on 1/2 strength R2A agar with 75 % artificial seawater were coccoid and mostly 0.8–1.2 µm and 1.0–1.2 µm in diameter, respectively. Cells of these two strains divided by means of binary fission, but cells were non-motile and no flagella or appendages were observed (Fig. 1d, e).

Growth under anaerobic conditions was determined after 2 weeks of incubation in an AnaeroPack (Mitsubishi Gas Chemical Co., Inc.) on marine agar 2216 and 1/2 strength R2A agar with 75 % artificial seawater. Catalase activity was determined by the observation of bubble formation in a 3 % H₂O₂ solution. Oxidase activity was determined by using cytochrome oxidase paper (Nissui Pharmaceutical Co., Ltd). API 20E, API 50CH and API ZYM strips (bioMérieux) were used to determine physiological and biochemical characteristics. The API 20E and API 50CH tests were read after 72 h incubation at 30 °C and the API ZYM tests were scored after 4 h incubation at 37 °C.

Determination of the respiratory quinone system and cellular fatty acid content were carried out as described previously (Katsuta *et al.*, 2005). DNA was prepared according to the method of Marmur (1961) from cells grown on marine agar 2216 and 1/2 strength R2A agar with 75 % artificial seawater. The DNA G+C content was determined by using the HPLC method of Mesbah *et al.* (1989). DNA–DNA hybridizations were performed with photobiotin-labelled probes in microplate wells as previously described (Ezaki *et al.*, 1989). The hybridization temperature was set at 50 °C. Hybridization was performed using five replications for each strain. Of the values obtained, the highest and lowest value for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values. The sensitivity of the five novel isolates to ampicillin and penicillin G was tested on marine agar 2216 and 1/2 strength R2A agar with 75 % artificial seawater using 8 mm paper discs (Advantec) at antibiotic concentrations of 1, 10, 100, 500 and 1000 µg ml⁻¹. Cell walls were prepared by the methods described by Schleifer & Kandler (1972) and the amino acids present in an acid hydrolysate of the cell walls were identified by TLC (Harper & Davis, 1979) and by HPLC, as their phenylthio-carbamoyl derivatives, with HPLC apparatus (model LC-10AD; Shimadzu) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries) (Yokota *et al.*, 1993).

An approximately 1500-bp fragment of the 16S rRNA gene was amplified from extracted DNA by using bacterial

Fig. 1. Transmission electron micrographs of negatively stained cells of strains 02PA-Ca-133^T (a–c), YM14-201^T (d) and H-MN57^T (e). Bars: (a–c), 500 nm; (d–e), 1 µm.

universal primers specific to the 16S rRNA gene: 27F and 1492R (Weisburg *et al.*, 1991). To ascertain the phylogenetic position of the novel isolates, the 16S rRNA gene sequences of strains 02PA-Ca-133^T, YM14-201^T, H-MN57^T, H-MN48 and MN1-156 were compared with sequences obtained from GenBank (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov>). Multiple alignments of the sequences were performed using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were not taken into consideration when the 1205 bases of the 16S rRNA gene nucleotides were compared. Phylogenetic relationships were determined by using maximum-likelihood (ML) method (Felsenstein, 1985) using the Ratchet model (Sikes & Lewis, 2001) of evolution in PAUP 4.0b10 (Swofford, 2002). A bootstrap analysis was performed by using 1000 trial replications to provide confidence estimates for tree topologies. The similarity values were calculated using MEGA3 (Kumar *et al.*, 2004).

An evolutionary tree based on the maximum-likelihood generated comparison of the 16S rRNA gene sequences revealed that strains 02PA-Ca-133^T, YM14-201^T, H-MN57^T, H-MN48 and MN1-156 form an independent clade within subdivision 4 (Fig. 2). The phylogenetic tree also showed that the five novel isolates were clustered within

subdivision 4 of the phylum ‘Verrucomicrobia’ with a bootstrap confidence value of 58.9 %. Analysis of the 16S rRNA gene sequences revealed that the sequence of strain 02PA-Ca-133^T had 98 % similarity to that of strain YM14-201^T and 96.4 % similarity to strains H-MN57^T, H-MN48 and MN1-156. Strain YM14-201^T shared 97.3 % gene sequence similarity with strains H-MN57^T, H-MN48 and MN1-156. The 16S rRNA gene sequence of strain H-MN57^T was 100 % similar to the sequences of strains H-MN48 and MN1-156. All other cultivated species of subdivision 4 with validly published names were more distantly related, showing 16S rRNA gene sequence similarities of less than 90 %.

DNA–DNA hybridization values between strain 02PA-Ca-133^T and strains YM14-201^T and H-MN57^T were 4.9 and 10.1 %, respectively. These results strongly suggest that strains 02PA-Ca-133^T, YM14-201^T and H-MN57^T should be classified as three separate species if they can also be distinguished by phenotypic traits (Wayne *et al.*, 1987). DNA–DNA relatedness values of 88–93 % between strains H-MN57^T, H-MN48 and MN1-156 indicate that these strains represent members of the same species.

As shown in Table 1, the predominant cellular fatty acids of the five novel strains were C_{16:0} (14.1–23.8 %), C_{16:1ω7c} (14.5–20.7 %) and anteiso-C_{15:0} (29.8–38.6 %).

Fig. 2. Maximum-likelihood phylogenetic tree showing the positions of strains 02PA-Ca-133^T, YM14-201^T, H-MN57^T, MN1-156 and H-MN48. The numbers at the branch nodes are bootstrap values based on 1000 replications. The sequence of *Escherichia coli* ATCC 11775^T was used as the outgroup. Bar, 0.05 substitutions per site.

Furthermore, on the basis of their fatty acid content, these five strains could be differentiated from *Coralimargarita akajimensis* 04OKA010-24^T, their closest phylogenetic taxon (Yoon *et al.*, 2007) indicating that strains 02PA-Ca-133^T, YM14-201^T, H-MN57^T, H-MN48 and MN1-156 probably represent an separate genus within subdivision 4 of the phylum '*Verrucomicrobia*'.

When the five novel isolates were grown in the presence of increasing concentrations (1–1000 µg ml⁻¹) of β-lactam antibiotics, they showed a remarkable resistance against ampicillin and penicillin G. Cell-wall samples of the novel strains were prepared by disrupting cells, followed by heating with 3 % SDS and then by washing and centrifugation. Amino acid analysis of the cell-wall hydrolysates indicated the absence of muramic acid and diaminopimelic acid in the cell wall, which suggests that the strains do not contain peptidoglycan in their cell walls.

The five novel strains also showed distinct phenotypic features that discriminated them from the cultivated members of subdivision 4 and phenotypic analysis separated the strains into three groups (Table 2); strain 02PA-Ca-133^T, strain YM14-201^T and strains H-MN57^T, H-MN48 and MN1-156. Strain 02PA-Ca-133^T is obligately aerobic with a

cell diameter of 0.5–0.7 µm, bears appendages and is motile by means of single or multiple flagella. Strain 02PA-Ca-133^T grows at 20–37 °C, with an optimum at around 30 °C, but can not grow at 4 °C. The strain hydrolyses agar, DNA and starch and produces acid from cellobiose, glucose and lactose. Positive results are obtained in tests for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase. In contrast, Strain YM14-201^T is facultatively anaerobic with a cell diameter of 0.8–1.2 µm and has no appendages and no flagella are observed. The strain grows at 15–37 °C, with an optimum around at 27 °C, but can not grow at 4 °C. DNA and aesculin are hydrolysed. Strain YM14-201^T gives a positive result in tests for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase and α-glucosidase. Strains H-MN57^T, H-MN48 and MN1-156 are obligately aerobic with a cell diameter of 1.0–1.2 µm and have no appendages or flagella. Growth of these strains takes place between 4 and 30 °C (strain H-MN48 can also grow at 37 °C), with an optimum at around 27 °C; no growth is seen at 45 °C. The strains are positive in tests for hydrolysis of ONPG (apart from strain H-MN48) and aesculin. Acid is produced from melibiose (but not in strains H-MN48 and MN1-156). Tests

Table 1. Cellular fatty acid contents of members of the genus *Pelagicoccus* gen. nov. and *Coralimargarita akajimensis* 04OKA010-24^T

Taxa: 1, strain 02PA-Ca-133^T; 2, strain YM14-201^T; 3, strain H-MN57^T; 4, strain H-MN48; 5, strain MN1-156; 6, *Coralimargarita akajimensis* 04OKA010-24^T (Yoon *et al.*, 2007). Data are expressed as percentages of total fatty acids. Fatty acids representing less than 1 % are not shown. —, Not detected; tr, trace.

Fatty acid	1	2	3	4	5	6
Saturated						
C _{13:0}	tr	—	—	—	—	—
C _{14:0}	5.8	4	4.4	3.7	4.9	24.2
C _{15:0}	5.9	1.7	4.5	5.7	5.0	1.7
C _{16:0}	23.3	23.8	14.3	15.6	14.1	3.3
C _{17:0}	2.1	tr	1.3	1.4	1.7	1.5
C _{18:0}	tr	1.2	1.4	1.3	1.7	15.6
Unsaturated						
C _{16:1} ω7c	15.1	14.5	20.7	18.5	19.7	tr
C _{18:1} ω9c	—	—	—	—	—	23.5
Branched						
iso-C _{14:0}	2.1	tr	1.1	1.2	1.3	8.2
iso-C _{16:0}	2.3	2.3	1.7	2.5	2.2	1.8
anteiso-C _{15:0}	29.8	37.5	38.1	38.6	35.1	3.0
2-Hydroxyl						
C _{13:0} 2-OH	—	5.5	5.7	6.3	6.5	—
3-Hydroxyl						
C _{12:0} 3-OH	tr	tr	tr	tr	tr	1.3
C _{16:0} 3-OH	tr	1.4	tr	—	tr	tr

for alkaline phosphatase and β-galactosidase are positive (but not for strain MN1-156).

Based on the results of the phylogenetic analysis and biochemical and physiological properties, the five isolates represent a new genus and three novel species within subdivision 4 of the phylum 'Verrucomicrobia'. The names *Pelagicoccus mobilis* gen. nov., sp. nov. (type strain 02PA-Ca-133^T), *Pelagicoccus albus* sp. nov. (type strain YM14-201^T) and *Pelagicoccus litoralis* sp. nov. (type strain H-MN57^T) are proposed for the novel isolates.

Description of *Pelagicoccus* gen. nov.

Pelagicoccus [Pe.la.gi.coc'cus. L. n. *pelagus* the open sea, the ocean; N.L. masc. n. *coccus* (from Gr. masc. n. *kokkos*) berry; N.L. masc. n. *Pelagicoccus* referring to a coccoid-shaped bacterium isolated from the sea].

Cells are cocci, Gram-negative and white-pigmented. Non-spore-forming. Nitrate is not reduced. The major respiratory quinone is MK-7. The G+C of the genomic DNA is 51–57 mol%. Predominant cellular fatty acids are C_{16:0}, C_{16:1}ω7c and anteiso-C_{15:0}. The type species is *Pelagicoccus mobilis*.

Description of *Pelagicoccus mobilis* sp. nov.

Pelagicoccus mobilis (mo.bi'lis. L. masc. adj. *mobilis* movable, mobile, referring to the ability to move by means of flagella).

Main characteristics are the same as those given for the genus. In addition, cells are obligately aerobic cocci that are 0.5–0.7 μm in diameter. Motile by means of single or multiple flagella. Appendages are found on some cells. Neither cellular gliding movement nor swarming growth is observed. Colonies grown on 1/2 strength R2A agar medium with 75 % artificial seawater are circular, convex and white. Temperature range for growth is 20–37 °C, the optimal temperature is between 28 and 30 °C, but no growth occurs at 4 or 45 °C. The pH range for growth is 7.0–9.0. NaCl is required for growth; tolerates up to 4 % (w/v) NaCl. Growth occurs in the presence of ampicillin (1–1000 μg ml⁻¹) and penicillin G (1–1000 μg ml⁻¹). Catalase reaction is negative, oxidase reaction is positive. Aesculin and urea are hydrolysed, but agar, DNA, starch and gelatin are not hydrolysed. Negative reactions in tests for acetoin, ONPG, tryptophan deaminase, citrate utilization, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide and indole production. Acid is produced from aesculin ferric citrate, salicin, cellobiose, lactose, sucrose, trehalose, starch, glycogen and gentiobiose, but not from glycerol, galactose, fructose, mannose, mannitol, sorbitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 5-ketogluconate, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xylopyranoside, glucose, sorbose, rhamnose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, maltose, melibiose, inulin, melezitose, raffinose, xylitol, gluconate or 2-ketogluconate. Tests for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase are positive, but esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are negative. The usual components of bacterial cell walls such as muramic acid and diaminopimelic acid can not be detected. Major fatty acid components (>1.0 %) include anteiso-C_{15:0} (29.8 %), C_{16:0} (23.3 %), C_{16:1}ω7c (15.1 %), C_{15:0} (5.9 %), C_{14:0} (5.8 %), iso-C_{16:0} (2.3 %), C_{17:0} (2.1 %) and iso-C_{14:0} (2.1 %). DNA G+C content of the type strain is 57.4 mol%.

The type strain, strain 02PA-Ca-133^T (= MBIC08004^T = IAM 15422^T = KCTC 13126^T), was isolated from seawater.

Description of *Pelagicoccus albus* sp. nov.

Pelagicoccus albus (al'bus. L. masc. adj. *albus* white, referring to the dull-white colour of colonies).

Main characteristics are the same as those given for the genus. In addition, cells are facultatively anaerobic cocci

Table 2. Phenotypic characteristics that differentiate the genus *Pelagicoccus* gen. nov. from other genera of subdivision 4 within the phylum 'Verrucomicrobia'

Taxa: 1, strain 02PA-Ca-133^T; 2, strain YM14-201^T; 3, strain H-MN57^T; 4, strain H-MN48; 5, strain MN1-156; 6, *Coralimargarita akajimensis* 04OKA010-24^T; 7, *Alterococcus agarolyticus* BCRC 19135^T; 8, *Opitutus terrae* DSM 11246^T. Data are from this study and earlier studies (Shieh & Jean, 1998; Chin *et al.*, 2001; Sakai *et al.*, 2003; Yoon *et al.*, 2007). +, Positive; w, weakly positive; –, negative; ND, no data.

Characteristic	1	2	3	4	5	6	7	8
Isolation source	Seawater	Seawater	Seawater	Seawater	Seawater	Seawater	Hot springs	Rice paddy soil
Cell diameter (µm)	0.5–0.7	0.8–1.2	1.0–1.2	1.0–1.2	1.0–1.2	0.5–1.2	0.8–0.9	0.4–0.6
Flagella	+	–	–	–	–	–	+	+
Appendage	+	–	–	–	–	–	–	–
Motility	+	–	–	–	–	–	+	+
O ₂ requirement	Obligately aerobic	Facultatively anaerobic	Obligately aerobic	Obligately aerobic	Obligately aerobic	Obligately aerobic	Facultatively anaerobic	Obligately anaerobic
Production of:								
Catalase	–	+	+	+	+	–	+	–
ONPG	–	+	+	–	+	+	ND	ND
Oxidase	+	+	+	+	+	+	+	–
Nitrate reduction	–	–	–	–	–	–	ND	+
Temperature range for growth (°C)	20–37	15–37	4–30	4–37	4–30	20–30	38–58	10–37
Growth at 4 °C	–	–	+	+	+	–	ND	–
Growth at 37 °C	+	+	–	+	–	–	ND	+
pH range	7.0–9.0	6.5–9.0	6.5–9.0	7.0–9.0	7.0–9.0	7.0–9.0	7.0–8.5	5.5–9.0
NaCl range (%)	1–4	3–7	1–4	1–4	3–4	1–5	2–2.5	0–3
Hydrolysis of:								
Aesculin	+	+	+	+	+	–	ND	ND
Agar	+	–	–	–	–	–	+	ND
DNA	+	+	–	–	–	w	–	ND
Starch	+	–	–	–	–	–	w	+
Urea	+	–	–	–	–	+	ND	–
Acid production from:								
D-Arabinose	–	–	–	–	–	–	–	+
Cellobiose	+	–	–	–	–	–	+	+
Galactose	–	–	–	–	–	w	+	+
Glucose	+	–	–	–	–	–	+	+
Lactose	+	–	–	–	–	–	+	+
Mannitol	–	–	–	–	–	+	–	+
Mannose	–	–	–	–	–	+	w	+
Melibiose	–	–	+	–	–	–	–	+
Xylose	–	–	–	–	–	–	+	–

Table 2. cont.

Characteristic	1	2	3	4	5	6	7	8
Enzyme activity of:								
N-acetyl- β -glucosaminidase	+	-	-	+	+	-	ND	ND
Acid phosphatase	+	+	-	-	-	+	ND	ND
α -Galactosidase	-	+	-	-	-	-	ND	ND
α -Glucosidase	-	+	-	-	-	-	ND	ND
Antibiotic resistance ($\mu\text{g ml}^{-1}$):								
Ampicillin (1–1000)	+	+	+	+	+	+	-	ND
Penicillin G (1–1000)	+	+	+	+	+	+	-	ND
Cell wall component:								
Muramic acid	-	-	-	-	-	-	ND	ND
meso-Diaminopimelic acid	-	-	-	-	-	-	ND	ND
DNA G + C content (mol%)	57.4	57.2	56.4	57	51.2	53.9	65.5–67.0	74
Major quinone	MK-7	MK-7	MK-7	MK-7	MK-7	MK-7	ND	ND

with a diameter of 0.8–1.2 μm . Neither cellular gliding movement nor swarming growth are observed. Colonies grown on 1/2 strength R2A agar medium with 75 % artificial seawater are circular, convex and white. The temperature range for growth is 15–37 °C, optimal growth is at between 25 and 27 °C; no growth occurs at 4 or 45 °C. The pH range for growth is 6.5–9.0. NaCl is required for growth and is able to tolerate up to 7 % (w/v) NaCl. Growth occurs in the presence of ampicillin (1–1000 $\mu\text{g ml}^{-1}$) and penicillin G (1–1000 $\mu\text{g ml}^{-1}$). Catalase- and oxidase-positive. DNA and aesculin are hydrolysed, but agar, starch, gelatin and urea are not. Gives a positive result in tests for acetoin, ONPG and tryptophan deaminase, but negative results for citrate utilization, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide and indole production. Acid is produced from aesculin ferric citrate and 5-ketogluconate, but not from glycerol, galactose, fructose, mannose, mannitol, sorbitol, trehalose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β -D-xylopyranoside, glucose, sorbose, rhamnose, dulcitol, inositol, methyl α -D-mannopyranoside, methyl α -D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, gluconate or 2-ketogluconate. Positive results in tests for alkaline phosphatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase and α -glucosidase, but negative results for leucine arylamidase, valine arylamidase, trypsin, esterase (C4), esterase lipase (C8), lipase (C4), cysteine arylamidase, chymotrypsin, β -glucuronidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. The usual components of bacterial cell walls such as muramic acid and diaminopimelic acid can not be detected. Major fatty acid components (> 1.0 %) include anteiso-C_{15:0} (37.5 %), C_{16:0} (23.8 %), C_{16:1} ω 7c (14.5 %), C_{13:0} 2-OH (5.5 %), C_{14:0} (4 %), iso-C_{16:0} (2.3 %), C_{15:0} (1.7 %), C_{16:0} 3-OH (1.4 %) and C_{18:0} (1.2 %). The DNA G + C content of the type strain is 57.2 mol%.

The type strain, YM14-201^T (=MBIC08272^T=IAM 15421^T=KCTC 13124^T), was isolated from seawater by using an *in situ* cultivation technique.

Description of *Pelagicoccus litoralis* sp. nov.

Pelagicoccus litoralis (li.to.ra'lis. L. masc. adj. *litoralis* pertaining to the coast).

Main characteristics are the same as those given for the genus. In addition, cells are obligately aerobic cocci with a diameter of 1.0–1.2 μm . Neither cellular gliding movement nor swarming growth are observed. Colonies grown on 1/2 strength R2A agar medium with 75 % artificial seawater are circular, convex and white. The temperature range for growth is 4–30 °C, optimal growth occurs at between 25 and 27 °C; no growth occurs at 45 °C. The pH range for growth is

6.5–9.0. NaCl is required for growth and is able to tolerate up to 4% (w/v) NaCl. Growth occurs in the presence of ampicillin (1–1000 µg ml⁻¹) and penicillin G (1–1000 µg ml⁻¹). Catalase- and oxidase-positive. Aesculin is hydrolysed, but agar, DNA, starch, gelatin and urea are not. Positive result in reactions for acetoin, ONPG and tryptophan deaminase, but negative result are obtained in tests for citrate utilization, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, hydrogen sulfide and indole production. Acid is produced from aesculin ferric citrate, melibiose, sucrose and 5-ketogluconate, but not from glycerol, galactose, fructose, mannose, mannitol, sorbitol, trehalose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, erythritol, D-arabinose, L-arabinose, ribose, D-xylose, L-xylose, adonitol, methyl β-D-xylopyranoside, glucose, sorbose, rhamnose, dulcitol, inositol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, cellobiose, maltose, lactose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, gluconate or 2-ketogluconate. Tests for alkaline phosphatase and β-galactosidase are positive, but negative results in tests for acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, leucine arylamidase, valine arylamidase, trypsin, esterase (C4), esterase lipase (C8), lipase (C4), cysteine arylamidase, chymotrypsin, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The usual components of bacterial cell walls such as muramic acid and diaminopimelic acid can not be detected. Major fatty acid components of the type strain (>1.0%) include anteiso-C_{15:0} (38.1%), C_{16:1ω7c} (20.7%), C_{16:0} (14.3%), C_{13:0} 2-OH (5.7%), C_{15:0} (4.5%), C_{14:0} (4.4%), iso-C_{16:0} (1.7%), C_{18:0} (1.4%), C_{17:0} (1.3%) and iso-C_{14:0} (1.1%). The DNA G+C content is 51.2–57 mol%.

The type strain, H-MN57^T (=MBIC08273^T=IAM 15423^T=KCTC 13125^T), was isolated from seawater by using an *in situ* cultivation technique.

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