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# *Oceanicoccus sagamiensis* gen. nov., sp. nov., a Gammaproteobacterium Isolated from Sea Water of Sagami Bay in Japan

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**A gram-negative, motile, coccoid- and amorphous-shaped, non-pigmented chemoheterotrophic bacterium, designated strain PZ-5$^T$, was isolated from sea water of Sagami Bay in Japan and subjected to a polyphasic taxonomic study. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the novel isolate could be affiliated with the class *Gammaproteobacteria*. Strain PZ-5$^T$ showed below 93.9% similarity with validly published bacteria and demonstrated the highest sequence similarity to *Dasania marina* KOPRI 20902$^T$ (93.9%). Strain PZ-5$^T$ formed a monophyletic group with *D. marina* KOPRI 20902$^T$. The DNA G+C content of strain PZ-5$^T$ was 49.8 mol%. The major isoprenoid quinone was Q-8 and predominant cellular fatty acids were $C_{15:0}$ ISO 2OH (19%), $C_{16:1}$ ω7c (17.4%), $C_{17:1}$ ω8c (16.2%), $C_{11:0}$ 3OH (7.5%), and $C_{15:1}$ ω8c (6.5%). Based on evidence from a polyphasic taxonomical study, it was concluded that the strain should be classified as representing a new genus and species of the class *Gammaproteobacteria*, for which the name *Oceanicoccus sagamiensis* gen. nov., sp. nov., (type strain PZ-5$^T$ =NBRC 107125$^T$ =KCTC 23278$^T$) is proposed.**

***Keywords*:** *Gammaproteobacteria*, marine environment, *Oceanicoccus sagamiensis* gen. nov., sp. nov.

Type order of the class *Gammaproteobacteria*, the order *Pseudomonadales*, was proposed by Orla-Jensen (1921) and includes two families, *Pseudomonadaceae* and *Moraxellaceae*, and the genus *Dasania* (Garrity *et al.*, 2005; Lee *et al.*, 2007). The family *Pseudomanadaceae* contains the genera *Pseudomonas*, *Azomonas*, *Azotobacter*, *Cellvibrio*, *Mesophilobacter*, *Rhizobacter*, *Rugamonas*, and *Serpens*. The family *Moraxellaceae* includes *Moraxella*, *Acinetobacter*, *Alkanindiges*, *Enhydrobacter*, and *Psychrobacter* (Bogan *et al.*, 2003; Garrity *et al.*, 2005). The genus *Dasania* was proposed as a member of the order *Pseudomonadales* (Lee *et al.*, 2007) but affiliation at the family level has not yet been determined. In this study, a novel aerobic bacterium, designated strain PZ-5$^T$, was isolated from seawater samples obtained from Sagami Bay in Japan. The strain PZ-5$^T$ showed highest sequence similarity to *Dasania marina* KOPRI 20902$^T$ (93.9%) in validated species and formed a monophyletic group with *D. marina* KOPRI 20902$^T$. Polyphasic taxonomical analysis of strain PZ-5$^T$ demonstrated that it represents a novel genus and species as *Oceanicoccus sagamiensis* gen. nov., sp. nov., within the class *Gammaproteobacteria.*

## Materials and Methods

### Isolation of bacterial strain and cultivation

An aerobic bacterium was isolated from seawater samples obtained from Sagami Bay in Japan (35°00' N, 139°20' E; depth, 100 m) during KT-09-11 (2-6 July 2009) of research ship 'Tansei Maru' (Ocean Research Institute, The University of Tokyo and Japan Agency for Marine-Earth Science and Technology [JAMSTEC]). The seawater samples (200 μl) were applied to 1/10-strength ZoBell agar (agar 15 g, polypeptone 0.5 g, yeast extract 0.1 g/L) containing 80% natural seawater and the agar plates were incubated at 15°C for 4 weeks. The bacterial strain PZ-5$^T$ was isolated from these plates and maintained on 1/2-strength marine agar 2216 (MA: Difco, USA) containing 2% NaCl at 15°C. The temperature (5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 37°C, and 45°C) and pH (5, 6, 7, 8, 9, and 10) ranges for growth were determined by incubating the isolate on 1/2 MA. The NaCl concentration for growth was determined on 1/2 MA medium without NaCl (agar 15 g, $MgCl_2$ 4.4 g, peptone 2.5 g, $Na_2SO_3$ 1.62 g, $CaCl_2$ 0.9 g, yeast extract 0.5 g, KCl 0.27 g, $NaHCO_3$ 0.8 g, ferric citrate 0.5 g, KBr 0.04 g, $SrCl_2$ 0.015 g, $H_3BO_3$ 0.01 g, $Na_2HPO_4$ 4 mg, $Na_2SiO_3$ 2 mg, NaF 1.2 mg, $NH_4NO_3$ 0.8 mg/ DW 1 L) by adding 0, 1, 2, 3, 4, 5, 8, 10, and 15% (w/v) NaCl.

### Morphological, physiological, and biochemical tests

Gram-staining was performed as described by Murray *et al.* (1994). Cell morphology and motility were observed by light microscopy (BX60; Olympus, Japan) and transmission electron microscopy (TEM). For TEM observation, cells were mounted on Formvar-coated copper grids and negatively stained with 1% (w/v) aqueous uranyl acetate. Grids were observed in a JEOL 1011 TEM operated at 100 kV. Growth under anaerobic conditions was determined after incubation for 4 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co., Japan) on 1/2 MA. Catalase activity was determined by bubble formation in a 3% $H_2O_2$ solution. Oxidase activity was determined using cytochrome oxidase test paper (Nissui Pharmaceutical Co., Japan). API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux, France) were used to determine physiological and biochemical characteristics. All suspension media for API test strips were supplemented with 2% (w/v) NaCl (final concentration). API 20E, API 20NE, API 50CH test strips were read after incubation for 5 days

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and the API ZYM test strip was read after incubation for 2 days at 15°C.

### 16S rRNA gene sequencing and phylogenetic analysis

A fragment of approximately 1,450 bp from the 16S rRNA gene was amplified from extracted DNA using bacterial universal primers specific to the 16S rRNA gene: 27F and 1492R (Weisburg *et al*., 1991). To ascertain the phylogenetic position of the novel strain PZ-5$^T$, its 16S rRNA gene sequence was compared with those obtained from GenBank (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov). Multiple alignments of sequences were performed using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were not considered when the 1,205 bases of the 16S rRNA gene nucleotides were compared. Aligned sequences were analyzed using MEGA 4 (Tamura *et al*., 2007). Evolutionary distances, [distance options according to the Kimura two-parameter model (Kimura, 1983)] and clustering with the neighbour-joining (NJ: Saitou and Nei, 1987) and maximum-likelihood (ML: Felsenstein, 1981) methods using the PAUP 4 program (Swofford, 2002) were determined using bootstrap values based on 1,000 replications (Felsenstein, 1985). Similarities were calculated using the same software values (MEGA 4).

### Chemotaxonomic investigation

Determination of respiratory quinone was carried out as described previously (Xie and Yokota, 2003). Bacterial cells grown on 1/2 MA for 4 weeks at 15°C were used for the analysis of fatty acid methyl esters, which were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990).

For the determination of DNA G+C content, DNA was prepared according to the method of Murmur (1961) and DNA base composition was determined by using the HPLC method of Mesbah *et al*. (1989).

### Nucleotide sequence accession numbers

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain PZ-5$^T$ is AB545809.

## Results and Discussion

### Phylogenetic analysis

An almost complete 16S rRNA gene sequence for strain PZ-5$^T$ was determined and a FASTA search in GenBank showed that the strain belongs to the class *Gammaproteobacteria.* Strain PZ-5$^T$ showed 96.3% and 96.2% 16S rRNA gene sequence similarity to marine gammaproteobacterium clone HTCC2143 (GenBank accession no. AY386333) and uncultured gammaproteobacterium clone 20m-45 (GU061297). However, all species with validly published names exhibited <94% sequence similarity to the determined sequence. Strain PZ-5$^T$ displayed the highest 16S rRNA gene sequences similarities to *Dasania marina* KOPRI 20902$^T$ (GenBank accession no. AY771747, 93.9%), *Spongiibacter marinus* HAL40b$^T$ (AM117932, 92.9%), *Melitea salexigens* 5IX/A01/131$^T$ (AY576729, 92.7%), *Haliea salexigens* 3X/A02/235$^T$ (AY576769, 91.8%), and *Congregibacter litoralis* KT71$^T$ (AAOA01000004. 89.4%). It also displayed 87.5% sequence similarity to *Pseudomonas aeruginosa* DSM 50071$^T$(X06684) (type genus of family *Pseudomonadaceae*). The NJ- and ML-trees based on the 16S rRNA gene sequences revealed that the strain formed a monophyletic clade with *D. marina* KOPRI 20902$^T$ and two unidentified strains within the class *Gammaproteobacteria* with bootstrap confidence values of 96% in the NJ method and 74% in the ML method (Fig. 1).

### Morphological, physiological and biochemical analysis

Cells of strain PZ-5$^T$ grown on 1/2 MA agar containing 2%

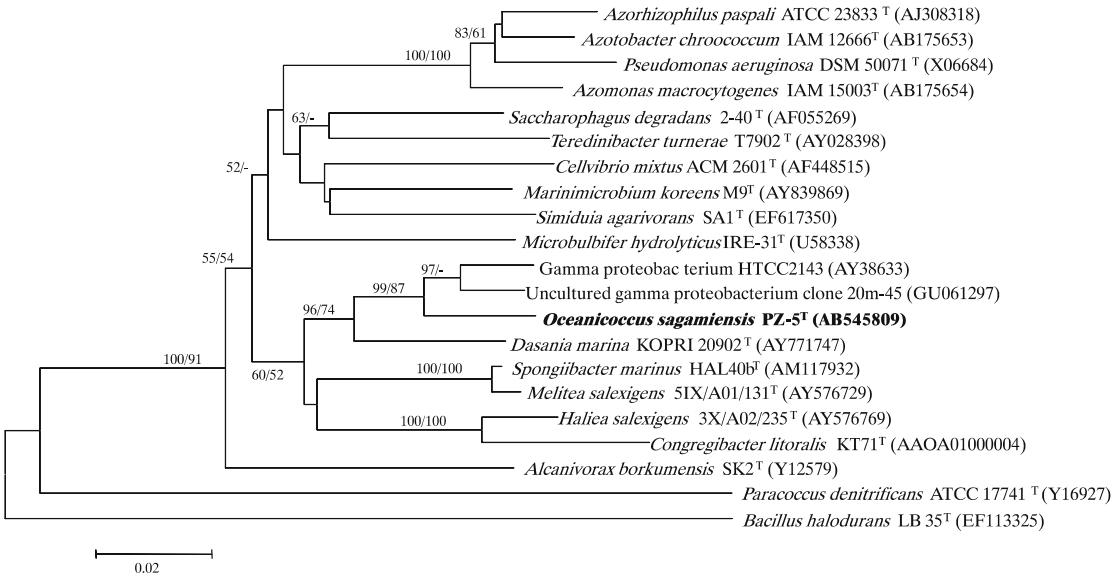


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of PZ-5$^T$ among the currently known and related species of the class *Gammaproteobacteria.* Numbers at nodes are bootstrap percentages derived from 1,000 replications (NJ/ML). The sequence of *Bacillus halodurans* LB 35$^T$ (EF113325) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position. -, indicates recovered nodes with <50% bootstrap values in the ML tree.

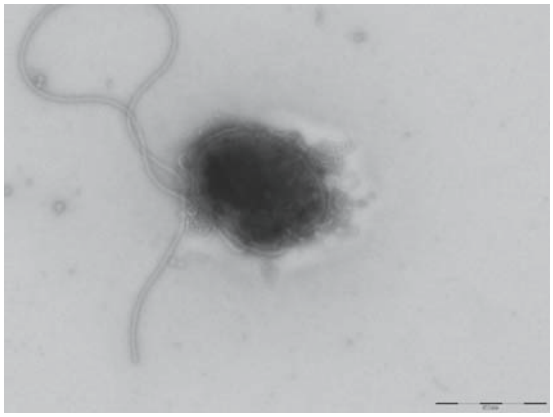

**Fig. 2.** Transmission electron micrograph of strain PZ-5$^T$, showing coccoid and amorphous-shaped cells with a flagellum. Bar, 500 nm.

NaCl at 15℃ for 4 weeks were coccoid and amorphous shaped, approximately 0.5-0.7 μm wide and 0.6-0.8 μm long. Colonies after 4 weeks of incubation on 1/2 marine agar were 1-2 mm in diameter. Cells were motile by means of a flagellum (Fig. 2). Spores were not formed. Cultural, physiological and biochemical characteristics of PZ-5$^T$ were compared to related genera within the class *Gammaproteobacteria* (Table 1). No growth was observed under anaerobic conditions in 1/2 MA with added 1% NaCl. Growth occurred only under aerobic conditions between 10-30℃. Optimal temperature and pH for the growth of PZ-5$^T$ was 15-20℃ and pH 6-8, respectively. DNA G+C content of the strain PZ-5$^T$ was 49.8% and the major respiratory quinone was ubiquinone-8. The strain required NaCl for growth and grew in 1-5% NaCl.

## Chemotaxonomic analysis

As shown in Table 2, the predominant cellular fatty acids of strain PZ-5$^T$ were $C_{15:0}$ ISO 2OH (19%), $C_{16:1}$ ω7c (17.4%), $C_{17:1}$ ω8c (16.2%), $C_{11:0}$ 3OH (7.5%), and $C_{15:1}$ ω8c (6.5%). The presence of unsaturated fatty acids $C_{15:1}$ ω8c (6.5%), $C_{15:1}$ ω6c (3.8%), and 3-hydroxy fatty acid $C_{11:1}$ 3OH (7.5%), distinguishes strain PZ-5$^T$ from type species of related genera.

## Strain PZ-5$^T$ showed features clearly different from related genera

Strain PZ-5$^T$ has a coccoid and amorphous shape, while related genera are rod shaped.

DNA G+C content of strain PZ-5$^T$ was 49.8 mol% which is much different from the genera *Dasania* (37 mol%), *Melitea* (57 mol%), *Spongiibacter* (57.7-69.1 mol%), *Haliea* (61.4-64.8 mol%) and *Congregibacter* (57.8 mol%). In the fatty acids analysis, PZ-5$^T$ showed a different fatty acid profile from related genera. Strain PZ-5$^T$ contains a substantial amount of unsaturated fatty acid $C_{15:1}$ ω8c (6.5%) while related genera only contain a small amount (≤0.7%) or none. Also PZ-5$^T$ showed a different fatty acid profile from related genera. Based on the results of the phylogenetic analysis and biochemical and physiological properties, the strain PZ-5$^T$ isolated from Sagami Bay in Japan represents a new genus and a novel species of the class *Gammaproteobacteria*, for which the name *Oceanicoccus sagamiensis* gen. nov., sp. nov., is proposed. Although the affiliation of the genus *Oceanicoccus* at the family level remains uncertain, the genus together with genera *Dasania*, *Melitea*, *Spongiibacter*, *Haliea* and *Congregibacter* may form a family, separate from *Pseudomonadaceae* and *Alteromonadaceae*.

## Description of *Oceanicoccus* gen. nov.

Oceanicoccus (O.ce.a.ni.coc'cus. L. masc. n. *oceanus* the great sea, outer sea, ocean; N.L. masc. n. *coccus* from Gr. n. *kokkos* a berry; unit; N.L. masc. n. *Oceanicoccus,* coccus from sea).

Cells are non-pigmented, coccoid and ellipsoid shape, Gram-negative and obligately aerobic. Cells are motile with a single flagellum and are non-spore-forming. The major respiratory quinone is ubiquinone 8. The DNA G+C content of the genomic DNA is 49.8 mol%. Predominant cellular fatty acids are $C_{15:0}$ ISO 2OH (19%), $C_{16:1}$ ω7c (17.4%), $C_{17:1}$ ω8c (16.2%) and $C_{11:0}$ 3OH (7.5%). The type species is *Oceanicoccus sagamiensis.*

## Description of *Oceanicoccus sagamiensis* sp. nov.

*Oceanicoccus sagamiensis* (sa.ga.mi.en'sis. N.L. adj. *sagamiensis* referring to Sagami Bay, the site of isolation).

It exhibits the following properties in addition to those given in the genus description. Cells are approximately 0.5-0.7 μm

**Table 1.** Differential characteristics of strain PZ-5$^T$ and related genera

Strain: 1, Strain PZ-5$^T$; 2, *Dasania* (data from Lee *et al*., 2007); 3, *Melitea* (Urios *et al*., 2008a); 4, *Spongiibacter* (Graeber *et al*., 2008: Hwang and Cho, 2009); 5, *Haliea* (Urios *et al*., 2008b: Urios *et al*., 2009: Lucena *et al*., 2010); 6, *Congregibacter* (Spring *et al*., 2009). ND, No data available, +/- means variable.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Shape | coccus/ amorphous | irregular rod | rod | rod | rod | pleomoorphic |
| Temperature for growth (℃) | 10-30 | 4-30 | 15-37 | 10-40 | 10-44 | 9-33 |
| Opt temperature (℃) | 15-20 | 17-22 | 30 | 20-35 | 25-30 | 28 |
| NaCl (%) | 1-5 | 1-9 | 0.7-7 | 1-9 | 0.4-15 | 1-7 |
| Catalase | - | + | + | + | + | + |
| Esterase | + | - | - | + | - | ND |
| *N*-Acetyl-β-glucosaminidase | - | - | - | +/- | +/- | ND |
| Utilization of | | | | | | |
| Arabinose | + | - | - | +/- | - | ND |
| Glucose | + | - | + | ND | +/- | - |
| DNA G+C content (mol%) | 49.8 | 37 | 57 | 57.7-69.1 | 61.4-64.8 | 57.8 |

**Table 2.** Cellular fatty acid content (%) of the PZ-5$^T$ and related genus type species
Strain: 1, Strain PZ-5$^T$; 2, *Dasania marina* KOPRI 20902$^T$ (Data from Lee *et al*., 2007); 3, *Melitea salexigens* 51X/A01/131$^T$(Urios *et al*., 2008a); 4, *Spongiibacter marinus* HAL40b$^T$ (Graeber *et al*., 2008); 5, *Haliea salexigens* 3X/A02/235$^T$ (Urios *et al*., 2008b); 6, *Congregibacter litoralis* KT71$^T$ (Spring *et al*., 2009). -, Not detected

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Saturated | | | | | | |
| $C_{10:0}$ | 2.1 | 1.4 | - | - | - | 0.5 |
| $C_{14:0}$ | 0.8 | 5.9 | 0.8 | 0.3 | 1.3 | 2.0 |
| $C_{16:0}$ | 1.9 | 18.4 | 3.9 | 2.4 | 2.0 | 5.4 |
| $C_{17:0}$ | - | - | 13.0 | 9.6 | 9.3 | 3.1 |
| $C_{18:0}$ | 0.7 | 1.8 | 0.5 | 0.4 | - | 0.6 |
| Unsaturated | | | | | | |
| $C_{15:1}$ ω6c | 3.8 | - | - | 0.4 | 5.8 | 2.0 |
| $C_{15:1}$ ω8c | 6.5 | - | - | 0.7 | - | 0.3 |
| $C_{16:1}$ ω7c | 17.4 | - | - | - | 21.2 | 23.1 |
| $C_{17:1}$ ω8c | 16.2 | - | 34.1 | 51.7 | 23.9 | 8.1 |
| $C_{18:1}$ ω7c | 1.4 | 4.1 | 11.4 | 7.8 | 17.5 | 29.4 |
| Hydroxy | | | | | | |
| $C_{10:0}$ 3-OH | 4.0 | 10.4 | 1.7 | 1.3 | 1.8 | 2.1 |
| $C_{11:0}$ 3-OH | 7.5 | - | 4.6 | 4.1 | 3.3 | 0.5 |
| $C_{15:0}$ ISO 2OH | 19.0 | - | - | - | - | - |
| * Summed feature 3 | - | 45.3 | 9.0 | 5.9 | - | - |

* Summed feature 3 comprises $C_{16:1}$ ω7c and/or $C_{15:0}$ ISO 2OH.

wide and 0.6-0.8 μm long. Temperature range for growth is 10-30°C, with optimal temperature growth of 15-20°C. No growth occurs above 30°C. The pH range for growth is 5.0-9.0 and with the optimum being 6-8. NaCl is required for growth and can be tolerated up to 5% (w/v). Cells are catalase-negative but oxidase-positive. Nitrate reduction to nitrite is positive. Alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, and naphthol-AS-BI-phosphohydrolase are positive, leucine arylamidase, and trypsin are weakly positive but lipase (C14), valine arylamidase, *N*-acetyl-β-glucosaminidase, *α*-galactosidase, *β*-galactosidase, *α*-glucosidase, cystine arylamidase, chymotrypsin, *β*-glucuronidase, *β*-glucosidase, *α*-mannosidase, and *α*-fucosidase are negative. Agar is not hydrolyzed. Acid is produced from glycerol, ribose, glucose, mannitol, esculin, sucrose, and melezitose. Indole production is positive. Esculin is hydrolyzed. Major cellular fatty acids are $C_{15:0}$ ISO 2OH, $C_{16:1}$ ω7c, $C_{17:1}$ ω8c, $C_{11:0}$ 3OH, $C_{17:1}$ ω8c, $C_{11:1}$ 3OH and $C_{15:1}$ ω8c. The DNA G+C content of the type strain is 49.8 mol%. The type strain, PZ-5$^T$ (=NBRC 107125$^T$ =KCTC 23278$^T$), was isolated from Sagami Bay in Japan (35°00' N, 139°20' E; depth 100 m).

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