

## Emendation of the Genus *Planococcus* and Transfer of *Flavobacterium okeanokoites* Zobell and Upham 1944 to the Genus *Planococcus* as *Planococcus okeanokoites* comb. nov.

YASUYOSHI NAKAGAWA,\* TAKESHI SAKANE, AND AKIRA YOKOTA†

*Institute for Fermentation, Osaka, Yodogawa-ku, Osaka 532, Japan*

The taxonomic position of *Flavobacterium okeanokoites* IFO 12536<sup>T</sup> (T = type strain) was determined by 16S rRNA gene sequencing and chemotaxonomic methods. Phylogenetic evidence derived from a 16S rRNA sequence analysis indicated that *F. okeanokoites*, which forms rod-shaped cells, belongs to the genus *Planococcus*, which forms spherical cells. A phylogenetically close relationship was supported by chemotaxonomic characteristics, such as the presence of menaquinone 7 and menaquinone 8 as major isoprenoid quinones, the presence of phosphatidylglycerol, bisphosphatidylglycerol, and phosphatidylethanolamine as cellular polar lipids, and the G+C content of the DNA (46.3 mol%). These data suggest that whether a cell is a rod or a coccus is not a generic criterion. Accordingly, we propose that *F. okeanokoites* should be transferred to the genus *Planococcus* and that the description of the genus *Planococcus* should be emended.

Bergey et al. (2) created the genus *Flavobacterium* for aerobic, yellow-pigmented rods which produce acid from carbohydrates weakly. The original description of this taxon was so vague that it included species that were gram negative, non-motile or motile by means of flagella, aerobic or facultatively anaerobic, and even gram positive. The heterogeneity of the genus has been reduced by reclassifying various *Flavobacterium* species into different or new genera (13, 21, 29, 30). Recently, the description of the genus *Flavobacterium* was emended to restrict it to organisms which contain menaquinone 6 (MK-6) and inhabit soil and freshwater (3), and consequently, the genus *Flavobacterium* has become a phylogenetically homogeneous group.

*Flavobacterium okeanokoites*, which was described by Zobell and Upham (32), is one of the misnamed flavobacteria. This organism was isolated from marine mud, and it possesses peritrichous flagella and therefore is treated as a species incertae sedis in *Bergey's Manual of Systematic Bacteriology* (12). Bauwens and De Ley (1) showed that *F. okeanokoites* did not belong to the genus *Flavobacterium* on the basis of DNA-rRNA hybridization results. Chemotaxonomic characteristics suggested that *F. okeanokoites* was similar to the genus *Bacillus*, even though it could not form spores (28).

We investigated the taxonomic position of *F. okeanokoites* by a polyphasic approach and, on the basis of our results, propose that *F. okeanokoites* should be transferred to the genus *Planococcus* and that the description of the genus *Planococcus* should be emended.

### MATERIALS AND METHODS

**Bacterial strains and cultivation.** The strains examined were *F. okeanokoites* IFO 12536<sup>T</sup> (= NCIMB 561<sup>T</sup>) (T = type strain), *Planococcus citreus* IAM 12541<sup>T</sup> (= IFO 15849<sup>T</sup>), and *Planococcus kocurii* IAM 12847<sup>T</sup> (= IFO 15850<sup>T</sup>). The organisms were cultivated aerobically at 28°C in medium containing (per liter) 10.0 g of Polypeptone (Wako Pure Chemical Industries, Osaka, Japan), 2.0 g of yeast extract (Difco Laboratories, Detroit, Mich.), 1.0 g of MgSO<sub>4</sub> · 7H<sub>2</sub>O, 30.0 g of NaCl, and 15 g of agar, if needed (pH 7.0). Cells were harvested by centrifugation at the stationary phase for PCR and chemotaxonomic experiments.

\* Corresponding author. Mailing address: Institute for Fermentation, Osaka, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan. Phone: 81 6 300 6555. Fax: 81 6 300 6814.

† Present address: Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

**Morphological and physiological tests.** Cells in the early exponential growth phase grown on solid media were used for morphological and physiological characterizations. The size, shape, and motility of cells were determined with a phase-contrast microscope and a scanning electron microscope (model JSM-5400; JEOL, Akishima, Japan). The samples used for scanning electron microscopy were prepared by the methods of Yokota et al. (31). Physiological tests were performed as described by Cowan and Steel (6). The ability to form spores was determined by using Schaeffer's medium (25) and soil extract agar, which contains 5.0 g of peptone (Difco), 3.0 g of beef extract (Difco), and 15 g of agar in 750 ml of tap water and 250 ml of soil extract.

**Respiratory quinone analysis.** Isoprenoid quinones were extracted from freeze-dried cells (200 mg) with chloroform-methanol (2:1, vol/vol) and were purified by thin-layer chromatography with *n*-hexane-diethyl ether (85:15, vol/vol) as the solvent. The menaquinone fraction was extracted with acetone, dried under a nitrogen gas stream, and then analyzed by high-performance liquid chromatography (HPLC) with a model LC-5A apparatus (Shimadzu, Kyoto, Japan), using a Zorbax octyldecyl silane column (4.6 by 150 mm).

**Cellular fatty acid analysis.** The procedures used to prepare cellular fatty acid methyl esters were the procedures described by Suzuki and Komagata (27). The fatty acid methyl ester composition was determined by gas chromatography-mass spectrometry with a model GC-17A, QP-5000 apparatus (Shimadzu).

**Polar lipid analysis.** Free lipids were extracted from 100 mg of dried cells,

TABLE 1. Phenotypic characteristics of *F. okeanokoites* IFO 12536<sup>T</sup>

Characteristic	Strain IFO 12536 <sup>T</sup>
Color of colonies	Bright orange
Morphology of cells	Rods
Gram stain reaction	Positive to variable
Spore formation	–
Catalase activity	+
Urease activity	–
Nitrate reduction	–
Hydrolysis of:	
Starch	–
Esculin	–
Tween 80	–
Acid production from:	
L-Arabinose	–
D-Galactose	–
Lactose	–
Maltose	–
Mannitol	–
Sucrose	–
D-Xylose	–
Decarboxylation of:	
L-Lysine	+
L-Ornithine	+

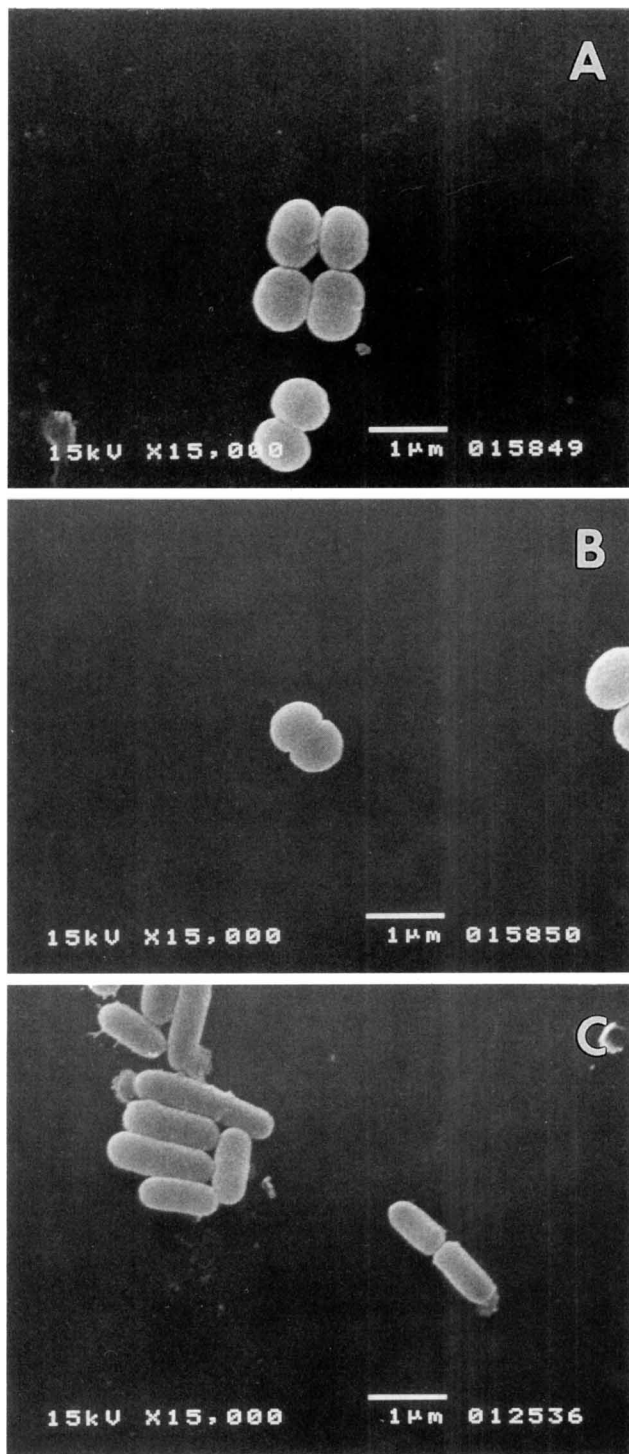


FIG. 1. Scanning electron micrographs of cells of *P. citreus* IAM 12541<sup>T</sup> (= IFO 15849<sup>T</sup>) (A), *P. kocurii* IAM 12847<sup>T</sup> (= IFO 15850<sup>T</sup>) (B), and *F. okeanokoites* IFO 12536<sup>T</sup> (C).

purified by the procedure of Minnikin et al. (19), and examined by two-dimensional thin-layer chromatography, using Kieselgel 60 F<sub>254</sub> plates (E. Merck, Darmstadt, Germany) as described by Yokota et al. (31).

**Peptidoglycan analysis.** Cell walls were prepared from 500 mg of dried cells by mechanical disruption with a model 201M ultrasonic oscillator (Kubota, Tokyo, Japan) and were purified as described by Schleifer and Kandler (26). The amino acids in complete cell wall hydrolysates were determined as their phenylthiocarbonyl derivatives by HPLC by using a model LC-6AD apparatus (Shimadzu)

and a Wakopak WS-PTC column (Wako Pure Chemical Industries) as recommended by the column manufacturer.

**DNA base composition analysis.** DNA was extracted by the methods of Marmur (17) and Saito and Miura (23) with the modifications described previously (20). The guanine-plus-cytosine (G+C) content of the DNA was determined by the method of Mesbah et al. (18).

**DNA-DNA hybridization.** Levels of DNA relatedness were determined by the photobiotin-microplate method of Ezaki et al. (7).

**PCR amplification, cloning, and sequencing of 16S ribosomal DNA.** The 16S rRNA gene was amplified by PCR (22) by using TaKaRa *Taq* (Takara Shuzo, Kyoto, Japan) and primers 9F and 1541R (20). The 1.5-kb amplified 16S ribosomal DNA fragment was purified by agarose gel electrophoresis and with a Prep-A-Gene DNA purification kit (Bio-Rad Laboratories, Hercules, Calif.). The methods used for cloning and sequencing of the purified fragment have been described previously in detail (20). The 5'-fluorescein-labeled oligonucleotide primers used were M13 Universal and Reverse Primer (Pharmacia), as well as primers 339F (5'-CTCCTACGGGAGGCAGCAG; same sequence as positions 339 to 357 [*Escherichia coli* numbering system]), 785F (5'-GGATTAGATACC CTGGTAGTC; same sequence as positions 785 to 805), 1099F (5'-GCAACG AGCGCAACCC; same sequence as positions 1099 to 1115), 536R (5'-GTATT ACCGCGGCTGCTG; complementary to positions 519 to 536), 802R (5'-TAC CAGGGTATCTAATCC; complementary to positions 785 to 802), and 1115R (5'-AGGGTTGCGCTCGTTG; complementary to positions 1100 to 1115).

**Phylogenetic analysis.** The 16S rRNA sequences of the strains examined and the sequences of reference organisms obtained from databases were aligned with the *E. coli* sequence (4) (for the accession numbers of the strains see Table 4). The CLUSTAL V software package (11) was used to generate evolutionary distances ( $K_{nuc}$  values [14]) and similarity values and to construct a phylogenetic tree by using the neighbor-joining method (24) and the  $K_{nuc}$  values. The positions at which secondary structures varied between strains (positions 66 to 103, 179 to 220, 447 to 487, 841 to 845, 1004 to 1036, 1134 to 1140, and 1256 to 1281) and the positions at which sequences were not determined in some reference organisms (positions 1431 to 1491) were excluded from the analysis. The total number of nucleotides compared was 1,136 after we eliminated all sites at which sequences were not determined in any organisms. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (8) with 1,000 replicates.

**Nucleotide sequence accession number.** The 16S ribosomal DNA sequence of *F. okeanokoites* IFO 12536<sup>T</sup> has been deposited in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession number D55729.

**RESULTS**

**Morphological and physiological characteristics.** *F. okeanokoites* IFO 12536<sup>T</sup> was a gram-positive to gram-variable rod-shaped organism whose cells were 0.4 to 0.8 µm wide and 1.0 to 20 µm long (Fig. 1). Most cells were less than 2.8 µm long. Motile cells were observed in the early growth phase, and these

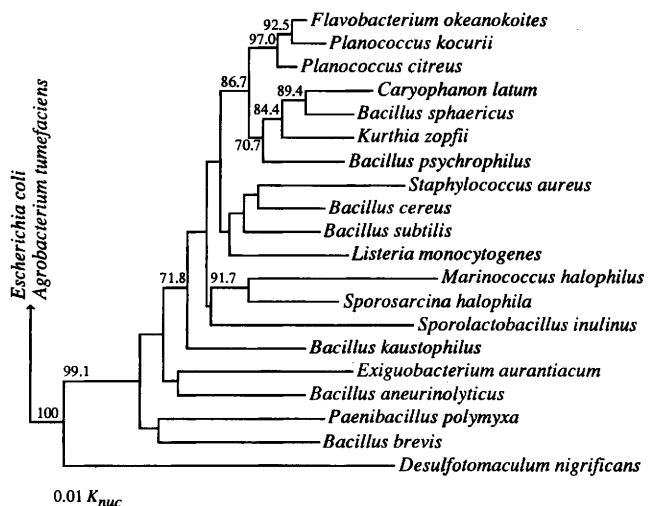


FIG. 2. Phylogenetic tree derived from 16S rRNA sequences of *F. okeanokoites* and related organisms. *E. coli* and *Agrobacterium tumefaciens* were used as the root organisms. Scale bar = 0.01  $K_{nuc}$  in nucleotide sequences. The lengths of the vertical lines are not significant. The numbers on the branches are the confidence limits (expressed as percentages) estimated by a bootstrap analysis performed with 1,000 replicates. Confidence limits less than 60% are not shown.

TABLE 2. Differential properties of *F. okeanokoites* and *Planococcus* species<sup>a</sup>

Species	Growth in the presence of:		Oxidase activity	Hydrolysis of gelatin	Acid produced from glucose	Major menaquinones	Major fatty acid(s) <sup>b</sup>	Phospholipids <sup>c</sup>	G+C content of DNA (mol%)
	No NaCl	15% NaCl							
<i>P. citreus</i>	+ <sup>d</sup>	+	-	+	+	MK-7, MK-8	ai15:0	PE, PG, BPG	48.52
<i>P. kocurii</i>	+	-	-	+ or -	+	MK-7, MK-8	ai15:0	PE, PG, BPG	39.43
<i>F. okeanokoites</i>	w	-	w	+	-	MK-8, MK-7	i14:0, i16:0	PE, PG, BPG	46.3

<sup>a</sup> Data from reference 10 and this study.

<sup>b</sup> i, iso branched; ai, anteiso branched; 14:0, tetradecanoic acid; 15:0, pentadecanoic acid; 16:0, hexadecanoic acid.

<sup>c</sup> PE, phosphatidylethanolamine; PG, phosphatidylglycerol; BPG, bisphosphatidylglycerol.

<sup>d</sup> +, positive; -, negative; w, weakly positive.

cells had peritrichous flagella (data not shown). Spore-forming cells were not observed in any medium. Some of the phenotypic characteristics of IFO 12536<sup>T</sup> are shown in Tables 1 and 2.

**Chemotaxonomic characteristics.** Some chemotaxonomic characteristics of *F. okeanokoites* IFO 12536<sup>T</sup> are summarized in Table 2. The major isoprenoid quinones of this organism were MK-7 and MK-8, which accounted for 34 and 66% of the total isoprenoid quinones, respectively. The cellular fatty acids were mainly branched fatty acids. IFO 12536<sup>T</sup> contained iso-tetradecanoic acid (i14:0) (33.9% of the total fatty acids) and iso-hexadecanoic acid (i16:0) (28.1%) as its major cellular fatty acids (Table 3). The cellular polar lipids of IFO 12536<sup>T</sup> were phosphatidylglycerol, bisphosphatidylglycerol, and phosphatidylethanolamine. The molar ratio of the amino acids glutamic acid, lysine, alanine, and aspartic acid in the cell wall was 1:1.2:1. No attempt was made to determine the chiral nature of the amino acids.

**16S rRNA sequence analysis.** The 16S rRNA sequence of *F. okeanokoites* IFO 12536<sup>T</sup> was determined from position 29 to position 1491. The levels of 16S rRNA similarity are shown in Table 4. The phylogenetic tree constructed by the neighbor-joining method and the  $K_{nuc}$  values shows that *F. okeanokoites* IFO 12536<sup>T</sup> should be included in the genus *Planococcus* (the similarity values range from 98.6 to 99.0%) (Fig. 2 and Table 4).

**DNA-DNA hybridization.** The results of DNA-DNA hybridization experiments performed with *F. okeanokoites* IFO 12536<sup>T</sup>, *P. citreus* IAM 12541<sup>T</sup>, and *P. kocurii* IAM 12847<sup>T</sup> are shown in Table 5. *F. okeanokoites* IFO 12536<sup>T</sup> did not exhibit species level DNA relatedness (i.e., >70% relatedness) with any of the other species examined.

## DISCUSSION

Cellular morphology has been used as a key feature to define genera. However, our phylogenetic analysis clearly revealed that *F. okeanokoites*, which forms rod-shaped cells but not coccoid cells, belongs to the genus *Planococcus*, which forms spherical cells (Fig. 1 and 2). A phylogenetic jumble of cocci

and rods has been observed in the genera *Bacillus* and *Staphylococcus* (5), the genera *Clostridium* and *Sarcina* (5), and the genera *Arthrobacter* and *Micrococcus* (15, 16). Koch et al. (16) proposed that *Micrococcus agilis* should be transferred to the genus *Arthrobacter* and that the description of the genus *Arthrobacter* should be emended to include species which form spherical cells. *F. okeanokoites* and the genus *Planococcus* share the following chemotaxonomic characteristics: MK-7 and MK-8 are the major isoprenoid quinones, phosphatidylethanolamine, phosphatidylglycerol, and bisphosphatidylglycerol are the cellular polar lipids, and branched fatty acids are the major cellular fatty acids (Tables 2 and 3). The G+C content of the DNA of *F. okeanokoites* IFO 12536<sup>T</sup> (46.3 mol%) is also similar to the G+C contents of members of the genus *Planococcus* (Table 2).

Levels of DNA relatedness of 15 to 27% for *F. okeanokoites* IFO 12536<sup>T</sup>, *P. citreus* IAM 12541<sup>T</sup>, and *P. kocurii* IAM 12847<sup>T</sup> indicate that these organisms belong to different species (Table 5). *F. okeanokoites* can also be differentiated from *P. citreus* and *P. kocurii* phenotypically (Table 2). *F. okeanokoites* IFO 12536<sup>T</sup> is characterized by having i14:0 and i16:0 as its major cellular fatty acids, whereas *Planococcus* species are characterized by having anteiso-pentadecanoic acid (ai15:0). *F. okeanokoites* IFO 12536<sup>T</sup> contains aspartic acid in the peptidoglycan of its cell walls, and this compound is not found in *Planococcus* species (10). Accordingly, we propose that *F. okeanokoites* should be transferred to the genus *Planococcus* as *Planococcus okeanokoites* comb. nov. A description of *Planococcus okeanokoites* is given below. The transfer of a rod-shaped species to the genus *Planococcus* means that the description of this genus must also be emended, as described below.

### Emended description of the genus *Planococcus* Migula 1894.

Cells are cocci that are 1.0 to 1.2  $\mu\text{m}$  in diameter and occur singly, in pairs, in threes, or in tetrads or are rods which are 0.4 to 0.8  $\mu\text{m}$  wide and 1.0 to 20  $\mu\text{m}$  long. Gram positive to gram variable. Motile. Motile cells possess several flagella. Spores are not formed. The color of the cell mass is yellow to orange. Chemoorganotrophs. Metabolism is respiratory. Aerobes. Catalase positive. Urease negative. Nitrate is not reduced to nitrite. Hydrolysis of esculin, starch, and Tween 80 is negative. The

TABLE 3. Cellular fatty acid compositions of *F. okeanokoites* and *Planococcus* species

Strain	Fatty acid composition (%) <sup>a</sup>															
	i14:0	14:0	i15:0	ai15:0	15:0	15:1	i16:0	i16:1	16:0	16:1	i17:0	ai17:0	17:0	17:1	i18:0	18:0
<i>P. citreus</i> IAM 12541 <sup>T</sup>	3.4	Tr <sup>b</sup>	1.5	59.0	5.0	Tr	5.9	4.7	3.7	4.1		10.9	5.5	3.2	Tr	1.7
<i>P. kocurii</i> IAM 12847 <sup>T</sup>	11.9	Tr	4.3	48.7	13.5	4.1	4.0	6.7	2.7	2.9		3.1	Tr	Tr		2.2
<i>F. okeanokoites</i> IFO 12536 <sup>T</sup>	33.9	Tr	2.9	14.0	Tr		28.1	11.7	4.7	2.8	Tr	Tr			Tr	1.8

<sup>a</sup> i, iso branched; ai, anteiso branched.

<sup>b</sup> Tr, trace (less than 1%).

TABLE 4. Similarity matrix for 16S rRNA sequences of *F. okeanokoites* and related organisms

Organism	Accession no.	% Similarity																
		<i>Flavobacterium okeanokoites</i>	<i>Planococcus kocurii</i>	<i>Planococcus citreus</i>	<i>Caryophanon latum</i>	<i>Bacillus sphaericus</i>	<i>Kurthia zopfii</i>	<i>Bacillus psychrophilus</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i> <sup>a</sup>	<i>Listeria monocytogenes</i>	<i>Marinococcus halophilus</i>	<i>Sporosarcina halophila</i>	<i>Sporolactobacillus inulinus</i>	<i>Bacillus kaustophilus</i>	<i>Exiguobacterium aurantiacum</i>	<i>Paenibacillus polymyxa</i>	<i>Desulfotomaculum nigrificans</i>
<i>Planococcus kocurii</i>	X62173	99.0																
<i>Planococcus citreus</i>	X62172	99.0	98.6															
<i>Caryophanon latum</i>	X70314	96.0	96.0	96.3														
<i>Bacillus sphaericus</i>	D16280	96.7	96.1	96.7	97.7													
<i>Kurthia zopfii</i>	X70321	96.8	96.5	96.3	96.8	97.0												
<i>Bacillus psychrophilus</i>	D16277	97.4	97.1	97.1	96.2	96.4	96.7											
<i>Staphylococcus aureus</i>	L37597	94.3	94.1	94.5	93.5	94.4	94.2	93.9										
<i>Bacillus subtilis</i>		96.0	95.7	96.5	95.0	95.4	94.7	95.1	95.3									
<i>Listeria monocytogenes</i>	S55472	95.8	95.3	96.0	94.1	94.7	94.9	95.1	94.6	95.7								
<i>Marinococcus halophilus</i>	X62171	93.5	93.3	93.7	91.6	92.7	92.3	92.7	91.3	93.1	92.3							
<i>Sporosarcina halophila</i>	X62175	95.3	95.1	95.8	93.6	93.8	93.8	94.7	93.3	95.3	95.0	94.4						
<i>Sporolactobacillus inulinus</i>	D16283	93.7	93.3	94.1	92.1	92.4	93.1	93.1	92.0	93.9	93.4	91.7	93.1					
<i>Bacillus kaustophilus</i>	X60618	95.7	95.2	95.9	94.1	94.7	93.8	94.5	92.7	94.9	94.1	92.3	94.4	93.1				
<i>Exiguobacterium aurantiacum</i>	X70316	93.8	93.2	93.6	93.4	93.1	93.3	93.1	91.6	92.8	92.2	90.7	92.4	91.2	93.1			
<i>Paenibacillus polymyxa</i>	D16276	93.3	93.1	93.3	92.3	92.6	92.3	92.7	91.2	92.0	91.2	91.5	92.3	91.9	92.6	91.7		
<i>Desulfotomaculum nigrificans</i>	X62176	88.7	88.4	89.0	89.2	89.0	87.9	87.9	87.7	88.3	87.6	87.2	87.9	86.2	89.7	87.6	88.8	
<i>Escherichia coli</i> <sup>b</sup>		82.8	82.4	82.4	82.7	82.6	83.0	83.6	82.5	82.6	82.8	81.8	83.0	82.7	83.8	82.6	83.3	82.3

<sup>a</sup> Data from reference 9.  
<sup>b</sup> Data from reference 4.

major isoprenoid quinones are MK-7 and MK-8. The cellular fatty acids are mainly branched fatty acids. The G+C content of the DNA is 39 to 52 mol%.

**Description of *Planococcus okeanokoites* comb. nov.** *Planococcus okeanokoites* (o.ke.a.no.ko.i'tes. Gr. masc. n. *oceanus*, the ocean; Gr. fem. n. *coites*, bed; M.L. fem. gen. n. *okeanokoites*, of the ocean bed). Cells are rods that are 0.4 to 0.8 µm wide and 1.0 to 20 µm long. Most cells are less than 2.8 µm long. Motile cells have peritrichous flagella. Spores are not formed. Gram positive to gram variable in medium containing NaCl. Gram negative in medium without NaCl. The color of the cell mass is usually bright yellow to bright orange. Chemoorganotrophs. Metabolism is respiratory. Strict aerobes. Catalase positive. Weakly oxidase positive. Urease negative. Nitrate is not reduced to nitrite. The following tests are positive: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, decomposition of casein, and liquefaction of gelatin. The following tests are negative: methyl red; Voges-Proskauer reaction; indole production; phenylalanine deaminase; hydrolysis of starch and cellulose; and decomposition of tyrosine, esculin, and Tween 80. Acid is not produced from D-glucose, D-galactose, L-arabinose, D-xylose, sucrose, maltose, lactose, or man-

nitol in Hugh-Leifson O-F medium. The optimum level of salinity for growth is 3 to 5% NaCl. No growth occurs in the presence of more than 7% NaCl. The optimum growth temperature is 20 to 37°C.

The molar ratio of the amino acids glutamic acid, lysine, alanine, and aspartic acid in the cell wall is 1:1:2:1. The major isoprenoid quinones are MK-8 and MK-7. The major cellular fatty acids are i14:0 and i16:0. The G+C content of the DNA of the type strain is 46.3 mol%.

The type strain is IFO 12536 (= NCIMB 561).

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TABLE 5. Levels of DNA relatedness for *F. okeanokoites* and *Planococcus* strains

Strain	% Reassociation with DNA from:		
	IAM 12541 <sup>T</sup>	IAM 12847 <sup>T</sup>	IFO 12536 <sup>T</sup>
<i>P. citreus</i> IAM 12541 <sup>T</sup>	100	22	27
<i>P. kocurii</i> IAM 12847 <sup>T</sup>	16	100	22
<i>F. okeanokoites</i> IFO 12536 <sup>T</sup>	15	26	100

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