Oceanicola granulosus gen. nov., sp. nov. and *Oceanicola batsensis* sp. nov., poly-β-hydroxybutyrate-producing marine bacteria in the order '*Rhodobacterales*'

Jang-Cheon Cho and Stephen J. Giovannoni

Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA

Correspondence
Stephen J. Giovannoni
steve.giovannoni@orst.edu

Three Gram-negative, chemoheterotrophic, non-motile, rod-shaped bacterial strains that accumulate poly-β-hydroxybutyrate granules were isolated from the Bermuda Atlantic Time-series Study site by high-throughput culturing methods and characterized by polyphasic approaches. DNA–DNA hybridization, DNA G+C content and phylogenetic analyses based on 16S rRNA gene sequences divided the three isolates into two distinct genospecies that were clearly differentiated by fatty acid profiles, carbon source utilization patterns, antibiotic susceptibility and biochemical characteristics. The strains utilized a wide range of substrates, including pentoses, hexoses, oligosaccharides, sugar alcohols, organic acids and amino acids. DNA G+C contents were 71·5, 70·9 and 67·3 mol% for strains HTCC2516^T, HTCC2523 and HTCC2597^T, respectively. The most dominant fatty acid was 18 : 1ω7*c* in strains HTCC2516^T and HTCC2523, and cyclo 19 : 0 in strain HTCC2597^T. The type strains HTCC2516^T and HTCC2597^T were clearly differentiated by the presence or absence of 12 : 0, 12 : 1ω11*c*, 14 : 0, 15 : 0 and methyl 18 : 1. Phylogenetic analyses indicated that the strains formed a distinct monophyletic lineage within the *Roseobacter* clade in the order '*Rhodobacterales*' of the *Alphaproteobacteria*, and which did not associate with any of the described genera. Genotypic and phenotypic differences of the isolates from the previously described genera support the description of *Oceanicola granulosus* gen. nov., sp. nov. with the type strain HTCC2516^T (=ATCC BAA-861^T=DSM 15982^T=KCTC 12143^T) and of *Oceanicola batsensis* sp. nov. with the type strain HTCC2597^T (=ATCC BAA-863^T=DSM 15984^T=KCTC 12145^T).

INTRODUCTION

The *Roseobacter* clade (synonym '*Roseobacter–Sulfitobacter–Silicibacter*' group; Wagner-Döbler *et al.*, 2003) in the order '*Rhodobacterales*' (Garrity & Holt, 2001), which was named after the genus *Roseobacter* by Giovannoni & Rappé (2000), is the second most abundant 16S rRNA gene clone type in marine environments (Rappé *et al.*, 2000; Giovannoni & Rappé, 2000). This bacterial group is relatively readily cultivable compared to more common marine bacterial lineages such as SAR11, SAR116, SAR86, SAR202 and marine *Actinobacteria*. In the last few years, a large number of strains from this group have been isolated from diverse marine environments (Connon & Giovannoni, 2002; Allgaier *et al.*, 2003), and many have been identified as novel genera and species (Doronina *et al.*, 2000; Labrenz *et al.*, 2000; Urbance *et al.*, 2001; Schaefer *et al.*, 2002). This group is currently comprised 11 chemoheterotrophic genera, *Antarctobacter* (Labrenz *et al.*, 1998), *Sagittula* (González *et al.*, 1997), *Ruegeria* (Uchino *et al.*, 1998), *Silicibacter* (Petursdottir & Kristjansson, 1997), *Leisingera* (Schaefer *et al.*, 2002), *Sulfitobacter* (Sorokin, 1995), *Octadecabacter* (Gosink *et al.*, 1997), *Jannaschia* (Wagner-Döbler *et al.*, 2003), *Ketogulonicigenium* (Urbance *et al.*, 2001), '*Marinosulfonomonas*' (Holmes *et al.*, 1997) and *Methylarcula* (Doronina *et al.*, 2000), and four bacteriochlorophyll *a*-containing genera, *Roseobacter* (Shiba, 1991), *Roseovarius* (Labrenz *et al.*, 1999), *Staleya* (Labrenz *et al.*, 2000) and *Roseovivax* (Suzuki *et al.*, 1999). The metabolism of isolates in this bacterial group has been reported to be very diverse, including such metabolic pathways as aerobic anoxygenic photosynthesis (Shiba, 1991; Allgaier *et al.*, 2003), oxidation or degradation of sulfite (Sorokin, 1995; Pukall *et al.*, 1999), methanesulfonic acid (Holmes *et al.*, 1997), dimethylsulfoniopropionate (Ledyard *et al.*,

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Abbreviations: BATS, Bermuda Atlantic Time-series Study; PHB, poly-β-hydroxybutyrate.

The GenBank accession numbers for the 16S rRNA gene sequences of strains HTCC2516^T, HTCC2523 and HTCC2597^T are AY424896, AY424897 and AY424898, respectively.

1993; González *et al.*, 2000, 2003), methylamine (Doronina *et al.*, 2000), methyl bromide (Schaefer *et al.*, 2002), lignin (González *et al.*, 1997), aromatic compounds (Buchan *et al.*, 2000) and production of allelopathic compounds (Lafay *et al.*, 1995), suggesting that they play an important role for oceanic nutrient cycling.

In this study, three strains were isolated from the Bermuda Atlantic Time-series Study (BATS) site using the high-throughput culturing method. Polyphasic analyses indicated that these isolates represent a novel genus within the *Roseobacter* clade of the order '*Rhodobacterales*'. Two new species, *Oceanicola granulosus* sp. nov. and *Oceanicola batsensis* sp. nov., in a new genus *Oceanicola* gen. nov. are proposed for strains HTCC2516^T and HTCC2597^T, respectively.

METHODS

Isolation. Collecting sea water samples at BATS, Sargasso Sea, Atlantic Ocean and high-throughput culturing were performed as described previously (Cho & Giovannoni, 2003b). The liquid cultures of three strains designated HTCC2516^T, HTCC2523 and HTCC2597^T were obtained after incubating dilution-to-extinction cultures for 21 d at 16 °C. These liquid cultures were spread and purified as single colonies on marine agar 2216 (Difco) after incubation for 10 days at 30 °C.

Microscopy. The strains were grown to late exponential phase in marine broth 2216 (Difco) at 30 °C and 150 r.p.m. on a rotatory shaker. Cell size and cell morphology were examined by both Safranin O-stained light microscopy and DAPI (4′,6-diamidino-2-phenylindole; Porter & Feig, 1980)-stained epifluorescence microscopy, under a Leica DMRB microscope equipped with a cooled CCD camera (ORCA-ER; Hamamatsu) and IPLab v3.5 scientific imaging software (Scanalytics). Motility was examined from wet mounts of exponential-phase cells under dark-field microscopy (DMRB; Leica). Accumulation of poly-β-hydroxybutyrate (PHB) was determined by the Sudan Black staining method (Smibert & Krieg, 1994) under a Leica light microscope. Negatively stained transmission electron micrographs were taken as described elsewhere (Cho & Giovannoni, 2003b).

Phenotypic characterization. Tests used for phenotypic characterizations have been detailed in previous studies (Cho & Giovannoni, 2003a, b). The following biochemical and phenotypic characteristics were examined: morphology of cells and colonies; Gram staining; motility; cell pigmentation; bacteriochlorophyll *a*; ranges and optima for temperature, pH and salinity; oxidase and catalase production; glucose acidification; arginine dihydrolase; β-galactosidase activity; denitrification; indole production; hydrolysis of urea, arginine and aesculin; accumulation of PHB; sodium requirement; utilization of sole carbon sources (47 carbon compounds); oxidation of carbon sources (SN2; Biolog); and susceptibility to antibiotics (14 antibiotics).

Chemotaxonomy. Cellular fatty acid methyl esters were prepared and analysed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). Fatty acid profiles were analysed by Microbial ID. Genomic DNA was extracted and purified using the Qiagen DNeasy tissue kit. The DNA G+C contents were analysed by HPLC according to Mesbah *et al.* (1989) using a Platinum EPS reverse-phase C18 column (150 mm, 4·6 mm, 5 μm pore size; Alltech). Phage λ DNA was used as standards throughout the analyses.

DNA–DNA hybridization. Percentages of genomic DNA relatedness among the HTCC strains were determined by dot-blot hybridizations. Probe DNA of strain HTCC2516^T was prepared using DIG High prime DNA labelling and detection starter kit I (Roche Molecular Biochemicals). Genomic DNAs from strains HTCC2516^T, HTCC2523 and HTCC2597^T were denatured by boiling for 10 min in 6× SSC (1× SSC: 0·15 M NaCl, 0·015 M sodium citrate) and transferred onto positively charged nylon membranes by UV cross-linking. Prehybridization, hybridization, stringency washing and detection were performed according to the manufacturer's instructions. The hybridization temperature was 52 °C and stringency washing was carried out in 0·5× SSC and 0·1 % SDS at 65 °C in a hybridization chamber. Densitometric analyses were carried out using the Personal Densitometer with ImageQuant imaging software (Amersham Biosciences).

Phylogenetic analyses. The 16S rRNA gene fragments of the marine strains were generated by PCR as described previously (Cho & Giovannoni, 2003b) and directly sequenced by the chain-termination method on an ABI 377 automated sequencer. Nearly full-length sequences of the 16S rRNA gene were aligned against a variety of other 16S rRNA gene sequences using the ARB software package (Ludwig *et al.*, 1998) and 1186 unambiguously aligned nucleotide positions were used for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Percentage sequence similarities were determined from distance matrices according to Jukes & Cantor (1969). Phylogenetic trees were inferred by three different algorithms: neighbour-joining with Kimura two-parameter model; maximum-parsimony with a heuristic search; and maximum-likelihood with a heuristic search, TBR-branching, and a Ti/Tv ratio that was estimated from the dataset (1·395). The tree topologies from neighbour-joining and maximum-parsimony were evaluated by bootstrap analyses based on 1000 resamplings.

RESULTS AND DISCUSSION

Cell and colony morphology

All isolates were Gram-negative (by Gram staining and KOH test), non-motile short rods, 0·7–2·1 μm long and 0·4–1·1 μm wide, dividing by binary fission (Fig. 1). The cell length and width of strain HTCC2597^T were smaller than those of strains HTCC2516^T and HTCC2523. PHB granules were always detected in the three HTCC strains by both negatively stained electron microscopy (Fig. 1) and Sudan Black staining. The typical morphology of Gram-negative cell membranes was observed in electron micrographs. Neither flagella nor endospores were observed on negatively stained cells. Colonies were faint yellow, 0·5–1·5 mm in diameter, uniformly circular, convex and opaque after growth on marine agar 2216 at 30 °C for 5 d.

Growth and physiological characteristics

No isolates grew under strict anaerobic conditions, even with prolonged incubations of 30 days. However, strains HTCC2516^T and HTCC2523 sustained their growth activity under microaerobic conditions, although their growth was poor. The temperature range for growth was 4–40 °C (optimum 28–30 °C). The pH range for growth was pH 5·5–9·5 (optimum 7·5–8·0) and the NaCl concentrations for growth were 0·25–10 % (w/v) (optimum 2·5–3·0 %). The

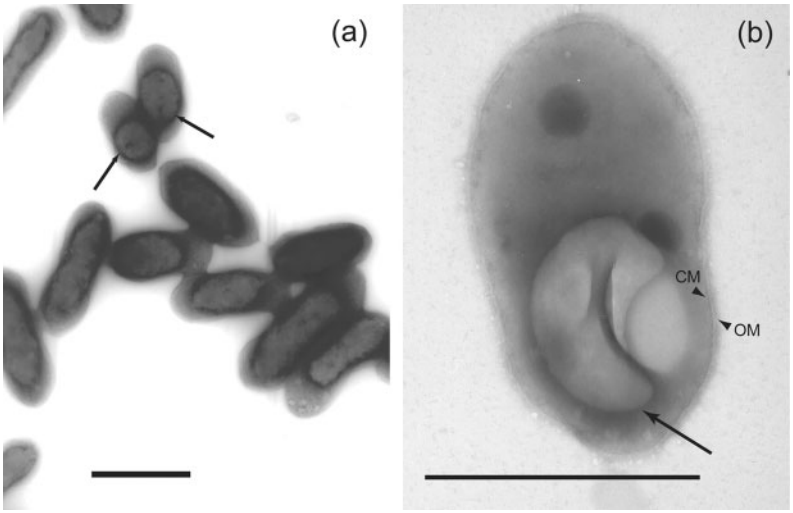

Fig. 1. Electron micrographs of negatively stained cells of strains HTCC2516^T (a) and HTCC2597^T (b). Arrows indicate PHB granules. OM, outer membrane; CM, cytoplasmic membrane. Scale bars, 1·0 μm.

minimum NaCl concentrations for supporting growth were 0·25 % in HTCC2516 and 0·75 % in HTCC2597.

All HTCC isolates were oxidase-positive. Biochemical tests for denitrification activity, arginine and gelatin hydrolysis, indole production and glucose acidification were negative in all the HTCC isolates studied. The strains produced neither acetone/methanol-extractable pigments nor bacteriochlorophyll *a*. Therefore, the energy metabolism of the isolates appears to be exclusively non-photosynthetic chemoheterotrophy. Major characteristics that differentiate the strains are represented in Table 1. Biochemical characteristics, carbon source utilization patterns and antibiotic susceptibility clearly differentiated the strains from each other. The strains utilized a wide range of substrates, including hexoses, oligosaccharides, organic acids and amino acids as their sole carbon sources (custom-made 48-well plate tests). All three strains utilized D-galactose, D-maltose, D-melezitose, citric acid, formic acid, propionic acid, L-glutamic acid, L-serine and L-arginine as sole carbon sources. However, none of the strains utilized DL-glyceraldehyde, D-xylose, D-fructose, L-rhamnose, D-melibiose, D-raffinose, D-sorbitol, adonitol, ethanol, glycerol, methanol, succinic acid, itaconic acid, D-malic acid, L-ornithine, L-proline, L-lysine, D-mannose, D-glucosamine, L-leucine or L-isoleucine. All strains were susceptible to kanamycin, carbenicillin, tetracycline, ampicillin, puromycin, vancomycin, rifampicin and benzylpenicillin, and were resistant to nalidixic acid and cycloheximide.

Phylogenetic analyses

Nearly complete 16S rRNA gene sequences (1418–1423 bp) were determined for three HTCC isolates and used for phylogenetic analyses. Strains HTCC2516^T and HTCC2523 shared 99·9 % sequence similarity, and represented 95·1 and 95·3 % similarity to HTCC2597^T, respectively. According

Table 1. Differential characteristics for the HTCC isolates studied

Strains: 1, *Oceanicola granulosus* HTCC2516^T; 2, *Oceanicola granulosus* HTCC2523; 3, *Oceanicola batsensis* HTCC2597^T.

Characteristic	1	2	3
Catalase	−	−	+
Urease	−	−	+
Aesculin hydrolysis	+	+	−
β-Galactosidase	+	+	−
Susceptibility to:			
Chloramphenicol	+	+	−
Streptomycin	−	+	+
Erythromycin	+	−	+
Gentamicin	−	−	+
Sole carbon source utilization			
D-Ribose	+	+	−
D-Arabinose	−	−	+
D-Glucose	+	+	−
L-Sorbose	−	−	+
Sucrose	+	+	−
β-Lactose	+	−	−
D-Trehalose	+	+	−
D-Cellobiose	+	+	−
D-Mannitol	−	−	+
N-Acetyl-D-glucosamine	+	+	−
Pyruvic acid	+	+	−
Gluconic acid	+	−	+
Malonic acid	−	−	+
Lactic acid	−	+	+
m-Inositol	−	+	+
L-Alanine	+	+	−
Glycine	−	−	+
DNA G+C mol%	71·5	70·9	67·3
DNA–DNA hybridization (with HTCC2516^T)	100	95·7	48·0

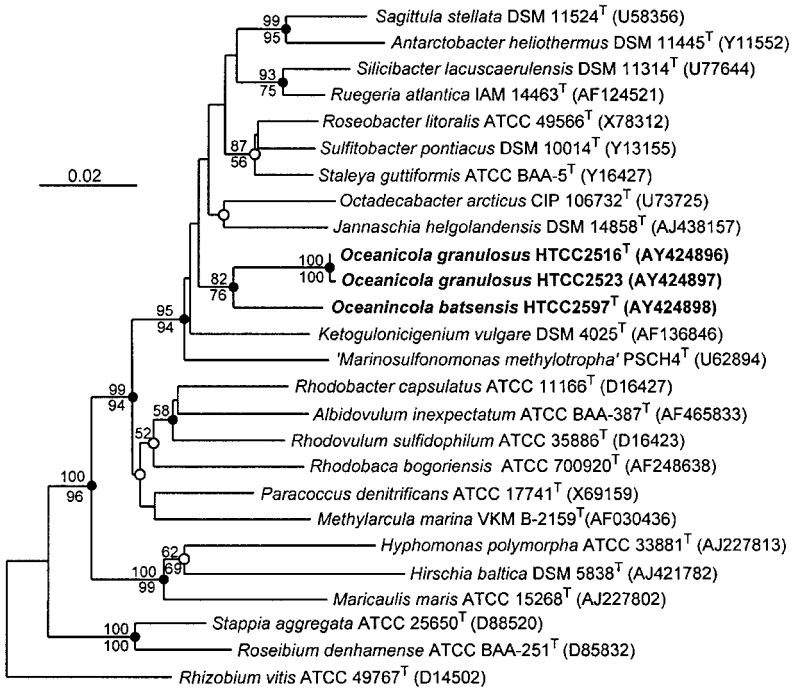

Fig. 2. Neighbour-joining tree showing relationships between the three HTCC isolates and representatives of the order '*Rhodobacterales*' within the *Alphaproteobacteria* inferred from 16S rRNA gene sequence analyses. Bootstrap proportions over 50 % from both neighbour-joining (above nodes) and maximum-parsimony (below nodes) are shown. The filled and open circles at each node indicate the nodes recovered reproducibly by all treeing methods and two treeing methods, respectively. *Rhizobium vitis* ATCC 49767^T in the order '*Rhizobiales*' was used as an outgroup to define the root of the tree. Scale bar, 0·02 substitutions per nucleotide position.

to the practical 3 % cut-off value for 16S rRNA divergence to demarcate species (Stackebrandt & Göebel, 1994), HTCC2516^T and HTCC2597^T represent two separate species. BLAST search results and phylogenetic analyses in the ARB database indicated that all strains belong to the order '*Rhodobacterales*' of the *Alphaproteobacteria*. Sequence comparisons to validly named bacteria indicated that the three strains were most closely related to species recently classified, *Ketogulonicigenium vulgarae* (92–94 %), *Jannaschia helgolandensis* (92–93 %) and *Octadecabacter arcticus* (92–93 %). Strain HTCC2597^T was most closely related to Sargasso Sea isolate GMDsbM4 (GenBank no. AY162095; Zengler *et al.*, 2002) with 99 % similarity. In all phylogenetic trees, two strains represented by HTCC2516^T formed one distinct subclade with 100 % bootstrap value and strain HTCC2597^T formed a separate branch (Fig. 2). The three HTCC isolates always formed a distant monophyletic clade with 76–82 % bootstrap support for a position within the order '*Rhodobacterales*' in different phylogenetic trees, and this clade was not closely associated with any other taxa in the order '*Rhodobacterales*' (Fig. 2). These phylogenetic analyses indicated that the HTCC isolates represent a novel genus-level lineage within the *Roseobacter* clade of the order '*Rhodobacterales*'.

DNA relatedness and chemotaxonomy

The DNA G+C content of the HTCC strains ranged from 67·3 to 71·5 mol% (Table 1). The G+C content of strain HTCC2597^T was 3–4 mol% lower than that of the other two strains. When the DNA of strain HTCC2516^T was used as a probe for DNA–DNA hybridization, strain HTCC2523 showed 95·7 % DNA relatedness to strain HTCC2516^T, while strain HTCC2597^T showed 48·0 % relatedness (Table 1). From the results of G+C mol% and DNA–DNA hybridization analyses, the strains were clearly categorized into two genospecies. The predominant fatty acids in the isolates were 18 : 1ω7*c*, cyclo 19 : 0, 16 : 0 and methyl 18 : 1 (Table 2). The type strains HTCC2516^T and HTCC2597^T of the two genospecies were differentiated by the presence or absence of several fatty acids, including 12 : 0, 12 : 1ω11*c*, 14 : 0, 15 : 0 and methyl 18 : 1, and by the proportions of 16 : 0, 18 : 1ω7*c* and 19 : 0 cyclo ω8*c*. The fatty acid profile of the HTCC isolates differed significantly from the phylogenetically related taxa in the order '*Rhodobacterales*' mainly by the proportions of 16 : 0, 18 : 1ω7*c*, methyl 18 : 1 and cyclo 19 : 0 (Table 3).

Taxonomic conclusions

Polyphasic approaches, including phenotypic data (Table 1), fatty acid profiles (Table 2), DNA G+C content (Table 1), DNA–DNA hybridization (Table 1) and 16S rDNA phylogenetic analyses (Fig. 2) support the placement of the HTCC isolates in a novel genus within the order '*Rhodobacterales*' and comprise two distinct species. Furthermore, comparisons of phenotypic and biochemical characteristics among the phylogenetically associated genera and species clearly demonstrated that the isolates are not closely related to any of the previously described related taxa (Table 3). Two strains, HTCC2516^T and HTCC2523, shared very similar phenotypic and genotypic characteristics, such as >99 % 16S rDNA sequence similarity and >95 % DNA–DNA hybridization, so they were regarded as members of the same species. Relatively low 16S rDNA sequence similarity (95 %) and DNA relatedness (48 %) between strains

Table 2. Cellular fatty acid composition (%) of *Oceanicola granulosus* and *Oceanicola batsensis*

Strains: 1, *Oceanicola granulosus* HTCC2516^T; 2, *Oceanicola granulosus* HTCC2523; 3, *Oceanicola batsensis* HTCC2597^T.

Fatty acid	1	2	3
10:0	0·1	0·1	–
3-OH 10:0	1·5	1·6	0·4
12:0	–	–	2·0
3-OH 12:0	1·6	1·9	–
12:1ω11*c*	–	–	4·9
14:0	–	–	1·5
15:0	–	–	0·9
16:0	11·9	13·2	15·0
iso 16:0	–	0·3	–
17:0	0·4	0·2	1·5
anteiso 17:0	0·2	0·3	–
cyclo 17:0	0·2	0·6	–
17:1ω8*c*	0·3	–	–
18:0	0·9	0·9	2·4
18:1ω7*c*	62·8	55·4	31·0
Methyl 18:1	8·1	10·9	–
cyclo 19:0	10·8	13·1	40·4
20:2ω6, ω9*c*	–	0·4	–
15:0 iso 2-OH + 16:1ω7*c*	1·2	1·1	–
Total	100·0	100·0	100·0

HTCC2516^T and HTCC2597^T differentiated the strains into independent species (Wayne *et al.*, 1987). The 4·2 mol% G+C difference between strains HTCC2516^T and HTCC2597^T supported the conclusion that both strains belong to the same genus, because a 10 % difference in G+C mol% is generally a criterion for differentiating genera (Stackebrandt & Liesack, 1993). Therefore, we placed these type strains as two separate species in the same genus. Consequently, on the basis of both phylogenetic and phenotypic distinction, we propose the *Oceanicola* gen. nov., containing the species *Oceanicola granulosus* sp. nov. and *Oceanicola batsensis* sp. nov.

Description of *Oceanicola* gen. nov.

Oceanicola (o.ce.a.ni′co.la. L. n. *oceanus* the ocean; L. masc. suffix *-cola* inhabitant; N.L. masc. n. *Oceanicola* inhabitant of the ocean).

Cells are Gram-negative, non-motile short rods that multiply by binary fission. Accumulate PHB granules. Neither flagella nor endospores were observed. Faint yellowish colonies are formed on marine agar. Bacteriochlorophyll was absent. Metabolism is chemoheterotrophic and obligately aerobic to microaerotolerant. Oxidase-positive. Denitrification, arginine and gelatin hydrolysis, indole production and glucose acidification were negative. Predominant fatty acids are 18:1ω7*c*, 16:0 and cyclo 19:0. The G+C content of genomic DNA is 67·3–71·5 mol% (by HPLC). Phylogenetically, the genus forms a distinct clade within the order '*Rhodobacterales*'. The genus contains two species, *Oceanicola granulosus* sp. nov. and *Oceanicola batsensis* sp. nov.

The type species of the genus is *Oceanicola granulosus*.

Description of *Oceanicola granulosus* sp. nov.

Oceanicola granulosus (gra.nu.lo′sus. N.L. masc. adj. *granulosus* granular).

The description of this species is the same as that for the genus. Cells are 1·1–2·1 μm wide and 0·5–1·0 μm long. Colonies are 0·5–1·0 mm in diameter, uniformly circular, convex and opaque. Temperature range for growth is 4–40 °C, optimum 28 °C. pH range for growth is 5·5–9·5, optimum 7·5–8·0. Grows at 0·25–10·0 % NaCl. Catalase- and urease-negative. Aesculin- and β-galactosidase-positive. The sole carbon source utilization patterns for differentiating strains are listed in Table 1 and the text. According to Biolog tests, the following substrates were oxidized by all strains: glycogen, Tween 80, *N*-acetyl-D-glucosamine, D-arabitol, D-cellobiose, D-galactose, gentiobiose, α-D-glucose, maltose, sucrose, D-trehalose, xylitol, pyruvic acid methyl ester, acetic acid, citric acid, formic acid, α-ketobutyric acid, α-ketoglutaric acid, L-lactic acid, propionic acid, L-alanine, L-glutamic acid, L-serine, inosine, uridine and 2,3-butanediol. Predominant fatty acids are 16:0 (12–13 %), 18:1ω7*c* (55–63 %), methyl 18:1 (8–11 %) and cyclo 19:0 (11–13 %).

DNA G+C content is 70·9–71·5 mol%. Isolated from the western Sargasso Sea, Atlantic Ocean. Type strain is strain HTCC2516^T (=ATCC BAA-861^T=DSM 15982^T=KCTC 12143^T). Reference strain: HTCC2523.

Description of *Oceanicola batsensis* sp. nov.

Oceanicola batsensis (ba.tsen′sis. N.L. masc. adj. *batsensis* from BATS, an abbreviation for Bermuda Atlantic Time-series Study site)

The description of this species is the same as the genus described above. Cells are 0·7–1·6 μm wide and 0·4–0·8 μm long. Colonies are 0·7–1·5 mm in diameter, uniformly circular, convex and opaque. Temperature range for growth is 4–40 °C, optimum 30 °C. pH range for growth is 6·0–9·0, optimum 7·5–8·0. Grows at 0·75–10·0 % NaCl. Catalase- and urease-positive. Aesculin- and β-galactosidase-negative. The sole carbon source utilization patterns for differentiating strains are listed in Table 1 and the text. According to Biolog tests, the following substrates were oxidized by the species: glycogen, Tween 40, L-arabinose, D-arabitol, D-galactose, mannitol, D-sorbitol, acetic acid, formic acid, D-galactonic acid lactone, D-gluconic acid, α-ketobutyric acid, α-ketoglutaric acid, L-lactic acid, malonic acid, propionic acid, D-saccharic acid, L-alanyl-glycine, L-glutamic acid, glycyl-L-glutamic acid, L-serine, threonine, putrescine, 2,3-butanediol and

Table 3. Differential characteristics of *Oceanicola granulosus* HTCC2516^T and *Oceanicola batsensis* HTCC2597^T from phylogenetically related type species in the '*Rhodobacterales*'

Strains: 1, *Oceanicola granulosus* HTCC2516^T (this study); 2, *Oceanicola batsensis* HTCC2597^T (this study); 3, *Jannaschia helgolandensis* DSM 14858^T (Wagner-Döbler *et al.*, 2003); 4, *Octadecabacter arcticus* CIP 106732^T (Gosink *et al.*, 1997); 5, *Staleya guttiformis* ATCC BAA-5^T (Labrenz *et al.*, 2000); 6, *Sulfitobacter pontiacus* DSM 10014^T (Sorokin, 1995; Labrenz *et al.*, 2000); 7, *Roseobacter litoralis* ATCC 49566^T (Shiba, 1991; Labrenz *et al.*, 1999); 8, *Ketogulonicigenium vulgarae* DSM 4025^T (Urbance *et al.*, 2001); 9, '*Marinosulfonomonas methylotropha*' PSCH4^T (Holmes *et al.*, 1997); 10, *Ruegeria atlantica* IAM 14463^T (Rüger & Höfle, 1992; Uchino *et al.*, 1998); 11, *Silicibacter lacuscaerulensis* DSM 11314^T (Petursdottir & Kristjansson, 1997; González *et al.*, 2003); 12, *Antarctobacter heliothermus* DSM 11445^T (Labrenz *et al.*, 1998); 13, *Sagittula stellata* DSM 11524^T (González *et al.*, 1997). Symbols: V, variable; W, weakly positive; ND, not determined. Colony colour: FY, faint yellow; CR, cream; WH, white; BG, beige; CL, colourless; BR, brown; BY; brownish yellow.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Rosette formation	−	−	−	−	+	+	−	−	+	+	−	+	+
Motility	−	−	−	−	+	+	+	−	−	−	−	−*	−
Colony colour	FY	FY	WH	WH	BG	CL	PI	BR	CL	BG	BR	BY	CR
Bacteriochlorophyll *a*	−	−	−	−	V	−	+	−	−	−	−	−	−
Gas vesicles	−	−	−	+	−	−	−	ND	−	ND	+	−	−
PHB	+	+	ND†	−	+	+	−	ND	ND	ND	−	+	+
Oxidase	+	+	W	−	+	+	+	+	+	ND	+	+	+
Catalase	−	+	+	+	W	+	+	+	+	+	+	+	+
Growth at 37 °C	+	+	−	−	−	−	−	−	+	−	+	+	+
Gelatin hydrolysis	−	−	−	−	−	−	+	ND	ND	−	ND	+	−
Nitrate reduction	−	−	−	−	+	ND	−	ND	+	+	+	+	−
Utilization of:													
Glucose	+	−	+	V	W	ND	+	+	+	+	−	+	+
Fructose	−	−	+	−	W	ND	ND	+	+	−	ND	+	+
Mannitol	−	+	+	ND	−	ND	ND	+	ND	+	ND	+	ND
Methanol	−	−	ND	−	−	ND	−	ND	+	ND	ND	−	+
Succinate	−	−	+	V	+	ND	+	+	+	+	ND	+	+
Butyrate	−	−	−	−	−	+	−	ND	ND	ND	ND	+	+
Citrate	−	−	+	V	−	ND	+	ND	+	+	−	+	+
Major fatty acids:													
3-OH 10 : 0	1·5	0·4	5·6	4	5·9	3·6	1·9	3·6	ND	−	1·6	−	−
3-OH 12 : 0	1·6	−	−	−	−	−	−	2·9	ND	7·7	4·0	−	−
3-OH 14 : 1	−	−	−	−	2·1	2·0	3·9	−	ND	1·9	−	−	−
16 : 0	11·9	15·0	−	6	3·9	8·1	1·1	33·5	ND	−	1·6	2·5	8·6
16 : 1ω7*c*	−	−	−	8	−	1·2	−	−	ND	−	0·3	0·8	−
18 : 0	0·9	2·4	10·8	−	0·7	−	1·3	2·9	ND	−	2·6	1·0	6·8
18 : 1ω7*c*	62·8	31·0	45·1	75‡	79·7	79·1	88·8	52‡	ND	80	81·7	83·2	§
methyl 18 : 1	8·1	−	5·7	−	−	−	−	−	ND	−	2·2	−	−
cyclo 19 : 0	10·8	40·4	25·1	−	−	−	−	−	ND	−	−	2·4	1·8
G+C content (mol%)	72	67	63	57	55	62	57	54	57	55	66	62	65

*Three strains of the species showed motility.
†Inclusion bodies were found in the cells.
‡18 : 1ω7*c* + 18 : 1ω9*t* + 18 : 1ω12*t*.
§Major fatty acid, but not quantified.

DL-α-glycerol phosphate. Predominant fatty acids are 16 : 0 (15 %), 18 : 1ω7*c* (31 %) and cyclo 19 : 0 (40 %).

DNA G+C content is 67·3 mol%. Isolated from the western Sargasso Sea, Atlantic Ocean. Type strain is strain HTCC2597^T (=ATCC BAA-863^T=DSM 15984^T=KCTC 12145^T).

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