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Croceibacter atlanticus gen. nov., sp. nov., A Novel Marine Bacterium in the Family *Flavobacteriaceae*

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Summary

A bright, saffron-colored marine bacterium HTCC2559^T was isolated from the Bermuda Atlantic Time Series station in the western Sargasso Sea, Atlantic Ocean by high throughput culturing methods and characterized by polyphasic approaches. Phenotypic data and phylogenetic analyses showed that the strain is a member of the family *Flavobacteriaceae*. The strain was Gram-negative, non-motile, chemoheterotrophic, strictly aerobic, NaCl-requiring, rod-shaped cells that contain carotenoid pigments but not flexirubin. Several kinds of macromolecules (gelatin, DNA, starch, casein, and elastin) were degraded and carbohydrates, sugar alcohols, organic acids, and amino acids were utilized as sole carbon sources. The dominant fatty acids were branched or hydroxy acids, and 3-OH i17:0, i15:0, i15:1, and i17:1 ω9c were abundant. The DNA G+C content of the strain is 34.8 mol%. Phylogenetic analyses using three treeing algorithms based on 16S rRNA gene sequences revealed that the strain formed a very distinct lineage that is allied closely with several seawater environmental clones in the family *Flavobacteriaceae*. Therefore, it is proposed from the polyphasic studies that strain HTCC2559^T (=ATCC BAA-628^T = KCTC 12090^T) belongs to a new genus and species named *Croceibacter atlanticus* gen. nov., sp. nov.

Key words: *Croceibacter atlanticus* – the family *Flavobacteriaceae* – seawater – 16S rRNA – polyphasic taxonomy – high throughput culturing

Introduction

Ecological studies based on cultivation or culture-independent approaches have revealed that the phylum *'Bacteroidetes'* ([14]; synonym as 'flavobacter-bacteroides' phylum, *Flavobacterium-Cytophaga* complex, *Cytophaga-Flavobacterium-Bacteroides* (CFB) group, and rRNA superfamily V) is one of the dominant groups of the domain *Bacteria* in the marine environment [6, 11]. The members of the *Cytophaga-Flavobacterium* cluster generally comprise 6–30% (up to 72%) of total bacterial communities in seawater as determined by fluorescence *in situ* hybridization (FISH) with CFB-specific oligonucleotide probes [12, 15]. They are well known for degrading macromolecules such as chitin, DNA, cellulose, starch, pectin, etc. [29], suggesting that they can play an important role in the detritus food chain and the microbial loop. CFB 16S rRNA gene clones are rare in environmental clone libraries made from seawater but *in situ* hybridization data suggest that they are abundant in bacterioplankton [19]. The cause of this discrepancy is probably mismatches between CFB 16S rRNA genes and the PCR primer EubA that was in common use in the 1990s for amplifying bacterial genes [32]. Undescribed marine members of the CFB phylum form a number of unique lineages that are obvious candidates for cultivation and taxonomic description.

Since Bernardet et al. [2] made an emended description of the family *Flavobacteriaceae*, three novel genera (*Arenibacter*, *Muricauda*, and *Aequorivita*) have been included in this family [4, 17, 22]. Now the family *Flavobacteriaceae* is composed of 21 well-defined genera and two misclassified organisms, i.e. [*Cytophaga*] *marinoflava* and [*Cytophaga*] *latercula*. Most of the recently

The GenBank accession number for the 16S rRNA gene sequence of strain HTCC2559^T is AY163576.

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classified taxa of the family *Flavobacteriaceae*, such as *Gelidibacter* and *Psychroserpens* [5], *Polaribacter* [16], *Psychroflexus* [7], *Salegentibacter* [24], *Cellulophaga* [18], *Arenibacter* [17], *Muricauda* [22], and *Aequorivita* [4] were cultivated from marine environments and are psychrophilic organisms, indicating that marine environments are an important habitat for the members of the family *Flavobacteriaceae*.

We have isolated several novel strains of CFB from the Sargasso Sea and the Oregon coast using the high throughput culturing approaches [10]. One of these isolates, strain HTCC2559^T ("HTCC" stands for the high throughput culture collection that is being maintained by our laboratory at Oregon State University, USA), was cultivated from water collected at a depth of 250 m from the Sargasso Sea and characterized by polyphasic taxonomy. Strain HTCC2559^T was shown by phylogenetic analysis to be related by similarities of over 99% to several marine environmental clones and formed a unique lineage with them in the family *Flavobacteriaceae*. Here we describe this strain by polyphasic approaches and propose its inclusion in a new genus and a species named *Croceibacter atlanticus* gen. nov., sp. nov.

Material and Methods

Strain isolation

A seawater sample was collected from a depth of 250 m at the Bermuda Atlantic Time Series station (BATS), in the western Sargasso Sea, Atlantic Ocean in August 2001. The seawater sample was diluted to 10 cells per ml in a low nutrient heterotrophic medium (LNHM, 0.2 μm-filtered and autoclaved seawater amended with 1.0 μM NH_4Cl and 0.1 μM KH_2PO_4) amended with 0.001% (w/v) of D-glucose, D-ribose, succinic acid, pyruvic acid, glycerol, N-acetyl D-glucosamine, and 0.002% (v/v) of ethanol [27]. The initial liquid culture of a strain designated HTCC2559^T was isolated using the high throughput approaches outlined in Connon and Giovannoni [10]. The liquid culture of strain HTCC2559^T was spread and purified as single colonies on marine agar 2216 (Difco) after incubation for 5 d at 25 °C. The strain described here was from the subset of HTCC strains that can be grown on agar. Most HTCC strains cannot be grown on agar. The strain was maintained as viable cultures on marine agar plates and slants at 4 °C, and also stored as 10% (v/v) glycerol suspensions in liquid nitrogen.

Phenotypic analysis

Unless otherwise indicated, standard methods for phenotypic characterization of the *Flavobacterium*-like strain were employed as described by Smibert and Krieg [30] and Bowman et al. [5, 7]. A catalase test was performed by addition of 3.0% hydrogen peroxide to fresh colonies, and oxidase activity was determined using Kovacs' solution. Other biochemical tests were carried out on API 20NE strips (BioMérieux) following the manufacturer's instructions.

Gliding motility was examined by both light microscopy and phase-contrast microscopy of hanging drop preparations. Motility was examined from wet mounts of exponential phase cells under dark field microscopy. Cell size and cell morphology were examined by DAPI (4′-6-diamidino-2-phenylindole) staining according to Porter and Feig [26] using an epifluorescence microscope (Leica, DMRB). For electron microscopy, exponential phase cells were concentrated by centrifugation, washed with phosphate buffered saline (pH 8.0) twice, fixed with 1.5% glutaraldehyde, and negatively stained with 2% aqueous ammonium molybdate (pH 6.3) on Formvar-filmed copper grids. Transmission electron microscopy was carried out using a Phillips CM12 transmission electron microscope operated at 60 kV in transmission mode.

The temperature range and optimum were tested at a range from 4 to 44 °C using marine agar. The pH range and optimum were examined at pH values 4.0 to 12.0 at 25 °C. The NaCl concentrations and optimum for growth were determined in a medium which contained: 1.0 g $MgCl_2{\cdot}6H_2O$, 5.0 g $MgSO_4 \cdot 7H_2O$, 0.7 g KCl, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.5 g NH_4Cl, 0.1 g KBr, 0.27 g KH_2PO_4, 0.04 g $SrCl_2 \cdot 6H_2O$, 0.025 g H_3BO_3, 5.0 g peptone, and 1.0 g yeast extract per 1 liter (pH 8.0) with the NaCl concentrations of 0–20% (w/v). Anaerobic growth and capnophilic activity were tested using the Oxoid Anaerobic system and the Merck Anaerocult mini C, respectively. Susceptibility to antibiotics was determined by the diffusion plate method. The following antibiotics were tested: chloramphenicol (25 μg), nalidixic acid (25 μg), kanamycin (30 μg), carbenicillin (25 μg), tetracycline (30 μg), streptomycin (50 μg), ampicillin (10 μg), puromycin (25 μg), erythromycin (15 μg), vancomycin (30 μg), rifampicin (50 μg), benzylpenicillin (100 U), gentamycin (10 μg), cyclohexamide (50 μg).

The bathochromic shift test was performed to detect flexirubin pigmentation using 20% (w/v) KOH solution [13]. Pigments were extracted using a methanol and acetone mixture (1:1) from cultures grown on marine agar 2216 for 5 d, and their absorption spectra were determined using a scanning UV/Visible spectrophotometer (Shimadzu, Biospec-1601).

Macromolecule degradation and carbon source utilization tests

Degradation of macromolecules was tested by incubating HTCC2559^T on marine agar containing macromolecules at 25 °C for 14 d. Casein and gelatin hydrolysis were tested using API 20NE strips. DNA and starch (0.2%, w/v) degradation were tested by the methods of Smibert and Krieg [30]. The following macromolecules were added to marine agar as top agar, and their hydrolysis was determined by formation of clear zones around the colonies: cellulose (paper); carboxymethylcellulose (1.5%, w/v); chitin (1.0%, w/v); elastin (0.2%, w/v); alginate (0.2%, w/v); carrageenan (1.0% w/v); and dextran (0.2%, w/v). Custom-made 48-well microplates containing 47 different carbon compounds at a final concentration of 0.2% (w/v, or v/v) were used for sole carbon source utilization tests. After incubating microtiter plates at 25 °C for 5 d, cellular growth and purity were examined by DAPI-stained epifluorescence microscopy. BIOLOG SN2 microplates were used to test oxidative utilization of 95 carbon sources.

Cellular fatty acids and DNA base composition

Cellular fatty acid methyl esters were prepared from the cultures grown on marine agar at 25 °C for 3 d, and analyzed using gas chromatography according to the instructions of the Microbial Identification System (MIDI). The samples were analyzed by Microbial ID, Inc. (Newark, DE, USA). The G+C content of the DNA was measured using an HPLC according to Mesbah et al. [25] with the Platinum EPS reverse-phase C18 column (150 mm, 4.6 mm, 5 μm pore size, Alltech).

Phylogenetic analyses

The 16S rRNA gene of strain HTCC2559^T was amplified by PCR using the slightly modified bacterial universal primers, 27F-B (5′-AGRGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) [20], and directly sequenced by the

chain termination method on an ABI 377 automated sequencer. Sequences were aligned using the ARB software package [21] and 1181 unambiguously aligned nucleotide positions were used for phylogenetic analyses in the PAUP* 4.0 beta 10 [33]. Phylogenetic trees were inferred by three tree-making algorithms: neighbour-joining with Kimura-2 parameter model; maximum parsimony with a heuristic search; and maximum likelihood with a heuristic search, TBR-branching, Ti/Tv ratio of 1.551181. The neighbour-joining tree and parsimony tree were evaluated by bootstrap analyses based on 1000 resamplings. A short sequence (1102 bp) of the sea-ice clone SIC.B8012 was added to the tree by using the parsimony insertion tool in ARB.

Results

Phenotypic characteristics

Strain HTCC2559^T was a Gram-negative (by Gram staining and KOH test), non-motile straight rod, 1.2–3.1 (average 1.9) μm long and 0.3–0.6 (average 0.4) μm wide, dividing by binary fission (Fig. 1a, 1b). No flagella were observed on the negatively stained cells. The strain contained neither endospores nor poly-β-hydroxybutyrate granules. Colonies on marine agar were 1.8–3.0 mm in diameter, bright orange or saffron-colored, uniformly circular, convex, opaque, and with a smooth surface. The strain was an obligately aerobic, NaCl-requiring chemoheterotroph. The major phenotypic characteristics of strain HTCC2559^T are shown in Table 1. No gliding motility was observed by hanging drop preparations. The strain grew under neither strictly anaerobic conditions nor microaerobic conditions. The temperature range for growth was 10–28 °C, with optimum growth at 20–23 °C. No growth was observed at 4 and 30 °C. The pH range for growth was pH 6.0–10.0, with optimum growth at pH 7.5–8.0. No growth was detected at pH 5.5 and 10.5. It was moderately halophilic, showing good growth at NaCl concentrations of 0.5–15% (w/v), with optimal growth at 3.0% (w/v) NaCl. The strain was catalase positive and oxidase negative. No denitrification activity was detected. It showed urease and arginine dehydrolase activity, but did not produce indole. Acid was not produced from glucose. The strain produced carotenoid pigments with wavelength absorbance spectral peaks at 318 and 483 nm. There was no difference in the spectral peaks between light-grown and dark-grown cultures. The bathochromic shift test results indicated that flexirubin pigments were absent.

Gelatin, DNA, starch, casein, and elastin were degraded after prolonged incubation for 2 weeks, but not aesculin, dextran, cellulose, alginate, chitin, and carrageenan. The strain utilized the following carbon sources as sole carbon sources; D,L-glyceraldehyde, D-arabinose, D-galactose, D-fructose, D-trehalose, D-maltose, D-melezitose, D-mannose, D-mannitol, D-sorbitol, pyruvic acid, succinic acid, gluconic acid, L-glutamic acid, L-ornithin, L-proline, L-alanine, L-serine, and L-leucine. However, D-ribose, D-xylose, D-glucose, L-rhamnose, L-sorbose, sucrose, β-lactose, D-cellobiose, D-melibiose, D-raffinose, adonitol, myo-inositol, methanol, ethanol, glycerol, N-acetyl-D-glucosamine, D-glucosamine, itaconic acid, citric acid, D-malic acid, malonic acid, formic acid, propionic acid, lactic acid, L-lysine, L-isoleucine, glycine, and L-arginine were not utilized as sole carbon sources. In the test using BIOLOG-SN2 microplates, the following carbon compounds were utilized; α-cyclodextrin, glycogen, Tween 40, L-arabinose, D-fructose, L-fucose, D-galactose, maltose, D-mannitol, D-mannose, D-psicose, D-sorbitol, D-trehalose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, D, L-lactic acid (weak), glycerol (weak), β-hydroxy butyric acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, propionic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-serine, L-threonine, inosine, uridine, and thymidine. The results of antibiotics susceptibility tests are given in Table 1.

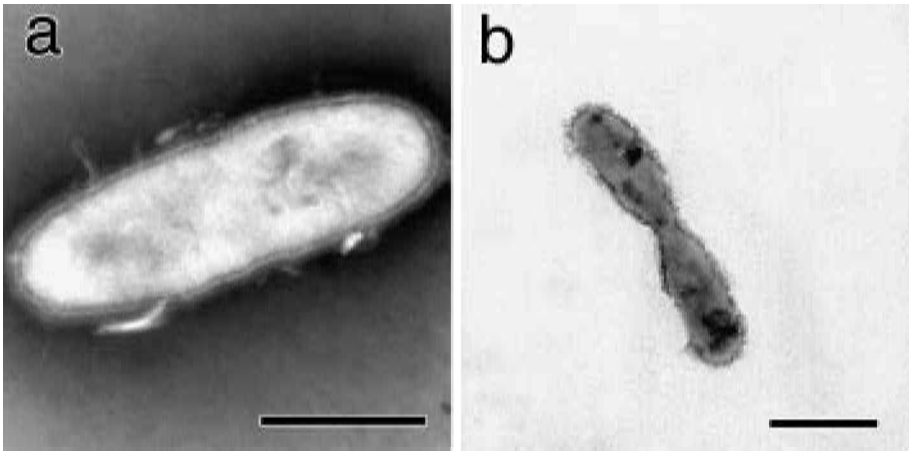

Fig. 1. Electron micrographs of negatively stained cells of stain HTCC2559^T. (a) Single cell (b) Dividing cells. The scale bars represent 1.0 μm.

Table 1. Major phenotypic characteristics of *Croceibacter atlanticus* HTCC2559^T.

Characteristics	Results
Catalase, arginine dehydrolase, urease	+
Oxidase, denitrification, indole, glucose acidification, β-galactosidase	–
Flagellation	–
Gliding motility	–
Pigmentation	+
Flexirubin-type pigment production	–
Capnophilic metabolism	–
Anaerobic growth	–
Growth at:	
10–28 °C, 0.5–15% NaCl, pH 6.0–10.0	+
Degradation of:	
Gelatin, DNA, starch, casein, elastin	+
Aesculin, dextran, cellulose, alginate, chitin, carrageenan	–
Susceptibility to:	
Chloramphenicol, nalidixic acid, tetracycline, erythromycin	+
Vancomycin, rifampicin, benzylphenicillin	+
Kanamycin, carbenicillin, streptomycin, ampicillin,	–
Puromycin, gentamycin, cycloheximide	–
Sole carbon source utilization:	
D,L-glyceraldehyde, D-arabinose, D-fructose, D-trehalose, D-mannitol,	+
Pyruvic acid, succinic acid, L-ornithin, L-proline, L-leucine,	+
D-ribose, D-glucose, L-sorbose, adonitol, glycerol	–
Citric acid, malonic acid, L-proline, L-lycine, L-arginine	–

Table 2. Celluar fatty acid composition of *Croceibacter atlanticus* HTCC2559^T.

Fatty acids		Percent of total
Saturated acids	(Total: 4.68)	
15:0		3.85
16:0		0.55
18:0		0.28
Branched acids	(Total: 38.91)	
i 15:0		13.30
a15:0		1.55
i 15:1		9.16
i 15:1		0.37
i 16:0		1.49
i 16:1		1.72
i 17:0		0.18
i 17:1 ω9c		9.36
a17:1 ω9c		1.30
i 18:0		0.48
Unsaturated acids	(Total: 3.49)	
17:1 ω8c		0.73
17:1 ω6c		1.17
18:1 ω9c		0.82
18:1 ω5c		0.77
Hydroxy acids	(Total: 41.14)	
2-OH 15:0		1.17
3-OH i15:0		4.26
3-OH 16:0		0.41
3-OH i16:0		4.41
2-OH 17:0		2.89
3-OH i17:0		28.00
Other acids	(Total: 6.38)	
2-OH i15:0 + 16:1 ω7c		5.11
Unknown		1.27
Total		94.6

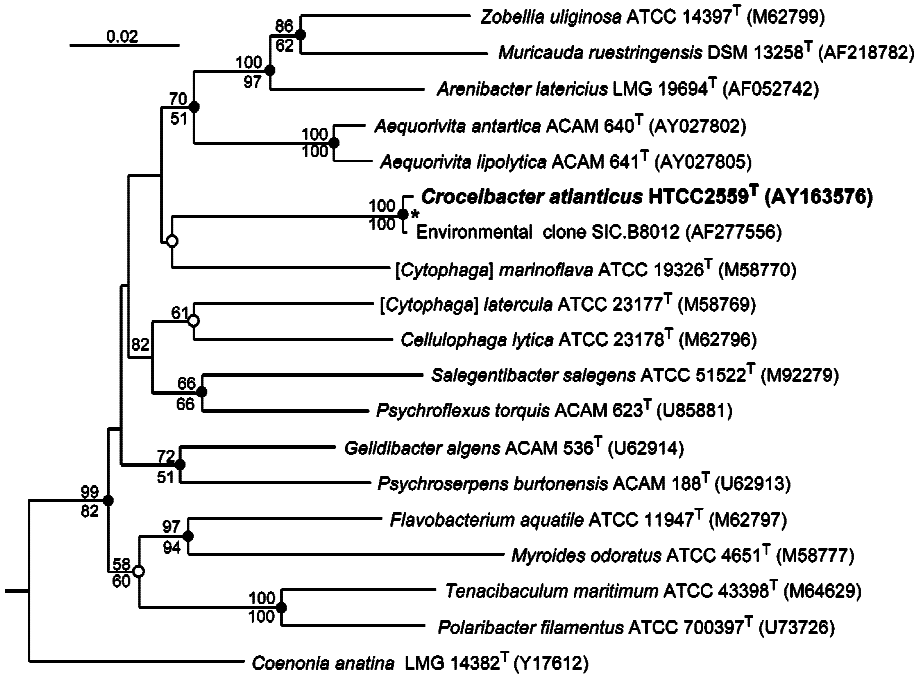

Fig. 2. Neighbour-joining tree showing relationships between strain HTCC2559^T and representatives of the family *Flavobacteriaceae* inferred from 16S rRNA gene sequence analyses. Bootstrap proportions over 50% from both neighbor-joining (above nodes) and maximum parsimony (below nodes) are shown. GenBank accession numbers are listed parenthetically. The closed circles and open circles at each node indicate recovered nodes in all treeing methods and recovered nodes in two treeing methods, respectively. The relatively short sequence (1102 bp) of environmental clone SIC.B8012 was inserted in the tree by using the parsimony insertion tool in ARB. The values at the node (*) were obtained by bootstrap analyses based on 897 unambiguously aligned nucleotide positions. The scale bar represents 0.02 substitutions per nucleotide position. *Coenonia anatina* (Y17612) was used as an outgroup to define the root of the tree.

Fatty acid profiles and DNA base composition

A total of 22 kinds of fatty acids, containing 15–18 carbon atoms, were observed (Table 2). The major fatty acid types were branched acids and hydroxy acids, comprising 80.1% of total fatty acids. The most dominant fatty acids were 3-OH i17:0, i15:0, i15:1, and i17:1 ω9c. The DNA G+C content of strain HTCC2559^T was 34.8 ± 0.1 mol% (mean ± SD; n = 3) determined by HPLC method.

Phylogeny based on 16S rDNA sequences

A nearly full sequence (1489 bp) of 16S rRNA gene sequence was determined for strain HTCC2559^T. The results of a preliminary BLAST network search and an ARB tree analyses indicated that the strain belongs to the family *Flavobacteriaceae*. The16S rDNA sequence of strain HTCC2559^T was most closely related to that of the sea-ice clone SIC.B8012 (AF277556, [8]) (99.5% similarity) and was also closely related to the sequences of several coastal mud clones KS27 (AF328176), KS16 (AF328169), KS28 (AF328177), and KS17 (AF328170) [23] (98.5–99.4% similarity). Comparative 16S rDNA sequence phylogenetic analyses with validly classified bacteria indicated that the strain was most closely related to [*Cytophaga*] *marinoflava* (90.3% similarity), *Aequorivita antartica* (90.2%), *Aequorivita lipolytica* (88.9%), and [*Cytophaga*] *latercula* (88.9%). The phylogenetic positions of strain HTCC2559^T were examined using three treeing algorithms; in all three phylogenetic trees, strain HTCC2559^T and sea-ice clone SIC.B8012 formed a distant branch with strong bootstrap support (Fig. 2). Although strain HTCC2559^T formed a clade with [*Cytophaga*] *marinoflava* in the neighbor-joining and maximum parsimony trees, the relationship was unclear because of very low bootstrap values. These phylogenetic analyses indicate a distant relationship between strain HTCC2559^T and other described members of the family *Flavobacteriaceae*.

Discussion

The phenotypic and genomic characteristics of strain HTCC2559^T, together with the phylogenetic analyses of the 16S rRNA gene sequence, met the minimal standards of the family *Flavobacteriaceae* [2]. The members of the family *Flavobacteriaceae* are Gram negative, short to moderately long rods, and cannot degrade crystalline cellulose [28]. The major cellular fatty acids are branched or hydroxy fatty acids, with high levels of i15:0 and 3-OH i17:0 as diagnostic features. The DNA G + C contents of this family range from 27 to 44 mol%. Strain HTCC2559^T was a Gram-negative, strictly aerobic, nonmotile, straight rod without a flagellum. It degraded gelatin, DNA, starch, casein, and elastin, but not cellulose. The G+C content of genomic DNA was 34.8 mol%. The major fatty acid types of the strain were branched or hydroxy fatty acids; i15:0 and 3-OH i17:0 are the most abundant fatty acids. Taking all characteristics of strain HTCC2559^T together, this bacterium had sufficient relatedness to classify it with the members of the family *Flavobacteriaceae*.

Classification of strain HTCC2559^T at genus-level based on phenotypic analyses alone was difficult because of the resemblances of phenotypes among genera in the family *Flavobacteriaceae*. Therefore, polyphasic approaches were carried out to overcome this problem. The cornerstone of polyphasic approaches, 16S rRNA gene sequence analysis, indicated that the strain formed a unique clade in the family *Flavobacteriaceae*. The most striking observation from phylogenetic analyses was that strain HTCC2559^T was related to then-uncultivated seawater clones by very high similarity values (98.5–99.5%). This indicates that the strain is a member of a potentially significant undescribed taxon in the family *Flavobacteriaceae*. The 16S rDNA sequence similarity of this strain to other genera in the family *Flavobacteriaceae* was only 87.3–90.3%, which indicates that the strain is well-differentiated phylogenetically and should be described as a novel genus in the family *Flavobacteriaceae*.

Strain HTCC2559^T was moderately differentiated from other genera of the family *Flavobacteriaceae* on the basis of phenotypic characteristics (Table 3). The property of the strain that best differentiated it from other members of the family *Flavobacteriaceae* was its temperature range. The members of the *Flavobacteriaceae* can be categorized into two groups from their growth temperature ranges and optima. Most members of the family grow optimally at 25–35 °C, while some genera (*Gelidibacter*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Salegentibacter*, and *Aequorivita*) are psychrophilic or psychrotolerant. Generally, psychrophilic members of the family can grow at 4 °C but not 30 °C, and other members of the family cannot grow at 4 °C but grow at 30 °C or higher. Strain HTCC2559^T grew neither at 4 °C nor at 30 °C, which differentiates the strain from the other members of the family *Flavobacteriaceae* (Table 3). The growth range of the strain indicates that the strain is well adapted to the environment from which it was isolated, because the annual range of temperatures at 250 m of the Sargasso Sea is approximately 15–26 °C [31]. In addition to the growth temperature range difference, strain HTCC2559^T could be differentiated from the genus *Aequorivita* because of distinctly different profiles of cellular fatty acids and some macromolecule degradation patterns; the genus *Aequorivita* has high amounts of a15:1 ω10c and a15:0, and low amount of 3-OH i17:0 [4]. The placement of the strain in the genus *Arenibacter* is also inadequate, judging from the degradation patterns of macromolecules. Similarly, strain HTCC2559^T could be differentiated from other members of the family *Flavobacteriaceae* by the differences of macromolecule degradation pattern, oxidase and catalase production, DNA G+C contents, and gliding motility. Considering all the data presented in this study, the novel strain HTCC2559^T belongs to a new genus and species within the family *Flavobacteriaceae*, thus we propose for it the name *Croceibacter atlanticus* gen. nov., sp. nov.

Table 3. Phenotypic characteristics differentiating *Croceibacter atlanticus* from other members of the family *Flavobacteriaceae*.

Characteristic	Species/Genera												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Habitat	M	M	M	M	M, S	M	M	M, S	M, S	M, S	M, S	M, S	FL, S
Pigmentation/Flexirubin	+/–	+/–	+/–	+/–	+/+	+/–	+/–	+/V	+/–	+/–	+/–	+/–	+/V
Gliding motility	–	–	–	–	+	+	–	+	–	V	+	–	+v
Reaction to oxygen	A	A	A	F	A	A	A	A	A	A	A	A	A
Sea water requirement	+	V	+	+	+	–	+	V	–	V	+	+	–
Growth at 4 °C/30 °C	–/–	+/–	–/+	–/+	–/+	–/+	–/+	V/+	+/+	+/V	+/V	+/–	V/+
Carbohydrate utilization	+	V	+	+	+	+	–	+	+	+	+	–	V
Nitrate reduction	–	–	ND	–	+	+	–	V	+	–	–	–†	V
Oxidase	–	ND	+	+	+	+	+	–v	+	+	–	–	V
Catalase	+	+	+	–	+	+	–	+	+	+	+	+	+
β-galactosidase	–	–	ND	ND	+	ND	+	ND	+	–	–*	V	V
Degradation of:													
Agar	–	–	–	–	+	–	+	+	–	–	–	–	V
Starch	+	V	–	–	V	+	–	+	+	+	+	–	+v
Aesculin	–	V	–	ND	+	+	ND	ND	+	V	–*	–	+v
Gelatin	+	+	–	–	+	ND	+	V	+	–†	V	V	+v
DNA	+	–	–	ND	+	ND	ND	V	+	+	V	–	V
G+C mol%	35	33–39	38–39	41	42–43	37	32	32–42	37–38	32–36	36–40	27–29	32–37

1. *Croceibacter atlanticus* HTCC2559^T, 2. *Aequorivita*, 3. *Arenibacter latericius*, 4. *Muricauda ruestringensis*, 5. *Zobellia*, 6. [*Cytophaga*] *marinoflava*, 7. [*Cytophaga*] *latercula*, 8. *Celluophaga*, 9. *Salegentibacter salegens*, 10. *Psychroflexus*, 11. *Gelidibacter*, 12. *Psychroserpens burtonensis*, 13. *Flavobacterium*.

Data taken from Bernardet et al. [2], Bowman and Nichols [4], Macián et al. [22], Ivanova et al. [17], Bruns et al. [9], Barbeyron et al. [1], Reichenbach [28], Johansen et al. [18], Bowman [3], Bowman et al. [5, 7], McCammon and Bowman [24], and this study.

Symbols and abbreviations: –, Negative; +, positive; V, variable; –v, most species negative; +v, most species positive; M, marine environment; S, saprophytic; FL, free-living; A, obligate aerobic; F, facultative anaerobic.

† Some strains of *Psychroflexus gondwanense* utilize gelatin.
* Not determined for *Gelidibacter mesophilus*.

Description of *Croceibacter* gen. nov.

Croceibacter atlanticus (Cro.cei.bac'ter. L. adj. *croceus* saffron-colored; N.L. masc. n. *bacter* rod N.L. masc.n. *Croceibacter* saffron-colored rod).

Gram-negative rod-shaped cells with rounded ends, dividing by binary fission. Does not exhibit motility and gliding motility. Colonies on marine agar are saffron-colored or bright orange, circular, and convex. Produces carotenoid pigments, but not flexirubin pigments. Strictly aerobic and chemoheterotrophic. Requires NaCl for growth. Catalase-, urease-, and arginine dehydrolase-positive, but oxidase-negative. Does not produce indole and acid from glucose. Degrades gelatin, DNA, starch, casein, and elastin. The major fatty acid types were branched acids and hydroxy acids. Phylogenetically it is a novel member of the family *Flavobacteriaceae*. The type and only species of the genus is *Croceibacter atlanticus*.

Description of *Croceibacter atlanticus* gen. nov., sp. nov.

Croceibacter atlanticus (at.lan'ti.cus. L. adj. *atlanticus* of the Atlantic Ocean, a species isolated from the Atlantic Ocean.)

Description is the same as the genus. Cells are 1.2–3.1 µm long and 0.3–0.6 µm wide, which occur singly or sometimes in pairs. Growth occurs at 10–28 °C, but not 4 or 30 °C. The pH and salinity ranges for growth are 6.0–10.0 and 0.5–15% (w/v). No denitrification activity was detected. Carbon sources utilization patterns are described in the text and Table 1. Susceptible to chloramphenicol, nalidixic acid, tetracycline, erythromycin, vancomycin, rifampicin, and benzylphenicillin, but resistant to kanamycin, carbenicillin, streptomycin, ampicillin, puromycin, gentamycin, cycloheximide. The predominant fatty acids are 3-OH i17:0, i15:0, i15:1, and i17:1 ω9c. The DNA G+C content of the species is 34.8 ± 0.1 mol% (HPLC method). The strain was isolated from the Bermuda Atlantic Time Series station in the western Sargasso Sea, Atlantic Ocean. The type strain is strain HTCC2559^T (=ATCC BAA-628^T = KCTC 12090^T).

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