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Karine Alain[1†], Laurent Intertaglia[1], Philippe Catala[1]

and Philippe Lebaron[1].

[1] Université Pierre et Marie Curie-Paris6, UMR7621, F-66650 Banyuls-sur-Mer, France ; CNRS, UMR7621, F-66650 Banyuls-sur-Mer, France.

† Present address : UMR6197, Laboratoire de Microbiologie des Environnements Extrêmes, IUEM, Technopôle Brest-Iroise, F-29280 Plouzané, France.

Correspondence : Philippe Lebaron

philippe.lebaron@obs-banyuls.fr

Phone number : +33-(0)4-68-88-73-00 Fax : +33-(0)4-68-88-16-99

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Footnote: The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Eudoraea adriatica* AS/06/20a^T is AM745437.

A scanning electron micrograph of cells of strain AS06/20a^T (Fig. S1) and graphs showing the effects of temperature, pH and salinity on the maximum growth rate (μmax) of the novel isolate (Fig. S2) are available in IJSEM online.

A novel aerobic, non-motile, Gram-negative bacterium designated AS06/20a^T, was isolated from coastal waters of the Adriatic Sea and subjected to a polyphasic taxonomic analysis. Cells were rod-shaped and formed non-pigmented punctiform colonies on agar plates. The novel isolate grew heterotrophically on a variety of carbon compounds, including organic acids, carbohydrates, polyols,

amino acids and complex organic substrates. Cells grew at temperature between 15-33°C (optimum 30°C), from pH 6.5 to 8.5 (optimum 7.5-8.0) and between 2 and 6% (w/v) NaCl (optimum 2%). The dominant fatty acids (> 5%) detected in strain AS06/20a^T were iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:1}\omega 9c$ and iso-$C_{17:0}$ 3-OH. The major respiratory quinone was MK-6. The G+C content of the genomic DNA was 38.9 mol%.

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain AS06/20a^T fell within the family *Flavobacteriaceae*, in the phylum *Bacteroidetes*. Comparative 16S rRNA gene sequence analysis indicated that strain AS06/20a^T was most closely related to the genera *Zeaxanthinibacter* and *Robiginitalea*, sharing 92 to 93% 16S rDNA sequence similarity with representatives of these genera. It is proposed, from the polyphasic evidence, that the strain should be placed into a novel genus, *Eudoraea* gen. nov., with *Eudoraea adriatica* sp. nov. as the type species. The type strain is AS06/20a^T (= DSM 19308^T = CIP 109577^T = OOB 358^T).

Bacteria belonging to the phylum *Bacteroidetes* are widely distributed in nature and have been found in almost every habitat of the biosphere, the lithosphere and the hydrosphere (Bernardet *et al*., 2002; Kirchman, 2002). They seem particularly common and abundant in marine surface waters, as indicated by fluorescence *in situ* hybridization analyses (Glöckner, 1999; Cottrell & Kirchman, 2000a; Simon *et al*., 2001; Alonso & Pernthaler, 2005). Cultured isolates of *Bacteroidetes* are all heterotrophs and especially efficient in degrading complex biomacromolecules such as protein, chitin, pectin, agar, starch and cellulose.

In marine ecosystems, the heterotrophic prokaryotes are the main consumers of dissolved organic material (DOM) and represent a very dynamic compartment in global biogochemical cycles. Uptake of DOM is the first step in the microbial loop that ultimately mineralizes over half of the organic matter formed by photosynthetic bacteria and algae (Cole *et al*., 1988). Although the importance of DOM uptake is well recognized, the relative contributions of the different phylogenetic groups to DOM uptake remain to be elucidated. Because of their abundance and heterotrophic metabolism, *Bacteroidetes* are thought to occupy a special place in the carbon cycling of oceans. They are obviously involved in the degradation and uptake of DOM, and likely to contribute to the degradation of the high molecular weight (HMW; > 1 kDa) fraction of the DOM (Kirchman, 2002). Indeed, the HMW fraction is composed of biopolymers; hydrolysis of these

macromolecules requires specific extracellular enzymes which are produced by numerous known *Bacteroidetes* members. Using a method combining microautoradiography and hybridization of fluorescent rRNA-targeted oligonucleotide probes to whole cells from natural marine assemblages (MICRO-FISH), Cottrell and Kirchman (2000b) demonstrated that *Bacteroidetes* represented, for the considered habitats, a large fraction of the community efficient in degrading chitin, *N*-acetylglucosamine and protein, part of the HMW fraction of the DOM. All these data together suggest strongly that, in marine environments, *Bacteroidetes* are key players in the degradation of the high molecular weight fraction of the DOM, which has been demonstrated to be more bioreactive and more bioavailable than the low molecular weight fraction (Amon & Benner, 1996). As relevant work on cultured representatives from marine origin is lacking, there is a great interest in isolating and describing phenotypic and genotypic characteristics of marine *Bacteroidetes* known only by their 16S rDNA sequence. For example, there are notably very few sequence data on enzymes catalyzing biopolymer hydrolysis (Kirchman, 2002). The isolation of strains is still the easiest way to access to their physiological and genomic capital. This is probably the reason why the number of strains of *Bacteroidetes* from marine ecosystems described the last five years has increased exponentially.

In this study, a novel marine bacterium belonging to the phylum *Bacteroidetes* is described. Based on the results of a polyphasic taxonomic analysis, the strain AS06/20a^T represents a novel species and genus, *Eudoraea adriatica* gen. nov., sp. nov.

In April 2006, marine surface waters were collected on the coast of the Adriatic Sea, Italy (44.690°N, 12.520°E). One subsample collected by 10 m depth was spread on a marine agar 2216 (MA; Difco) plate, and then incubated at 25°C. After 2 weeks, a small non-pigmented colony was picked and purified by repeated streaking on MA plates. It was referenced as strain AS06/20a^T. Stock cultures were stored at −80°C in marine broth 2216 (MB; Difco) supplemented with 5% (v/v) DMSO or 35% (v/v) glycerol, until characterization.

The almost complete 16S rRNA gene (1393 bp) of the strain was double-strand sequenced from one single colony, as described elsewhere (Agogué *et al*., 2005). This sequence was compared to those in available

databases by use of the BLAST program (Altschul *et al*., 1990) and then aligned to its nearest neighbours using the CLUSTALX program (Thompson *et al*., 1997). Alignments were refined manually using the SEAVIEW program (Galtier *et al*., 1996). Phylogenetic trees were constructed by the PHYLIP (PHYlogeny Inference Package) version 3.63 software (http://evolution.genetics.washington.edu/phylip/getme.html) on the basis of evolutionary distance (neighbour-joining method with Jukes and Cantor corrections) (Saitou and Nei, 1987) and maximum likelihood (Felsenstein, 1981). The robustness of the inferred topologies was assessed by bootstrap analyses based on 1000 bootstrap resamplings for the neighbour-joining and 100 replications for the maximum likelihood method (Felsenstein, 1985). The 16S rRNA gene-based analysis located the strain AS06/20a^T within the phylum *Bacteroidetes*. The novel isolate was affiliated to the family *Flavobacteriaceae*, one of the main phyletic lines of the phylum *Bacteroidetes* (Reichenbach, 1989; Bernardet *et al*., 1996; Bernardet *et al*., 2002) (Fig. 1). The results of different phylogenetic reconstructions performed with different treeing algorithms were in accordance with each other. The novel isolate formed a distinct lineage together with *Zeaxanthinibacter enoshimensis* TD-ZE$_3^T$ and *Robiginitalea biformata* HTCC 2501^T, but this lineage did not cluster robustly with any of the recognized genera in the family. Within this lineage, the novel isolate showed only a distant relatedness to its nearest phylogenetic neighbours, the most closely genus being *Zeaxanthinibacter* (93% 16S rDNA sequence similarity), followed by *Robiginitalea* (92%). These low 16S rDNA sequence similarities between the strain AS06/20a^T and the genera described so far suggest that the novel isolate represents a new genus within the family *Flavobacteriaceae*.

The DNA G+C content was determined, by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), by HPLC analysis of deoxyribonucleosides as described by Mesbah *et al*. (1989). The G+C content of strain 26III/A02/215^T was 38.9 mol%.

Colonies on MA were non-pigmented and cream in colour. They were circular, punctiform, opaque, with an entire edge and possessed a smooth surface. After 1 week incubation, colonies were less than 1 mm in diameter. Morphological characteristics of the cells were determined by light microscopy (Olympus AX70) and by transmission electron microscopy (Hitachi H-7500) after negative staining with uranyl acetate (Raguénès *et al*., 1997). Gram-staining was determined using standard procedures. Gliding motility was

determined using the hanging drop method on cells grown at 30°C in low nutrient medium (0.1% (w/v) marine broth solidified with 1% agar), as described in Bernardet *et al*. (2002). In summary, cells of strain AS06/20a^T were Gram-negative, non-motile, straight rods of 1.48-2.99 μm in length (mean 2.22 ± 0.54 μm, n=8) and 0.61-0.78 μm in width (mean 0.70 ± 0.07 μm, n=8) in the mid-exponential phase of growth (see Supplementary Fig. S1 in IJSEM online). Gliding motility was not observed under the conditions tested. Cells swell into balloon-like shapes during the late stationary growth phase.

In order to analyse respiratory quinones and polar lipids, strain AS06/20a^T was grown for 5 days on MB medium at 30°C, and checked for purity. Analyses of isoprenoid quinones and polar lipids were carried out by the Identification Service (Dr. Brian Tindall, DSMZ culture collection, Braunschweig, Germany). As described for the other members of the family *Flavobacteriaceae*, the main respiratory quinone was menaquinone 6 (MK-6; 83%) (Bernardet *et al*., 2002); Menaquinone 7 was also detected (MK-7; 17%). Phosphatidylethanolamine was the only major phospholipid identified in strain AS06/20a^T. Three other major unidentified polar lipids, two containing amino groups, were also present.

The determination of the whole-cell fatty acid composition was performed on cultures grown at 30°C for 72h on marine agar 2216. This analysis was carried out at the DSMZ according to the standard protocol of the Microbial Identification System (MIDI Inc., Del. USA, 2001). Extracts were analysed using a Hewlett Packard model HP6890A gas chromatograph equipped with a flame-ionization detector as described by Kämpfer & Kroppenstedt (1996). Results are summarized in Table 1. The dominant fatty acids in strain AS0620a^T were iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:1}\omega 9c$, iso-$C_{17:0}$ 3-OH and the fatty acids summed in feature 3.

Unless stated otherwise, physiological characterization was carried out aerobically in marine broth medium (MB 2216; Difco), in triplicate, and incubation were done in the dark and under agitation. Growth was routinely monitored by measuring the increase in optical density at 600 nm using a spectrophotometer. Cell numbers were determined by flow cytometry in order to calculate calibration curves 'Cell numbers = f(OD_{600})'. Growth rates were calculated using linear regression analysis of four to nine points along the logarithmic portions of the resulting growth curves. Growth temperature was tested over the range 9-44°C

(i.e. 9, 15, 20, 25, 30, 33, 37, 44°C). The temperature range for growth was 15-33°C, with optimum growth rate at 30°C (see Supplementary Fig. S2a in IJSEM online). No growth was observed at 9 and 37°C. The pH range for growth was tested at 30°C in MB medium, buffered and adjusted to the required pH as described elsewhere (Alain *et al*., 2002). The pH range for growth was rather narrow (Fig. S2b); Growth was observed from pH 6.5 to pH 8.5, the optimum being around pH 7.5-8.0. Salt tolerance was tested at 30°C in MB medium prepared with various concentrations of NaCl (0.02, 0.5, 1, 2, 3, 4, 5, 6, 7 and 9% w/v). The isolate required NaCl for growth (Fig. S2c). This is congruent with the fact that this strain was isolated from brackish waters of the North Adriatic Sea. Growth was observed at salt concentrations ranging from 2 to 6% (w/v) NaCl, the optimum salinity being around 2%. No growth was observed at 1 and 7% (w/v) NaCl.

Strain AS06/20a^T was found to be aerobic. Conventional phenotypic tests including those for oxidase, catalase, tween esterase and nitrate reductase were performed according to standard methods (Smibert & Krieg, 1994). The results are given in Table 1. Biochemical tests were performed at 30°C using api®ZYM (bioMérieux) and Biolog GN2 microplates (Oxoid). These tests were inoculated with cells grown on MA plates, swabbed from the surface of the agar plates and then suspended in ASW ½ (diluted artificial seawater) to the density specified by the manufacturer. Supplementary biochemical tests were also performed using api®20NE strips (bioMérieux), following the manufacturer's instructions. The data obtained are given in Table 1. Testing for oxidation of carbon sources with Biolog GN2 plates indicated that the strain was able to oxidize a range of sugars, organic acids and amino acids. To confirm these results and to test for the capability of the strain to catabolize different substrates as sole carbon and energy source, with oxygen as a terminal electron acceptor, the strain was grown aerobically, in the dark, on a mineral medium supplemented with one substrate. The defined medium had the following composition (l^{-1}): phosphate buffer, 30 mM; NaCl 20 g, $MgCl_2.6H_2O$ 3 g, $CaCl_2.2H_2O$ 1.0 g, NH_4Cl 0.3 g, KCl 0.5 g, Na_2SO_4 3 g, $NaNO_3$ 1 g; trace element solution, 1 ml; selenite-tungstate solution, 1 ml; vitamin solution, 1 ml. The strain was found to grow chemoorganoheterotrophically on a variety of carbon compounds, including pentoses, hexoses, polyols, organic acids, amino acids and complex organic substrates (Table 1).

Antibiotic sensitivity tests were performed by using susceptibility discs (Biorad) or filter-paper discs impregnated with different antibiotics. Discs were placed on MA plates spread with a culture of the isolate

and were then incubated at 30°C for one week. Susceptibility was scored as positive at zone diameters above 10 mm. The results are summarized in Table 1.

The phenotypic and genotypic characteristics of the novel isolate generally met the minimal standards for the family *Flavobacteriaceae* (Bernardet *et al*., 2002). Nevertheless, strain AS06/20a^T differed from other *Flavobacteriaceae* genera, with validly published names, in terms of a number of phylogenetic, genotypic, chemotaxonomic, morphological and physiological features (Table 2). In brief, in addition to the phylogenetic distance, the novel taxon can be distinguished from its closest relatives by some of its phenotypic features, including its absence of colonial pigmentation, the narrow NaCl and pH ranges for growth and its restricted degrading-capabilities regarding to the tested macromolecules. In addition to these phenotypic differences, the novel isolate is unambiguously distinct from its two closest neighbours by its genomic DNA G+C content which is more than 5% lower than those of *Zeaxanthinibacter enoshimensis* and *Robiginitalea biformata*. In conclusion, in view of all the above-mentioned distinctive features, we propose that the isolate AS06/20a^T should be assigned as the type strain of a novel genus and species, for which the name *Eudoraea adriatica* gen. nov., sp. nov. is proposed.

Description of *Eudoraea* gen. nov.

Eudoraea (N. L. fem. n. *Eudora* was a sea goddess of the Greek mythology; N. L. fem. n. *Eudoraea*, named after *Eudora*).

Cells are Gram-negative, non-spore forming, non-motile, non-gliding rods. Pigments are not produced. Aerobic and chemoorganoheterotrophic. Catalase- and oxidase- positive. Mesophilic. Neutrophilic. Requires NaCl for growth. The predominant quinone is MK-6. Polar lipids include phosphatidylethanolamine. Major fatty acids are iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:1}\omega 9c$, iso-$C_{17:0}$ 3-OH and summed feature 3. The G+C content of the DNA is close to 39 mol%. Phylogenetically, the genus *Eudoraea* belongs to the phylum *Bacteroidetes*, family *Flavobacteriaceae*, showing a distant relatedness to the marine genera *Zeaxanthinibacter* and *Robiginitalea*. The type species is *Eudoraea adriatica*.

Description of *Eudoraea adriatica* sp. nov.

Eudoraea adriatica (a.dri.a'ti'ca. L. fem. adj. *adriatica*, of the Adriatic Sea, where the type strain was isolated). In addition to the characters described for the genus, the following properties apply. Colonies on MA medium are

punctiform, cream in colour, opaque and smooth. Optimal growth occurs at 30°C, with a growth range from 15 to 33°C. The pH and NaCl ranges are 6.5-8.5 (optimum 7.5-8.0) and 2-6% (w/v) (optimum, 2% w/v NaCl), respectively. Aesculin is hydrolysed but agar, cellulose, gelatin, starch and urea are not. β-galactosidase activity is positive. Glucose is not fermented. Nitrate is not reduced. A variety of carbon compounds are used as sole carbon sources, including pentoses, hexoses, polyols, organic acids, amino acids and complex substrates (Table 1). The G+C content of the type strain AS06/20a^T is 38.9 mol%.

The type strain, AS06/20a^T (DSM 19308^T, CIP 109577^T, OOB 358^T), was isolated from coastal waters of the Adriatic Sea, Italy (44.690°N, 12.520°E).

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TABLES and FIGURES

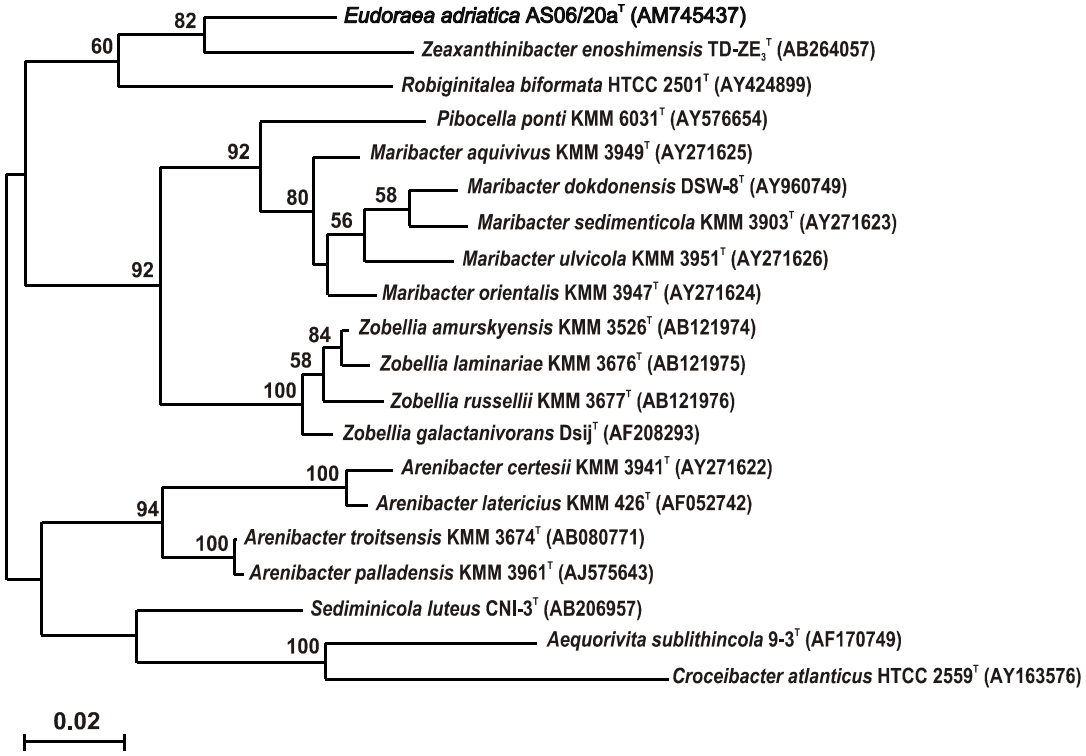


Fig. 1. **Phylogenetic tree based on 16S rRNA gene sequences of strain AS06/20a^T and representative members of related genera within the family *Flavobacteriaceae***. Sequence data of reference strains were obtained from the GenBank/EMBL and/or RDP databases. Accession numbers are indicated in parentheses. The topology shown corresponds to an unrooted tree obtained by the maximum likelihood algorithm, established using the PHYLIP package. Bootstrap values (from 100 replicates) are indicated at the branch nodes. The positioning of the novel isolate was confirmed by the neighbour-joining method. The scale bar indicates 2.0 nt substitutions per 100 nt.

Table 1. Phenotypic and genotypic characteristics of strain AS06/20a^T.

Legend: +, positive; ─, negative; PE, phosphatidylethanolamine; L, unidentified, polar lipid; AL, unidentified aminolipid.

Characteristic	***Eudoraea adriatica* strain AS06/20a^T**
Temperature range for growth (°C) [optimum]	15-33 [30]
NaCl range for growth (%) [optimum]	2-6 [2]
pH range for growth [optimum]	6.5-8.5 [7.5-8.0]
Biochemical properties (API 20NE)	
Catalase, oxidase, aesculin hydrolysis, β-galactosidase production	+
Denitrification, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis	─
Enzymatic activities (APIZYM)	
Alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase	+
Lipase (C14), cystine arylamidase, trypsin, α-galactosidase, α-glucosidase, α-fucosidase	─
Substrate assimilation	
D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, *m*-inositol, acetic acid, citric acid, pyruvic acid, propionic acid, capric acid, adipic acid, malic acid, phenylacetic acid, L-arginine, yeast extract	+
Formic acid, tween 40, tween 80, D-maltose	─
Degradation of macromolecules	
Aesculin	+
Agar, cellulose (filter paper), gelatin, starch, urea	─
Susceptibility to antibiotics	
Vancomycin, rifampicin, tetracycline, polymyxin B	+
Oxacillin, penicillin, ciprofloxacin	─
Fatty acid composition (%)*	iso-$C_{15:1}$ G (15.97%), iso-$C_{15:0}$ (29.37%), iso-$C_{15:0}$ 3-OH (7.27%), iso-$C_{17:1}\omega9c$ (8.96%), iso-$C_{17:0}$ 3-OH (14.13%) and summed feature 3 [§] (11.57%).
Polar lipids	PE, L, AL1, AL2
Quinones	MK-6 (83%), MK-7 (17%)
DNA G+C content (mol%)	38.9

[§], Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

* Fatty acids comprising more than 5% of the total content are listed. The following fatty acids were present in lesser amounts: iso-$C_{13:0}$ (0.48%), $C_{14:0}$ (0.17%), anteiso-$C_{15:0}$ (0.72%), $C_{15:1}\omega6c$ (1.06%), iso-$C_{16:0}$ (0.25%), $C_{16:0}$ (0.67%), $C_{15:0}$ 2-OH (0.30%), $C_{15:0}$ 3-OH (1.33%), iso-$C_{17:0}$ (0.17%), $C_{17:1}\omega8c$ (0.48%), $C_{17:1}\omega6c$ (0.83%), iso-$C_{16:0}$ 3-OH (1.38%), $C_{16:0}$ 3-OH (0.37%), $C_{18:1}\omega5c$ (0.70%), $C_{17:0}$ 3-OH (0.20%) and three unknown fatty acids designated by their equivalent chain-length [ECL 11.54 (0.21%), ECL 13.56 (2.26%) and ECL 16.58 (1.15%)].

Table 2. Characteristics differentiating *Eudoraea* from related genera of the family *Flavobacteriaceae*.

Genera: 1, *Eudoraea adriatica* AS06/20a^T (this study); 2, *Zeaxanthinibacter enoshimensis* TD-ZE$_3$^{T}; 3, *Robiginitalea biformata* HTCC 2501^T; 4, *Pibocella ponti* KMM 6031^T; 5, *Maribacter sedimenticola* KMM 3903^T; 6, *Zobellia galactanivorans* Dsij^T; 7, *Arenibacter latericius* KMM 426^T; 8, *Sediminicola luteus* CNI-3^T.

Legend: +, positive; ─, negative; ND, not determined; Y, yellow; O, orange; DO, dark orange; w, weak. The percentage of 16S rDNA sequence similarity is calculated in reference to the 16S rDNA sequence of the novel isolate AS06/20a^T.

Characteristic	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Oxidase/catalase	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/ w
Pigmentation	─	Y	O	Y	Y	Y	DO	Y
Flexirubin pigments	─	─	─	─	─	+	─	─
Gliding motility	─	+	─	+	+	+	─	─
Growth in/at								
8% NaCl	─	─	+	+	+	─	─	─
7% NaCl	─	+	+	+	─	─	+	+
1% NaCl	─	ND	+	+	+	+	+	+
4°C	─	─	─	+	+	─	─	─
10°C	─	─	+	+	+	─	+	+
42°C	─	─	+	─	─	+	─	─
Hydrolysis of								
Agar	─	─	ND	─	+	+	─	ND
Gelatin	─	+	─	+	+	+	─	+
Starch	─	+	+	+	─	+	─	+
Cellulose	─	─	─	─	─	ND	─	─
Aesculin	+	+	+	ND	ND	+	─	ND
Urease activity	─	─	─	─	─	─	+	─
β-galactosidase	+	+	─	ND	─	+	+	ND
Nitrate reduction	─	─	─	─	─	+	+	+
DNA G+C content (mol%)	38.9	46.5	56.4	35.5	37	43	37-38	38
16S rDNA similarity (%)	100	93	92	90	91	91	91	90

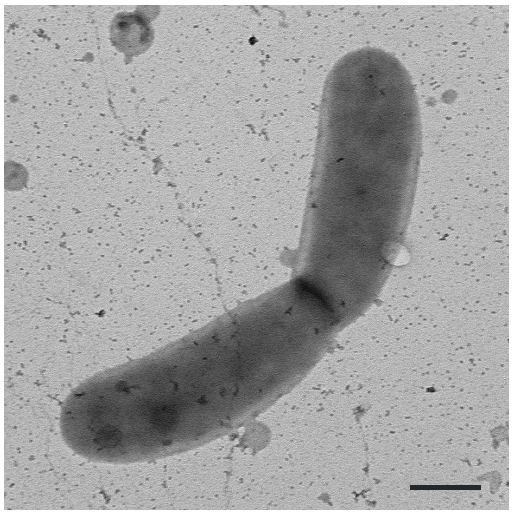
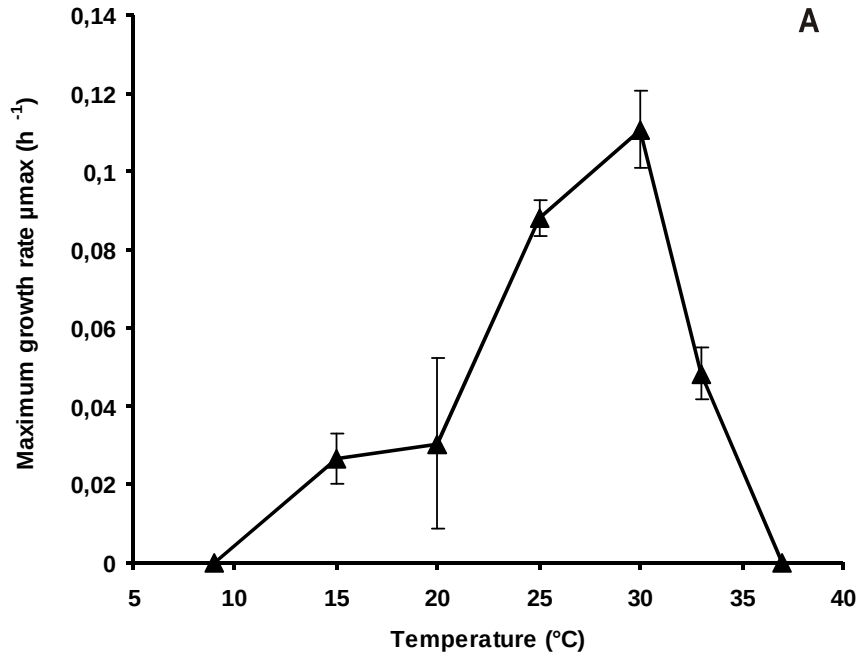
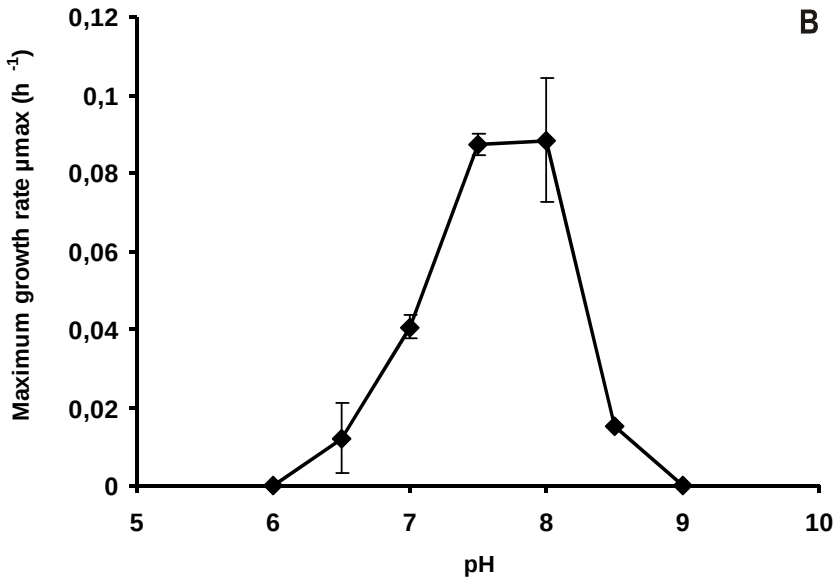
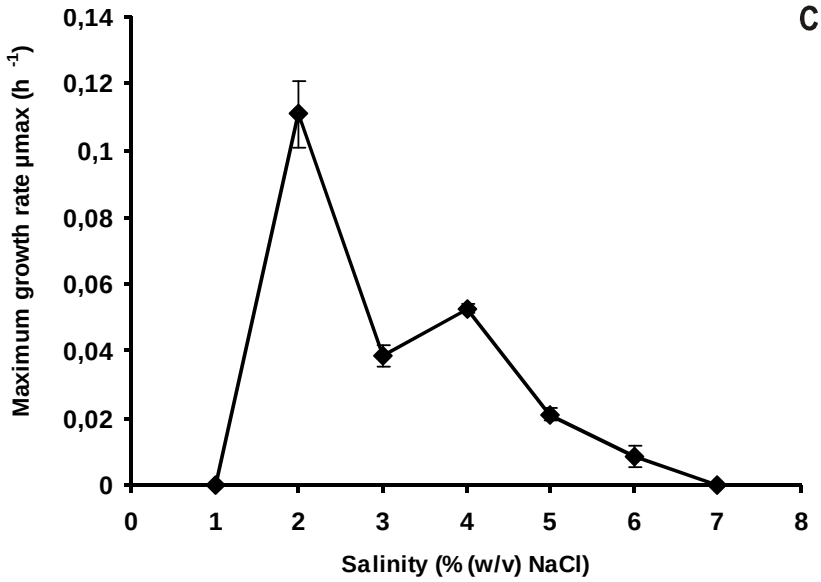


Fig. S1. Transmission electron micrograph of cells of strain AS06/20a^T in a state of division. Cells were grown at 30°C on marine broth and harvested during the mid-exponential phase of growth. The preparation was negatively stained with uranyl acetate. Bar, 0.5 μm.

Fig. S2. Effects of temperature (A), pH (B) and salinity (C) on the maximum growth rate of strain AS06/20a^T. Bars indicate confidence intervals.


A
Maximum growth rate µmax (h -1)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
5
10
15
20
25
30
35
40
Temperature (°C)


B
Maximum growth rate µmax (h -1)
0,12
0,1
0,08
0,06
0,04
0,02
0
5
6
7
8
9
10
pH


C
Maximum growth rate µmax (h -1)
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
0
1
2
3
4
5
6
7
8
Salinity (% (w/v) NaCl)

382