***Vibrio lentus* sp. nov., isolated from Mediterranean oysters**

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Twelve phenotypically similar marine bacteria have been studied by means of ribotyping, DNA–DNA hybridization and cultural and physiological characterization. Phylogenetic analysis has been performed of the 16S and 23S rRNA genes of two representative strains. Phylogenetically, they belong to the *Vibrio*/*Photobacterium* branch of the γ -Proteobacteria and they share all of the properties that define the genus *Vibrio*. The strains represent a new *Vibrio* species that is phenotypically similar to *Vibrio splendidus*. However, resistance to the vibriostatic agent O129 and production of acid from several carbohydrates allow differentiation between *V. splendidus* and the proposed new species. The DNA G+C content of the proposed type strain is 44.0 mol %. The name *Vibrio lentus* sp. nov. is proposed for the new species and strain 40M4^T (= CECT 5110^T = DSM 13757^T) is the type strain.

Keywords: γ -Proteobacteria, marine bacteria, *Vibrio lentus* sp. nov., 16S rDNA phylogeny, ribotyping

INTRODUCTION

The family *Vibrionaceae* (Baumann & Schubert, 1984) comprises bacteria inhabiting aquatic environments, especially marine and estuarine waters, where they are frequently associated with organisms ranging from plankton to fish. Currently, this family includes, among others, the marine genera *Vibrio* (Baumann *et al.*, 1984), *Photobacterium* (Baumann & Baumann, 1984) and *Listonella* (MacDonell & Colwell, 1985), and it has experienced several taxonomic rearrangements in the last two decades. In particular, the number of recognized species in the genus *Vibrio* has increased dramatically, from 18 recognized species in *Bergey's Manual of Systematic Bacteriology* (Baumann *et al.*, 1984) to more than 40 currently recognized species. The second edition of *The Prokaryotes* (Farmer & Hickman-Brenner, 1992) added nine new *Vibrio* species and 13 new species were described later (Borrego *et al.*, 1996; Cerdà-Cuellar *et al.*, 1997; Ishimaru *et al.*, 1995, 1996; Iwamoto *et al.*, 1996; Lambert *et al.*, 1998; Lunder *et al.*, 2000; Pujalte *et al.*,

1993; Raguénès *et al.*, 1997; Sawabe *et al.*, 1998; Shieh *et al.*, 2000; Urdaci *et al.*, 1991; Yumoto *et al.*, 1999). Some species are symbionts, whereas others are known pathogens of humans as well as of marine animals. It is also known that these fermentative Gram-negative bacteria constitute an important percentage of the culturable heterotrophic bacteria associated with marine bivalves, especially oysters and mussels (Kueh & Chan, 1985; Prieur *et al.*, 1990; Olafsen *et al.*, 1993; Pujalte *et al.*, 1999).

In a previous numerical taxonomic study, our group analysed the halophilic, facultatively anaerobic, Gram-negative bacteria isolated from oysters and seawater from the Spanish Mediterranean coast. This study revealed that a considerable number of phenotypes corresponding to the family *Vibrionaceae* could not be ascribed to any known species (Ortigosa *et al.*, 1994) and required further genetic characterization.

In the present study, a group of 12 isolates that phenotypically resembled *Vibrio splendidus* were analysed by performing ribotyping and DNA–DNA hybridization, by analysing DNA G+C content and cultural and physiological features and by phylogenetic studies of 16S and 23S rRNA. The results have shown that the group represents a new species of *Vibrio*, for which the name *Vibrio lentus* sp. nov. is proposed.

Abbreviations: ADH, arginine dihydrolase; TCBS, thiosulfate/citrate/bile salts/sucrose.

The GenBank/EMBL accession numbers for the 16S rRNA gene sequences of *Vibrio lentus* CECT 5110^T and CECT 5293 are AJ278881 and AJ278880, respectively.

METHODS

Bacterial strains and growth conditions. Twelve environmental strains that clustered in phenon 2 in a previous study (Ortigosa *et al.*, 1994) were isolated from Mediterranean oysters on plates of Marine agar 2216 (MA; Difco). Cultures were maintained on semi-solid MA stabbs at room temperature and were grown on MA or Marine broth 2216 (MB; Difco) at 22 °C. Reference strains of *V. splendidus* biotype 1, ATCC 33125^T, and *V. splendidus* biotype 2, ATCC 33870, were also used. Three of the 12 strains studied here have been deposited in the Colección Española de Cultivos Tipo (CECT) and the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ): 4OM4^T (= CECT 5110^T = DSM 13757^T), 3OM16 (= CECT 5111 = DSM 13758) and 3OM21 (= CECT 5293 = DSM 13759).

Phenotypic characterization. Phenotypic profiles were determined of the 12 environmental isolates, including the proposed type strain, 4OM4^T (= CECT 5110^T). Cultivation was done at 22 °C and commercial media, except for MA and MB, were supplemented with NaCl up to 2% (w/v). Cell morphology, size and motility were examined on wet mounts by phase-contrast microscopy; flagellar arrangement was determined after staining MA-grown cells by the method of Heimbrook *et al.* (1989). The ability to reduce nitrate to nitrite was tested on nitrate broth plus 1% NaCl (Smibert & Krieg, 1981). The oxidation/fermentation test was performed in O/F medium (Difco) plus half-strength artificial sea water with 1% (w/v) glucose. Fermentation and acid production from other sugars were also tested in O/F medium (Difco). Tests for swarming, Thornley's arginine dihydrolase (ADH) and nutritional screening on multi-inoculated basal medium plates were performed by using previously described methods (Baumann & Baumann, 1981). The alkalization/decarboxylation of arginine, lysine and ornithine in Møller broth (Decarboxylase medium base; Difco), oxidase test, Voges-Proskauer test and growth and acid production from sucrose on thiosulfate/citrate/bile salts/sucrose (TCBS) agar were performed by the methods of Lee & Donovan (1985). Hydrolysis of casein, gelatin, Tween 80, lecithin, starch, sodium alginate and DNA were tested according to Ortigosa *et al.* (1994). Indole production was tested on MB after 48 h. Growth at different temperatures was tested in MB at 4 (7 d), 13, 28, 30, 37 and 40 °C. Luminescence was observed in the dark on MA. Growth at different salinities was tested in STB (1% tryptone, 0.3% yeast extract plus NaCl; pH 7.0). Sensitivity to O129 (150 µg per disc) was determined with Oxoid discs.

Determination of G + C content. Genomic DNA was isolated by the method of Marmur (1961). The G + C contents (mol%) of genomic DNA were determined by thermodenaturation using the method of Mandel & Marmur (1968), including DNA from *Escherichia coli* ATCC 11775^T (G + C content, 51 mol%) as a standard.

Extraction of genomic DNA, amplification and sequencing of DNA. Isolation of genomic DNA and amplification of almost full-length 16S rRNA gene fragments were performed according to methods described elsewhere (Macián *et al.*, 2001). The 23S rRNA gene was amplified *in vitro* using two primer sets: 616Valt/985R and 118V/504R. The sequence, target position (*E. coli* numbering; Brosius *et al.*, 1981) and annealing temperature for each of the primers were: 616Valt, 5'-AGAGTTTGATYMTGGCTCAG-3', 8–27 (16S rRNA gene), 56 °C; 985R, 5'-CCGGTCTCTCGTACT-3', 2660–2675 (23S rRNA gene), 52 °C; 118V, 5'-TCYGAATGGGNAAC-3', 121–136 (23S rRNA gene), 46 °C; 504R, 5'-

SWGTTTCGRVAWGGGA-3', 25–49 (5S rRNA gene), 44 °C. Sequencing of rDNA was done by using a LICOR automated sequencer (MWG Biotech).

Sequence data analysis. Sequences were added to the 16S and 23S rRNA sequence databases of the Technical University Munich using the program package ARB (Ludwig & Strunk, 1997). The ARB tools were used for automated sequence alignment. The alignment was checked by eye and corrected manually using the sequence editor ARB_EDIT. Phylogenetic analyses were performed by applying the maximum-parsimony (full dataset of 20000 sequences, ARB, parsimony), distance-matrix (all available γ -proteobacteria as well as selected references from other major phylogenetic groups, ARB, PHYLIP as implemented in ARB; Felsenstein, 1982) and maximum-likelihood (known selected references of γ -proteobacteria, fastDNAm1 as implemented in ARB, Maidak *et al.*, 1996) methods on different datasets that varied with respect to the inclusion of variable sequence positions (Ludwig *et al.*, 1998).

DNA–DNA hybridization. Two methods were used for DNA–DNA hybridization experiments. Firstly, intraspecific hybridizations were done with the 12 environmental isolates. DNA was extracted by the guanidinium thiocyanate method of Pitcher *et al.* (1989). DNA was transferred under vacuum (Milliblot-V system; Millipore) to a non-charged nylon membrane (Qiabran; Qiagen) and further slot-blot hybridization was done as described by Aznar *et al.* (1992). Total DNA of strain 4OM4^T (= CECT 5110^T) labelled with digoxigenin (DIG oligonucleotide 3'-end labelling kit; Boehringer) was used as a probe. Hybridization was done at 65 °C and hybrid detection was performed by chemiluminescence (DIG luminescent detection kit; Boehringer). Afterwards, a more accurate DNA–DNA hybridization method was applied to four of the 12 environmental isolates, including the proposed type strain, as well as *V. splendidus* biotype 1 ATCC 33125^T and *V. splendidus* biotype 2 ATCC 33870, the closest phylogenetic neighbours. DNA was extracted according to the method of Brenner *et al.* (1982). *In vitro* labelling of the DNA of strain 4OM4^T (= CECT 5110^T) with tritium-labelled nucleotides was performed by the random primer method and hybridization was carried out at 60 °C by the S₁ nuclease/trichloroacetic acid method (Grimont *et al.*, 1980) with adsorption of S₁-resistant DNA onto nitrocellulose filters (Sartorius). The temperature at which 50% of the reassociated DNA became hydrolysable by S₁ nuclease (*T*_m) was determined by the method of Crosa *et al.* (1973). The difference between the *T*_m of the homoduplex (in the homologous reaction) and the *T*_m of a heteroduplex (in a heterologous reaction) is an estimate of divergence between two DNAs.

Ribotyping. Two restriction enzymes, *Kpn*I and *Sal*I (Gibco-BRL), were used for ribotyping of the isolates. Chromosomal DNA (5 µg) was digested as recommended by the manufacturer. DNA restriction fragments were separated by electrophoresis in 1% (w/v) agarose gels (Sigma) with Tris/acetate electrophoresis buffer, as described by Sambrook *et al.* (1989). A Hybaid blot processing pump was used to transfer DNA onto a non-charged nylon membrane (Qiabran). After the transfer, DNA fragments were fixed by baking for 30 min at 80 °C and hybridized to an rDNA-specific probe using methods described previously (Aznar *et al.*, 1993). Photodigoxigenin-labelled phage λ HindIII DNA fragments (Boehringer) were used as size markers. A 19 bp oligonucleotide complementary to a highly conserved region of the bacterial 23S rRNA gene was used as a probe: 1038

(3'-GTAGCGAAATTCCTTGTCG-5'), complementary to positions 1930-1948 (Brosius *et al.*, 1981). The probe was labelled with digoxigenin using the DIG oligonucleotide 3'-end labelling kit (Boehringer) as described previously by Aznar *et al.* (1993). Hybridization was done at 48 °C. Hybridization patterns were recorded as described by Arias *et al.* (1997) and analysed by using the software package GELCOMP 4.0 (Applied Maths).

RESULTS AND DISCUSSION

Phenotypic characterization

The Mediterranean oyster isolates shared the properties of the genus *Vibrio*. The cells were Gram-negative. The strains were facultative anaerobes, able to produce acid from glucose under anaerobic conditions. No growth occurred without addition of NaCl to the culture medium. The strains were oxidase- and catalase-positive and reduced nitrates to nitrites. The G+C content of the DNA was 44.0 mol%. This value is in the known range for the genus *Vibrio* (39-50 mol%; Baumann *et al.*, 1984).

Cells were rods, 1.5-3 µm long by 0.8-1 µm wide, motile by one polar flagellum. After 24 h incubation on MA at 22 °C, plates showed colonies less than 1 mm in diameter, in most cases about 0.3-0.5 mm in diameter. Some of the strains needed more than 24 h to yield visible colonies on MA; for example, colonies of strain 3OM16 (= CECT 5111) were not larger than 0.2 mm diameter after 3 d of incubation. Colonies on MA were round, opaque, non-pigmented and did not swarm. Strains grew between 4 and 30 °C, but not at 37 °C or higher, and they were not luminescent. After 48 h incubation, colonies on TCBS agar were green (negative for acid production from sucrose). All strains produced acid but not gas from D-glucose, D-galactose, trehalose, D-mannose, D-mannitol, D-cellobiose, maltose and melibiose. Thornley's ADH test was positive. Voges-Proskauer, Møller's ADH, lysine and ornithine decarboxylases were negative. The amount of acid produced by some strains in the decarboxylase medium was low, thus becoming greyish, but the O/F test was clearly fermentative. All strains hydrolysed starch, gelatin and Tween 80 and most of them also hydrolysed casein and DNA. Only two of the 12 strains were negative for casein hydrolysis. Both of them hydrolysed sodium alginate, whereas the other 10 isolates were negative for that trait. Variable responses were obtained for most of the sole carbon sources tested, since five of the 12 strains required growth factors, as could be deduced from the lack of growth in any of the nutritional tests. All 12 strains were resistant to the vibriostatic agent O129 (150 µg per disc), which is a phenotypic characteristic quite uncommon among the vibrios.

Ribotyping analysis

The 12 environmental isolates exhibited identical *KpnI* ribotypes, consisting of eight fragments ranging from 23.1 to 1.7 kb. The number of fragments, indicating the

minimum number of rRNA operons in the chromosome of these strains, is in accordance with the number described for other *Vibrio* species (Aznar *et al.*, 1993; Lan & Reeves, 1998). When *SalI* was used, two ribotypes (A and B) were obtained. Eleven of the 12 strains showed an identical pattern (A) consisting of 10 fragments, whereas strain 3OM16 (= CECT 5111) showed a ribotype pattern (B) quite similar to A, but lacking the three fragments of higher molecular mass. Ribotype A showed fragments ranging from 23.1 to 2.5 kb. Pattern B consisted of fragments between 7.0 and 2.5 kb.

Ribotyping analysis confirmed that *V. lentus* strains are highly homogeneous. The 12 strains were all isolated from the same type of host and geographical origin (oyster samples from the Western Mediterranean coast) and corresponded to two successive samplings, in March and April 1990.

Phylogenetic analysis

The almost complete 16S and 23S rRNA gene sequences of strains 4OM4^T (= CECT 5110^T) and 3OM21 (= CECT 5293) were determined. Comparative analysis confirmed the affiliation of the newly isolated strains to the genus *Vibrio*. Analyses were performed applying three alternative treeing methods, and the results were congruent with respect to the positioning of the new isolates. Figs 1 and 2 show the phylogenetic positions of strains 4OM4^T (= CECT 5110^T) and 3OM21 (= CECT 5293) within the genus *Vibrio*. These two strains shared 98% 16S rDNA sequence similarity and formed a cluster with *V. splendidus* biotypes 1 and 2. The 16S rDNA sequence similarities of strain 4OM4^T (= CECT 5110^T) to *V. splendidus* biotype 1 ATCC 33125^T and *V. splendidus* biotype 2 ATCC 33870 were 98.6 and 98.0%. The levels of 16S rDNA similarity between strain 3OM21 (= CECT 5293) and *V. splendidus* biotype 1 ATCC 33125^T and *V. splendidus* biotype 2 ATCC 33870 were 98.5 and 97.9%. The level of 23S rDNA sequence similarity between strains 4OM4^T (= CECT 5110^T) and 3OM21 (= CECT 5293) was 99.8%. The levels of similarity between the sequences of these two strains and the sequence of *V. splendidus* biotype 1 ATCC 33125^T were 97.4% to 4OM4^T (= CECT 5110^T) and 97.2% to 3OM21 (= CECT 5293). These high levels of both 16S and 23S rDNA sequence similarity are above the limit of intraspecies variability (97%) proposed by Stackebrandt & Goebel (1994) for 16S rDNA sequences and do not allow discrimination between *V. splendidus* and our strains at the species level.

DNA-DNA hybridization

Intraspecific slot-blot DNA-DNA hybridization experiments were done on a non-charged nylon membrane with the 12 environmental isolates, using digoxigenin-labelled DNA of strain 4OM4^T (= CECT 5110^T) as a probe. In all cases, the similarities were

Fig. 1. Phylogenetic tree derived from maximum-parsimony analysis of the 16S rRNA gene sequences of *V. lentus* strains CECT 5110^T and CECT 5293 and other related species of the family *Vibrionaceae*. Bar, 5% estimated sequence divergence. *Ph.*, *Photobacterium*.

higher than 90%, indicating that our strains constituted a tight DNA genomic group.

Additional DNA–DNA hybridization experiments were carried out using the *S*₁ nuclease/trichloroacetic

acid method (Grimont *et al.*, 1980). Hybridizations were performed with four environmental isolates, strains 3OM16 (= CECT 5111), 3OM28, 4OM4^T (= CECT 5110^T) and 4OM8; tritium-labelled DNA of strain 4OM4^T (= CECT 5110^T) was used as the probe.

Fig. 2. Phylogenetic tree derived from distance-matrix analysis of the 23S rRNA gene sequences of *V. lentus* strains CECT 5110^T and CECT 5293 and other related species of the genus *Vibrio*. Bar, 5% estimated sequence divergence.

The lowest DNA similarity (96%) among the environmental isolates was observed for strain 3OM28 and a value of 100% was observed for strain 3OM16

(= CECT 5111), thus confirming the results obtained with the slot-blot hybridization. Using the S_1 nuclease hybridization protocol, both biotypes of *V. splendidus*, the most closely related *Vibrio* species as deduced from the phylogenetic analysis, were hybridized with the proposed type strain, 4OM4^T (= CECT 5110^T). DNA similarities between strain 4OM4^T (= CECT 5110^T) and *V. splendidus* biotype 1 ATCC 33125^T and *V. splendidus* biotype 2 ATCC 33870 were 59.0 and 48.0%; ΔT_m values were 7.0 and 9.7 °C, respectively. From these results, and taking into consideration the criteria recommended by Wayne *et al.* (1987), it can be concluded that the Mediterranean oyster strains are a distinct genospecies within the genus *Vibrio*.

Phenotypic differentiation from other *Vibrio* species

Although our isolates represent a new genospecies within the genus *Vibrio*, their physiological characteristics are similar to those of *V. splendidus*. However, there are some differences between our isolates and both biotypes of *V. splendidus*. Resistance to O129, utilization of L-arginine as sole carbon source and production of acid from D-galactose and melibiose allow differentiation between the proposed new species

Table 1. Phenotypic characteristics that distinguish *V. lentus* CECT 5110^T from *V. splendidus* and other lysine and ornithine decarboxylase-negative *Vibrio* species described since 1992

Taxa are indicated as: 1, *V. lentus*; 2, *V. splendidus* biotype 1; 3, *V. splendidus* biotype 2; 4, *V. mytili*; 5, *V. navarrensis*; 6, *V. penaeicida*; 7, *V. ichthyenteri*; 8, *V. tapetis*; 9, *V. scophthalmi*; 10, *V. pectenicida*; 11, *V. halitocoli*; 12, *V. rumoiensis*; 13, *V. aerogenes*; 14, *V. wodanis*. Data were taken from Borrego *et al.* (1996), Cerdà-Cuellar *et al.* (1997), Farmer & Hickman-Brenner (1992), Ishimaru *et al.* (1995, 1996), Lambert *et al.* (1998), Lunder *et al.* (2000), Pujalte *et al.* (1993), Sawabe *et al.* (1998), Shieh *et al.* (2000), Urdaci *et al.* (1991) and Yumoto *et al.* (1999). Characters are scored as: +, positive; –, negative; v, variable (result for type strain given in parentheses); ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Motility	+	+	+	+	+	+	+	+	+	+	–	–	+	+
ADH*	+ ^a /– ^b	+ ^a /v ^b	– ^a /– ^b	+ ^a /– ^b	– ^a /– ^b	– ^b	– ^a /– ^b	– ^a /– ^b	+	–	–	–	+	–
Indole	v (+)	+	+	–	+	v	–	+	–	–	+	–	+	v
Gas from glucose	–	–	–	+	–	–	–	ND	–	–	–	ND	+	–
Growth on/at:														
TCBS	+	+	+	ND	+	ND	+	+	+	–	–	ND	ND	–
6% NaCl	+	v	v	+	+	–	+	–	–	+	–	+	+	v
4 °C	v (+)	v	–	–	ND	–	–	+	–	v	–	+	–	+
Hydrolysis of:														
Gelatin	+	+	+	–	+	+	–	+	–	+	–	–	+	+
Tween 80	+	+	+	+	ND	+	ND	+	ND	+	–	+	+	+
Starch	+	+	+	–	+	v	–	+	–	+	–	+	+	+
Alginate	v (–)	v	–	–	ND	v	–	–	ND	+	+	–	ND	v
Acid production from:														
D-Galactose	+	–	–	+	+	–	–	+	ND	–	ND	ND	+	+
D-Mannose	+	+	–	–	ND	+	+	+	ND	–	ND	+	+	+
Melibiose	v (–)	–	–	–	ND	–	–	–	ND	–	ND	ND	ND	–
Arabinose	–	–	–	+	–	–	–	–	ND	–	–	+	–	ND
Sucrose	–	v	–	+	+	+	+	–	+	–	–	+	+	v
D-Mannitol	+	+	+	ND	+	–	–	–	ND	ND	+	+	+	v
m-Inositol	–	–	–	–	ND	–	–	–	ND	–	–	–	+	ND
Utilization of:														
L-Arabinose	–	–	–	v	–	ND	ND	–	ND	–	–	ND	+	ND
D-Xylose	–	–	–	+	–	–	–	ND	ND	–	–	ND	+	ND
L-Rhamnose	–	–	–	–	–	ND	ND	–	–	+	ND	ND	ND	ND
Sucrose	–	v	–	+	+	–	v	–	+	v	–	ND	+	ND
L-Arginine	–	+	–	ND	ND	ND	ND	ND	ND	–	ND	ND	–	ND
Sensitivity to O129 (150 µg)	–	+	+	+	+	+	+	+	+	+	+	+	–	+

* Assayed by the Thornley method (a) and/or the Møller method (b).

and *V. splendidus* biotype 1 ATCC 33125^T. *V. splendidus* biotype 2 ATCC 33870 is sensitive to O129 and negative for Thornley's ADH test and does not produce acid from D-galactose, D-mannose or melibiose.

In addition to the phenotypic traits that appear in Table 1, we list below other differences that allow the Mediterranean oyster isolates to be distinguished from other lysine and ornithine decarboxylase-negative *Vibrio* species, described since publication of the latest edition of *The Prokaryotes* (Farmer & Hickman-Brenner, 1992). *Vibrio mytili* produces gas from D-glucose and acid from salicin and utilizes D-xylose as sole carbon source (Pujalte *et al.*, 1993). *Vibrio tapetis* is unable to grow at temperatures above 22 °C (Borrego *et al.*, 1996). *Vibrio wodanis*, one of the recently proposed *Vibrio* species, can be differentiated by pigment production, acid production from cellobiose and temperature preferences (Lunder *et al.*, 2000): our isolates are able to grow at 30 °C while *V. wodanis* does not grow at temperatures above 25 °C. *Vibrio pectenica* swarms and does not produce acid from cellobiose and it utilizes L-rhamnose (Lambert *et al.*, 1998). *Vibrio rumoiensis* utilizes lactose as sole carbon source and does not produce acid from cellobiose (Yumoto *et al.*, 1999). *Vibrio aerogenes* is oxidase-negative, produces acid from xylose and trehalose and utilizes L-arabinose, D-xylose and *m*-inositol as sole carbon sources (Shieh *et al.*, 2000). *Vibrio ichthyenteri*, which does not produce acid from cellobiose (Ishimaru *et al.*, 1996), is one of the three *Vibrio* species, the others being *Vibrio penaeicida* and *Vibrio trachuri*, that do not have 16S rRNA sequences available. However, there are differences enough to ensure that our isolates are not the same as any of these species (Table 1). *V. trachuri* (not included in Table 1) is Thornley's ADH- and ONPG-negative and lysine decarboxylase-positive and produces acid from sucrose and sorbitol (Iwamoto *et al.*, 1996).

Description of *Vibrio lentus* sp. nov.

Vibrio lentus (len'tus. L. adj. *lentus* slow).

Gram-negative rods, facultatively anaerobic, motile by a polar flagellum. Colonies are regular in shape and unpigmented. Swarming has not been detected. Glucose metabolism is fermentative without gas production. Tests for catalase, oxidase and nitrate reduction to nitrite are positive. Resistant to the vibriostatic agent O129 (150 µg per disc). No growth occurs without the addition of NaCl to the culture medium. Growth occurs between 4 and 30 °C. Not luminescent. All strains are positive for Thornley's ADH test. Indole is positive in most strains. All strains hydrolyse starch, gelatin and Tween 80. Acid is produced from D-glucose, D-galactose, D-trehalose, D-mannose, maltose, cellobiose, D-mannitol and melibiose; none of the strains produce acid from L-arabinose, D-xylose, L-rhamnose, D-raffinose, sucrose, D-galacturonate, methyl α -D-glucopyranoside, eryth-

ritol, adonitol, D-sorbitol, *m*-inositol or salicin. Strains that do not require growth factors use D-galactose, D-mannose, maltose, cellobiose, *N*-acetyl D-glucosamine, glycerol, D-mannitol, pyruvate, fumarate, DL-lactate, glycine, L-serine, L-threonine, L-glutamate, L-alanine, aspartate and glutamine as sole carbon sources. Most of the strains that do not require growth factors also use D-glucose, D-fructose, D-trehalose, citrate, *trans*-aconitate, α -ketoglutarate, succinate, DL-malate, L-citrulline and L-histidine as sole carbon sources. None of the strains utilizes the following substrates as sole carbon and energy sources: L-arabinose, D-xylose, L-rhamnose, sucrose, lactose, D-melibiose, D-raffinose, salicin, amygdalin, D-galacturonate, glucosamine, D-sorbitol, *m*-inositol, DL-glycerate, D-saccharate, methanol, propionate, DL- β -hydroxybutyrate, *p*-hydroxybenzoate, L-leucine, L-arginine, L-tyrosine, γ -aminobutyrate, L-ornithine, L-lysine, sarcosine and putrescine.

The type strain is strain 4OM4^T (= CECT 5110^T = DSM 13757^T). In addition to the characteristics given in the species description, the type strain is indole-positive. It hydrolyses casein and DNA, but not alginate. It does not produce acid from D-mannitol or melibiose. It utilizes D-fructose, D-galactose, D-mannose, maltose, *N*-acetyl D-glucosamine, glycerol, D-mannitol, pyruvate, citrate, *trans*-aconitate, α -ketoglutarate, succinate, fumarate, DL-malate, DL-lactate, glycine, L-serine, L-threonine, L-glutamate, L-alanine, L-citrulline, aspartate, glutamine and L-histidine as sole carbon sources. In addition to the negative traits given above for all 12 strains, the type strain is negative for utilization of D-ribose, D-glucose, D-trehalose, D-gluconate, D-glucuronate and acetate. The mean G + C content is 44.0 mol %. Isolated from oysters from the Mediterranean coast of Valencia (Spain).

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