***Thalassomonas viridans* gen. nov., sp. nov., a novel marine γ -proteobacterium**

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A new genus and species are proposed for two halophilic, strictly aerobic, chemo-organotrophic, marine bacterial strains. These bacteria are Gram-negative, motile rods isolated from oysters cultivated off the Mediterranean coast at Valencia (Spain). They produce green/blue-green diffusible pigment. The G+C content of the DNA of the proposed type strain (XOM25^T) is 48.4 mol%. A 16S rRNA gene sequence analysis of the two strains has shown that the new isolates represent a branch within the γ -Proteobacteria, close to the genus *Colwellia*. The type species of the new genus is *Thalassomonas viridans* gen. nov., sp. nov., with the type strain XOM25^T (= CECT 5083^T = DSM 13754^T).

Keywords: γ -Proteobacteria, marine bacteria, *Thalassomonas viridans* gen. nov., sp. nov., green pigment, 16S rDNA phylogeny

INTRODUCTION

Most of the readily culturable, heterotrophic, marine bacteria belong to the γ -Proteobacteria, a branch that includes the fermentative genera *Vibrio*, *Photobacterium*, *Moritella* and *Colwellia*, the non-fermentative or weakly fermentative facultative anaerobes *Shewanella* and *Ferrimonas* and strict aerobes distributed among the halophilic/halotolerant genera *Alcanivorax*, *Alteromonas*, *Cycloclasticus*, *Fundibacter*, *Glaciecola*, *Halomonas*, *Idiomarina*, *Marinobacter*, *Marinomonas*, *Marinospirillum*, *Microbulbifer*, *Neptunomonas*, *Oceanospirillum* and *Pseudoalteromonas* and some authentic *Pseudomonas* species.

Of these genera, at least 22 species are known to produce pigmented colonies or diffusible compounds ranging in colour from yellow/orange (*Cycloclasticus pugetii*, *Pseudoalteromonas aurantia*, *Pseudoalteromonas citrea*, *Pseudoalteromonas peptidolytica*, *Pseudoalteromonas piscicida*, *Vibrio fischeri*, *Vibrio logei*) to red (*Colwellia psychrerythraea*, *Glaciecola punicea*, *Pseudoalteromonas bacteriolytica*, *Pseudoalteromonas denitrificans*, *Pseudoalteromonas rubra*, *Vibrio gazogenes*), pink (*Glaciecola pallidula*, some

shewanellae), violet/yellow (*Pseudoalteromonas luteoviolacea*), brown/black (*Marinomonas mediterranea*, *Neptunomonas naphthovorans*, *Pseudoalteromonas nigrifaciens*, *Shewanella colwelliana*, *Vibrio nigripulchritudo*) and green (*Oceanospirillum linum*, *Pseudoalteromonas tunicata*). Pigment production seems to be involved in antibiotic/antilarval activities (Holmström & Kjelleberg, 1999; Holmström *et al.*, 1992; Lemos *et al.*, 1991) and autotoxicity (Baumann *et al.*, 1984). It is possible that the active components are produced by the same pathway or by a branch of the pathway leading to pigment production (Holmström *et al.*, 1998).

Two strains of halophilic, strictly aerobic bacteria producing a deep green to blue-green, diffusible and unstable pigment were isolated from oysters close to the coastal area of Valencia, Spain. In a previous numerical taxonomic study (Ortigosa *et al.*, 1994), strains XOM5 and XOM25^T were phenotypically characterized and remained as a clearly isolated, unique phenon after clustering. They showed a marked loss of viability within a few days in culture, and were deposited at the Colección Española de Cultivos Tipo as CECT 5082 and CECT 5083^T and at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) as DSM 13753 and DSM 13754^T, respectively.

We have characterized both strains by determining the complete sequence of the 16S rRNA genes, the G+C

The GenBank/EMBL accession numbers for the 16S rRNA sequences of *Thalassomonas viridans* CECT 5082 and CECT 5083^T are AJ294747 and AJ294748, respectively.

content of the DNAs and cultural, physiological and biochemical features. Their phenotypic characteristics and the results of phylogenetic analyses indicate a moderate relationship to the genus *Colwellia* (within the γ -*Proteobacteria*) that is sufficiently distant to constitute a new genus.

METHODS

Bacterial strains and growth conditions. Two environmental strains (XOM5 and XOM25^T) that constituted phenon 29 in the previous study by Ortigosa *et al.* (1994) were isolated from Mediterranean oysters, on Marine agar 2216 (MA; Difco) plates. Cultures were maintained on semi-solid MA stabs at room temperature and were also suspended in filtered, aged, sterile sea water at room temperature in the dark. They were grown routinely on MA or Marine broth 2216 (MB; Difco) at 24–26 °C.

Phenotypic characterization. The phenotypic profiles of the two environmental strains were determined. Cultivation was done at 24–26 °C. Pigment production, swarming and colony morphology were examined after growing the cells on MA plates. Cell morphology and motility were examined on wet mounts by phase-contrast microscopy. The flagellar arrangement was examined after staining MA-grown cells by the method of Heimbrook *et al.* (1989). The Gram reaction was determined by using the KOH lysis method of Buck (1982). The ability to reduce nitrate to nitrite was tested in nitrate broth plus 1% (w/v) NaCl (Smibert & Krieg, 1994) and in denitrification medium (Baumann & Baumann, 1981). The oxidation/fermentation test was performed in O/F medium (Difco) rehydrated with half-strength artificial sea water (ASW) plus 1% (w/v) glucose, glycerol or *N*-acetylglucosamine. Tests for Thornley's arginine dihydrolase and nutritional screening on basal medium plates, as well as on Baumann's basal medium supplemented with 0.1 g yeast extract l⁻¹, were performed by using methods described previously (Baumann *et al.*, 1984). The alkalization/decarboxylation of arginine, lysine and ornithine was determined in Møller broth (decarboxylase medium base; Difco) and the oxidase test and growth on thiosulfate/citrate/bile salts/sucrose (TCBS) agar were performed using the methods of Lee & Donovan (1985). The catalase test (10 vols H₂O₂) was performed according to Smibert & Krieg (1994). Indole production was tested on MB after 48 h. Hydrolysis of casein, gelatin, Tween 80, lecithin, starch, sodium alginate and agar was tested according to Ortigosa *et al.* (1994). The range of salinity supporting growth of the strains was tested by using five media: (i) salt-tolerance broth [STB: 1% (w/v) tryptone, 0.3% (w/v) yeast extract with 0, 2, 4, 6, 8 and 10% (w/v) NaCl]; (ii) STB with 2 or 4% (w/v) NaCl and 0.1% (w/v) MgCl₂; (iii) STB with 2% (w/v) NaCl and 0.1% (w/v) CaCl₂; (iv) STB with 2% (w/v) NaCl plus 0.1% (w/v) MgCl₂ and 0.1% (w/v) CaCl₂; (v) MB supplemented with enough NaCl to achieve 4, 6, 8 and 10% (w/v) total NaCl. Growth at different temperatures was tested in both liquid (MB) and solid (MA) media at 4 °C (7 d) and at 13, 20, 28, 30, 37 and 40 °C. Sulfite oxidation was tested on solid medium by following the method described by González *et al.* (1999).

Cellular fatty acid analysis. For the analysis of whole-cell fatty acids, cells were grown for 24 h at 28 °C on MA and approximately 40 mg bacterial cells was harvested from the Petri dish. Analysis of the fatty acids by GLC was performed

at the DSMZ by using a method described previously (Kämpfer & Kroppenstedt, 1996).

DNA base composition. The G+C content (mol%) of genomic DNA was determined from the midpoint value of the thermal denaturation profile (Marmur & Doty, 1962) obtained with a model UV-Vis 551S spectrophotometer (Perkin-Elmer) at 260 nm. This instrument was programmed for temperature increases of 1.0 °C min⁻¹. The G+C content of reference DNA from *Escherichia coli* NCTC 9001^T was taken to be 51 mol% (Owen & Pitcher, 1985).

Extraction of genomic DNA and amplification and sequencing of rDNA. Isolation of genomic DNA was carried out according to Lewington *et al.* (1987). The genomic DNA of bacterial strains was used for the *in vitro* amplification of almost full-length 16S rRNA gene fragments by using the following primer set: 5'-AGAGTTTGATYMTGGCTC-AG-3' (*E. coli* positions 8–27; Brosius *et al.*, 1981) and 5'-CAKAAAGGAGGTGATCC-3' (positions 1529–1546). Amplifications were performed with a Hybaid OmniGene temperature controller (MWG-Biotech) as follows. The following components were added to a 0.2 ml reaction tube: 1 µl (0.1–1 µg) genomic DNA solution, 50 pmol each of the appropriate primers, 10 mM dNTP mix (Qiagen), 10 µl 10 × PCR buffer [Tris/HCl, (NH₄)₂SO₄, 15 mM MgCl₂; pH 8.7] and 1.5 U thermostable *Taq* polymerase (Qiagen). The total volume was adjusted to 100 µl with sterile water. After initial heating at 94 °C for 3 min, 30 cycles consisting of denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s and extension at 72 °C for 1 min, increasing by 5 s per cycle, were performed, followed by a final elongation step at 72 °C for 10 min. The resulting PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using a LICOR automated sequencer (MWG Biotech). Cycle-sequencing protocols based upon the chain-termination technique were applied using the Thermo Sequenase fluorescent labelled primer cycle-sequencing kit of Amersham.

Sequence data analysis. Sequences were added to the 16S rRNA sequence databases of the Technical University of Munich by using the program package ARB (Ludwig & Strunk, 1997). 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 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; fastDNAmI 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).

RESULTS AND DISCUSSION

Phenotypic characteristics

Strains XOM5 and XOM25^T are Gram-negative, strictly aerobic and rod-shaped (approx. 1.5–2 µm long × 0.8–1 µm wide); each cell was motile by means of one polar flagellum. The strains grew on MA as regular, opaque colonies that did not swarm or luminesce. They produced a diffusible green to blue-green pigment when grown in MB (only on the surface)

or on MA, lecithin and starch media at 13–30 °C, but not at 37 °C. The colour was stable for no longer than 48 h; it then faded. When the strains grew in liquid medium, they formed a compact pellicle on the top of the liquid and no turbidity was observed below the surface.

The isolates were strictly halophilic, requiring sea-water-based media for growth; they were unable to grow in STB (10 g tryptone l⁻¹, 3 g yeast extract l⁻¹) containing 2–4% NaCl or supplemented with 0.1% divalent cations (Mg²⁺ and Ca²⁺) alone or in combination, but showed good growth on MB/MA and Baumann's ASW-based media (Baumann *et al.*, 1984). They were able to grow on MB containing up to 4% NaCl, but no growth was observed at ≥ 6% NaCl. The organisms were chemo-organotrophs, unable to ferment sugars under anaerobic conditions, as determined on O/F medium with half-strength ASW. The catalase and oxidase reactions were positive. Nitrate was not reduced to nitrite. No growth or gas production was observed on Baumann's denitrification medium. The strains grew at temperatures ranging from 13 to 37 °C but not at 4 or 40 °C. No growth was observed on TCBS agar. No hydrolytic activities were detected on alginate or agar. They hydrolysed gelatin, casein, starch and lecithin. Tween 80 was hydrolysed slightly after prolonged incubation (more than 7 d). The strains were negative for the following enzymatic activities: arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, indole production from tryptophan and sulfite oxidation.

Although the strains were originally reported as being capable of growing without added growth factors (Ortigosa *et al.*, 1994), they are currently unable to grow on basal medium without added yeast extract; thus, the results reported here for nutritional screening were obtained on Bauman's basal medium supplemented with 1 g yeast extract l⁻¹. The strains used the following compounds as sole carbon and energy sources: cellobiose, amygdalin, propionate, L-tyrosine, L-citrulline, glutamine and histidine. Other carbon sources allowing weak growth were D-glucose, D-fructose, maltose, sucrose, fumarate and L-leucine. Neither of the strains utilized the following substrates: L-arabinose, D-xylose, D-galactose, D-mannose, L-rhamnose, sucrose, lactose, melibiose, salicin, arbutin, D-gluconate, D-glucuronate, D-galacturonate, N-acetyl D-glucosamine, saccharate, glycerate, glycerol, D-mannitol, D-sorbitol, *m*-inositol, citrate, aconitate, α-ketoglutarate, DL-malate, acetate, DL-lactate, DL-β-hydroxybutyrate, *p*-hydroxybenzoate, glycine, L-serine, L-threonine, L-glutamate, L-alanine, γ-amino-butyrate, L-ornithine, sarcosine and putrescine.

Fatty acid composition

Thirty-one fatty acids, containing 9–20 carbon atoms, were detected in the extracts (Table 1). The predominant fatty acids were straight-chain fatty acids, and only minor quantities of iso-branched fatty acids

Table 1. Cellular fatty acid composition of *Thalassomonas viridans* strains

Values are percentages of total fatty acid content. The numbers of carbon atoms and double bonds are indicated. The following fatty acids were present at levels of < 1% in both strains: 10:0, 10:0 3-OH, i11:0 3-OH, 13:0, i12:0 3-OH, i15:0, i16:0, i17:0, 17:1ω6c, 18:1ω9c and 18:0. Fatty acid 9:0 was present at < 1% in strain CECT 5082 but was absent from strain CECT 5083^T. Fatty acids i12:0 and 20:1ω7c were present at < 1% in strain CECT 5083^T but were absent from CECT 5082.

Fatty acid	CECT 5082	CECT 5083 ^T
11:0	1.10	0.90
11:0 3-OH	4.20	3.00
12:0	1.56	1.97
12:0 3-OH	4.99	6.06
13:0 3-OH/i15:1*	1.33	0.89
14:0	2.30	3.07
15:1ω8c	5.77	4.54
15:1ω6c	1.20	0.40
15:0	11.14	5.96
16:0	11.19	13.65
16:1ω7c/i15 2-OH*	21.23	28.38
17:1ω8c	19.68	14.07
17:0	4.71	3.11
18:1ω7c	3.18	6.12

* Fatty acids 13:0 3-OH/i15:1 and 16:1ω7c/i15 2-OH could not be separated in the analysis, so the values represent the summed features.

were detected. Saturated and monounsaturated fatty acids, namely 15:0, 16:0 and 17:1ω8c, were the most abundant, together with a mixture of 16:1ω7c and i15:0 2-OH. Hydroxy fatty acids (11:0 3-OH and 12:0 3-OH) were found in both strains in appreciable amounts. Neither of the strains produced polyunsaturated fatty acids.

G + C content

The G + C content (determined by the thermal denaturation method) of the DNA of the type strain (XOM25^T = CECT 5083^T) was 48.4 mol %.

16S rRNA gene sequencing and phylogenetic analysis

The almost complete sequences of the 16S rRNA genes were determined for the two strains. Phylogenetic analyses were performed by applying three alternative treeing methods, and the results were congruent with respect to the positioning of the new isolates. The sequences of the two strains were almost identical. There were differences in only two positions.

Comparative analysis of the sequences confirmed the affiliation of the strains to the γ-Proteobacteria. The next closest (though only moderately related)

Fig. 1. Phylogenetic tree derived from maximum-parsimony analysis of the 16S rRNA gene sequences of *Thalassomonas viridans* strains CECT 5083^T and CECT 5082 and other related genera of the γ -Proteobacteria. Bar, 5% estimated sequence divergence.

Table 2. Levels of 16S rDNA sequence similarity between *Thalassomonas viridans* strains CECT 5082 and CECT 5083^T and related organisms

Taxon	1	2	3	4	5	6	7	8	9	10	11
1. <i>Colwellia rossensis</i>											
2. <i>Colwellia maris</i>	98.2										
3. <i>Colwellia psychrerythraea</i>	97.3	97.3									
4. <i>Colwellia demingiae</i>	96.5	96.8	96.5								
5. <i>Colwellia psychrotropica</i>	97.6	97.8	95.8	95.7							
6. <i>Colwellia hornerae</i>	96.6	97.0	96.1	95.5	95.8						
7. <i>Thalassomonas viridans</i> CECT 5082	93.5	94.2	94.6	94.9	93.3	94.2					
8. <i>Thalassomonas viridans</i> CECT 5083 ^T	93.4	94.1	94.5	94.8	93.2	94.1	99.9				
9. <i>Idiomarina zobellii</i>	89.7	90.2	89.8	90.0	90.6	90.1	90.6	90.6			
10. <i>Idiomarina abyssalis</i>	89.8	90.3	89.9	90.0	90.6	90.0	90.6	90.5	96.9		
11. <i>Glaciecola punicea</i>	85.6	85.6	85.8	85.4	85.3	85.6	85.1	85.0	85.9	85.8	
12. <i>Glaciecola pallidula</i>	86.8	87.7	87.6	87.3	87.2	86.9	86.6	86.5	86.6	86.2	91.6

neighbours were *Colwellia* species (Fig. 1). The corresponding overall 16S rRNA sequence similarity values are indicated in Table 2. The range of 16S rRNA gene sequence similarity values to *Colwellia* species was 93.2–94.8%, the highest similarity being shown by the *Colwellia demingiae* sequence. The *Colwellia* assemblage contains several other marine genera, including *Idiomarina* and *Glaciecola*. The sequence similarity was 90.5–90.6% to *Idiomarina* species and 85.0–86.6% to members of the genus *Glaciecola*.

Conclusions

Two strains of heterotrophic marine bacteria were isolated from Mediterranean oysters (*Ostrea edulis*) cultivated off the coast near Valencia (Spain). The organisms were strictly aerobic, halophilic, negative in the oxidation/fermentation test, green-pigmented, motile, oxidase- and catalase-positive, Gram-negative

rods with a DNA G + C content of 48.4 mol%. It is evident from the phylogenetic analysis and the 16S rDNA sequence similarity to other species (94.8% or less) that the two isolates represent a separate group amongst the γ -Proteobacteria, moderately related to the genera *Alteromonas*, *Colwellia*, *Glaciecola*, *Idiomarina* and *Pseudoalteromonas*. They can be differentiated phenotypically from these organisms by the traits reported in Table 3. Members of *Idiomarina* differ from our isolates by the absence of pigmentation, the ability to grow at 4 °C, salinity requirements and, notably, the fact that they are rich in iso-branched fatty acids. Representatives of the genus *Colwellia* differ notably from our isolates in that they possess a lower G + C ratio (approx. 10% lower), have a fermentative metabolism, reduce nitrates to nitrites, are strict psychrophilic bacteria and produce poly-unsaturated fatty acids. It is concluded, therefore, that the oyster isolates can be separated from other described marine bacterial species by using a combi-

Table 3. Characteristics that distinguish *Thalassomonas viridans* gen. nov., sp. nov. from other heterotrophic, oxidative, marine genera

Data are from the following references: Baumann *et al.* (1972), Bowman *et al.* (1998a, b), Deming *et al.* (1988), Holmström *et al.* (1998), Ivanova *et al.* (2000), Raguénès *et al.* (1997), Yumoto *et al.* (1998). Species are indicated as: 1, *Thalassomonas viridans*; 2, *Colwellia psychrerythraea*; 3, *Colwellia demingiae*; 4, *Colwellia hornerae*; 5, *Colwellia rossensis*; 6, *Colwellia psychrotrophica*; 7, *Colwellia maris*; 8, *Alteromonas macleodii*; 9, *Alteromonas infernus*; 10, *Glaciecola punicea*; 11, *Glaciecola pallidula*; 12, *Pseudoalteromonas tunicata*; 13, *Idiomarina abyssalis*; 14, *Idiomarina zobellii*. Characteristics are scored as: +, positive; –, negative; ND, no data available; NG, no growth; TR, trace amounts of fatty acid (< 0.1 % of total fatty acid content); v, variable response. PUFA, Polyunsaturated fatty acids.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pigment	Green	Red	–	–	–	–	–	–	–	Red	Pink	Green	–	–
Growth in 6 % NaCl	–	ND	–	–	–	+	ND	+	+	+	+	+	+	+
Growth at 4 °C	–	+	+	+	+	+	+	–	–	+	+	+	+	+
Growth at 30 °C	+	–	–	–	–	–	–	+	+	–	–	ND	+	+
Fermentative on carbohydrates*	– ^a	+ ^b	+ ^c	+ ^d	+ ^a	+ ^c	–	–	–	–	–	–	–	–
Hydrolysis of:														
Gelatin	+	v	–	–	–	–	+	+	+	–	–	+	+	+
Tween 80†	–	+	–	+	NG	+	+	+	ND	v	+	ND	+	+
Nitrate reduction	–	+	+	+	+	+	+	–	–	–	–	ND	ND	ND
Use of:														
D-Fructose	+	–	–	–	+	–	–	+	+	–	–	–	–	–
Maltose	+	–	–	–	–	–	–	+	+	–	–	+	–	–
Sucrose	+	–	–	–	–	–	–	+	+	–	–	–	–	–
D-Xylose, galactose	–	–	–	–	–	–	–	+	+	–	–	–	ND	ND
Melibiose, D-mannitol	–	–	–	–	–	–	–	+	+	–	–	–	–	–
D-Gluconate	–	–	–	–	+	–	ND	+	+	–	–	ND	ND	ND
Glycerol	–	–	–	+	+	–	–	+	+	–	+	–	+‡	–
Acetate	–	+	+	+	+	+	+	+	ND	–	+	ND	+	ND
L-Aconitate	–	+	+	+	+	+	ND	v	ND	–	+	ND	ND	ND
L-Alanine	–		v		+	+	ND	+	ND	–	–	ND	+	+
Fatty acids (% of total):														
i15:0	< 1.0	–	–	–	–	–	ND	ND	ND	ND	ND	ND	33.7	40.6
i17:0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	ND	ND	ND	ND	ND	ND	11.9	12.5
17:1 ω 8c	14–19.7	0–1.3	TR	5.6	< 1.0	4.5	ND	ND	ND	ND	ND	ND	< 1.0	1.1
PUFA	–	5.5–9.5	1.7–2.2	4.1	6.0	< 1.0	ND	ND	ND	ND	ND	ND	–	–
DNA G + C content (mol %)	48.4	35–38	37	39	38	42	39.4	44–47	48.1	44–46	40	42–43	50.4	48

* Fermentative metabolism on D-glucose, N-acetylglucosamine and glycerol (a), D-glucose and N-acetylglucosamine (b), N-acetylglucosamine (c) or glycerol (d) is indicated.

† After less than 7 d of incubation.

‡ Genus description gives contradictory results for this trait (Ivanova *et al.*, 2000).

nation of phenotypic characteristics. Thus, we propose the creation of a new genus, *Thalassomonas* gen. nov., containing strains XOM5 and XOM25^T as *Thalassomonas viridans* sp. nov.

Description of *Thalassomonas* gen. nov.

Thalassomonas (Tha.las'so.mo.nas. Gr. n. *Thalasso* the sea; Gr. n. *monas* a unit; N.L. n. *Thalassomonas* a monad from the sea).

Gram-negative, strictly aerobic, chemo-organotrophic, halophilic bacteria. Oxidase- and catalase-positive, motile by single polar flagella. Cells are rod-shaped and approximately 1.5–2 µm long and 0.8–1

µm wide. No growth without sea water or the addition of combined marine salts to the medium. Mesophilic. The temperature range for growth is 13–37 °C; no growth at 4 or 40 °C. Gelatin, casein, starch and lecithin, but not alginate or agar, are hydrolysed. Nitrate is not reduced to nitrite. Sulfite is not oxidized. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and indole-production activities are not detected. The predominant cellular fatty acids are saturated and monounsaturated straight-chain fatty acids. The DNA G + C content of the type strain of the type species, CECT 5083^T (= XOM25^T), is 48.4 mol%. The genus is affiliated to the γ -*Proteobacteria* and contains one species, *Thalassomonas viridans*, which is the type species.

Description of *Thalassomonas viridans* sp. nov.

Thalassomonas viridans (vi.ri'dans. L. adj. *viridans* green).

In addition to the characteristics that define the genus, it has the characteristics described below. Strains grow on MA as regular, opaque colonies that produce a green/blue-green, unstable, diffusible pigment. They grow in MB with 2–4% NaCl but not with 6% or more. No growth is observed in STB 2–4% NaCl, in STB 2–4% NaCl plus MgCl₂, in STB 2% NaCl plus CaCl₂ or in STB 2% NaCl plus MgCl₂ and CaCl₂. Strains hydrolyse Tween 80 only after prolonged incubation (more than 7 d). Organic growth factors are required. Strains are able to grow on basal medium supplemented with 0.1 g yeast extract l⁻¹, using the following compounds as sole carbon and energy sources: cellobiose, amygdalin, propionate, L-tyrosine, L-citrulline, glutamine and histidine. Growth is weak on D-glucose, D-fructose, maltose, sucrose, fumarate and L-leucine. Neither of the strains utilizes the following substrates: L-arabinose, D-xylose, D-galactose, D-mannose, L-rhamnose, sucrose, lactose, melibiose, salicin, arbutin, D-gluconate, D-glucuronate, D-galacturonate, N-acetyl D-glucosamine, saccharate, glycerate, glycerol, D-mannitol, D-sorbitol, *m*-inositol, citrate, aconitate, α -ketoglutarate, DL-malate, acetate, DL-lactate, *p*-hydroxybenzoate, glycine, L-serine, L-glutamate, L-alanine, γ -aminobutyrate, sarcosine and putrescine. The major fatty acids are 15:0, 16:0 and 17:1 ω 8*c*. Isolated from oysters harvested on the Mediterranean coast of Vinaroz, Spain.

The type strain, XOM25^T (= CECT 5083^T = DSM 13754^T), was isolated from oyster homogenate. The species also includes strain XOM5 (= CECT 5082 = DSM 13753). The type strain XOM25^T differs from XOM5 by the following traits: it is able to use D-ribose, succinate, DL- β -hydroxybutyrate, L-threonine, L-arginine and L-ornithine as sole carbon and energy sources.

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REFERENCES

- Baumann, P. & Baumann, L. (1981). The marine gram-negative eubacteria: genera *Photobacterium*, *Beneckea*, *Alteromonas*, *Pseudomonas* and *Alcaligenes*. In *The Prokaryotes*, vol. 2, pp. 1302–1331. Edited by M. P. Starr, H. G. Trüper, A. Balows & H. Schlegel. Berlin: Springer.
- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.
- Baumann, P., Gauthier, M. J. & Baumann, L. (1984). Genus *Alteromonas* Baumann, Baumann, Mandel and Allen 1972. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 343–352.
- Bowman, J. P., Gosink, J. J., McCammon, S. A., Lewis, T. E., Nichols, D. S., Nichols, P. D., Skerratt, J. H., Staley, J. T. & McMeekin, T. A. (1998a). *Colwellia demingiae* sp. nov., *Colwellia hornerae* sp. nov., *Colwellia rossensis* sp. nov. and *Colwellia psychrotropica* sp. nov.: psychrophilic Antarctic species with the ability to synthesize docosahexaenoic acid (22:6 ω 3). *Int J Syst Bacteriol* **48**, 1171–1180.
- Bowman, J. P., McCammon, S. A., Brown, J. L. & McMeekin, T. A. (1998b). *Glaciecola punicea* gen. nov., sp. nov. and *Glaciecola pallidula* gen. nov., sp. nov.: psychrophilic bacteria from Antarctic sea-ice habitats. *Int J Syst Bacteriol* **48**, 1213–1222.
- Brosius, J., Dull, T. J., Sleeter, D. D. & Noller, H. F. (1981). Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* **148**, 107–127.
- Buck, J. D. (1982). Nonstaining (KOH) method for determination of Gram reactions of marine bacteria. *Appl Environ Microbiol* **44**, 992–993.
- Deming, J. W., Somers, L. K., Straube, W. L., Swartz, D. G. & MacDonell, M. T. (1988). Isolation of an obligately barophilic bacterium and description of a new genus, *Colwellia* gen. nov. *Syst Appl Microbiol* **10**, 152–160.
- Felsenstein, J. (1982). Numerical methods for inferring phylogenetic trees. *Q Rev Biol* **57**, 379–404.
- González, J. M., Kiene, R. P. & Moran, M. A. (1999). Transformation of sulfur compounds by an abundant lineage of marine bacteria in the α -subclass of the class *Proteobacteria*. *Appl Environ Microbiol* **65**, 3810–3819.
- Heimbrook, M. E., Wang, W. L. & Campbell, G. (1989). Staining bacterial flagella easily. *J Clin Microbiol* **27**, 2612–2615.
- Holmström, C. & Kjelleberg, S. (1999). Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiol Ecol* **30**, 285–293.
- Holmström, C., Rittschof, D. & Kjelleberg, S. (1992). Inhibition of settlement by larvae of *Balanus amphitrite* and *Ciona intestinalis* by a surface-colonizing marine bacterium. *Appl Environ Microbiol* **58**, 2111–2115.
- Holmström, C., James, S., Neilan, B. A., White, D. C. & Kjelleberg, S. (1998). *Pseudoalteromonas tunicata* sp. nov., a bacterium that produces antifouling agents. *Int J Syst Bacteriol* **48**, 1205–1212.
- Ivanova, E. P., Romanenko, L. A., Chun, J. & 7 other authors. (2000). *Idiomarina* gen. nov., comprising novel indigenous deep-sea bacteria from the Pacific Ocean, including descriptions of two species, *Idiomarina abyssalis* sp. nov. and *Idiomarina zobellii* sp. nov. *Int J Syst Evol Microbiol* **50**, 901–907.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Lee, J. V. & Donovan, T. J. (1985). *Vibrio*, *Aeromonas* and *Plesiomonas*. In *Isolation and Identification of Microorganisms of Medical and Veterinary Importance*, pp. 13–33. Society for Applied Bacteriology Technical Series no. 21. Edited by C. H. Collins & J. M. Grange. London: Academic Press.
- Lemos, M. L., Dopazo, C. P., Toranzo, A. E. & Barja, J. L. (1991). Competitive dominance of antibiotic-producing marine bacteria in mixed cultures. *J Appl Bacteriol* **71**, 228–232.
- Lewington, J. S. D., Greenaway, B. J. & Spillane, B. J. (1987). Rapid small scale preparation of bacterial genomic DNA suitable for cloning and hybridization analysis. *Lett Appl Microbiol* **5**, 51–53.

- Ludwig, W. & Strunk, O. (1997). ARB: a software environment for sequence data. <http://www.mikro.biologie.tu-muenchen.de/pub/ARB/documentation/arb.ps>
- Ludwig, W., Strunk, O., Klugbauer, S., Klugbauer, N., Weizenegger, M., Neumaier, J., Bachleitner, M. & Schleifer, K.-H. (1998). Bacterial phylogeny based on comparative sequence analysis. *Electrophoresis* **19**, 554–568.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The Ribosomal Database Project (RDP). *Nucleic Acids Res* **24**, 82–85.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Ortigosa, M., Garay, E. & Pujalte, M. J. (1994). Numerical taxonomy of aerobic, Gram-negative bacteria associated with oysters and surrounding seawater of the Mediterranean coast. *Syst Appl Microbiol* **17**, 589–600.
- Owen, R. J. & Pitcher, D. (1985). Current methods for estimating DNA base composition and levels of DNA-DNA hybridization. In *Chemical Methods in Bacterial Systematics*, pp. 67–93. Edited by M. Goodfellow & E. Minnikin. London: Academic Press.
- Raguénès, G. H. C., Peres, A., Ruimy, R., Pignet, P., Christen, R., Loaec, M., Rougeaux, H., Barbier, G. & Guezennec, J. G. (1997). *Alteromonas infernus* sp. nov., a new polysaccharide-producing bacterium isolated from a deep-sea hydrothermal vent. *J Appl Microbiol* **82**, 422–430.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Yumoto, I., Kawasaki, K., Iwata, H., Matsuyama, H. & Okuyama, H. (1998). Assignment of *Vibrio* sp. strain ABE-1 to *Colwellia maris* sp. nov., a new psychrophilic bacterium. *Int J Syst Bacteriol* **48**, 1357–1362.