Brevundimonas mediterranea sp. nov., a non-stalked species from the Mediterranean Sea

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Six strains of Gram-negative, rod-shaped, non-spore-forming bacteria were isolated from the Mediterranean Sea. 16S rRNA gene sequence analysis indicated that the strains were affiliated within the alphaproteobacterial genus *Brevundimonas*, with *Brevundimonas intermedia* (99.4%) and *Brevundimonas vesicularis* (99.2%) as their closest relatives. This affiliation was supported by chemotaxonomic data (major polar lipids: phosphatidyl diacylglycerol, sulfoquinovosyl diacylglycerol and phosphatidyl glucopyranosyl diacylglycerol; major fatty acids: C_{18:1}, C_{16:0}, C_{16:1}, C_{15:0}, C_{17:1}ω8c, 11-Me-C_{18:1}ω5t). The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the strains from all recognized *Brevundimonas* species. The strains therefore represent a novel species, for which the name *Brevundimonas mediterranea* sp. nov. is proposed, with the type strain V4.BO.10^T (= LMG 21911^T = CIP 107934^T).

Two species originally described as *Pseudomonas diminuta* and *Pseudomonas vesicularis* were reclassified by Segers *et al.* (1994) into a new genus *Brevundimonas* with *Brevundimonas diminuta* as the type species of the genus. In 1999 several species were transferred from the genus *Caulobacter* to *Brevundimonas* and the description of the genus *Brevundimonas* was emended (Abraham *et al.*, 1999). *Brevundimonas* species are characterized and differentiated from *Caulobacter* species by the presence of certain glyco- and polar lipids, outer-membrane proteins and an increased salt tolerance, but members of the genus show diverse cell morphologies and substrate specificities. Although some marine *Brevundimonas* strains have been described (Stahl *et al.*, 1992; Yokoyama *et al.*, 1996; Abraham *et al.*, 2002),

no marine *Brevundimonas* species has been named to date. Here we describe six strains isolated from marine water samples, which we propose are representatives of a novel species, *Brevundimonas mediterranea* sp. nov.

Sampling

Marine water samples were taken aboard the ship *Thetys II* (Institute National des Sciences de l'Univers INSU - CNRS/ France) at two stations located in the north-western basin of the Mediterranean Sea on a transect between Nice and Corsica. Stations S1 [5.5 nautical miles (about 10 km) from the coast, nautical coordinates 43° 37' 48" N 7° 26' 06" E] and station S2 [28 nautical miles (about 52 km) from the coast, nautical coordinates 43° 25' 00" N 7° 52' 00" E] were sampled in early April 1995. Samples were collected with 12 litre Niskin bottles during the early afternoon and were stored in HCl-washed polycarbonate tanks until return to the institute. Plating was performed in triplicate either directly on Difco marine broth (MB) agar (representing the total biocoenosis) or using either the filtrate (representing the free-living biocoenosis) or the resuspended residue (representing the particle-bound biocoenosis) from 1 µm Nucleopore-filtered marine water samples (Fritz, 2000). After 2 weeks of dark incubation at ambient temperature, plate counts were performed and bacterial colonies were isolated.

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Abbreviations: LMW-RNA, low-molecular-mass RNAs (tRNAs and rRNAs); OTU, operational taxonomic unit; PGL, phosphatidyl glucopyranosyl diacylglycerol; RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains V4.BO.07, V4.BO.10^T, LMG 9567t1, LMG 11070, LMG 9564, V4.BO.05, V4.BO.22, V4.BO.18, V4.BE.56, V4.BO.27, V4.BE.49 and V4.BP.05 are AJ227800, AJ227801, AJ244647–AJ244649 and AJ244704–AJ244710.

Strain details, transmission electron micrographs, protein profiles and RAPD profiles are available as supplementary material in IJSEM Online.

Isolation and morphological diagnosis

Plates occasionally showed an abundance of a distinct type of bacteria with unique colony morphology. Bacterial colonies were cream-white in colour with a central brownish spot and had a slimy consistency; depending on age, colonies measured between 2 and 8 mm in diameter and contributed to more than 50 % of all colony-forming units in all triplicates of some investigated water samples (Fritz, 2000). Colony-forming units with this distinct, highly characteristic morphology were observed in water samples from both stations, from both free-living and particle-bound biocoenosis and from various depths. Six strains (V4.BE.49, V4.BE.56, V4.BO.10^T, V4.BO.18, V4.BO.22 and V4.BO.27) with the described colony morphology originating from water samples from both stations and various depths were purified to macroscopic and microscopic homogeneity (see Supplementary Table A for details of their properties, available in IJSEM Online). Microscopically, the cells consisted of flexible, slightly bent long rods with a length of 1.5–4 µm and a diameter of 0.5–1 µm (Supplementary Fig. A). These six strains are hereafter referred to as operational taxonomic unit (OTU) H (Fritz, 2000).

16S rRNA gene sequencing

16S rRNA gene sequence analysis, conducted as described by Abraham *et al.* (1999), showed that the six OTU H strains were very similar, with sequence similarities

exceeding 99.8 %, and that they formed a distinct lineage within the genus *Brevundimonas* (Fig. 1). The closest recognized relatives of strain V4.BO.10^T were the unpigmented prosthecate strain *Brevundimonas intermedia* ATCC 15262^T and the red-pigmented *Brevundimonas vesicularis* LMG 2350^T, with respective sequence similarities of 99.4 and 99.2 %. Slightly more distantly related were *Brevundimonas aurantiaca* DSM 4731^T (98.9 %) and the recently discovered *Brevundimonas nasdae* GTC 1043^T (99.0 %) (Li *et al.*, 2004). To provide further detail of the taxonomic relations within the genus *Brevundimonas*, the 16S rRNA gene sequences of three hitherto phylogenetically unaffiliated *Brevundimonas* strains, LMG 9564, LMG 9567t1 and LMG 11070 (Segers *et al.*, 1994), were determined. Among these strains, the unpigmented *Brevundimonas* sp. LMG 11070 appeared to be a close relative of OTU H, whereas the red-pigmented strains LMG 9564 and LMG 9567t1 showed close phylogenetic relationships to *B. aurantiaca* and *B. vesicularis*, respectively. In addition, strain LMG 19834, characterized by Mergaert *et al.* (2001), showed a close relationship to OTU H. Thus OTU H, LMG 11070 and LMG 19834 formed a monophyletic cluster within the genus *Brevundimonas*. This topology was supported by maximum parsimony and maximum likelihood and in 93 % of the neighbour-joining bootstrap calculations. Within this cluster, strain V4.BO.18 formed a subcluster together with strains LMG 11070 and LMG 19834, due to a 3 nt exchange (i.e. 0.2 %) common to these three strains in the 1416 bp sequence investigated.

Fig. 1. Similarity dendrogram based on the comparison of 16S rRNA gene sequences. The dendrogram was calculated with the method of Fitch & Margoliash (1967) after distance correction as described by Jukes & Cantor (1969) and as implemented in the ARB (MacOSX port) and PHYLIP program packages (Felsenstein, 1989; Hines & Dyall-Smith, 2003; Ludwig *et al.*, 2004), respectively. To compensate for multiple nucleotide substitutions, only columns at which all nucleotides were unambiguously determined and one nucleotide had an abundance of 50 % or more were included in the calculation. A nucleotide sequence stretch spanning 21 nt between the *Escherichia coli* homologous positions 1256 and 1278, which is present in the genus *Caulobacter* but is missing in the genus *Brevundimonas* (Abraham *et al.*, 1999; Anzai *et al.*, 2000; Fritz, 2000), was been included in the calculation. Additional dendrograms calculated with maximum-likelihood and neighbour-joining bootstrap analyses (1000 bootstraps) proved the robustness of the presented overall tree topology.

Fig. 2. Similarity dendrograms from protein fingerprints (a), LMW-RNA profiles (b) and RAPD-PCR fingerprints (c). Starting from a digital file of the scanned gel, bands were manually assigned using the program RFLPSCAN 3.0 (Scanalytics). After applying appropriate band tolerances to correct for imperfections resulting from the electrophoresis process in individual lanes, a binary representation of presence/absence patterns of matched gel bands was constructed. Further evaluation was by use of the program packages TREECON 1.15 (Van de Peer & De Wachter, 1994) and PHYLIP 3.6 (Felsenstein, 2004). A distance matrix was calculated according to the formula $D_{xy} = (N_x + N_y) / (N_x + N_y + N_{xy})$ (Link *et al.*, 1995), where D_{xy} is the distance between two pairwise-compared lanes x and y , N_x is the number of bands present in lane x but not in lane y , N_y is the number of bands present in y but not in x and N_{xy} is the number of bands present in both lanes. A total of 1000 bootstraps was calculated. The tree construction method was UPGMA. Only bootstrap values above than 90 % are shown. Abbreviations: *B. aur.*, *Brevundimonas aurantiaca*; *B. dim.*, *Brevundimonas diminuta*; *B. int.*, *Brevundimonas intermedia*; *B. ves.*, *Brevundimonas vesicularis*.

Fingerprinting

Fingerprinting data targeted at the protein, RNA and genomic DNA levels supported the suggestion that OTU H was a homogeneous taxonomic entity. As shown in Fig. 2, UPGMA clustering of binary presence/absence patterns based on matched gel bands reliably distinguished OTU H strains from their closest recognized relatives using all investigated fingerprint techniques.

PAGE of whole-cell proteins revealed that all OTU H strains with the exception of V4.BO.18 possessed indistinguishable protein profiles (Fig. 2a; Supplementary Fig. B). The profiles of V4.BO.18 and LMG 11070 differed slightly from the other protein profiles of OTU H in the region between 75 and 100 kDa. By contrast, the protein profiles from *B. aurantiaca* DSM 4731^T, *B. vesicularis* LMG 2350^T and *B. intermedia* ATCC 15262^T were more heterogeneous and appeared clearly different from those of the OTU H strains.

As shown by Fritz (2000), all six OTU H strains possessed identical low-molecular-mass RNA (LMW-RNA) profiles, which differed at least in the size of one distinct band to all recognized *Brevundimonas* species. Höfle (1998) indicated that identical LMW-RNA profiles point to a degree of relatedness at the species level. Indeed, the type strains of *B. vesicularis*, *B. intermedia* and *B. aurantiaca* shared an identical LMW-RNA profile [Fig. 2b; see scanned gel in Fritz (2000), p. 126], but were not related at the species level, as shown by DNA–DNA hybridization data (Table 1) and various other approaches (Abraham *et al.*, 1999; Li *et al.*, 2004). This demonstrates that LMW-RNA profiles can be

used to differentiate *Brevundimonas* strains above species but below the genus level. Consequently, LMW-RNA profile analysis supports the view of an affiliation of OTU H within a distinct *Brevundimonas* species (Fritz, 2000). The LMW-RNA profile of strain LMG 11070 differed from that of the OTU H strains.

Randomly amplified polymorphic DNA (RAPD) fingerprints, targeted at the clonal structure below species level, were conducted as described by Ziemke *et al.* (1997). The monophyly of OTU H was supported in 95.8 % of 1000 bootstrap analyses (Fig. 2c; Supplementary Fig. C). However, the RAPD fingerprints of OTU H were similar but not identical. Instead, OTU H could be differentiated into three RAPD fingerprint types. Type 1 consisted of three strains

Table 1. Genomic DNA–DNA hybridization values (%) for type strains from selected *Brevundimonas* species

Strain	1	2	3	4
1. <i>B. vesicularis</i> LMG 2350 ^T	100			
2. <i>B. aurantiaca</i> DSM 4731 ^T	35	100		
3. V4.BO.10 ^T	33	33	100	
4. <i>B. intermedia</i> ATCC 15262 ^T	ND*	33†	27	100†

*A DNA–DNA hybridization value ('stringent conditions') of 34.4 % was reported by Li *et al.* (2004). The other hybridization values measured by Li *et al.* (2004) under stringent conditions are in good agreement with the corresponding hybridization values determined in our study.

†Data taken from Abraham *et al.* (1999).

isolated from the oligotrophic station, strains V4.BO.10^T, V4.BO.22 and V4.BO.27, but not strain V4.BO.18. Type 2 consisted of both strains from the eutrophic station, V4.BE.49 and V4.BE.56. V4.BO.18 had a unique RAPD fingerprint type. Thus OTU H consisted of three clonal lineages. Strain LMG 11070 could not be affiliated with any of the three fingerprint types.

G+C content, genome size and DNA–DNA hybridization

The DNA G+C contents of OTU H ranged between 67.1 and 67.5 mol% (Supplementary Table A). This is slightly higher than the G+C contents measured for *B. intermedia* ATCC 15262^T (66.1 mol%), *B. vesicularis* LMG 2350^T (66.2 mol%) and *B. aurantiaca* DSM 4731^T (65.6 mol%). For strain LMG 11070, Segers *et al.* (1994) determined a G+C content of 67.0 mol%. The genome size of strain V4.BO.10^T, as determined by the method of Gillis *et al.* (1970), was 2.4×10^9 Da. Similar genome sizes were determined for *B. intermedia* ATCC 15262^T (2.2×10^9 Da), *B. vesicularis* LMG 2350^T (2.3×10^9 Da) and *B. aurantiaca* DSM 4731^T (2.4×10^9 Da). DNA–DNA hybridization data are shown in Table 1. For strain V4.BO.10^T, no DNA relatedness exceeding 33% was measured, which is well below the generally accepted value of 70% for intraspecies strains (Wayne *et al.*, 1987).

Lipid analysis

For OTU H, fatty acids have been reported to be similar to those of *B. intermedia* and the polar lipids were phosphatidyl diacylglycerol (720, 734, 746, 748, 760, 762, 774 and 788 Da), sulfoquinovosyl diacylglycerol (820 and 834 Da) and phosphatidyl glucopyranosyl diacylglycerol (PGL; 1411, 1413, 1425, 1427, 1439, 1453 Da) (Abraham *et al.*, 1997). The cellular fatty acids of strain V4.BO.10^T comprised C_{14:0} (0.6%), C_{15:0} (5.4%), 3-OH-C_{12:0} (2.0%), C_{16:0} (16.1%), C_{17:0} (6.1%), C_{18:0} (0.9%), C_{16:1} (8.4%), C_{17:1ω6c} (7.8%), C_{17:1ω8c} (9.9%), C_{18:1} (36.7%), 11-Me-C_{18:1ω5t} (4.2%) and ECL 17.897 (1.6%), similar to those of *Brevundimonas* sp. LMG 11070, and with larger amounts of C_{15:0} and C_{17:1ω8c} and smaller amounts of C_{18:1} compared with *B. intermedia* and *B. vesicularis* (Abraham *et al.*, 1997). OTU H differed from the recently described *B. nasdae* (Li *et al.*, 2004) by the presence of considerable amounts of C_{15:0}, larger amounts of summed feature 4 fatty acids, the presence of fatty acid ECL 17.897, larger amounts of 11-Me-C_{18:1ω5t} and summed feature 7 fatty acids and the lack of C_{19:0} cyclo ω8c.

Substrate utilization

Good growth of the OTU H strains was obtained at 30 °C and NaCl concentrations between 1 and 3% (w/v). Further details are given in the species description. Strain LMG 11070 did not tolerate NaCl concentrations above 1% (w/v). No nitrogen fixation was detected, as tested by growth

experiments in Burk's nitrogen-free medium (Smibert & Krieg, 1981), which was adjusted to an NaCl concentration of 3% (w/v).

The results from substrate specificity tests, as determined by API galleries (bioMérieux) and from Biolog substrate utilization tests, are given in detail in the species description. Criteria for differentiation on the basis of substrate utilization patterns between the OTU H strains as well as closely related *Brevundimonas* species and isolates are given in Table 2.

As revealed by API ZYM tests, the OTU H strains exhibited similar enzyme activity patterns (see Table 3 and the species description). The strains could be differentiated from *B. vesicularis* by the presence of β-glucosidase activity and from *B. intermedia* by the lack of chymotrypsin and β-galactosidase activity.

Conclusions and discussion

The data presented here suggest a close affiliation of the genetically and phenetically similar six OTU H strains at the species level. As revealed by 16S rRNA gene sequence data, RAPD and protein fingerprints, strain V4.BO.18 differed slightly from all other OTU H strains, but phenotypic data did not permit a clear-cut distinction of this strain above the species level. Consequently, we propose to affiliate strain V4.BO.18 with the remaining OTU H strains at the species level. The taxonomic status of strain LMG 11070, however, remains unclear. Strain LMG 11070 is similar to OTU H with respect to protein fingerprints, cellular lipid content and 16S rRNA gene sequences. However, LMG 11070 differs from the OTU H strains in a number of phenotypic traits, including LMW-RNA profiles, the lack of tolerance of NaCl concentrations above 1% and substrate utilization patterns (Table 2). Further investigations are required to clarify the taxonomic status of this strain. With regard to strain LMG 19834, Mergaert *et al.* (2001) reported its inability to reduce nitrate to N₂ and that, in contrast to the OTU H strains, LMG 19834 formed stalks. As we did not investigate this strain in detail, we cannot determine whether strain LMG 19834 and OTU H are related at the species level.

Although generally regarded as non-marine bacteria, reports on *Brevundimonas* strains isolated from marine habitats have accumulated (Stahl *et al.*, 1992; Yokoyama *et al.*, 1996; Abraham *et al.*, 2002). Two marine *Brevundimonas* sp. strains, MCS 17 and MCS 24 (Stahl *et al.*, 1992; Abraham *et al.*, 2002), for which 16S rRNA gene sequence data have been published, showed relatively distant phylogenetic relationships (96.1–96.7% similarity) with OTU H (Abraham *et al.*, 1999) (Fig. 2). During sampling in the Mediterranean Sea, from a total of 227 isolated strains, three further *Brevundimonas* strains were isolated (Fritz, 2000). The red-pigmented strains V4.BO.05 and V4.BO.07 (designated OTU H' in Fritz, 2000) were closely related to *B. vesicularis*, whereas the brown-pigmented strain V4.BP.05 (designated

Table 2. Differentiation of selected *Brevundimonas* species and strains according to substrate utilization patterns and pigmentation

Strains were cultivated in a mineral salts medium with addition of trace elements and vitamins, but without any carbon and energy source. Substrate utilization patterns were determined with the API 20NE, API 50CH and API Biotype 100 galleries (bioMérieux). Data from Biolog analyses are not included. +, Detected in all strains; –, not detected in any strain; + –, detected in some strains, including the type strain; – +, detected in some strains, excluding the type strain; ND, not determined. Data for *B. vesicularis* and strain LMG 11070 were taken from Segers *et al.* (1994).

Characteristic	OTU H	LMG 11070	<i>B. intermedia</i>	<i>B. vesicularis</i>
Pigmentation	–	–	–	+ –
D-Galactose	– +	+	+	+
β -Hydroxybutyrate	+ –	ND	+	+
Fumarate	+ –	ND	+	+ –
L(–)-Malate	+ –	ND	+	+ –
Maltotriose	+ –	ND	+	ND
L-Proline	+ –	ND	+	+
Cellobiose	+	ND	+	–
Salicin	+	+	+	–
Rhamnose	– +	ND	–	– +
L-Serine	– +	ND	–	– +
Adipate	– +	–	+	–
L-Alanine	– +	–	+	–
D-Fructose	+	–	+	–
Gentiobiose	+	–	+	–
Propionate	+	–	+	–
Arbutin	+	+	–	–
Amygdalin	+	–	–	–
L-Arabinose	+ –	–	–	–
Trehalose	+	–	–	–
Turanose	+ –	–	–	–
Caprate	–	+	–	–
D-Arabinose	–	–	+	–
Lactose	–	–	+	–
D-Mannose	–	–	+	–
D-Xylose	–	–	+	–
L-Aspartate	–	ND	+	– +
Galacturonate	–	ND	+	–
D-Glucosamine	–	ND	+	–
Lactulose	–	ND	+	–
Melibiose	–	ND	+	–
Methyl β -glucopyranoside	–	ND	+	–
D-Alanine	–	ND	–	– +

OTU H* in Fritz, 2000) was similar but not identical to *Brevundimonas subvibrioides*. However, as revealed by plate counts, no abundance of colony-forming units with similar morphologies to these strains was detected in any of the water samples investigated by Fritz (2000). Analyses of a clone library from excised DGGE bands derived from RNA extracts from the Mediterranean water samples revealed the presence of a clone with a partial 16S rRNA gene sequence similar to *Brevundimonas alba* (I. Fritz, unpublished, accession number AJ508416), suggesting an abundance of further *Brevundimonas* species in marine water samples.

Based on the data presented, we propose that the six OTU H strains cannot be affiliated within any recognized *Brevundimonas* species. The closest recognized relative of the OTU H strains is *B. intermedia*, which differs on the basis of morphology, genome size, presence of chymotrypsin and β -galactosidase activity and absence of PGLs of 1411 Da (Abraham *et al.*, 1997). The OTU H strains differ from *B. vesicularis* by having thicker cells, the presence of β -glucosidase, smaller amounts of C_{15:0}, C_{16:1} and C_{17:1}ω8, but larger amounts of C_{18:1}, the presence of phosphatidylglycerols of 720, 734, 746, 760 and 788 Da

Table 3. Enzyme activity patterns of selected *Brevundimonas* species and strains

w, Weak activity; otherwise, enzyme activities are scored as in Table 2. Data for strain LMG 11070 and *B. vesicularis* were taken from Segers *et al.* (1994).

Enzyme	OTU H	LMG 11070	<i>B. intermedia</i>	<i>B. vesicularis</i>
α -Glucosidase	w*	+	+	+
β -Glucosidase	+	+	+	—
Naphthol-AS-BI-phosphohydrolase	+	ND	+	ND
Valine arylamidase	+	ND	+	+
Chymotrypsin	—	ND	+	—
β -Galactosidase	—	—	+	—

*Negative for strain V4.BO.18.

and the presence of PGL of 1411 Da. OTU H can be distinguished from the recently described *B. nasdae* GTC 1043^T (Li *et al.*, 2004) by the presence of C_{15:0} and ECL 17.897, larger amounts of 11-Me-C_{18:1} ω 5t and the lack of C_{19:0} cyclo ω 8c in the cellular fatty acids, a higher G + C content, the ability to grow at NaCl concentrations of 4% (w/v), the ability to utilize fructose but not succinate and *n*-capric acid as well as the presence of urease activity in some strains. Furthermore, the OTU H strains can be differentiated from their closest recognized relatives by DNA–DNA hybridization, 16S rRNA gene sequencing, LMW-RNA profile analysis, protein fingerprints and substrate utilization patterns. Therefore, we propose to include the six OTU H strains within a novel species, *Brevundimonas mediterranea* sp. nov., with V4.BO.10^T as the type strain.

Description of *Brevundimonas mediterranea* sp. nov.

Brevundimonas mediterranea (me.di.ter.ra'ne.a. N.L. fem. adj. *mediterranea* of the Mediterranean Sea).

The description is as that of the genus with the following additions. Colonies measure 2–5 mm in diameter on MB agar, have a soft and slimy consistency with a glistening surface and smooth margin, a slightly concave shape and are cream-white coloured with a characteristic slightly brownish spot in the centre. Pigmentation is not present. Flexible, non-stalked, occasionally bent rods with a length of 1.5–4 μ m and a diameter of 0.5–1.0 μ m. Some strains but not the type strain may possess a branching cell morphology.

Alkaline and acid phosphatase, esterase (C4), esterase lipase (C8), β - and weak α -glucosidase, leucine arylamidase, valine arylamidase and trypsin activities are always positive. Activities for the following enzymes are not detected: chymotrypsin, cystine arylamidase, α -fucosidase, α -galactosidase, β -galactosidase, β -glucuronidase, lipase (C14), α -mannosidase and *N*-acetylglucosaminidase. Urease activity is present in some strains but not the type strain. Gelatin is not liquefied. Aesculin is hydrolysed. As determined by API galleries, the following substrates are utilized by all strains: amygdalin, arbutin, cellobiose, D-fructose, gentiobiose, α -D-glucose,

β -hydroxybutyric acid, maltose, salicin and starch. The following substrates are used as sole carbon and energy sources by some strains including the type strain: L-arabinose, fumarate, L-glutamate, DL-lactate, L(–)-malate, maltotriose, L-proline, propionate and turanose. The following substrates were utilized by some strains but not by the type strain: adipate, L-alanine, rhamnose and L-serine. The following substrates are not utilized: D(+)–malate, malonate, maltitol, mannitol, D-mannose, melezitose, melibiose, methyl α -D-glucopyranoside, methyl α -galactopyranoside, methyl β -galactopyranoside, methyl β -glucopyranoside, methyl α -D-mannoside, methyl β -xyloside, mucate, palatinose, phenylacetate, 3-phenylpropionate, protocatechuate, putrescine, (–)-quinic acid, D-raffinose, D(–)-ribose, D-saccharate, D-sorbitol, L-sorbose, D-tagatose, D(–)-tartrate, L(+)–tartrate, meso-tartrate, trehalose, tricarballoylate, trigonelline, tryptamine, tyrosine, L-xylitol, xylitol, D-xylose and L-xylose.

As measured by Biolog tests, Tween 40 and α -cyclodextrin serve as electron donors for the artificial electron acceptor tetrazolium violet and thus may be metabolized by all strains. Some strains including the type strain use acetic acid, alaninamide, γ -aminobutyric acid, 2-aminoethanol, L-asparagine, 2,3-butanediol, dextrin, D-galactonic acid lactone, glycyl L-aspartic acid, glycyl L-glutamic acid, hydroxy-L-proline, methylpyruvate, monomethyl succinate, L-ornithine, L-pyroglutamic acid, sebacic acid, L-threonine and Tween 80 as electron donors. L-Alanyl glycine is utilized by some strains but not by the type strain. None of the following are used as electron donors by any strain: *N*-acetyl-D-galactosamine, bromosuccinic acid, DL-carnitine, formic acid, D-glucosaminic acid, glucose 1-phosphate, glucose 6-phosphate, glucuronamide, DL- α -glycerol phosphate, α -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, inosine, α -ketobutyric acid, α -ketoglutaric acid, α -ketovaleric acid, L-leucine, phenylethylamine, L-phenylalanine, D-psicose, D-serine, succinamic acid, succinic acid, sucrose, thymidine, uridine and urocanic acid.

NaCl is not strictly required but promotes growth at

optimal concentrations. The optimum NaCl concentration for growth is 1 % (w/v), with good growth at 0–3 % and no growth at 6 % or above. Optimal growth temperature is 30 °C. The species grows between 15 and 37 °C, but not above 42 °C. Some strains including the type strain show weak growth at 7 °C. Nitrogen is not fixed. Neither nitrate nor nitrite is utilized as a terminal electron acceptor with the exception of strain V4.BO.18, which is capable of reducing nitrate to nitrite but not to nitrogen. Polar lipids are 18:1/14:0-, 18:1/15:0-, 18:1/16:1-, 18:1/16:0-, 18:1/17:1-, 18:1/18:1-, 19:1/16:0- and 19:1/18:1-PG, 18:1/17:0-SQDG and PGLs of 1411, 1413, 1425, 1427, 1439 and 1453 Da. The main fatty acids of the cellular hydrolysate are C_{18:1}, C_{16:0}, C_{16:1}, C_{15:0}, C_{17:1}ω8c, C_{17:1}ω6c, C_{17:0} and 11-Me-C_{18:1}ω5t; the main hydroxy-fatty acid is 3-OH-C_{12:0}. The G+C content is between 67.1 and 67.5 mol%. Main ubiquinone is Q-10 (Abraham *et al.*, 1999). DNA–DNA relatedness between strain V4.BO.10^T and *B. intermedia* ATCC 15262^T, *B. vesicularis* LMG 2350^T and *B. aurantiaca* DSM 4731^T is 27, 33 and 33 %, respectively.

Strain V4.BO.10^T (=LMG 21911^T=CIP 107934^T) is the type strain. The strains were isolated from water samples from the western Mediterranean Sea near Nice.

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