Marinomonas brasilensis sp. nov. isolated from the coral *Mussismilia hispida* and reclassification of *Marinomonas basaltis* as a later synonym of *Marinomonas communis*

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Abstract

A Gram-negative, aerobic bacterium, designated R-40503^T was isolated from mucus of the reef builder coral, *Mussismilia hispida,* located in the São Sebastião Channel, São Paulo, Brazil. Phylogenetic analyses revealed that strain R-40503^T belongs to the genus *Marinomonas*. The 16S rRNA gene sequence similarity of R-40503^T was above 97 % with the type strains of *Marinomonas vaga*, *M. basaltis*, *M. communis* and *M. pontica*, and below 97 % with all other the *Marinomonas* type strains. Strain R-40503^T showed less than 35 % DNA-DNA hybridization (DDH) similarity with the type strains of the phylogenetically closest *Marinomonas* species, demonstrating it should be classified into a novel species. Amplified Fragment Length Polymorphism (AFLP), chemotaxonomic and phenotypic analyses provided further evidence for the classification of the new species. Concurrently, a close genomic relationship between *M. basaltis* and *M. communis* was observed. The type strains of these two species showed 78 % DDH similarity and 63 % AFLP pattern similarity. Their phenotypic features were very similar, and their DNA G+C content was identical (46.3 mol%). Collectively, these data demonstrates unambiguously the synonymy of *Marinomonas basaltis* and *Marinomonas communis.* Several phenotypic features can be used to discriminate *Marinomonas* species. The novel strain R-40503^T is clearly distinguishable species from its neighbours. For instance, it shows oxidase and urease activity, utilizes L-asparagine, has the fatty acid C12:1 3-OH (but lacks C10:0 and C12:0). The name *Marinomonas brasilensis* sp. nov. is proposed (type strain is R-40503^T = LMG 25434^T = CAIM 1459^T). The DNA G+C content of the type strain R-40503^T is 46.5 mol%.

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67 *Mussismilia hispida* is one of the major reef-builders corals along the
68 northeastern Brazilian coast, and it also has the widest geographic distribution
69 among its genus (from Maranhão to Santa Catarina states, ca. 5000 km) (Leão
70 & Kikuchi, 2005). The ability of *Mussismilia* to survive in different regions
71 indicates its adaptation to wide environmental gradients, such as temperature,
72 water turbidity and pollution. However, recent studies have revealed that *M.*
73 *hispida* and *M. braziliensis* are threatened by extinction (Castro *et al*., 2010;
74 Francini-Filho *et al.*, 2008). Microorganisms appear to play a key role in coral
75 health. Microorganisms and the coral compose the holobiont (Rosenberg *et al.,*
76 2007). The holobiont microbiota appears to protect its host by providing
77 nourishment and antibiotics (Raina *et al.,* 2009; Shnit-Orland & Kushmaro,
78 2009). It is also recognized that the holobiont harbours a vast microbial
79 diversity. In the last 10 years a growing number of studies have focused on the
80 characterization of the coral microbiota diversity and ecology (Alves *et al*., 2009;
81 Dinsdale *et al*. 2008; Rohwer *et al*. 2001).

82

83 The genus *Marinomonas* was created in 1983 to accommodate *Alteromonas*
84 *communis* and *Alteromonas vaga* (Baumann *et al*., 1972), which were distinct
85 from the other species of *Alteromonas* (van Landschoot & De Ley, 1983). The
86 genus *Marinomonas* comprises 15 species, mainly originating from sea-water of
87 different geographical locations. *M. communis* and *M. vaga* (Baumann *et al.,*
88 1972; van Landschoot & De Ley, 1983), were isolated from the Pacific Ocean,
89 *M. pontica* (Ivanova *et al.,* 2005) from the Black Sea, *M. dokdonensis* (Yoon *et*
90 *al.,* 2005) from the East Sea of Korea, and *M. mediterranea* (Solano &

Sanchez-Amat, 1999) and *M. aquimarina* (Macián *et al.,* 2005) from the Mediterranean Sea. *M. polaris* (Gupta *et al.,* 2006) and *M. ushuaiensis* (Prabagaran *et al.,* 2005) were isolated from the subantarctic region, while *M. primoryensis* (Romanenko *et al.,* 2003) and *M. arctica* (Zhang *et al*., 2008) were isolated from sea-ice. *M. ostreistagni* (Lau *et al.,* 2006) and some *M. aquimarina* strains (Macián *et al.,* 2005) were isolated from oysters. *M. basaltis* (Chang *et al*., 2008) and *M. arenicola* (Romanenko *et al*., 2009) were isolated from marine sediment, while *M. balearica* and *M. pollencensis* (Espinosa *et al*., 2009) were isolated from seagrass *Posidonia oceanica.*

In the present study, one isolate (R-40503^T), obtained from mucus of apparently healthy coral *Mussismilia hispida,* located in the rocky shore of Grande beach (coordinate 23°50´25´´S; 045°24´59´´W) in São Sebastião Channel, São Paulo, Brazil, in the summer of 2005, during a survey of the heterotrophic bacterial diversity associated with cnidarians in São Paulo (Brazil) (Chimetto *et al*., 2008, 2009), was investigated using a polyphasic taxonomic approach. The strain was isolated using the nitrogen-free (NFb) selective medium supplemented with 3 % NaCl after 4 days of incubation at 28 °C.

Five strains (R-236, R-237, R-249, R-256, and R-278) isolated at the time of collection as described in Chimetto *et al*. (2008) clustered together in this new taxa by 16S rRNA gene sequences, but only one strain (R-278 = R-40503^T) maintained viability. The 16S rRNA gene sequence of R-40503^T (1425 nt), accession number GU929940, was obtained as described previously (Chimetto *et al*., 2008, 2009). The raw sequence data were transferred to the ChromasPro ver. 1.34 software (Technelysium Pty. Ltd, Tewantin, Australia) where

consensus sequences were determined. The sequence was aligned with sequences from EMBL using the ClustalW software (Chenna *et al*., 2003). Pairwise similarities were calculated with the BioNumerics 4.61 software (Applied Maths, Sint-Martiens-Latem Belgium), using an open gap penalty of 100 % and a unit gap penalty of 0 %. Similarity matrices and phylogenetic trees were constructed using the MEGA ver. 4.0 (Tamura *et al*., 2007) and the BioNumerics 4.61 software (Applied Maths, Belgium). Trees were drawn using the Neighbour-Joining (Saitou & Nei, 1987) and Maximum Parsimony methods (Eck & Dayhoff, 1966). The robustness of the topologies of the trees were checked by bootstrap replications (Felsenstein, 1985). The gene sequence data obtained in this study are also available through our website TAXVIBRIO (http://www.taxvibrio.lncc.br/).

The novel strain R-40503^T was closely related to *M. vaga,* with 97.9% 16S rRNA gene sequence similarity. R-40503T had 97.2% 16S rRNA gene sequence similarity towards *M. basaltis, M. communis, M. aquimarina* (**Fig. 1 and Supplementary Figure S2)**. DNA-DNA hybridizations were performed between the novel strain R-40503^T and the type strains of the closest phylogenetic neighbours, i.e. *M. vaga*, *M. basaltis, M. communis* and *M. aquimarina* (**Table 1**), using the microplate method described by Ezaki *et al*. (1989) with minor modifications (Willems *et al*., 2001). Hybridizations were performed at 40.7 °C in the presence of 50 % formamide. Reciprocal reactions were performed for every DNA pair and their variation was within the limits of this method (Goris *et al.,* 1998). The DDH relatedness between R-40503^T and the tested type strains was below 70 % (Table 1). The DDH demonstrated that it

142 represents a novel species in the genus *Marinomonas* (Wayne *et al*., 1987;
143 Stackebrandt & Ebers, 2006). The DDH relatedness between *Marinomonas*
144 *basaltis* LMG 25279^T and *Marinomonas communis* LMG 2864^T was above 70 %
145 (i.e. 78 %), which suggests that these species are synonymous. Chang *et al*.
146 (2008) obtained 56.2 % DDH similarity between the same pair of type strains,
147 but the additional data of the present study (see below) support the value of 78
148 %. The authenticity of *M. basaltis* LMG 25279^T (GU929941) and *M. communis*
149 LMG 2864^T used in this study were verified by means of their 16S rRNA
150 sequences**.** The sequences of both type strains (1501 nt for LMG 25279^T and
151 1499 nt for LMG 2864^T showed 100 % similarity with those deposited in the
152 GenBank *M. basaltis* J63^T (EU143359) and *M. communis* LMG 2864^T
153 (DQ011528) respectively, indicating the authenticity of the LMG strains **(Figure**
154 **1)**. The 16S rRNA gene sequence similarity between *M. basaltis* LMG 25279^T
155 and *M. communis* LMG 2864^T was 98.7 %. Giving further support for the
156 synonymy between, *Marinomonas basaltis* LMG 25279^T and *Marinomonas*
157 *communis* LMG 2864^T had identical GC contents and related AFLP patterns.
158 DNA G+C contents were determined for R-40503^T, *M. basaltis* LMG 25279^T and
159 *M. communis* LMG 2864^T by HPLC as described previously (Mesbah *et al*.,
160 1989). The DNA G+C content of strain R-40503^T was 46.5 mol% (**Table 1**) and
161 46.3 mol% of the LMG strains.
162
163 AFLP analysis was performed for strain R-40503^T, *M. basaltis* LMG 25279^T, *M.*
164 *communis* LMG 2864^T, *M. vaga* LMG 2845^T and three *M. aquimarina* strains
165 (**Supplementary Figure S1**), as reported by Beaz Hidalgo *et al*. (2008) and
166 Thompson *et al.* (2001). Briefly, 1 µg of DNA was digested with *Taq*I (5'TCGA3')

and *Hind*III (5'AAGCTT3') (Amersham Pharmacia Biotech, Sweden), and subsequently ligated with double-stranded adaptors complementary to the ends of the restriction fragments, with T4 ligase (Amersham Pharmacia Biotech), to generate template DNA for PCR amplification. A selective PCR was then performed with the primers H01-6FAM (5'GACTGCGTACCAGCTTA3', labeled at the 5' end with the fluorescent dye 6-FAM) and T13 (5'GTTTCTTATGAGTCCTGACCGAG3'), using the conditions described by Thompson *et al*. (2001), in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, USA). Separation of the selective PCR products was performed using a capillary ABI Prism 3130XL DNA sequencer (Applied Biosystems). The level of reproducibility was controlled by generating the AFLP pattern of *Marinomonas brasilensis sp*. nov. R-40305^T three times, starting from different subcultures. Normalization of the resulting electrophoretic patterns was performed using the Gene Mapper 4.0 software (Applera Co., Norwalk, CT). For subsequent analysis fragments of 20 to 600 base pairs were transferred into the BioNumerics™ 4.61 software (Applied Maths, Belgium). For numerical analysis, the zone from 40- and 580-bp was used. Similarity values were calculated using the Dice coefficient (tolerance value of 0.15 %), and a dendrogram was constructed using the UPGMA algorithm. The similarity between the patterns of R-40503^T ranged from 93.0 to 94.4 %. The similarity level chosen to delineate the AFLP clusters was 63 %, as previously proposed by Beaz Hidalgo *et al*. (2008). Strains with AFLP profiles showing more than 63 % similarity can be considered as members of the same species. The AFLP data supported the DDH data obtained in this study. R-40503^T showed at most 46 % pairwise band pattern similarity with its closest phylogenetic neighbours, being below the cut-

192 off similarity level of 63 %, while the type strains of *M. basaltis* and *M.*
193 *communis* constituted a distinguishable cluster with 69 % mutual AFLP pattern
194 similarity **(Figure S1)**. AFLP has been reported as a widely applicable technique
195 with high discriminatory power and reproducibility (Janssen *et al*., 1996;
196 Savelkoul *et al*., 1999). It was proven to be useful for discrimination at the
197 species and intraspecies levels for *Aeromonas*, *Acinetobacter*, *Campylobacter*,
198 *Xanthomonas* (Savelkoul *et al*., 1999), *Vibrionaceae* (Thompson *et al*., 2001),
199 *Bradyrhizobium* (Willems *et al*., 2001), *Arcobacter* (On *et al*., 2003) and *Pantoea*
200 (Brady *et al*., 2007). The present study provides enough evidence to consider
201 *M. basaltis* (Chang *et al*., 2008) a later synonym of *M. communis* (Baumann *et*
202 *al.,* 1972; van Landschoot & De Ley (1983).
203
204 Phenotypic characteristics were determined inj order to demonstrate that the
205 novel strain R-40503^T belongs to a new species. Phenotypic analysis of the
206 novel strains and the type strains of the closest phylogenetic *Marinomonas*
207 species i.e. *M. vaga*, *M. basaltis, M. communis* and *M. aquimarina.* Analysis of
208 fatty acid methyl esters was carried out as described by Huys *et al.* (1994).
209 Cells for fatty acid analysis were grown on MA (Difco) for 24 h at 28 °C under
210 aerobic conditions. Phenotypic characterization was performed using the API
211 ZYM, API 20E and API 20NE kits (bioMérieux, France), and the Biolog GN2
212 microwell plates (Biolog Inc., USA), according to the manufacturer's instructions
213 with minor modifications. Cell suspensions for inoculation of the API tests were
214 prepared in a 3 % (w/v) NaCl solution, and those for the Biolog GN2 microwell
215 plates showed turbidity (transmission) of 20 %. Cells for the suspensions were
216 grown on Biolog medium for 24 h at 28 °C under aerobic conditions. The results
217 of the tests were recorded after 24 to 48 h of incubation at 28 °C. Growth at

different temperatures (4–42 °C) was determined by incubation on TSA (Difco) for 72 h. Growth at different salt concentrations (0–14 % NaCl) was determined by incubation on TSA (Difco) at 28 °C for 72h. Catalase activity was determined by adding young cells to a drop of a 3 % H_2O_2 solution and observation of 0_2 production. Oxidase activity was tested using 1% N,N,N',N'-tetramethyl *p*-phenylenediamine (Kovacs, 1956).

The novel strain R-40503^T species was differentiated from its closest phylogenetic neighbours by several phenotypic features (**Table 2**). It grew in medium containing 13 % NaCl, used tween 80, sucrose and L-asparagine but not α-ketoglutaric acid, L-aspartic acid, L-serine, L-ornithine and bromo succinic acid. It had oxidase activity, and was not able to grow at 40 ˚C (**Table 2**). This novel strain could be differentiated from its neighbours on the basis of the presence of the fatty acids $C_{12:1}$ 3-OH and the absence of the fatty acids $C_{10:0}$ and $C_{12:0.}$ The major cellular fatty acids of R-40503^T were $C_{18:1}$ ω7*c* (48.8 %), summed feature 3 ($C_{15:0}$ iso 2-OH and/or $C_{16:1}$ ω7*c*) (19 %), $C_{16:0}$ (10.5 %) and $C_{10:0}$ 3-OH (8 %) (**Supplementary Table S1**). Phenotypic features of *M. basaltis* and *M. communis* were very similar, except for some features, namely *M. communis* utilized saccharose, D-fructose, succinamic acid, urocanic acid and putrescine and had urease activity, whereas *M. basaltis* did not. Some results of the phenotype of *M. basaltis* obtained in this study are in contrast with those reported by Chang *et al*. (2008). They reported no growth in less than 1 % or more than 7 % NaCl, no esterase (C4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase activities, but activities for trypsin and N-acetyl-β-glucosaminidase, and assimilation of L-arabinose, L-aspartic acid and glycerol.

However, in this study, growth was observed at 0.5 – 11 % NaCl, as well as activities for esterase (C 4), esterase lipase (C8) and naphthol-AS-BI-phosphohydrolase. Trypsin and N-acetyl-β-glucosaminidase activities, and assimilation of L-arabinose, L-aspartic acid and glycerol were not observed. In our hands, no significant phenotypic or genotypic differences were found between *M. communis* and *M. basaltis*.

Based on the phylogenetic, genomic and phenotypic data, the new species *M. brasilensis* sp. nov. is proposed to encompass the strain R-40503^T (= LMG 25434^T = CAIM 1459^T).

Description of *Marinomonas brasilensis* sp. nov.

Marinomonas brasilensis (bra.si.len'sis. N.L. fem. adj. *brasilensis* of or belonging to Brazil).

Cells are Gram-negative, aerobic, halophilic, motile, straight rods approximately 1 µm wide and 1.5–3 µm long. Catalase- and oxidase- positive. Colonies on MA are circular, undulate, convex, smooth, beige in colour and 1 mm in size after 1 day of incubation at 28 °C. Prolific growth occurs between 20 and 35 °C and at NaCl concentrations (w/v) ranging from 1 to 11 %. No growth is observed in 0 % NaCl or in ≥ 14 % NaCl, and at ≤ 7 °C or at ≥ 40 °C. The strain has alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, urease and tryptophane deaminase enzyme activities, but it does not have lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine

decarboxylase, ornithine decarboxylase and gelatinase activities. It produces acetoin (Voges Proskauer reaction), but no H_2S or indol. It does not ferment glucose, mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin and arabinose. It is negative for nitrate reduction to nitrite or N_2 gas. It is capable to assimilate citrate, tween 40, tween 80, D-fructose, α-D-glucose, D-mannose, sucrose, monomethyl succinate, DL-lactic acid, D-saccharic acid, succinic acid, alaninamide, L-asparagine, L-glutamic acid, L-proline, inosine, uridine, and it is positive for hydrolysis of esculin. It has weak reaction for assimilation of α-cyclodextrin, L-arabinose, cellobiose, turanose, α-hydroxy butyric acid, α-keto butyric acid, urocanic acid and glycerol. It is negative for assimilation of dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, D-arabitol, *i*-erythritol, L-fucose, D-galactose, gentiobiose, *m*-inositol, α-lactose, α-D-lactose lactulose, maltose, D-mannitol, D-melibiose, β-methyl D-glucoside, psicose, D-raffinose, L-rhamnose, D-sorbitol, D-trehalose, xylitol, methyl pyruvate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, β-hydroxy butyric acid, γ-hydroxy butyric acid, p-hydroxy phenylacetic acid, itaconic acid, α-keto glutaric acid, α-keto valeric acid, malonic acid, propionic acid, quinic acid, sebacic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-alanine, L-alanylglycine, L-aspartic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, thymidine, phenyl ethylamine, putrescine, 2-amino ethanol, 2,3-butanediol, DL-α-glycerol phosphate, glucose-1-phosphate, glucose-6-phosphate,

potassium gluconate, capric acid, adipic acid, malate, and trisodium citrate. The main cellular fatty acids are $C_{18:1}$ ω7*c*, summed feature 3 ($C_{15:0}$ iso 2-OH and/or $C_{16:1}$ ω7*c*), $C_{16:0}$ and $C_{10:0}$ 3-OH corresponding to 86 % of the total FAME profile. The following fatty acids are present in small amounts: unknown fatty acid ECL 11.799 (5 %) $C_{12:1}$ 3-OH (3.6 %), $C_{18:0}$ (2.2 %) and $C_{14:0}$ (1.8 %) (**Supplementary Table S1**). The phenotypic profile of *M. brasilensis* sp. nov. is at present based on one strain. The DNA G+C content of the type strain is 46.5 mol%. The type strain R-40503^T (= LMG 25434^T = CAIM 1459^T) was isolated from mucus of the endemic coral *Mussismilia hispida* located in the São Sebastião channel, São Paulo, Brazil.

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Figure

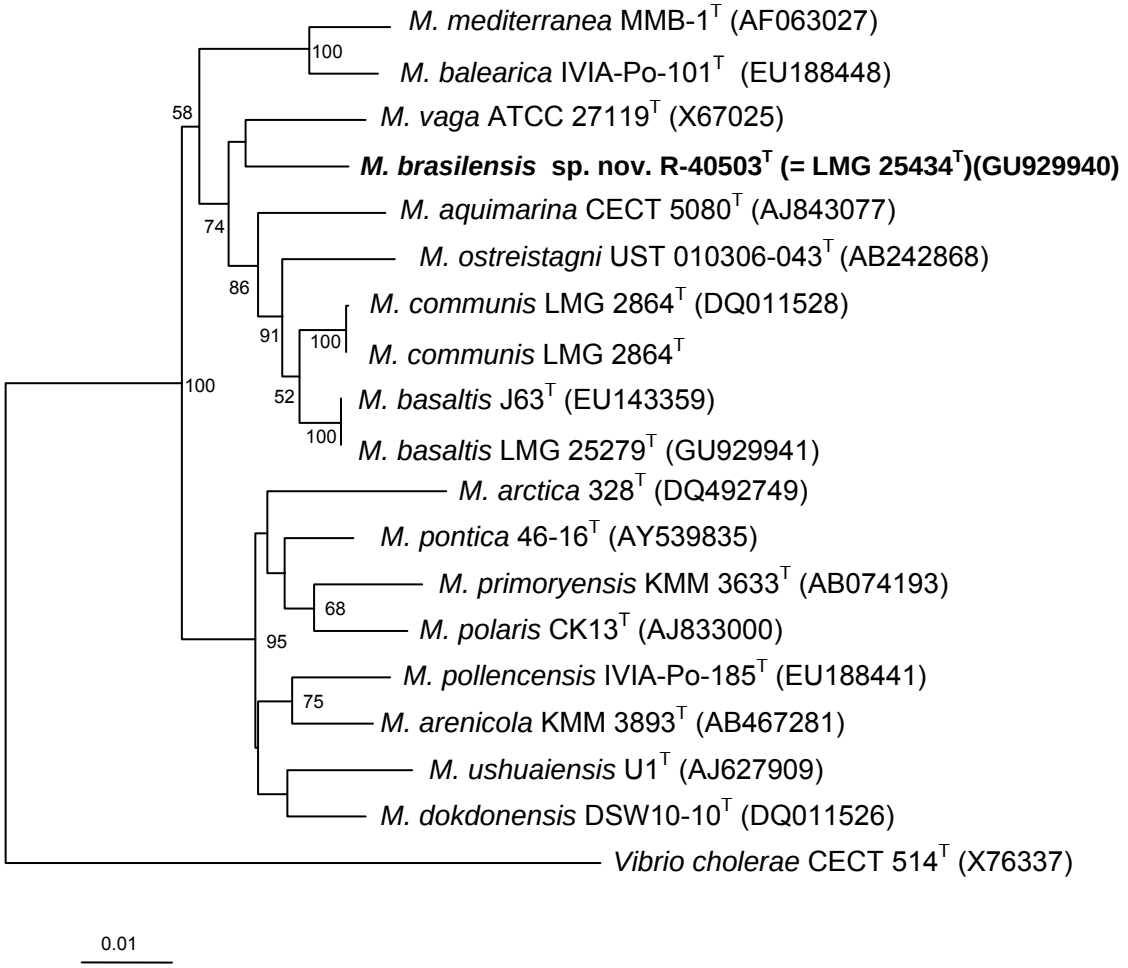


Figure 1. Neighbour-joining phylogenetic tree showing the phylogenetic position of *M. brasilensis* sp. nov. based on 16S rRNA gene sequences. The evolutionary distances were computed by BioNumerics 4.61 software (Applied Maths, Belgium). Bootstrap values (> 50 %) based on 1000 repetitions are shown. *Vibrio cholerae* was used as outgroup. Bar, 1 % estimated sequence divergence.

519

520 ## Tables

521

Table 1. DNA-DNA hybridization data, 16S rRNA gene sequence similarities and DNA G+C contents of *M. brasilensis* sp. nov. and phylogenetically related *Marinomonas* species

Strain	G+C content (mol%)	16S rRNA Similarity (%)	DNA-DNA relatedness values (%):				
		1	1	2	3	4	5
1. *M. brasilensis* sp. nov. R-40503^T (= LMG 25434^T)	46.5	100	100	42	23	22	17
2. *M. vaga* LMG 2845^T	47.5	97.9	27	100	16	15	21
3. *M. basaltis* LMG 25279^T	46.3	97.2	18	19	100	84	13
4. *M. communis* LMG 2864^T	46.3	97.2	16	21	73	100	12
5. *M. aquimarina* LMG 25236^T	49	96.7	5	3	12	11	100

522

Table 2. Phenotypic differences between *Marinomonas brasilensis* sp. nov. and its phylogenetic closest neighbours.

Species: **1**, *M. brasilensis* R-40503^T (= LMG 25434^T); **2**, *M. vaga* LMG 2845^T; **3,** *M. basaltis* LMG 25279^T; **4**, *M. communis* LMG 2864^T; **5**, *M. aquimarina* LMG 25236^T. Data for the reference species were obtained in this study, except when indicated. Abbreviations: +, positive; -, negative; w, weak reaction, NA, not available. All data were obtained in this study (except some data of *M. aquimarina* LMG 25236^T) using the same laboratory conditions.

Characteristic	1	2	3	4	5
Growth with NaCl (%w/v):					
12	+	+	-	-	+
13	w	+	-	-	w
Growth at (°C)					
40	-	w	+	+	+
Activity of:					
Oxidase	+	-	+	+	+
Urease	+	+	-	+	+
Utilization of:					
Tween 80	+	w	-	-	-[a]
Sucrose	+	+	-	-	-[a]
α-D-glucose	+	w	+	+	NA
Alaninamide	+	+	-	-	NA
L-asparagine	+	-	+	+	NA
L-arabinose	w	-	-	-	-[a]
Cellobiose	w	-	w	w	-[a]
Glycerol	w	-	-	-	-[a]

Turanose	w	+	-	-	NA
α-hydroxy butyric acid	w	+	+	+	NA
α-ketobutyric acid	w	-	+	+	NA
Methyl pyruvate	-	-	w	+	+[a]
α-ketoglutaric acid	-	+	-	-	+[a]
L-aspartic acid	-	+	-	-	+[a]
L-serine	-	+	+	+	+[a]
L-ornithine	-	+	-	-	+[a]
Putrescine	-	w	-	+	-[a]
Bromo succinic acid	-	+	-	-	NA
Glycyl-L-aspartic acid	-	w	-	-	NA

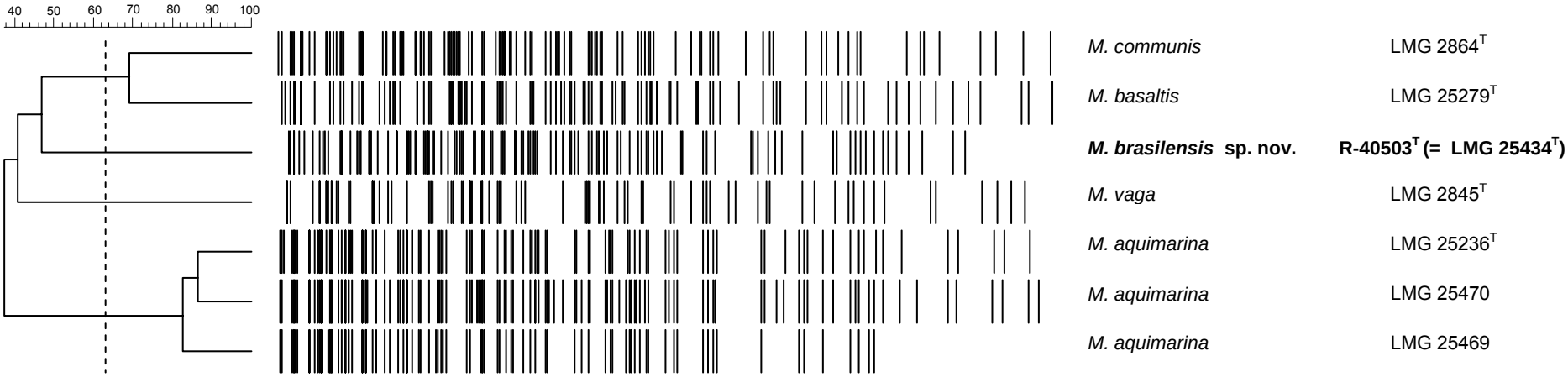
[a] Data from Marcian *et al*. (2005).

Supplementary data

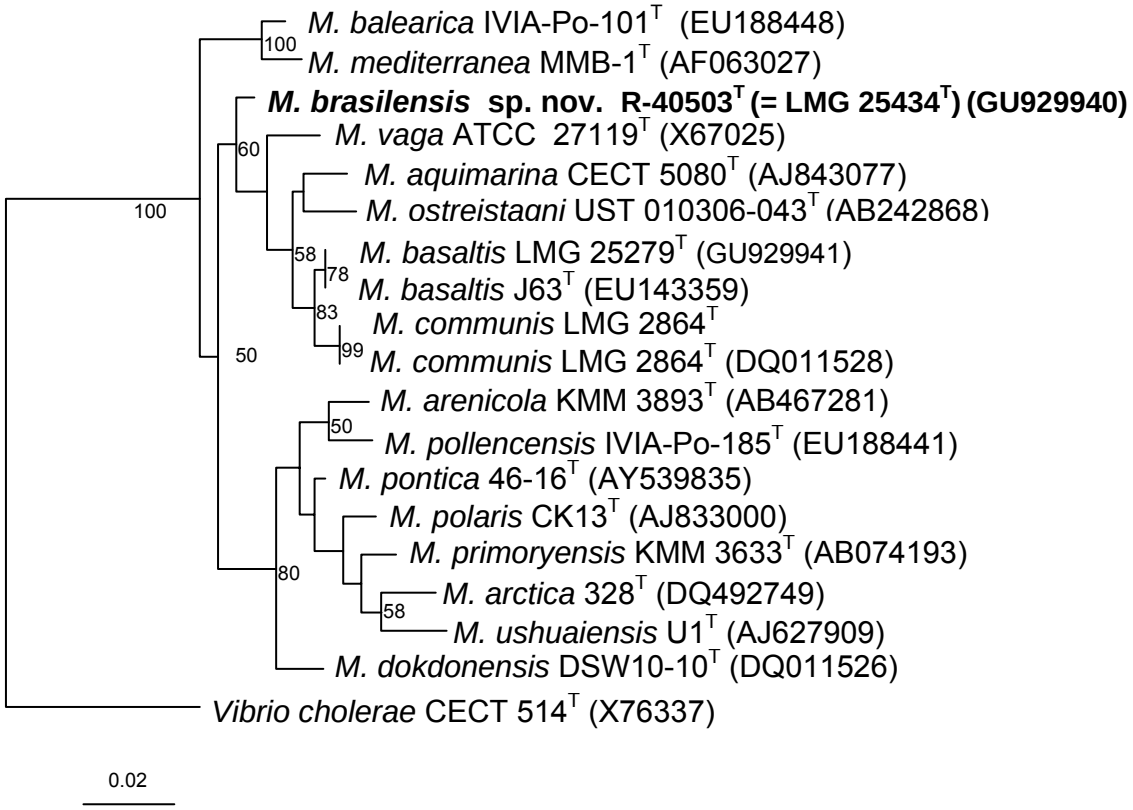
Supplementary Table S1. Cellular fatty acid contents of *Marinomonas brasilensis* sp. nov. and phylogenetically related *Marinomonas* species.

Taxa: **1**, *M. brasilensis* R-40503^T (= LMG 25434^T); **2**, *M. vaga* LMG 2845^T; **3,** *M. basaltis* LMG 25279^T; **4**, *M. communis* LMG 2864^T; **5**, *M. aquimarina* LMG 25236^T. Summed feature 3 comprises $C_{15:0}$ iso 2-OH and/or $C_{16:1}\omega7c$. Data are expressed as percentages of total fatty acids. Fatty acids representing <1 % are not shown. All data were obtained in this study on the same laboratory conditions.

Fatty acid	1	2	3	4	5
$C_{10:0}$	-	2.9	4.3	2.6	3.4
$C_{10:0}$ 3-OH	8	14.2	13.9	14.3	7.6
$C_{12:0}$	-	2.5	5.4	5.4	3.5
$C_{12:1}$ 3-OH	3.6	-	-	-	-
$C_{14:0}$	1.8	2.1	2.5	2	1.6
$C_{16:0}$	10.5	10.5	8.5	7.5	11.4
$C_{18:0}$	2.2	1.7	-	1.3	1.4
$C_{18:1}\omega6c$	-	-	8.6	-	-
$C_{18:1}\omega7c$	48.8	45.8	27.6	42.3	47
Summed feature 3	19	18.7	26.4	23.9	22.5
Unknown 11.799	5	1.7	-	-	-



Supplementary Figure S1. AFLP DNA fingerprints of *M. brasilensis* sp. nov. R-40503^T and strains of phylogenetically related *Marinomonas* species. The dendrogram was constructed with the UPGMA method after calculation of the band pattern similarity (%) using the DICE coefficient. The cut-off similarity level used to delineate AFLP clusters is 63 %. Strains with AFLP profiles showing more than 63 % similarity can be considered as members of the same species.



Supplementary Figure S2. Maximum Parsimony phylogenetic tree showing the phylogenetic position of *M. brasilensis* sp. nov. based on 16S rRNA gene sequences. The evolutionary distances were computed by BioNumerics 4.61 software (Applied Maths, Belgium). Bootstrap values (≥ 50 %) based on 100 repetitions are shown. *Vibrio cholerae* was used as outgroup. Bar, 2 % estimated sequence divergence.