Marinobacterium coralli sp. nov., isolated from mucus of coral (*Mussismilia hispida*)

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A Gram-negative, aerobic bacterium, designated R-40509^T, was isolated from mucus of the reef builder coral (*Mussismilia hispida*) located in the São Sebastião Channel, São Paulo, Brazil. The strain was oxidase-positive and catalase-negative, and required Na^+ for growth. Its phylogenetic position was in the genus *Marinobacterium* and the closest related species were *Marinobacterium sediminicola*, *Marinobacterium maritimum* and *Marinobacterium stanieri*; the isolate exhibited 16S rRNA gene sequence similarities of 97.5–98.0 % with the type strains of these species. 16S rRNA gene sequence similarities with other type strains of the genus *Marinobacterium* were below 96 %. DNA–DNA hybridizations between strain R-40509^T and the type strains of the phylogenetically closest species of the genus *Marinobacterium* revealed less than 70 % DNA–DNA relatedness, supporting the novel species status of the strain. Phenotypic characterization revealed that the strain was able to grow at 15–42 °C and in medium containing up to 9 % NaCl. The isolate could be differentiated from phenotypically related species by several features, including its ability to utilize D-alanine, L-alanine, bromosuccinic acid, β-hydroxybutyric acid and α-ketovaleric acid, but not acetate or L-arabinose. It produced acetoin (Voges–Proskauer), but did not have esterase lipase (C8) or catalase activities. It possessed $C_{18:1}\omega7c$ (35 %), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 25 %) and $C_{16:0}$ (22 %) as major cellular fatty acids. The DNA G+C content was 58.5 mol%. The name *Marinobacterium coralli* sp. nov. is proposed to accommodate this novel isolate; the type strain is R-40509^T (=LMG 25435^T =CAIM 1449^T).

Coral reefs are the most diverse marine biomes on Earth. They are constructed by a variety of invertebrates (e.g. corals, algae and sponges), serving as nurseries for marine life. These corals may secrete copious amounts of mucopolysaccharide material that contributes to the suspended organic matter in reefs (Wild *et al.*, 2004). Coral mucus plays an important role as a carrier of energy and nutrients to a range of planktonic and benthic consumers (Ducklow & Mitchell, 1979). Moreover, it represents an important resource for microbial growth in reef ecosystems (Brown & Bythell, 2005). Corals harbour diverse microbial communities and are better seen as holobionts (i.e. coral host + zooxanthellae + microbes) (Brown & Bythell, 2005; Rosenberg *et al.*, 2007). In these holobionts, mutualistic interactions such as microbial nitrogen and carbon fixation are key processes that allow bioproductivity in the reefs. The key role of bacteria in coral health is well-documented by various examples of symbiotic and pathogenic relationships (Rosenberg *et al.*, 2007). Bacteria living in coral mucus or tissue may act as a first line of defence for their holobiont hosts and protect them against diseases (Ritchie, 2006; Reshef *et al.*, 2006; Shnit-Orland & Kushmaro, 2009).

In the present study, a Gram-negative, aerobic bacterium (R-40509^T), isolated from mucus of the coral *Mussismilia hispida*, one of the main reef builders of the South Atlantic Ocean, was investigated using a polyphasic taxonomic approach. Strain R-40509^T was isolated in the summer of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain R-40509^T is GU183820.

A supplementary table is available with the online version of this paper.

2006 from apparently healthy coral located in the São Sebastião Channel at Preta beach (23° 49′ 10″ S 45° 24′ 37″ W), São Paulo, Brazil. It was obtained on marine agar medium (MA; Difco) after incubation for 48 h at 28 °C as described previously by Chimetto *et al.* (2008, 2009). It was tentatively allocated to the genus *Marinobacterium*, which comprises 11 species, at the time of writing, from different sources and locations. *Marinobacterium georgiense* originated from marine pulp mill effluent enrichment cultures (González *et al.*, 1997). *Marinobacterium stanieri* (Baumann *et al.*, 1983; Satomi *et al.*, 2002) and *Marinobacterium jannaschii* (Bowditch *et al.*, 1984; Satomi *et al.*, 2002) were isolated from coastal seawater, and *Marinobacterium litorale* and '*Marinobacterium marisflavi*' (Kim *et al.*, 2007, 2009a) were from seawater of the Yellow Sea. *Marinobacterium halophilum* (Chang *et al.*, 2007) and *Marinobacterium lutimaris* (Kim *et al.*, 2010) were isolated from tidal flats (Getbol), whereas *Marinobacterium rhizophilum* originated from roots of plants inhabiting a coastal tidal flat (Kim *et al.*, 2008). *Marinobacterium nitratireducens* and *Marinobacterium sediminicola* were isolated from sea sediment (Huo *et al.*, 2009), whereas *Marinobacterium maritimum* was isolated from Arctic marine sediment (Kim *et al.*, 2009b).

Bacterial genomic DNA and 16S rRNA gene sequences were obtained as described previously (Thompson *et al.*, 2001; Chimetto *et al.*, 2008, 2009). Raw sequence data were transferred to the ChromasPro v.1.34 software (Technelysium, Tewantin, Australia), where consensus sequences were determined. Sequences were aligned using the CLUSTAL W software (Chenna *et al.*, 2003). Pairwise similarities were calculated with the software BioNumerics 4.5 (Applied Maths) using an open gap penalty of 100 % and a unit gap penalty of 0 %. Similarity matrices and phylogenetic trees were reconstructed using the software MEGA4.0 (Tamura *et al.*, 2007) and BioNumerics 4.5. Trees were drawn using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Eck & Dayhoff, 1966) and minimum evolution methods (Rzhetsky & Nei, 1992). The robustness of the tree topologies was checked by 1000 bootstrap replications (Felsenstein, 1985). The gene sequence data obtained in this study are also available through the website TAXVIBRIO (http://www.taxvibrio.lncc.br/).

DNA–DNA hybridization experiments were performed using the microplate method described by Ezaki *et al.* (1989), with minor modifications (Willems *et al.*, 2001). Hybridizations were performed at 46 °C in the presence of 50 % formamide in four replicates. Reciprocal reactions were performed for every DNA pair in four replicates and their variation was within the limits of this method (Goris *et al.*, 1998). DNA G+C contents were determined by HPLC as described previously (Mesbah *et al.*, 1989). Analysis of fatty acid methyl esters was carried out as described by Huys *et al.* (1994). For fatty acid analysis, cells of the novel strain and the type strains of the closest known species (*M. sediminicola*, *M. maritimum* and *M. stanieri*) were grown on MA (Difco) for 24 h at 28 °C under aerobic conditions. Phenotypic characterization was performed using the API ZYM, API 20E and API 20NE kits (bioMérieux), and the Biolog GN2 microwell plates, according to the manufacturers' instructions, but with minor modifications, i.e. cell suspensions for inoculation of the API tests were prepared in a 3 % (w/v) NaCl solution and those for the Biolog GN2 microwell plates showed a turbidity (transmission) of 20 %. Cells for the suspensions were grown on Biolog medium for 24 h at 28 °C under aerobic conditions. Tests were read after 24–48 h of incubation at 28 °C. Growth at different temperatures (4–45 °C) and salt concentrations (0–14 % NaCl; determined at 28 °C) was determined by incubation on TSA (Difco) for 72 h. Catalase activity was determined by adding young cells to a drop of a 3 % H_2O_2 solution and observing whether O_2 was produced. Oxidase activity was tested using 1 % *N*,*N*,*N*′,*N*′-tetramethyl *p*-phenylenediamine (Kovacs, 1956).

Based on 16S rRNA gene sequence analyses, the phylogenetic position of strain R-40509^T (16S rRNA gene sequence of 1349 nt) was in the genus *Marinobacterium* and, more precisely, in a robust phylogenetic subcluster containing the species *M. sediminicola*, *M. maritimum* and *M. stanieri* (Fig. 1). The 16S rRNA gene sequence similarities between strain R-40509^T and the type strains of these species were 98.0–97.5 %, whereas they were below 96 % with the type strains of other known species of the genus *Marinobacterium*.

DNA–DNA hybridization experiments were performed between strain R-40509^T and the type strains of the closest phylogenetic neighbours (i.e. *M. sediminicola*, *M. maritimum* and *M. stanieri*) (Table 1). The DNA–DNA relatedness values for R-40509^T with these species were

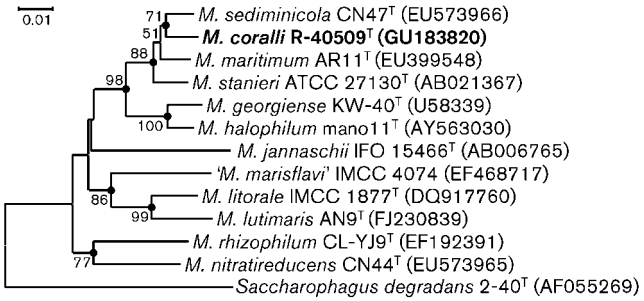

Fig. 1. Neighbour-joining tree showing the phylogenetic position of *Marinobacterium coralli* sp. nov. based on 16S rRNA gene sequences. Evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). Phylogenetic analyses were conducted using the MEGA4 software. Bootstrap values (>50 %) based on 1000 resamplings are shown. Solid circles indicate that bootstrap values >60 % were recovered in the maximum-parsimony and the minimum evolution trees. *Saccharophagus degradans* 2-40^T was used as outgroup. Bar, 1 % estimated sequence divergence.

Table 1. DNA–DNA hybridization data, 16S rRNA gene sequence similarities (%) and DNA G+C contents of *Marinobacterium coralli* sp. nov. and related species of the genus *Marinobacterium*

Strain	G+C content (mol%)	16S rRNA similarity*	DNA–DNA relatedness values (%) with strain:			
			1	2	3	4
1. *M. coralli* sp. nov. R-40509^T	58.5	100	100	29	30	20
2. *M. maritimum* LMG 25352^T	57.9†	97.8	42	100	39	24
3. *M. sediminicola* LMG 25280^T	56.3‡	98.0	28	30	100	15
4. *M. stanieri* LMG 6847^T	56.0	97.5	24	26	22	100

*Similarity values with *M. coralli* sp. nov. R-40509^T.
†Kim *et al.* (2009b).
‡Huo *et al.* (2009).

35 % with *M. maritimum* LMG 25352^T, 29 % with *M. sediminicola* LMG 25280^T and 22 % with *M. stanieri* LMG 6847^T. It can be concluded that strain R-40509^T represents a novel species in the genus *Marinobacterium* (Wayne *et al.*, 1987; Stackebrandt & Ebers, 2006). The DNA G+C content of strain R-40509^T was 58.5 mol% (Table 1).

Table 2. Phenotypic differences between *Marinobacterium coralli* sp. nov. and its closest phylogenetic neighbours

Strains: 1, *M. coralli* sp. nov. R-40509^T; 2, *M. maritimum* LMG 25352^T; 3, *M. sediminicola* LMG 25280^T; 4, *M. stanieri* LMG 6847^T. Data for reference strains were obtained in this study except where indicated. +, Positive; −, negative; W, weakly positive; ND, not determined.

Characteristic	1	2	3	4
Growth with NaCl (%, w/v)				
0.5	+	+	+	−
9	+	−	−	+
10	−	−	−	+
Growth at (°C):				
7	−	+	−	−
42	+	−	+	−
Esterase lipase (C8)	−	W	−	+
Catalase	−	+	W	+
Acetoin production (Voges–Proskauer)	+	+	W	W
Tween 80 hydrolysis	W	−	−	+*
Utilization of:				
Acetate	−	−	+	−*
DL-Lactic acid	+	W	W	−*
L-Arabinose	−	W	W	−†
L-Glutamic acid	+	W	−	+†
L-Proline	+	W	W	+†
D-Alanine	+	−	−	−†
L-Alanine	+	−	−	+†
β-Hydroxybutyric acid	+	−	−	+†
Bromosuccinic acid	+	−	−	ND
α-Ketovaleric acid	+	−	−	ND
Fatty acid methyl ester composition‡				
$C_{10:0}$	3.0	4.0	3.5	−
$C_{16:0}$	22.0	18.5	16.8	17.6
$C_{17:0}$ cyclo	−	1.5	−	−
Unknown fatty acid ECL 11.799	−	−	1.8	1.0

*Kim *et al.* (2009b).
†Baumann *et al.* (1983).
‡Fatty acid data are expressed as percentages of the total amount of fatty acids. Only fatty acids that differ between the strains are shown. Fatty acids representing <1 % are not shown.

Phenotypic characteristics were determined for the novel isolate and the type strains of the phylogenetically most closely related species of the genus *Marinobacterium*. The isolate can be differentiated from its closest phylogenetic neighbours by several phenotypic features (Table 2). The isolate grew in medium containing 9 % NaCl and utilized D-alanine, L-alanine, bromosuccinic acid, β-hydroxybutyric acid and α-ketovaleric acid, but not acetate or L-arabinose. It produced acetoin (Voges–Proskauer), but did not have esterase lipase (C8) or catalase activities. The presence of fatty acids $C_{10:0}$ and $C_{17:0}$ cyclo and the unknown fatty acid ECL 11.799 can also be used to differentiate the isolate from its closest phylogenetic neighbours (Table 2). The major cellular fatty acids of R-40509^T were $C_{18:1}\omega7c$ (35 %), summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$; 25 %) and $C_{16:0}$ (22 %) (Supplementary Table S1 available in IJSEM Online).

Based on the polyphasic analysis presented in this study, it is proposed that strain R-40509^T represents a novel species, *Marinobacterium coralli* sp. nov.

Description of *Marinobacterium coralli* sp. nov.

Marinobacterium coralli (co.ral′li. L. gen. n. *coralli* of coral, from which the organism was isolated).

Cells are Gram-negative, moderately halophilic, aerobic, motile, straight rods approximately 1 μm wide and 2–5 μm long. Catalase-negative and oxidase-positive. Colonies on MA are circular, slightly undulate, convex, smooth, beige translucent in colour and 0.8 mm in size after 1 day of incubation at 28 °C. Prolific growth occurs between 20 and 40 °C and at NaCl concentrations ranging from 1 to 7 % (w/v). No growth is observed in 0 % NaCl, in ⩾10 % NaCl, at ⩽7 °C or at ⩾43 °C. Shows alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities and produces acetoin (Voges–Proskauer). Capable of assimilating methyl pyruvate, monomethylsuccinate, *cis*-aconitic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, succinic acid, bromosuccinic acid, succinamic acid, D-alanine, L-alanine, L-alanylglycine, L-glutamic acid, L-proline and phenylethylamine. Weak reactions were observed for hydrolysis of Tween 40 and Tween 80 and for assimilation of *N*-acetyl-D-glucosamine, citric acid, alaninamide, L-asparagine and L-phenylalanine. Negative for: esterase lipase (C8), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, gelatinase and tryptophan deaminase activities; H_2S and indole production; reduction of nitrate and nitrite to N_2 gas; fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin and arabinose; and assimilation of citrate, α-cyclodextrin, dextrin, glycogen, *N*-acetyl-D-galactosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, *myo*-inositol, α-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, itaconic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, glucuronamide, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, L-hydroxyproline, L-leucine, L-ornithine, L-pyroglutamic acid, D-serine, L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, 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 $C_{18:1}\omega7c$, summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$) and $C_{16:0}$. The following fatty acids are present in small amounts: $C_{10:0}$ 3-OH, $C_{12:0}$ and $C_{10:0}$ (Supplementary Table S1 available in IJSEM Online).

The type strain is R-40509^T (=LMG 25435^T =CAIM 1449^T), isolated from mucus of the endemic coral *Mussismilia hispida* located in the São Sebastião channel, SP, Brazil. The DNA G+C content of the type strain is 58.5 mol%. The phenotypic profile of *M. coralli* is at present based on one strain. As more strains of this species are isolated and tested, the profile may change slightly.

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