Photobacterium rosenbergii sp. nov. and Enterovibrio coralii sp. nov., vibrios associated with coral bleaching

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Six new Vibrio-like isolates originating from different species of bleached and healthy corals around Magnetic Island (Australia) were investigated using a polyphasic approach. Phylogenetic analyses based on 16S rRNA, recA and rpoA gene sequences split the isolates in two new groups. Strains LMG 22223T, LMG 22224, LMG 22225, LMG 22226 and LMG 22227 were phylogenetic neighbours of Photobacterium leiognathi LMG 4228T (95.6 % 16S rRNA gene sequence similarity), whereas strain LMG 22228T was related to Enterovibrio norvegicus LMG 19839T (95.5 % 16S rRNA gene sequence similarity). The two new groups can be distinguished from closely related species on the basis of several phenotypic features, including fermentation of D-mannitol, melibiose and sucrose, and utilization of different compounds as carbon sources, arginine dihydrolase activity, nitrate reduction, resistance to the vibriostatic agent O/129 and the presence of fatty acids 15 : 0 iso and 17 : 0 iso. The names Photobacterium rosenbergii sp. nov. (type strain LMG 22223T = CBMAI 622T = CC1T) and Enterovibrio coralii sp. nov. (type strain LMG 22228T = CBMAI 623T = CC17T) are proposed to accommodate these new isolates. The G+C contents of the DNA of the two type strains are respectively 47.6 and 48.2 mol%.

Coral reefs are very important ecosystems for the marine environment as they harbour a great diversity of organisms. They are also relevant for the economy of several countries via tourism and fishing. In addition, coral reefs provide protection to coastal areas and may be a source of new bioactive compounds. The bacterial biodiversity associated with corals is poorly known (but see Rosenberg & Loya, 2004; Rosenberg & Falkovitz, 2004; Rohwer & Kelley, 2004). Rohwer et al. (2001, 2002) examined the bacterial community associated with different species of apparently healthy corals from Panama and Bermuda by both culture-dependent and culture-independent techniques. These authors found a high diversity of bacteria, including representative species of Bacillus, Clostridium, cyanobacteria, the Cytophaga-Flavobacterium-Bacteroides group and Proteobacteria. More than 80 % of the 1178 cloned 16S rRNA gene sequences originating from coral sources had not been allocated to recognized bacterial species as they had less than 93 % sequence similarity (Rohwer et al., 2002).

Coral reefs have experienced a tremendous decline in recent decades (Hoegh-Guldberg, 2004). Global climate changes, sea-water pollution as a result of aquaculture, oil spills and urban sewage, coral bleaching and other infectious diseases are the main causes of this decline (Hoegh-Guldberg, 2004; Hughes et al., 2003; Knowlton & Rohwer, 2003; Rosenberg & Loya, 2004; Rosenberg & Ben-Haim, 2002; Sutherland et al., 2004). Kushmaro et al. (1996) suggested that bleaching is, in fact, the result of an infectious disease. Bacterial infections of corals caused by Vibrio shilonii (=Vibrio mediterranei) (Kushmaro et al., 2001) and Vibrio

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, recA and rpoA gene sequences determined in this study are shown in Table 1.

Neighbour-joining trees showing relationships among Vibrio-like species on the basis of rpoA and recA gene sequence analysis are available as supplementary material in IJSEM Online.
coralliilyticus (Ben-Haim et al., 2003) were subsequently reproduced in the laboratory. V. coralliilyticus also causes disease in fish and shellfish (B. Austin, D. A. Austin, R. Sutherland, F. L. Thompson and J. Swings, unpublished results).

In the present study we analysed the taxonomic position of six new isolates originating from different species of bleached and healthy corals from Magnetic Island (Australia) in 2002. The taxa identified share the main phenotypic features of Vibrio, but 16S rRNA, recA and rpoA gene sequences clearly suggest that they represent two novel species for which we propose the names Photobacterium rosenbergii sp. nov. and Enterovibrio coralli sp. nov.

Details of sources of strains are given in Table 1. Strains included in this study were grown on tryptone soy agar (TSA; Oxoid) supplemented with 2 % NaCl (v/v) at 28 °C for 24 h unless stated otherwise. Colony morphology was examined on cultures grown on thiosulphate/citrate/bile salts/sucrose (TCBS; Difco) agar by using a stereoscopic microscope. Cell morphology was examined on wet mounts via a phase-contrast microscope.

Gene sequences of 16S rRNA, recA and rpoA were generated on an ABI Prism 3100 DNA sequencer (Applied Biosystems) and analysed as described by Thompson et al. (2001, 2004a), but with minor modifications. The sequences of recA primers used for amplification and sequencing were recA-01-F (5'-TGARAARCARTTYGGTAAAGG; position 222), recA-02-R (5'-TCRCCNTTRA-GCTRTACC; 1040), recA-03-F (5'-TYGGGBTAGTGYTYGGTACC; 767) and recA-04-R (5'-GGGTACCRAAC-ATCACVC; 769). These primers were designed using the 16S rRNA gene sequences from Vibrio cholerae n16961_o1, Vibrio parahaemolyticus o3k6_rimd2210633, Vibrio vulnificus cmcp6, Escherichia coli (cft073, o157h7_edl933, o157h7_rimd0509952 and k12_mg1655), Shigella flexneri o157h7_rimd0509952 and k12_mg1655), and Salmonella enterica (ct18 and ty2_typhi 3), Salmonella typhimurium (lt2sgsc1412_atcc700720), Yersinia pestis (co92 and kim) and Shewanella oneidensis (mr1). The primers recA-01-F and recA-02-R are useful to amplify recA fragments of all recognized strains of Vibrio-like species. Sequence accession numbers are given in Table 1.

Overall, consensus sequences were obtained by at least two reads of the same region of the gene. The consensus sequences were transferred into BIONUMERICS 3.5 software (Applied Maths) and phylogenetic trees were constructed based on the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods. The G+C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984).

Phenotypic characterization of the isolates was performed using API 20E, API ZYM (bioMérieux) and Biolog GN2 metabolic fingerprinting (Biolog) following the manufacturers’ instructions. Standard phenotypic tests were performed as described by Baumann et al. (1984), Farmer & Hickman-Brenner (1992) and Vandamme et al. (1998). Antibiograms were carried out using the disc diffusion methodology of Acar & Goldstein (1996) using commercial discs (Oxoid). The inhibition zone of each antibiotic was measured for strains grown on Iso-sensitest agar (Oxoid) supplemented with 1-5 % (w/v) NaCl for 24 h at 28 °C. Analysis of fatty acid methyl esters was carried out as described by Huys et al. (1994).

The 16S rRNA gene sequences of strains LMG 22223T (1505 nt) and LMG 22227 (1505 nt) were nearly identical, having more than 99-5 % similarity (Fig. 1). LMG 22223T and LMG 22228T were most closely related to Photobacterium leiognathi LMG 4228T (95-6 %) and Vibrio calviensis LMG 21294T (95-8 %), respectively. LMG 22227T and Enterovibrio norvegicus LMG 19839T had 95-5 % 16S rRNA gene sequence similarity. Clearly, V. calviensis should be transferred to Enterovibrio, but this remains to be done in future studies.

Table 1. Strains included in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>16S rRNA gene</th>
<th>recA</th>
<th>rpoA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterovibrio coralli sp. nov.</strong></td>
<td></td>
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</tr>
<tr>
<td>LMG 22223T (= CBMAI 623T = CC17T)</td>
<td>Water extract of bleached Merulina ampliata</td>
<td>AJ842343</td>
<td>AJ842347</td>
<td>AJ842530</td>
</tr>
<tr>
<td><strong>Photobacterium rosenbergii sp. nov.</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>LMG 22223T (= CBMAI 622T = CC1T)</td>
<td>Tissue extract of bleached Pachyseris speciosa</td>
<td>AJ842344</td>
<td>AJ842358</td>
<td>AJ842542</td>
</tr>
<tr>
<td>LMG 22224 (= R-21412 = CC6)</td>
<td>Water extract of bleached M. ampliata</td>
<td>ND</td>
<td>AJ842359</td>
<td>AJ842543</td>
</tr>
<tr>
<td>LMG 22225 (= R-21417 = CC11)</td>
<td>Water extract of apparently healthy P. speciosa</td>
<td>AJ842345</td>
<td>AJ842360</td>
<td>AJ842544</td>
</tr>
<tr>
<td>LMG 22226 (= R-21428 = CC22)</td>
<td>Water extract of bleached Barabattoa amicorum</td>
<td>ND</td>
<td>AJ842361</td>
<td>AJ842545</td>
</tr>
<tr>
<td>LMG 22227 (= R-21429 = CC23)</td>
<td>Tissue extract of bleached B. amicorum</td>
<td>AJ842346</td>
<td>ND</td>
<td>AJ842546</td>
</tr>
</tbody>
</table>

ND, Not determined.
Novel Enterovibrio and Photobacterium species

The rpoA gene sequences (928 nt; corresponding to about 94% of the coding region of this locus) of strains LMG 22223^T, LMG 22224, LMG 22225, LMG 22226 and LMG 22227 were nearly identical, having more than 99.6% similarity (see Supplementary Fig. A in IJSEM Online). The closest phylogenetic neighbours of LMG 22223^T and LMG 22228^T were Photobacterium phosphoreum ATCC 11040^T (D25310) and Vibrio salmonicida NCMB 2262^T (X70643). 5% of the coding region of this locus) of strains LMG 22228^T were identical.

The closest phylogenetic neighbours of LMG 22223^T and similarity (see Supplementary Fig. A in IJSEM Online). LMG 22223^T had 94% of the coding region of this locus) of strains LMG 22228^T had 88-2 and 85% rpoA gene sequence similarity to Enterovibrio norvegicus LMG 19839^T and Grimontia hollisae LMG 17719^T (AJ514909), respectively. Enterovibrio norvegicus LMG 19839^T and the novel strain LMG 22223^T had 95.5% rpoA sequence similarity. This low level of similarity suggests that LMG 22223^T and LMG 22228^T represent two novel phylogenetic branches within the current families Photobacteriaceae and Enterovibriaceae (Thompson et al., 2004b). The usefulness of the rpoA gene as an alternative phylogenetic marker for bacterial classification has been proposed recently (Zeigler, 2003; Gevers et al., 2004; Lerat et al., 2003). The rpoA gene is a single-copy and ubiquitous gene in the bacterial genomes sequenced to date. According to Zeigler (2003) this gene evolves as slowly as the 16S rRNA gene. It seems to be resistant to lateral gene transfer (Lerat et al., 2003) and is indeed a chronometer for Vibrio-like species (unpublished results).

Strains LMG 22224, LMG 22225 and LMG 22226 had identical recA gene sequences (797 nt) (see Supplementary Fig. B in IJSEM Online). LMG 22223^T had 94.5% recA gene sequence similarity to the other conspecific strains, but only 83.9% to its closest phylogenetic neighbour, Photobacterium leiognathi. LMG 22228^T had 88.2 and 85% recA gene sequence similarity to Enterovibrio norvegicus LMG 19839^T and Grimontia hollisae LMG 17719^T, respectively. Collectively, 16S RNA, recA and rpoA gene sequence data indicate that the coral isolates represent two novel species for which we propose the names Photobacterium rosenbergii and Enterovibrio coralii.

Enterovibrio coralii sp. nov. can be differentiated from Enterovibrio norvegicus on the basis of various phenotypic features. Enterovibrio coralii utilizes cellobiose, melibiose and L-histidine and reduces nitrate, but Enterovibrio norvegicus does not. Enterovibrio norvegicus produces indole, but Enterovibrio coralii does not. Enterovibrio coralii produces alcohol dehydrogenase and ferments D-mannitol, whereas Enterovibrio norvegicus, G. hollisae and V. calviensis do not. Enterovibrio coralii utilizes glycogen, D-fructose and D-trehalose, but V. calviensis does not. Enterovibrio coralii is resistant to O/129 (150 μg per disc), fusidic acid (10 μg per disc) and streptomycin (10 μg per disc), but V. calviensis is not. Photobacterium rosenbergii sp. nov. ferments D-mannitol and melibiose, whereas other Photobacterium species do not. Photobacterium rosenbergii utilizes citrate, cellobiose, melibiose, lactose, formate, propionate, D-raffinose, aconitate, D-alanine, L-alanine and L-histidine, whereas other Photobacterium species do not. Photobacterium species produce acetoin, but Enterovibrio coralii does not. None of the Photobacterium species produces the fatty acids 15:0 iso and 17:0 iso that are found in Photobacterium rosenbergii.

Description of Photobacterium rosenbergii sp. nov.

Photobacterium rosenbergii (ro.sen.ber’gi.i. N.L. gen. n. rosenbergii of Rosenberg, after the Israeli microbiologist Eugene Rosenberg).

Cells are Gram-negative, motile and oxidase-positive. Strains grow on the selective medium TCBS. Cells are 2–4 μm long and 1–2 μm wide after 1 day at 28 °C in TSA. Colonies are convex, round (1 cm in diameter), beige and opaque with entire and smooth margins after 2 days at 28 °C on TSA. Forms yellow colonies with raised margins on TCBS. Prolific growth occurs between 20 and 30 °C and at NaCl concentrations (w/v) of 2–6%. No growth is observed at 4 or 40 °C or in 0 or 8% NaCl. Utilizes the...
following carbon compounds as sole energy sources: dextrin, glycogen, Tweens 40 and 80, N-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, α-D-glucose, maltose, D-mannitol, D-mannose, D-melibiose, D-raffinose, methyl β-D-glucoside, psicose, sucrose, D-trehalose, methyl pyruvate, monomethyl succinate, acetic acid, cis-aconitic acid, citric acid, formic acid, p-hydroxyphenylacetic acid, α-ketobutyric acid, α-ketoglutaric acid, DL-lactic acid, propionic acid, succinic acid, bromosuccinic acid, alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycy1 L-aspartic acid, glycy1 L-glutamic acid, L-histidine, L-ornithine, L-serine, L-threonine, inosine, uridine, thymidine, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. Does not utilize adonitol, L-arabinose, D-arabitol, α-D-lactose lactulose, xylitol, D-galactonic acid, γ-hydroxybutyric acid, itaconic acid, α-ketocapric acid, malonic acid, D-saccaric acid, sebacic acid, hydroxy-L-proline, L-leucine, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, phenylglycine, 2-aminoethanol or 2,3-butenediol. Ferments glucose, D-mannitol, sucrose, melibiose and amygdalin. Does not ferment sorbitol or arabinose and is negative for acetoin and indole production. Reduces nitrate and is positive for arginine dihydrolase, β-galactosidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase. Lysine and ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase are negative. The following features are variable within the species (the reaction for the type strain is indicated in parentheses): utilization of α-cyclodextrin (+), N-acetyl-D-galactosamine (−), i-erythritol (−), L-fucose (−), gentiobiose (−), myo-inositol (+), α-lactose (−), D-raffinose (−), L-rhamnose (+), D-sorbitol (−), turanose (+), D-galacturonic acid (−), D-glucuronic acid (−), D-gluconaminic acid (−), D-glucuronic acid (+), α-hydroxybutyric acid (−), β-hydroxybutyric acid (−), quinic acid (−), succinic acid (−), glucuronide (−), L-phenylalanine (−), L-proline (+), L-pyroglutamic acid (+) and putrescine (−) as sole carbon sources. Fermentation of L-rhamnose and inositol, as well as lipase, α-galactosidase and α-glucosidase activity are variable, but positive for the type strain. Resistant to O/129 (150 µg per disc), fusidic acid (10 µg), streptomycin (10 µg), tetracycline (30 µg), penicillin G (10 µg) and chloramphenicol (30 µg). The most abundant fatty acids are summed feature 3 (41–44%; comprising 16:1o7c and/or 15 iso 2-OH), 18:1o7c (17–19%), 16:0 (10–15%), 17:0 iso (3–6%), 14:0 (3–4%), 15:0 iso (2–4%), summed feature 2 (2–3%; comprising 14:0 3-0H, 16:1 iso I, an unidentified fatty acid with equivalent chain-length of 10-28A and/or 12:0 ALDE), 12:0 3-OH (2–3%), 12:0 (2%), 17:1o9c iso (1–2%), 15:0 (1–2%), 13:0 iso (1%), 17:0 (1%), 17:1o8c (1%), 15:0 iso 3-OH (1%) and 16:1o7c alcohol (1%). The G+C content of the DNA ranges from 47-6 to 47-9 mol%.

The type strain is strain LMG 22223T (= CBMAI 622T = CC1T).

Description of Enterovibrio coralii sp. nov.

Enterovibrio coralii (co.ra’li.i. L. gen. sing. n. coralii of coral).

Gram-negative, motile, oxidase-positive. Strains grow on the selective medium TCBS. Cells are 1 µm in diameter after 1 day at 28°C in TSA. Colonies are umbonate, round (5 mm in diameter), beige and transparent with entire and smooth margins after 2 days at 28°C on TSA. Forms small, green colonies (2 mm in diameter) with raised margins on TCBS. Prolific growth occurs between 20 and 30°C and at NaCl concentrations (w/v) of 2–6%. No growth is observed at 4 or 40°C or in 0 or 8% NaCl. Utilizes the following compounds as sole carbon sources: dextrin, glycogen, N-acetyl-D-glucosamine, cellobiose, D-fructose, D-galactose, D-glucose, α-D-glucose, α-D-lactose lactulose, malto, D-mannitol, sucrose, melibiose and amygdalin. Does not ferment sorbitol or arabinose and is negative for acetoin and indole production. Reduces nitrate and is positive for arginine dihydrolase, β-galactosidase, alkaline phosphatase, esterase, esterase lipase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase. Lysine and ornithine decarboxylase, urease, tryptophan deaminase, gelatinase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase and α-fucosidase are negative. The following features are variable within the species (the reaction for the type strain is indicated in parentheses): utilization of α-cyclodextrin (+), N-acetyl-D-galactosamine (−), i-erythritol (−), L-fucose (−), gentiobiose (−), myo-inositol (+), α-lactose (−), D-raffinose (−), L-rhamnose (+), D-sorbitol (−), turanose (+), D-galacturonic acid (−), D-glucuronic acid (−), D-gluconaminic acid (−), D-glucuronic acid (+), α-hydroxybutyric acid (−), β-hydroxybutyric acid (−), quinic acid (−), succinic acid (−), glucuronide (−), L-phenylalanine (−), L-proline (+), L-pyroglutamic acid (+) and putrescine (−) as sole carbon sources. Fermentation of L-rhamnose and inositol, as well as lipase, α-galactosidase and α-glucosidase activity are variable, but positive for the type strain. Resistant to O/129 (150 µg per disc), fusidic acid (10 µg), streptomycin (10 µg), tetracycline (30 µg), penicillin G (10 µg) and chloramphenicol (30 µg). The most abundant fatty acids are summed feature 3 (41–44%; comprising 16:1o7c and/or 15 iso 2-OH), 18:1o7c (17–19%), 16:0 (10–15%), 17:0 iso (3–6%), 14:0 (3–4%), 15:0 iso (2–4%), summed feature 2 (2–3%; comprising 14:0 3-0H, 16:1 iso I, an unidentified fatty acid with equivalent chain-length of 10-28A and/or 12:0 ALDE), 12:0 3-OH (2–3%), 12:0 (2%), 17:1o9c iso (1–2%), 15:0 (1–2%), 13:0 iso (1%), 17:0 (1%), 17:1o8c (1%), 15:0 iso 3-OH (1%) and 16:1o7c alcohol (1%). The G+C content of the DNA ranges from 47-6 to 47-9 mol%.

The type strain is strain LMG 22223T (= CBMAI 622T = CC1T).
10:928 and/or 12:0 ALDE), 12:0 3-OH (2%), 18:0 (2%), 16:0 is (1%) and 17:1o8c (1%).

The type strain is LMG 22228T (= CBMAI 623T = CC17T). The G+C content of the DNA of LMG 22228T is 48–2 mol%.

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References


