Photobacterium jeanii sp. nov., isolated from corals and zoanthids

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Four novel isolates (R-40508^T, R-40507, R-40903 and R-21419) were obtained from different cnidarian species (*Phyllogorgia dilatata*, *Merulina ampliata* and *Palythoa caribaeorum*) from different places in Brazil and Australia. The novel isolates formed a tight phylogenetic group based on 16S rRNA, *recA*, *topA*, *ftsZ*, *mreB* and *rpoA* gene sequences. Their closest phylogenetic neighbours were the type strains of *Photobacterium leiognathi*, *P. rosenbergii* and *P. halotolerans*, sharing 97.1–97.5 % 16S rRNA gene sequence similarity. DNA–DNA hybridization between a representative strain (R-40508^T) and the type strains of these *Photobacterium* species revealed less than 20 % relatedness, showing that the new isolates belong to a novel species. Several phenotypic features allow the differentiation of the novel species from its closest phylogenetic neighbours. It has gelatinase and lipase activity and can utilize melibiose, but it cannot grow on 6 % NaCl. In addition, the novel species has the fatty acid iso-$C_{16:0}$, but lacks the fatty acids $C_{17:0}$, $C_{17:0}$ cyclo, iso-$C_{17:0}$, $C_{17:1}\omega8c$ and iso-$C_{17:1}\omega9c$. The name *Photobacterium jeanii* sp. nov. is proposed for this species, with the type strain R-40508^T (=LMG 25436^T =CAIM 1817^T). The G+C content of the type strain is 45.5 mol%.

There has been growing interest in coral microbiology in recent years, particularly because of increasing awareness of the role of corals in the marine environment and their extinction (Rohwer *et al.*, 2001; Dinsdale *et al.*, 2008; Sussman *et al.*, 2009; Shnit-Orland & Kushmaro, 2009). One of the main causes of coral extinction seems to be infectious disease, caused mainly by vibrios (Rosenberg *et al.*, 2007). Coral bacteria may also have a positive effect on the coral holobiont. Bacteria living in the coral mucus and tissue may be a first line of defence for their holobiont hosts, protecting them against disease (Ritchie, 2006). *Photobacterium* species associated with corals have been shown to be able to produce antibiotics that would protect the coral holobiont or would allow a competitive advantage (Ritchie, 2006). Other beneficial effects on the coral holobiont may include nitrogen fixation (Chimetto *et al.*, 2008), provision of a food resource (Kooperman *et al.*, 2007) and chitin decomposition (Ducklow & Mitchell, 1979). *Photobacterium rosenbergii* was one of the recently described species associated with healthy and diseased corals in Australia (Thompson *et al.*, 2005a). This species has also been found in association with healthy and diseased corals in Brazil (Chimetto *et al.*, 2009).

In the present study, a detailed polyphasic taxonomic analysis was performed on four novel isolates obtained in different studies in order to determine their exact taxonomic position (Table 1). They appeared to be related

Abbreviation: DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *recA*, *topA*, *ftsZ*, *mreB* and *rpoA* gene sequences of strains of *Photobacterium jeanii* sp. nov. are GU065209–GU065227 and AJ842701, as detailed in Supplementary Table S1.

Details of sequence accession numbers and strain-dependent phenotypic properties are available as supplementary material with the online version of this paper.

Table 1. Strains of *Photobacterium jeanii* sp. nov.

Isolate	Source	Locality	Year
R-40507 =LMG 25437	Mucus of apparently healthy *Palythoa caribaeorum*	Portinho beach in São Sebastião Channel, São Paulo, Brazil	2006
R-40508^T =LMG 25436^T =CAIM 1817^T	As above	Preta beach in São Sebastião Channel, São Paulo, Brazil	2006
R-40903	Mucus of apparently healthy *Phyllogorgia dilatata*	Abrolhos Bank, Bahia, Brazil	2007
R-21419	Apparently healthy *Merulina ampliata*	Magnetic Island, Australia	2002

to *Photobacterium* species on the basis of 16S rRNA gene sequence analysis (Alves *et al.*, 2010; Chimetto *et al.*, 2009; Thompson *et al.*, 2005b).

All isolates were obtained using marine agar (MA) at 28 °C after 48 h of incubation. Sequences of the 16S rRNA gene and genes encoding a recombination repair protein (*recA*), topoisomerase I (*topA*), a cell-division protein (*ftsZ*), an actin-like cytoskeleton protein (*mreB*) and the RNA polymerase alpha subunit (*rpoA*) were obtained as described previously (Chimetto *et al.*, 2008, 2009). Primers used for gene amplification and sequencing are described in Thompson *et al.* (2001b, 2005b) and Sawabe *et al.* (2007). Raw sequence data were transferred to ChromasPro version 1.34 (http://www.technelysium.com.au/ChromasPro.html), where consensus sequences were determined. Pairwise similarities of these sequences with sequences from the EMBL database were calculated with the BioNumerics 4.5 software package (Applied Maths) using an open gap penalty of 100 % and a unit gap penalty of 0 %. Sequences were aligned using CLUSTAL W. Similarity matrices and phylogenetic trees were constructed using the MEGA version 4.0 software (Tamura *et al.*, 2007) and BioNumerics 4.5 software (Applied Maths). Trees were drawn using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Eck & Dayhoff, 1966) methods. The robustness of each topology was checked by 1000 bootstrap replications (Felsenstein, 1985). The gene sequence data obtained in this study are available through the open-access website TAXVIBRIO (http://www.taxvibrio.lncc.br/). The GenBank accession numbers for the 16S rRNA, *ftsZ*, *mreB*, *recA*, *rpoA* and *topA* gene sequences determined in this study are listed in Supplementary Table S1, available in IJSEM Online. DNA–DNA hybridization (DDH) experiments were performed using Ezaki's microplate method as described in detail previously (Ezaki *et al.*, 1989; Willems *et al.*, 2001). Hybridization was conducted at 40 °C in the presence of 50 % formamide. Reciprocal reactions were performed for

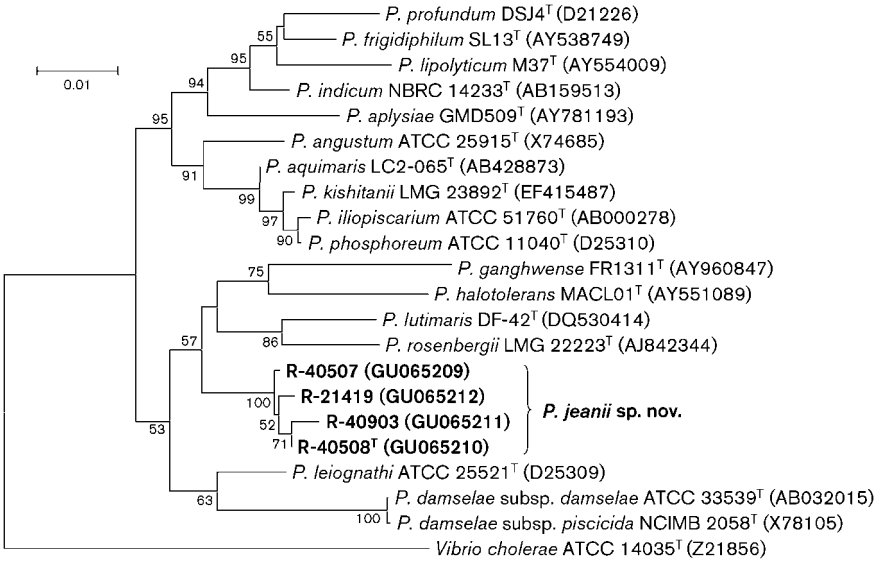

Fig. 1. Neighbour-joining phylogenetic tree of *Photobacterium* species based on 16S rRNA gene sequences (1466 nt) showing the position of the novel strains (*Photobacterium jeanii* sp. nov.). The optimal tree with the sum of branch lengths of 0.30919266 is shown. Evolutionary distances were computed using the Jukes–Cantor method. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). Phylogenetic analyses were conducted in MEGA4. Bootstrap values (>50 %) based on 1000 resamplings are shown. *Vibrio cholerae* ATCC 14035^T was used as an outgroup. Bar, 1 % estimated sequence divergence.

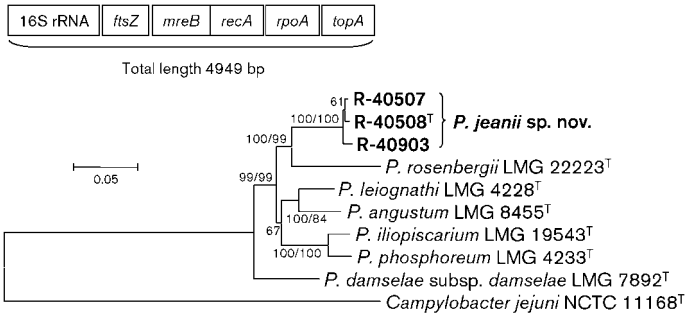

Fig. 2. Neighbour-joining phylogenetic tree based on concatenated 16S rRNA, *ftsZ*, *mreB*, *recA*, *rpoA* and *topA* gene sequences (4949 nt) showing the position of the novel strains (*P. jeanii* sp. nov.). Evolutionary distances were computed using the Jukes–Cantor method. Codon positions included were first + second + third + non-coding. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (pairwise deletion option). Phylogenetic analyses were conducted in MEGA4. Bootstrap values (>50 %) based on 1000 resamplings are shown for the neighbour-joining/maximum-parsimony methods. *Campylobacter jejuni* NCTC 11168^T was used as an outgroup. Bar, 5 % estimated sequence divergence.

Table 2. Phenotypic differences between *P. jeanii* sp. nov. and related *Photobacterium* species

Strains/species: 1, *P. jeanii* sp. nov. (four strains); 2, *P. leiognathi* (unless indicated, data from Baumann & Baumann, 1984; Nogi *et al.*, 1998); 3, *P. rosenbergii* (Thompson *et al.*, 2005a); 4, *P. lutimaris* (Jung *et al.*, 2007); 5, *P. halotolerans* (Rivas *et al.*, 2006); 6, *P. ganghwense* FR1311^T (Park *et al.*, 2006); 7, *P. lipolyticum* KCTC 10562^T (Yoon *et al.*, 2005); 8, *P. phosphoreum* LMG 4233^T (Nogi *et al.*, 1998; Ast *et al.*, 2007; Yoshizawa *et al.*, 2009); 9, *P. angustum* CIP 75.10^T (Baumann & Baumann, 1984; Nogi *et al.*, 1998; Yoshizawa *et al.*, 2009); 10, *P. aquimaris* LC2-065^T (Yoshizawa *et al.*, 2009); 11, *P. indicum* LMG 22857^T (Xie & Yokota, 2004; Ast *et al.*, 2007); 12, *P. profundum* (Nogi *et al.*, 1998). +, Positive; −, negative; W, weak; V, variable; ND, no data available. All taxa are negative for Gram stain and lysine and ornithine decarboxylases. Data in parentheses are for type strains.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Growth at:												
6 % (w/v) NaCl	−	+	+	+	+	+	−	−	+	−	ND	ND
4 °C	−	−	−	+	+	+	+	+	−	−	+	+
Optimum growth temperature (°C)	20–35	(26)	20–30	25–30	28	35	25–28	18	25	10–25	25	10
Catalase	+	(−)	(+)*	W	+	+	+	+	−	+	−	+
Oxidase	+	(+)	+	+	+	+	+	+	−	−	−	+
API ZYM results												
Esterase (C4)	+	+	+	+	−	+	+	W	W	−	ND	ND
Esterase lipase (C8)	+	(+)*	+	+	−	+	+	−	W	−	ND	ND
Lipase (C14)	+	−	(+)	−	(+)*	−	−	−†	−†	−	ND	+†
Valine arylamidase	W	−	+	−	−	−	−	W	−	−	ND	ND
Trypsin	+	(W)*	−	−	−	−	−	ND	ND	−	ND	ND
Naphthol-AS-BI-phosphohydrolase	+	(+)*	+	+	−	+	+	ND	ND	+	ND	ND
α-Galactosidase	−	−	(+)	−	(−)*	−	−	−	−	W	ND	ND
α-Glucosidase	+	(−)*	(+)	−	(+)*	+	−	ND	ND	−	ND	ND
API 20E results												
Arginine dihydrolase	+	+	+	+	−	+	−	−	−	−	+	+
Citrate utilization	−	−	+	+	+	+	−	−	+	−	−	−
Tryptophan deaminase	−	(−)*	−	−	W	−	ND	−	ND	−	W	ND
Indole production	−	(−)	−	+	−	+	+	−	−	ND	+	+
Acetoin production	(W)	+	−	(−)*	−	−	−	−	+	ND	−	+
Gelatinase	+	−	−	−	+	+	−	−	V	−	−	ND
Inositol fermentation/oxidation	−	−	(+)	+	−	+	−	−	−	ND	−	−
Melibiose fermentation/oxidation	+	−	+	−	−	−	−	−	−	ND	−	−
Amygdalin fermentation/oxidation	−	(−)*	+	(+)*	−	+	ND	−	ND	ND	−	ND
Arabinose fermentation/oxidation	−	−	−	−	+	−	−	−	−	ND	−	−
DNA G+C content (mol%)	45.1–45.5	40–44	47.6–47.9	48.3	49.8	44	47	39.1	40–42	42.9	40	42

*Data obtained in this study for *P. leiognathi* LMG 4228^T, *P. rosenbergii* LMG 22223^T, *P. lutimaris* LMG 25278^T or *P. halotolerans* LMG 22194^T.
†Data from Seo *et al.* (2005).

every DNA pair and their variation was within the limits of this method (Goris *et al.*, 1998). The G+C content of the DNAs was determined using 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 were grown on TSA (Difco) supplemented with 2 % NaCl for 24 h at 28 °C. Phenotypic characterization was performed as described previously (Thompson *et al.*, 2001a, 2006). Type strains of known *Photobacterium* species were included in these analyses as positive controls.

16S rRNA gene sequence analysis revealed that the four isolates formed a tight monophyletic group affiliated to the genus *Photobacterium* (Fig. 1). The four novel isolates formed a tight cluster with more than 99 % 16S rRNA gene sequence similarity. The closest neighbours of the novel isolates were the type strains of *Photobacterium leiognathi* (97.4 % sequence similarity), *P. rosenbergii* and *P. halotolerans* (both 97.1 % sequence similarity), *P. lutimaris* and *P. angustum* (both 97.0 % similarity). The 16S rRNA gene sequence similarity towards other *Photobacterium* species with validly published names was below 96.5 %. Phylogenetic analysis based on 16S rRNA, *recA*, *topA*, *ftsZ*, *mreB* and *rpoA* gene sequences (4949 nt in total) confirmed that the isolates form a tight group related to *P. rosenbergii* (Fig. 2). The novel isolates shared less than 95 % concatenated gene sequence similarity with their closest neighbour, indicating clearly that they belong to a novel *Photobacterium* species.

DDH experiments were performed with all four isolates and the type strains of the closest phylogenetic neighbours. Relatedness between the novel isolates varied between 84 and 97 %, showing that they belong to a single species. The novel isolate R-40508^T had only 19, 15, 11 and 9 % DDH relatedness with *P. lutimaris* LMG 25278^T, *P. rosenbergii* LMG 22223^T, *P. leiognathi* LMG 4228^T and *P. halotolerans* LMG 22194^T, respectively. Standard deviations of all hybridization experiments were below 10 %. Clearly, the DDH data demonstrate that the four isolates represent a novel species of the genus *Photobacterium*.

Several phenotypic features can be used to differentiate the novel species from its closest phylogenetic neighbours. The novel species has gelatinase and lipase activity and it can ferment melibiose, but it cannot grow on 6 % NaCl (Table 2). In addition, the novel species lacks the fatty acids $C_{17:0}$, $C_{17:0}$ cyclo, iso-$C_{17:0}$, $C_{17:1}\omega8c$ and iso-$C_{17:1}\omega9c$, which are commonly found in *Photobacterium* and *Vibrio* species (Table 3). For instance, iso-$C_{17:0}$ is found in *P. leiognathi*, *P. rosenbergii* and *P. lutimaris*, whereas $C_{17:0}$ is found in *P. leiognathi* and *P. halotolerans*. Phenotypic and chemotaxonomic variation was observed among the strains of the novel species, suggesting a good representation of the phenotype of the novel group (Supplementary Table S2). Based on the polyphasic analysis presented in this study, we propose to classify the four isolates in the novel species *Photobacterium jeanii* sp. nov. Another species of the genus has been described as '*Photobacterium swingsii*' (Gomez-Gil *et al.*, 2011), although the paper describing this organism is currently in press and the name has not yet been validly published, hence our use of a first name in forming the epithet.

Table 3. Cellular fatty acid contents of the novel isolates (*P. jeanii* sp. nov.) and related taxa of the genus *Photobacterium*

Strains: 1, *P. jeanii* sp. nov. R-40508^T, R-40507, R-40903 and R-21419 (range of values); 2, *P. leiognathi* LMG 4228^T; 3, *P. rosenbergii* LMG 22223^T; 4, *P. lutimaris* LMG 25278^T; 5, *P. halotolerans* LMG 22194^T. Data were obtained in this study and are expressed as percentages of total fatty acids. Fatty acids representing <1 % in all strains are not shown; −, not detected or <1 %.

Fatty acid	1	2	3	4	5
$C_{12:0}$	2.8–3.6	3.9	3.2	2.5	5.5
$C_{12:0}$ 3-OH	2.5–3.9	2.6	3.5	2.3	6.5
$C_{14:0}$	3.4–4.5	4.2	3.0	2.5	−
iso-$C_{15:0}$	0–2.0	1.0	2.0	1.6	−
$C_{16:0}$	19.5–21.9	26.0	12.5	17.5	18.5
iso-$C_{16:0}$	1.9–3.5	−	−	−	2.0
$C_{17:0}$	−	1.6	−	−	1.5
$C_{17:0}$ cyclo	−	10.6	−	−	−
iso-$C_{17:0}$	−	1.2	6.0	1.8	−
$C_{17:1}\omega8c$	−	−	−	−	1.6
iso-$C_{17:1}\omega9c$	−	−	1.5	−	−
$C_{18:1}\omega6c$	0–10.9	−	9.3	5.0	−
$C_{18:1}\omega7c$	8.5–15.0	10.0	8.5	16.0	22.5
Summed feature 2*	2.4–3.5	3.2	3.5	2.6	4.5
Summed feature 3*	37.2–45.0	30.5	39.0	42.0	27.0

*Summed features represent groups of two or three fatty acids that cannot be separated by the MIDI System. Summed feature 2 comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain-length of 10.928 and/or $C_{12:0}$ ALDE; summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$.

Description of *Photobacterium jeanii* sp. nov.

Photobacterium jeanii (jea′ni.i. N.L. gen. n. *jeanii* of Jean, after the Belgian microbiologist Jean Swings).

Colonies are convex, round (1 mm in diameter), beige and opaque with entire and smooth margins after 2 days at 28 °C on MA. Cells are small coccobacilli, Gram-negative, motile and catalase- and oxidase-positive, 2–2.5 μm long and 1–2 μm wide after 1 day at 28 °C on MA. Green colonies with poor growth appear on the selective medium TCBS. Prolific growth occurs between 20 and 35 °C and at NaCl concentrations of 0.5–2 % (w/v) in TSA. No growth appears at 4, 7 or 42 °C or in 0 or 6 % NaCl. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase (weak reaction), trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase (except R-40903), α-glucosidase, *N*-acetyl-β-glucosaminidase (except R-21419 and R-40508^T), arginine dihydrolase, acetoin production (weak for R-40508^T and R-40903) and gelatinase. Cells ferment glucose, meli-

biose and sucrose (except R-40507 and R-40903) and reduce nitrate to nitrite. Cystine arylamidase, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities are absent. Citrate is not utilized and H_2S and indole are not produced. Mannitol (except R-40903), inositol, sorbitol, rhamnose, amygdalin and arabinose are not fermented. The DNA G+C content of the type strain is 44.5 mol%. The most abundant cellular fatty acids are summed feature 3 (iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$), $C_{16:0}$, $C_{18:1}\omega7c$ and $C_{18:1}\omega6c$. The following fatty acids are detected in small amounts: $C_{14:0}$, iso-$C_{16:0}$, $C_{12:0}$, $C_{12:0}$ 3-OH, summed feature 2 ($C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I, an unidentified fatty acid with an equivalent chain-length of 10.928 and/or $C_{12:0}$ ALDE) and iso-$C_{15:0}$.

The type strain is R-40508^T (=LMG 25436^T =CAIM 1817^T), isolated from mucus of the zoanthid *Palythoa caribaeorum* in the São Sebastião channel, Brazil.

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