Description of *Catellibacterium caeni* sp. nov., reclassification of *Rhodobacter changlensis* Anil Kumar *et al.* 2007 as *Catellibacterium changlense* comb. nov. and emended description of the genus *Catellibacterium*

Jin-Wei Zheng,[1]† Yi-Guang Chen,[2,3]† Jun Zhang,[1] Ying-Ying Ni,[1] Wen-Jun Li,[2,4] Jian He[1] and Shun-Peng Li[1]

Correspondence
Wen-Jun Li
wjli@ynu.edu.cn
Jian He
hejian@njau.edu.cn

[1]Key Laboratory for Microbiological Engineering of Agricultural Environment of Ministry of Agriculture, Nanjing Agricultural University, Nanjing 210095, PR China

[2]Key Laboratory of Microbial Diversity in Southwest China Ministry of Education, Yunnan Institute of Microbiology, Yunnan University, Kunming 650091, PR China

[3]College of Biology and Environmental Science, Jishou University, Jishou 416000, PR China

[4]Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China

A novel non-sporulating, non-motile, catalase- and oxidase-positive, strictly aerobic, Gram-negative, rod-shaped bacterial strain, designated DCA-1^T, was isolated from activated sludge collected from a butachlor wastewater treatment facility. The strain was able to degrade about 85 % of 100 mg butachlor l^{-1} within 5 days of incubation. Growth occurred in the presence of 0–6 % (w/v) NaCl [optimum, 1 % (w/v) NaCl] and at pH 5.5–9.0 (optimum, pH 7.0) and 15–35 °C (optimum, 25–30 °C). Vesicular internal membrane structures and photoheterotrophic growth were not observed. The major respiratory quinone was ubiquinone 10 (Q-10) and the major cellular fatty acids were $C_{18:1}\omega7c$ and 11-methyl $C_{18:1}\omega7c$. The genomic DNA G+C content of strain DCA-1^T was 62.5 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparison revealed that strain DCA-1^T was a member of the family *Rhodobacteraceae* and was related most closely to the type strain of *Catellibacterium aquatile* (96.5 % sequence similarity). The combination of phylogenetic analysis, phenotypic characteristics and chemotaxonomic data supports the suggestion that strain DCA-1^T represents a novel species of the genus *Catellibacterium*, for which the name *Catellibacterium caeni* sp. nov. is proposed. The type strain is DCA-1^T (=CGMCC 1.7745^T =DSM 21823^T). In addition, based on the characterization data obtained in this study, it is proposed that *Rhodobacter changlensis* should be reclassified as *Catellibacterium changlense* comb. nov. (type strain JA139^T =DSM 18774^T =CCUG 53722^T =JCM 14338^T). An emended description of the genus *Catellibacterium* is also presented.

The genus *Catellibacterium*, belonging to the family *Rhodobacteraceae*, was first proposed by Tanaka *et al.* (2004) with the description of *Catellibacterium nectariphilum*. Recently, a second species, *Catellibacterium aquatile*, was proposed by Liu *et al.* (2010), with an emended description of the genus. During an investigation of the microbial diversity of a butachlor [*N*-(butoxymethyl)-2-chloro-*N*-(2,6-diethylphenyl)acetamide] wastewater treatment facility in Jiangsu Province, China, a butachlor-degrading, Gram-negative bacterium, designated strain DAC-1^T, was isolated from activated sludge. Based on the results of a polyphasic taxonomic study, this strain is considered to represent a novel species of the genus *Catellibacterium*.

Strain DCA-1^T was isolated from an activated sludge sample by plating 1 : 10 serial dilutions of the sample on Luria–Bertani (LB) agar (Atlas & Parks, 1993) supplemented with

†These authors contributed equally to this work.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DCA-1^T is FJ386516.

Three supplementary figures are available with the online version of this paper.

100 mg butachlor l^{-1} at 30 °C for 5 days. After primary isolation, the strain was purified by repeated streaking and subculturing (four or five times) on LB agar plates and examining the cultures by light microscopy. The isolate was preserved both on LB agar slants at 4 °C and as 20 % (v/v) glycerol stocks at −80 °C. Three reference strains, *C. aquatile* A1-9^T, *C. nectariphilum* JCM 11959^T and *Rhodobacter changlensis* JCM 14338^T, which were employed as controls in phenotypic tests (including chemotaxonomic studies), were obtained from the Institute of Microbiology, Chinese Academic Sciences (Beijing, China), and the Japan Collection of Microorganisms (Wako, Saitama, Japan). Unless indicated otherwise, morphological, physiological, biochemical, molecular and chemotaxonomic studies were performed with cells grown on LB/10 agar (1.0 g tryptone, 0.5 g yeast extract, 1.0 g NaCl and 15 g agar l^{-1}, pH 7.0) at 30 °C.

Cell morphology was examined by using light microscopy (BH-2; Olympus) and transmission electron microscopy (JEOL; Japan). Internal membrane structures were viewed with a transmission electron microscope after the cells had been processed as described by Hanada *et al.* (2002). Gram staining and the KOH lysis test were carried out according to Smibert & Krieg (1994) and Gregersen (1978), respectively. Growth was tested at 4 °C and at 5–50 °C (in increments of 5 °C) and at pH 5.0–11.0 (in increments of 0.5 pH units) on LB/10 agar as well as in LB/10 medium. Tolerance of NaCl was tested on LB/10 agar as well as in LB/10 medium at 0–8 % (w/v) NaCl (in increments of 0.5 %). Indole production and the Voges–Proskauer test were assessed as described by Smibert & Krieg (1994). Decomposition of starch, Tweens 20 and 80 and tyrosine was determined as described by Cowan & Steel (1965). Nitrate reduction was determined as described by Lányí (1987). Utilization of various carbon compounds was tested in a minimal medium [per litre deionized water: 1 g $(NH_4)_2SO_4$, 0.05 g yeast extract, 0.5 g KH_2PO_4, 0.4 g $MgSO_4.7H_2O$, 0.4 g NaCl, 0.075 g $CaCl_2.2H_2O$, 5 ml 0.1 % ferric citrate solution, 1 ml trace element solution (per litre deionized water: 0.1 g $ZnSO_4.7H_2O$, 0.03 g $MnCl_2.4H_2O$, 0.3 g H_3BO_4, 0.2 g $CoCl_2.6H_2O$, 0.01 g $CuCl_2.2H_2O$, 0.02 g $NiCl_2.6H_2O$, 0.03 g $Na_2MoO_4.2H_2O$)]. Carbon sources were added at a final concentration of 4.0 g l^{-1}. Growth was examined by measuring the turbidity of cultures incubated at 30 °C in 150 ml Erlenmeyer flasks containing 40 ml medium. Growth under anaerobic conditions was determined on LB agar supplemented with 0.5 % (w/v) glucose with or without 0.1 % (w/v) nitrate by using the GasPak Anaerobic System (BBL) according to the manufacturer's instructions. Phototrophic growth was assessed in minimal medium in completely filled screw-capped bottles under incandescent illumination (2000 lx); sodium succinate, sodium malate, sodium pyruvate, sodium citrate and glucose (4.0 g l^{-1}) were added as electron donors. The ability of the strain to degrade butachlor was determined according to the methods described by Chakraborty & Bhattacharyya (1991). *In vivo* pigment-absorption spectrum analysis was examined as described by Yoon *et al.* (2004) using a UV-Vis spectrophotometer (UV-2450; Shimadzu). Observation of motility and tests of catalase and oxidase activities were conducted as described previously (Chen *et al.*, 2007). Other enzyme activities and physiological and biochemical tests were also conducted by using API ZYM and API 20NE strips (bioMérieux) according to the manufacturer's instructions.

Strain DCA-1^T was strictly aerobic and the cells were Gram-negative, non-motile, non-sporulating rods (Supplementary Fig. S1, available in IJSEM Online). Colonies were pale yellow, convex and circular with entire margins. The strain grew in presence of 0–6 % (w/v) NaCl (optimum 1 %), at 15–35 °C (optimum 25–30 °C) and at pH 5.5–9.0 (optimum pH 7.0). Photoheterotrophic growth was not observed. Vesicular internal membrane structures and photosynthetic pigments were not detected (Supplementary Fig. S1). Strain DCA-1^T was able to degrade about 85 % of 100 mg butachlor l^{-1} initially added to the minimal medium within 5 days (Supplementary Fig. S2). Detailed morphological, physiological and biochemical characteristics of strain DCA-1^T are summarized in the species description and in Table 1.

The 16S rRNA gene sequence was amplified by PCR and sequenced as described by Cui *et al.* (2001). Pairwise sequence similarity was calculated by using a global alignment algorithm, implemented at the EzTaxon server (Chun *et al.*, 2007). Phylogenetic analysis was performed by using the software package MEGA version 4.1 (Tamura *et al.*, 2007) after multiple alignment of the sequence data with CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated by using distance options according to Kimura's two-parameter model (Kimura, 1980) and clustering was performed with the neighbour-joining method (Saitou & Nei, 1987). Maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) trees were generated by using the treeing algorithms contained in the PHYLIP package (Felsenstein, 2002). Confidence values for the branches of phylogenetic trees were determined by using bootstrap analyses (based on 1000 resamplings) (Felsenstein, 1985).

An almost-complete 16S rRNA gene sequence (1435 nt) was determined. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain DCA-1^T was a member of the family *Rhodobacteraceae* and was related most closely to the type strain of *C. aquatile* (96.5 % sequence similarity); less than 95.5 % 16S rRNA gene sequence similarity was observed with other species of the *Rhodobacteraceae*. In the neighbour-joining phylogenetic tree, strain DCA-1^T formed a separate lineage with the type strains of *C. aquatile*, *R. changlensis* (95.4 %; Anil Kumar *et al.*, 2007) and *C. nectariphilum* (94.3 %) (Fig. 1). Moreover, *R. changlensis* was also robustly included in the clade formed by the type strains of *Catellibacterium* species. The topology was similar to those of phylogenetic trees reconstructed by using the maximum-likelihood and maximum-parsimony methods (Supplementary Fig. S3).

Table 1. Phenotypic characteristics that differentiate strain DCA-1^T from closely related type strains

Strain: 1, DCA-1^T (*Catellibacterium caeni* sp. nov.); 2, *C. aquatile* A1-9^T; 3, *C. nectariphilum* JCM 11959^T; 4, [*R.*] *changlensis* JCM 14338^T (*Catellibacterium changlense* comb. nov.). All data were obtained in this study. All strains are non-sporulating, non-motile and Gram-negative and are positive for activity of catalase, esterase (C4), esterase lipase (C8), leucine arylamidase and oxidase, but negative for anaerobiosis, indole production, fermentation of glucose, hydrolysis of gelatin, nitrate reduction, activity of *N*-acetyl-β-glucosaminidase, arginine dihydrolase, cystine arylamidase, α-fucosidase, α-galactosidase, β-galactosidase (*p*-nitrophenyl-β-D-galactopyranosidase), β-glucuronidase, lipase (C14), α-mannosidase and trypsin and utilization of benzoate, xylose, trehalose and phenylacetic acid.

Characteristic	1	2	3	4
Colony pigmentation	Pale yellow	White	White to beige	Yellowish brown
Motility	−	+	−	−
Photoheterotrophic growth	−	−	−	+
Ranges for growth				
Temperature (°C)	15–35	10–40	20–35	5–35
pH	5.5–9.0	5.5–9.0	6.0–8.0	6.5–9.0
NaCl (%, w/v)	0–6	0–1	0–2.5	0–4
H_2S production	−	+	−	−
Hydrolysis of:				
Starch	−	−	+	−
Tween 20	−	+	−	−
Tween 80	−	+	+	−
Tyrosine	−	+	−	−
API ZYM results				
Alkaline phosphatase	+	−	+	+
Valine arylamidase	−	−	+	+
α-Chymotrypsin	−	−	−	+
Acid phosphatase	+	+	+	−
Naphthol-AS-BI-phosphohydrolase	+	−	−	−
α-Glucosidase	+	+	−	−
β-Glucosidase	−	+	−	−
API 20NE results				
Urease	−	−	−	+
Hydrolysis of aesculin	−	+	−	−
Utilization of:*				
Glucose	+	−	−	+
Arabinose	−	+	−	+
Sucrose	+	−	−	+
Fructose	+	−	−	+
Galactose	+	−	−	+
Maltose	+	−	−	+
Glutamate	−	−	+	+

*Utilization of various carbon sources was tested in minimal medium with each carbon source added at a final concentration of 4.0 g l^{-1}.

Therefore, it would appear that, on the basis of the phylogenetic data, strain DCA-1^T represents a novel species of the genus *Catellibacterium* according to accepted criteria (Stackebrandt & Goebel, 1994), and that *R. changlensis* is highly related to the genus *Catellibacterium*.

DNA was isolated according to the method of Hopwood *et al.* (1985) and the G+C content was determined by using the thermal denaturation method (Mandel & Marmur, 1968). Analysis of respiratory quinones was carried out by the Identification Service of the DSMZ and Dr Brian Tindall (DSMZ, Braunschweig, Germany). Fatty acids were determined as described by Sasser (1990) using the Microbial Identification System (MIDI; Microbial ID).

The chemotaxonomic data obtained for strain DCA-1^T were consistent with the assignment of the strain to the genus *Catellibacterium* (Tanaka *et al.*, 2004; Liu *et al.*, 2010). The DNA G+C content of strain DCA-1^T was 62.5 mol%. The strain contained ubiquinone 10 (Q-10) (87.4 %) as the predominant respiratory quinone. The fatty acid profile of strain DCA-1^T was similar to those of the type strains of the two *Catellibacterium* species and *R. changlensis*, although there were differences in the proportions of some components (Table 2). The major fatty acids

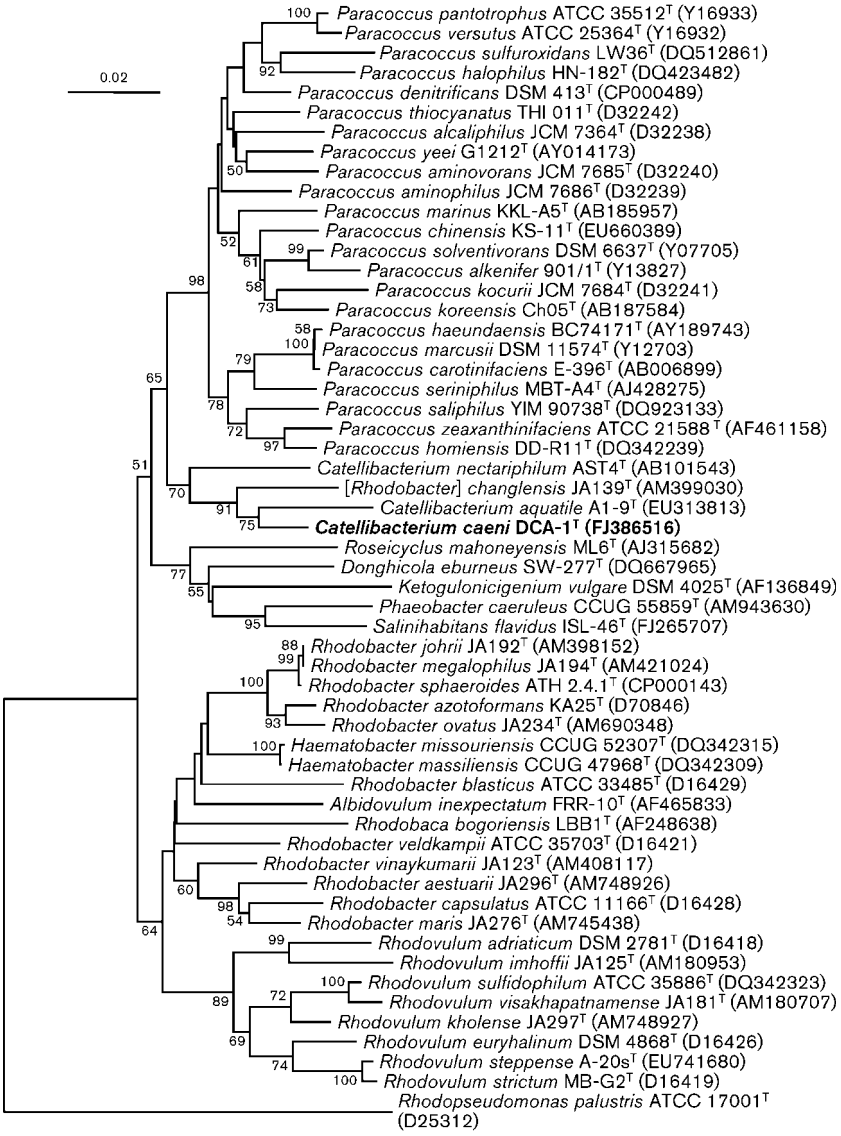

Fig. 1. Phylogenetic tree showing the position of strain DCA-1^T among related taxa based on 16S rRNA gene sequences reconstructed by using the neighbour-joining method. Numbers at nodes are bootstrap percentages (>50 %) based on a neighbour-joining analysis of 1000 resampled datasets. Bar, 2 substitutions per 100 nucleotide positions.

(>10 % of the total) of strain DCA-1^T were $C_{18:1}\omega7c$ (67.7 %) and 11-methyl $C_{18:1}\omega7c$ (13.3 %).

On the basis of the results of the phylogenetic analysis and of morphological and chemotaxonomic investigations, strain DCA-1^T represents a novel species of the genus *Catellibacterium*, for which the name *Catellibacterium caeni* sp. nov. is proposed.

Rhodobacter changlensis was proposed by Anil Kumar *et al.* (2007) based on the fact that the type strain had some characteristics typical of genus *Rhodobacter*, such as the presence of intracytoplasmic membrane structures and carotenoids and phototrophic growth, but there was strong evidence from our phylogenetic analysis that *R. changlensis* was highly related to members of the genus *Catellibacterium* and was clearly separated from other members of the genus *Rhodobacter* in phylogenetic trees (three tree-making methods). Additionally, the fatty acid profile and DNA G+C content of *R. changlensis* JCM 14338^T were similar to those of the type strains of the two *Catellibacterium* species and strain DCA-1^T. Accordingly, it is also proposed that *Rhodobacter changlensis* should be reclassified as *Catellibacterium changlense* comb. nov. This proposal necessitates an emended description of the genus *Catellibacterium*.

Description of *Catellibacterium caeni* sp. nov.

Catellibacterium caeni (cae′ni. L. gen. n. *caeni* of sludge).

Cells are non-sporulating, non-motile, catalase- and oxidase-positive, strictly aerobic, Gram-negative rods, approximately 0.6–0.7 μm wide and 1.8–4.5 μm long. Colonies are pale yellow-pigmented, convex and non-translucent with circular margins, 1–2 mm in diameter after incubation on LB/10 agar at 30 °C for 2–3 days. No diffusible pigments are produced. Growth occurs in 0–6 % (w/v) NaCl (optimum, 1 %) at pH 5.5–9.0 (optimum,

Table 2. Cellular fatty acid contents of strain DCA-1^T and closely related type strains

Strain: 1, DCA-1^T (*C. caeni* sp. nov.); 2, *C. aquatile* A1-9^T; 3, *C. nectariphilum* JCM 11959^T; 4, [*R.*] *changlensis* JCM 14338^T (*C. changlense* comb. nov.). Data are percentages of total fatty acids. Fatty acids that represented <1.0 % for all strains are omitted. –, Not detected.

Fatty acid	1	2	3	4
$C_{10:0}$ 3-OH	5.3	–	3.0	4.5
$C_{14:0}$	0.2	2.0	0.8	0.4
$C_{16:0}$	1.3	15.8	6.8	1.2
$C_{16:0}$ 2-OH	0.2	4.5	1.5	0.2
$C_{17:0}$	0.1	1.0	2.4	0.5
$C_{18:0}$	1.9	16.7	9.1	3.4
$C_{18:1}\omega7c$	67.7	49.0	57.2	71.4
$C_{18:1}\omega9c$	–	3.7	1.6	–
11-Methyl $C_{18:1}\omega7c$	13.3	5.7	1.3	1.3
$C_{18:0}$ 3-OH	2.1	0.8	2.9	0.1
$C_{19:0}$ cyclo $\omega8c$	–	–	9.9	–
10-Methyl $C_{19:0}$	1.8	–	0.7	0.5
Summed feature 3*	3.1	–	0.7	7.0
Summed feature 7*	2.0	–	1.3	6.1

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$. Summed feature 7 contained unknown ECL 18.846 and/or $C_{19:1}\omega6c$.

pH 7.0) and 15–35 °C (optimum, 25–30 °C). Vesicular internal membrane structures and photosynthetic pigments are not present. Photoheterotrophic growth is not observed. Negative for urease activity, nitrate reduction, indole and H_2S production and decomposition of starch, Tweens 20 and 80 and tyrosine. The following compounds are utilized as carbon sources: fructose, D-galactose, glucose, maltose, sucrose, 2,3-butanediol, glycerol, methanol, D-sorbitol, propionic acid, acetate, citrate, formate, malate, pyruvate and succinate. The following substances are not utilized: L-arabinose, lactose, trehalose, xylose, glutamate, adonitol, D-mannitol, D-ribose, benzoate and phenylacetic acid. Major fatty acids (>10 % of the total) are $C_{18:1}\omega7c$ and 11-methyl $C_{18:1}\omega7c$. The major isoprenoid quinone is Q-10. The DNA G+C content of the type strain is 62.5 mol%. Additional physiological, biochemical and chemotaxonomic properties are listed in Tables 1 and 2.

The type strain, DCA-1^T (=CGMCC 1.7745^T =DSM 21823^T), was isolated from activated sludge in a butachlor wastewater treatment facility in Jiangsu Province, China.

Description of *Catellibacterium changlense* Anil Kumar *et al.* 2007 comb. nov.

Catellibacterium changlense (chang.len′se. N.L. neut. adj. *changlense* of or pertaining to Changla Pass, the location from where the type strain was isolated).

Basonym: *Rhodobacter changlensis* Anil Kumar *et al.* 2007.

The description is the same as that given for *Rhodobacter changlensis* by Anil Kumar *et al.* (2007) with some changes listed in Tables 1 and 2. The type strain is JA139^T (=DSM 18774^T =CCUG 53722^T =JCM 14338^T).

Emended description of the genus *Catellibacterium* Tanaka *et al.* 2005 emend. Liu *et al.* 2010

The formal description as given by Tanaka *et al.* (2004) and emended by Liu *et al.* (2010) remains correct except that some species are photoheterotrophic.

Acknowledgements

We are grateful to Dr Susanne Verbarg and Dr B. J. Tindall (DSMZ) for the analysis of quinones, Mr Zheng Liu-Qiang for technical support and Dr Z.-P. Liu for providing the type strain *C. aquatile* A1-9^T. This work was supported by the National High Technology Research and Development Program of China (2006AA10Z402), the National Basic Research Program of China (no. 2010CB833800), the Natural Science Foundation of Jiangsu Province, China (BK2008331), and the Chinese National Technology Platform Programs (2005DKA21201-2).

References

Anil Kumar, P., Srinivas, T. N. R., Sasikala, Ch. & Ramana, Ch. V. (2007). *Rhodobacter changlensis* sp. nov., a psychrotolerant, phototrophic alphaproteobacterium from the Himalayas of India. *Int J Syst Evol Microbiol* **57**, 2568–2571.

Atlas, R. M. & Parks, L. C. (1993). *Handbook of Microbiological Media.* Edited by L. C. Parks. Boca Raton, FL: CRC Press.

Chakraborty, S. K. & Bhattacharyya, A. (1991). Degradation of butachlor by two soil fungi. *Chemosphere* **23**, 99–105.

Chen, Y.-G., Cui, X.-L., Pukall, R., Li, H.-M., Yang, Y.-L., Xu, L.-H., Wen, M.-L., Peng, Q. & Jiang, C.-L. (2007). *Salinicoccus kunmingensis* sp. nov., a moderately halophilic bacterium isolated from a salt mine in Yunnan, south-west China. *Int J Syst Evol Microbiol* **57**, 2327–2332.

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria.* London: Cambridge University Press.

Cui, X.-L., Mao, P.-H., Zeng, M., Li, W.-J., Zhang, L.-P., Xu, L.-H. & Jiang, C.-L. (2001). *Streptimonospora salina* gen. nov., sp. nov., a new member of the family *Nocardiopsaceae. Int J Syst Evol Microbiol* **51**, 357–363.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Felsenstein, J. (2002). PHYLIP (phylogeny inference package), version 3.6a. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.

Hanada, S., Takaichi, S., Matsuura, K. & Nakamura, K. (2002). *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. *Int J Syst Evol Microbiol* **52**, 187–193.

Hopwood, D. A., Bibb, M. J., Chater, K. F., Kieser, T., Bruton, C. J., Kieser, H. M., Lydiate, D. J., Smith, C. P. & Ward, J. M. (1985). *Genetic Manipulation of Streptomyces: a Laboratory Manual*. Norwich: John Innes Foundation.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

Lányí, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.

Liu, Y., Xu, C.-J., Jiang, J.-T., Liu, Y.-H., Song, X.-F., Li, H. & Liu, Z.-P. (2010). *Catellibacterium aquatile* sp. nov., isolated from freshwater and emended description of the genus *Catellibacterium* Tanaka *et al.* 2004. *Int J Syst Evol Microbiol* **60**, 2027–2031.

Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*. MIDI Technical Note 101. Newark, DE: MIDI, Inc.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetic analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tanaka, Y., Hanada, S., Manome, A., Tsuchida, T., Kurane, R., Nakamura, K. & Kamagata, Y. (2004). *Catellibacterium nectariphilum* gen. nov., sp. nov., which requires a diffusible compound from a strain related to the genus *Sphingomonas* for vigorous growth. *Int J Syst Evol Microbiol* **54**, 955–959.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Yoon, J.-H., Yeo, S.-H. & Oh, T.-K. (2004). *Hongiella marincola* sp. nov., isolated from sea water of the East Sea in Korea. *Int J Syst Evol Microbiol* **54**, 1845–1848.